

# Plant Based Protein Sources and Extraction



Ayten Gizem Özbek and Seda Ersus Bilek\*

Food Engineering Department, Ege University, Turkey

Received: 📅 April 05, 2018; Published: 📅 April 18, 2018

\*Corresponding author: Seda Ersus Bilek, Food Engineering Department, Ege University, Turkey

## Abstract

In the context of accelerating interest in novel protein sources, plants gain importance day by day. Vegans, vegetarians, people with gluten-restricted dietary necessities are looking forward to new protein sources from novel plant products. However, concerning protein extraction methods from plants have many influential points to consider. This mini-review below represents the current situation for protein extractions and implements solutions to some drawbacks in plant protein extraction issues. Hopefully, the solutions below will be a guide for industrial and technologic aspects of plant protein extraction.

## Introduction

Consuming an adequate amount of high-quality protein in daily nutrition is one of the key features of healthy lives. Shortage of protein in nourishment may lead metabolic diseases [1]. A climbing trend in the requirement of plant-based protein sources can be easily seen due to medical needs as lactose intolerance or lifestyle choice as being vegan or vegetarian. The population of vegetarian, vegan and the number of people who faces with problems depending on proteins based on animals are increasing. Vegetarian is a person who avoids eating for various reasons as health, religious or not being cruel to animals. And vegans do not consume or use meat and any other products such as fish, eggs, cheese or leather [2]. In the USA, Brasilia, Austria, and India have vegans and vegetarians in terms of 4, 8, 3 and 40% of their countries population, respectively [3]. To achieve enough amounts of protein resources, a number of studies have been taking place for years. Additionally, the price of animal-based proteins may cost much higher than plant-based proteins. Therefore, plant-based protein sources give an affordable alternative in the countries with tremendous prices for milk and dairy products [4].

Plant proteins have a wide ranged usage area. Plant-derived pharmaceuticals (as experimental drug ZMapp to heal disease caused by Ebola Virus [5],) are also considered for finding new plant protein sources. Plant-protein-based delivery systems for encapsulating bioactive ingredients in foods are also the new usage areas [6]. In terms of extending demand of plant protein sources for various numbers of reasons, it is clear that new researchers are

needed for finding novel plant materials available for extraction efficiency.

Soybeans are the main commercial source for gaining plant-based proteins. Additionally, new alternative possible sources are added to literature and new consumer goods have already been taken place in supermarkets. Searching for a novel plant as a new resource, led researchers to think various plants such as hemp [7], quinoa [8], potatoes [9], rice [10], maize [11], chickpeas [12], lentil Joshi, 2012, peas [13], sesame, peanuts, walnuts, hazelnuts, wheat and so on. Researchers have been considering even aquatic flora [14] as a candidate source. However, even they may contain high protein content; digestibility is another issue to consider while they may have low bioavailability. Another issue related to some of these sources is allergen molecules. So far, 15 major antigens that bind Ig-E antibodies have been found in soybean plant and products made from soybean [15]. Gluten allergy is closely related to celiac disease and nutrition including gluten raises up the symptoms of the disease. Besides that, some researchers indicate that eating habits including gluten may exasperate severity of schizophrenia and cerebellar ataxia [16]. Additionally, growing trend of avoiding GMO products in society forces companies to find alternative new resources.

To overcome lack of protein sources, extraction methods from plants have been studied for many years. Classification and purification of proteins in the extract or the product is possible by using analytical methods such as 2-dimensionalelectrophoresis

(2-DE). During this process, challenges can be seen in many ways [17]. Secondary metabolites and proteases are found in excessive amounts. Due to these components, protein extraction, separation, and identification may be affected. In terms of proteomic studies, a clear extract is necessary to prevent unwanted migrations of unwanted metabolites while 2-DE are made. These unwanted migrates may affect migration of proteins. Therefore, they need to be swept away from extract as much as possible [18].

