APPLICATION OF ULTRASOUND TECHNIQUE IN THE EXTRACTION OF PROTEINS FROM CHICKPEA (*CICER ARIETINUM L.)*

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GENERAL INFORMATION

Received date: 13/03/2024

Revised date: 17/05/2024

Accepted date: 20/07/2024

KEYWORD

Chickpea 1;

Protein 2;

Ultrasound 3;

ABSTRACT

This study explores the potential of chickpea protein as a nutritious alternative to animal protein. The study investigated the effects of water/powder ratio, ultrasound, and coagulation methods on chickpea protein recovery efficiency. The results showed that a water/powder ratio of 8/1 was optimal for protein extraction. In addition, when comparing the thermal precipitation method (60°C) and the acid precipitation method (5% acetic acid), the method of using heat was more efficient. Finally the study demonstrated that ultrasound, especially the Degas mode at 40Hz, significantly improved protein recovery with an efficiency of 43.76% and 43.05g of precipitated protein.

1. INTRODUCTION

Chickpea (*Cicer arietinum L.)* was a high-nutrient crop with a carbohydrate content (40–60%), protein (15–22%), essential fats (4–8%), and various minerals and vitamins (Madurapperumage et al., 2021; Hevryk et al., 2020). The fat composition includes palmitic (10.8%), oleic (33.5%), linoleic acid (49.7%), and linolenic (2.4%), with antioxidant compounds tocopherols (230.3 mg/100g oil) and carotenoids (46.3 µg/100g flour) (Ferreira et al., 2019 ; Şengül et al., 2020). Moreover, chickpeas were rich in essential amino acids (Dutta et al., 2022) and had a low glycemic index, which helps regulate blood glucose levels (Kim et al., 2016).

The fatty acid composition of the seeds enhances value, as fats impact the structure, shelf life, flavor, aroma, and nutritional composition of chickpea-derived food products (Madurapperumage et al., 2021). Chickpeas had been shown to lower cholesterol and blood sugar levels. Therefore, chickpeas can be included in a healthy diet to promote overall health and reduce the risk of cardiovascular diseases and diabetes (Rehm et al., 2023).

The growing global population has led to a growing demand for plant-based protein, with chickpeas emerging as a significant alternative protein source globally. From a nutritional perspective, these proteins had diverse biological activities, containing essential amino acids and protein digestibility. Technologically, they were odorless, had a neutral flavor, and a suitable light color to be used as raw materials for developing new products such as noodles, bread, cookies, and sausages (Boukid et al., 2021). Chickpea protein has various functions, such as high solubility, good water and oil absorption, emulsification, foaming, gelation, and being rich in essential amino acids (Nkhata et al., 2014; Grasso et al., 2022). This protein can be extracted through dry or wet methods. A few basic dry and wet methods for increasing protein levels are alkaline/acid extraction, salt extraction, isoelectric precipitation, and membrane filtration (Boukid et al., 2021). The protein extraction method from chickpeas was studied from chickpea by isoelectric extraction method (Glusac et al., 2020). Investigate extraction strategies using current equipment for regional used to lower the initial investment expenses.

It was possible to achieve protein precipitation through a variety of methods. One method involves using heat to induce protein precipitation (Lyu et al., 2021). You could also use mechanical methods to induce protein precipitation. The mechanical protein precipitation method used vortex flow to mechanically induce conformational change and protein precipitation (Lyu et al., 2021). In addition, studies also used acid protein extraction to recover proteins (Boukid et al., 2021). To support the extraction process better, researchers also used ultrasound during the protein extraction process (Ly et al., 2018).

2. METHODOLOGY

2.1. Material

Chickpeas were purchased at Tan Hiep market, Bien Hoa City, Dong Nai Province and imported from Canada. It's cleaned and checked worm in the laboratory of Dong Nai University of Technology before use.

2.2. Methods

The determination of the water-to-bean ratio and chickpea protein recovery efficiency was performed as follows: accurately weigh the chickpeas, soak them for 2 hours with water-to-flour ratios of 6/1; 7/1; 8/1; 9/1. Then grind and filter the mixture, extract the milk solution, and discard the residue. During filtration, regrind and filter the residue multiple times to extract all soluble substances in the milk solution. Boil the milk solution at 60°C until complete precipitation, continue to filter to collect the protein; and dry at 50°C to reach moisture equilibrium.

The protein content and protein recovery efficiency of the product were analyzed in this experiment.

Effect of precipitation method on protein recovery:

The method applied was protein precipitation using heat (60°C) and acid (5% acetic acid) to compare the amount of recovered protein. After complete precipitation, continue filtering, recover the protein, and dry it at a temperature of 50°C to a constant weight.