Extraction procedure development of a protein obtains many factors to think about. Depends on the part of the plant, extraction methods and solvents vary. As long as minimizing the tissues as much as possible is the key factor of having a larger amount of bulk protein; some challenging features (fiber-rich content, adhesive structure, hardly disrupting parts) are needed to be considered. Cell walls of plant cells consist of complex polysaccharides which add extra problems for squeezing tissues. Liquid nitrogen usage with mortar and pestle is a useful way to lower protein degradation during tissue disruption. Protein degradation can also be lowered by cooling and adding buffer solutions in homogenization environment. Addition of quartz sand facilitates obtaining finer powder [19].

New applications of extraction processes open up innovative ideas. One of the most important issues in that concept, considering the most convenient and appropriate extraction protocol depending on the aim of extraction. As an example, usage of toxic chemicals in extraction or precipitation procedures may lead to increase costs for cleaning procedure and also some chemicals may be forbidden due to regulatory rules. In this concept, we may need to take advantage of assisting applications above.

Microwave-assisted extraction (MAE) systems have a marvelous potential due to the new research. Decreased solvent consumption, thermally save applications, increased yields depending on a lowered number of extraction steps are the benefits of this system. Non-ionizing electromagnetic waves (which are between 300 MHz and 300 GHz) are known as microwaves and in the spectrum, they take place between X-ray and Infrared rays. Transferring absorbed electromagnetic energy to heat energy is possible by using electromagnetic waves. The theory behind MAE depends preventing loss of energy captured by environment-which is the drawback of conventional heating [20]. As long as MAE provides targeted and selective heating mechanism (with ionic conduction and dipole rotation), it is more favorable than traditional heating conventional heating methods.

Enzyme-assisted protein extraction (EAE) depends on disrupting cell wall and increasing yield in mild conditions. Therefore, it is more environmental-friendly method than using harsh chemicals. Specific enzymes for extraction methods have been developed for extraction of proteins from tea leaves, Leguminosae gums, oilseed meals and so on. A significant peak in yields has been shown in research when it is compared with chemical-based methods [21].

Ultrasound treatment before extraction process is responsible for reducing size in the target which increases surface area. Speeding up the extraction process and having an increased yield is possible after ultrasound treatment. Ultrasound application procedure stimulates hydration process by using the power of cavitations by using bubbles in hydrophobic surfaces.

Secondary metabolite removal is another way to obtain more pure protein extracts. Secondary metabolites are low molecular weight compounds and around 50,000 structures have been enlightening [23]. Definition of secondary metabolites demonstrates a compound whose biosynthesis is restricted to selected plant groups. They can be providing floral scent with volatile compounds, the color of the petals with pigments or protection systems made from toxic chemicals to avoid pathogens and herbivores [24]. Some examples of secondary metabolites can be given as phenols, flavonoids, tannins, lignins, stilbenes [25]. Most of the times, including these type of compounds in a food product, protein isolate and so on is mostly unfavorable due to taste, odor, appearance and health-related reasons [26]. Additionally, 2-DE requires samples without secondary metabolites. Removal procedure can be affected by species, tissue-specificity, age, and or developmental stage. These compounds can be removed before or after protein extraction. Using TCA/acetone precipitation, organic solvents are used to clean the extract. These give a result of white or light colored pellet [19,27]. Air-dried acetone powder is used for extracted in aqueous buffers after obtaining a pellet. However, this removal procedure comes out with drawbacks such as lessen yield of extraction. Other strategy-removing secondary metabolites during the extraction by using aqueous buffers including EDTA, DTT or 2-ME, a protease inhibitor cocktail, and poly vinyl poly pyrrolidone (PVPP). PVP(P) compounds can be cleaned up by using a centrifuge. Unfortunately, co precipitating contaminants are hardly removed by using organic solvents.

Therefore, this strategy fits tissues which can be easy extracted such as young tissues [19].