The influence of ultrasound treatment on the protein recovery efficiency of chickpeas:

The experimental samples were sequentially treated with ultrasound in normal, soft, and degas modes at a frequency of 40Hz and with different waveforms (Fig. 2). We did not treat the control sample with ultrasound waves. The powdered beans were preliminary processed, and then the milk solution (chickpea milk) was boiled at 60°C combined with ultrasound until the solution completely precipitated, then filtered. The protein was collected and dried at 50°C to a constant weight.

Method of data analysis and processing:

The Kjeldahl method determined the total protein content. Use concentrated sulfuric acid to digest the sample, converting the nitrogen into ammonium sulfate. Utilize concentrated alkali to extract the ammonia from the ammonium sulfate in the nitrogen distillation machine; this results in the formation of ammonium hydroxide, which then undergoes quantification using acid.

The moisture content of the sample was determined by drying to a constant weight at 105°C using an OHAUS-MB23 moisture balance.

Between-group variances were analyzed using one-way analysis of variance (ANOVA).

Here's how to express the formula for product recovery efficiency:

Protein recovery efficiency (%) = Amount of protein obtained / Initial mass of substance

Amount of protein obtained: the mass of the product obtained after the production process.

Initial mass of substance: volume of input materials before proceeding with the production process.

3. FINDINGS AND DISCUSSION

3.1. The influence of water-to- chickpea ratio on the recovery efficiency of chickpea protein

The water-to-flour ratio was an important factor influencing the protein recovery efficiency of chickpeas. The proper water ratio helps the flour absorb water fully and quickly to reach a doughy state, creating favorable conditions for proteins and other water-soluble substances.

When the water-to-flour was too low, the chickpea milk mixture can become too thick, making it difficult to separate the protein. The experimental results (Table 1) show that the amount of precipitate obtained with a water-to-flour ratio of 6:1 was only about 37.37g; increasing the amount of water increases the recovery rate of the precipitate, reaching 47.05g with a ratio of 8:1. Conversely, a ratio that was too high will reduce the viscosity of the mixture, but if the appropriate filter cloth was not selected during filtration, some material may be easily lost based on the amount of filtered water (a ratio of 9:1 - with 46.13g of precipitate obtained).

Table 1. The difference in water/chickpea flour ratio affects the amount of protein obtained

Ratio of water/chickpea flour	Amount of protein obtained (g)
6/1	$37.37^{c} \pm 0.181$
7/1	$43.49^{b} \pm 0.449$
8/1	$47.05^{a} \pm 0.214$
9/1	$46.13^{a} \pm 0.326$

Values in the same column followed by different characters had a significant difference in meaning p = 0,05

Table 2: The effect of water/chickpea meal ratio on protein recovery efficiency.

Ratio of water/chickpea flour	Recovery efficiency (%)
6/1	$33.12^{b} \pm 0.181$
7/1	$38.4^{a} \pm 0.449$
8/1	$39.84^{a} \pm 0.214$
9/1	$38.3^{a} \pm 0.326$

Values in the same column followed by different characters had a significant difference in meaning p = 0,05

Table 2 clearly shows that the water/flour ratio also influences the protein recovery efficiency of chickpea flour. Different water ratios will yield different protein amounts from the same amount of raw materials. The research results show that when adding water from the ratio of 6/1 - 8/1, the protein recovery efficiency increases from 33.12% to 39.84% and decreases to 18.3% when increasing the water amount to the ratio of 9/1.

Therefore, the 8/1 water to soybean ratio was the most suitable for extracting chickpea protein for high protein recovery efficiency (39.84%) and high solid yield (47.05g).

3.2. The impact of the precipitation method on the protein recovery amount

Carry out a survey of 2 experimental samples with 2 precipitation methods, namely heat precipitation and organic acid precipitation. The experimental results show that with the heat precipitation method, the amount of precipitated protein was 49g, while using the organic acid precipitation method results in a lower amount of precipitate (32.93g) (Figure .1).

The data in Figure. 1 shows a significant difference in the protein content of the product when using two different methods of precipitation. The protein content in the corresponding product of the heat precipitation method and the acid precipitation method were 17.97g and 13.07g, respectively.

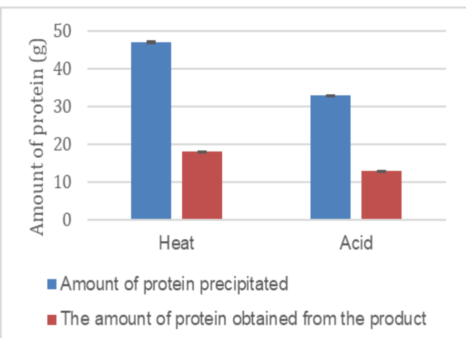

Figure .1. The influence of precipitation method on the amount of recovered protein

In addition, the heat precipitation method was superior to the organic acid precipitation method not only in terms of the protein yield, low cost but also in that it does not take much time to implement and does not leave behind the sour odor of acid. Therefore, the heat method was suitable for precipitating chickpea protein.

3.3. The influence of ultrasound treatment on the recovery efficiency of chickpea protein

The experiment surveyed 4 samples: normal, soft, degas treated with 40Hz ultrasonic waves, and heat samples not treated with ultrasonic waves. The experimental results show that changing the treatment mode (different waveforms at the same frequency) results in a significant difference in the amount of precipitate obtained. The precipitate amounts for the normal, soft, and degas samples were 35.36g, 39.22g, and 43.05g respectively, while the heat sample (using only heat and not treated with ultrasonic waves) yielded a significantly higher precipitate of over 47g (Figure. 2).

Although the yield of extract was the highest among the experimental samples, the protein recovery efficiency of the heat sample (38.23%) was lower than the ultrasound-treated degas sample (43.76%). Thwas was because the ultrasound waves transmitted into the liquid with successive cycles of compression and rarefaction had created gas bubbles, which burst under pressure and temperature, accelerating the protein diffusion process from the raw material and thereby increasing the protein extraction efficiency.

Therefore, the ultrasonic treatment method in degas mode combined with temperature achieved a high protein content recovery efficiency of 43.76%, making it the most suitable treatment for recovering protein from chickpea flour.

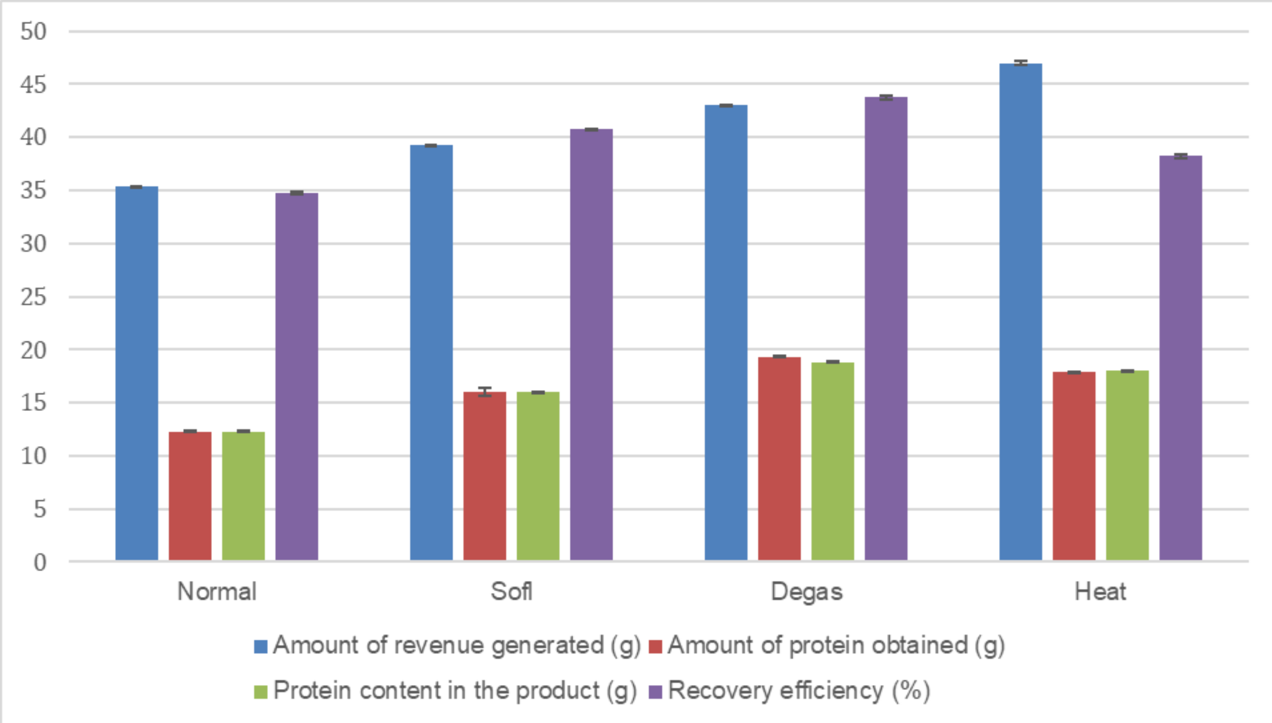

Figure. 2. The influence of ultrasound treatment on the recovery efficiency of chickpea protein

4. CONCLUSION

The study provides a positive outlook on the potential of chickpea protein as a nutrient-rich food source. The results show that the recovery efficiency of chickpea protein obtained in the research project reached 43.76%.

The study determined the extraction parameters of protein from chickpea seeds by adjusting the water/flour ratio at 8/1, using heat precipitation method at 60°C. These were important parameters to achieve high protein recovery efficiency.

The study also showed better temperature recovery of proteins when combined with the use of live ultrasound at the 40Hz degassing step.

With these results, we hope that the chickpea protein product can be further developed and widely applied in the food industry, contributing to improving human health, especially in providing essential nutrients for pregnant women. This research also opens up opportunities for studies related to the use of chicken peas as a source of high-quality and nutritious protein in the future.

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