After removing secondary metabolites, another limitation comes up with the bulk of protein. Solubilization of the protein is important to move on with rest of the experiment. 2-DE separation, measuring the amount of protein and other levels of the process may need a solubilized protein. Re-solubilization can be done with SDS. Anionic nature of SDS does not directly affect isoelectric focusing of the gel [28]. Long incubations with lysis buffers (including urea, thiourea, MDTT, CHAPS, carrier ampholytes) and shaking is another way to re-solve bulk proteins [19].

## Conclusion

Plants as sources of protein bring new opportunities to people who prefer or need to eliminate or decrease animal-based protein consumption. In this concept, we tried to implement some problems and key factors to rise up yield and overcome the problems depend on the extraction process. Hopefully, future studies will bring

additional novel species and sources to fulfill the requirement of wide-ranged plant proteins for food and many other industries.

## References

- Cox MM, Nelson DL (2008) Lehninger principles of biochemistry. WH Freeman, USA.
- Anonymous (2018a).
- Anonymous (2018b) Dünyadaki Vejetaryen& Vegan Nüfusu.
- Mäkinen OE, Wanhalinna V, Zannini E, Arendt EK (2016) Foods for special dietary needs: Non-dairy plant-based milk substitutes and fermented dairy-type products. *Critical reviews in food science and nutrition* 56(3): 339-349.
- Sack M, Hofbauer A, Fischer R, Stoger E (2015) The increasing value of plant-made proteins. *Current opinion in biotechnology* 32: 163-170.
- Wan ZL, Guo J, Yang XQ (2015) Plant protein-based delivery systems for bioactive ingredients in foods. *Food & function* 6(9): 2876-2889.
- Wang XS, Tang CH, Yang XQ, Gao WR (2008) Characterization, amino acid composition and in vitro digestibility of hemp (*Cannabis sativa L.*) proteins. *Food Chemistry* 107(1): 11-18.
- Abugoch L, Castro E, Tapia C, Añón MC, Gajardo P, Villarroel A (2009) Stability of quinoa flour proteins (*Chenopodium quinoa Willd.*) during storage. *International journal of food science & technology* 44(10): 2013-2020.
- Giuseppin MLF, Laus MC, Schipper J (2015) US Patent Application No.14/413,058.
- Morita T, Kiriya S (1993) Mass production method for rice protein isolate and nutritional evaluation. *Journal of food science* 58(6): 1393-1396.
- Shukla R, Cheryan M (2001) Zein: the industrial protein from corn. *Industrial crops and products* 13(3): 171-192.
- Paredes López O, Ordorica Falomir C, Olivares Vázquez MR (1991) Chickpea protein isolates: physicochemical, functional and nutritional characterization. *Journal of Food Science* 56(3): 726-729.
- Sumner AK, Nielsen MA, Youngs CG (1981) Production and evaluation of pea protein isolate. *Journal of Food Science* 46(2): 364-366.
- Boyd CE (1968) Fresh-water plants: a potential source of protein. *Economic Botany* 22(4): 359-368.
- Natarajan S, Khan F, Song Q, Lakshman S, Cregan P, et al. (2016) Characterization of soybean storage and allergen proteins affected by environmental and genetic factors. *Journal of agricultural and food chemistry* 64(6): 1433-1445.
- Sümer SAG, Harmandar FA, Uyar S, Çekin AH (2015) Non-Celiac Gluten Susceptibility.
- Gevaert K, Vandekerckhove J (2000) Protein identification methods in proteomics. *Electrophoresis* 21(6): 1145-1154.
- Isaacson T, Damasceno CM, Saravanan RS, He Y, Catalá C, Saladié M, Rose JK (2006) Sample extraction techniques for enhanced proteomic analysis of plant tissues. *Nature Protocols* 1(2): 769.
- Wang W, Tai F, Chen S (2008) Optimizing protein extraction from plant tissues for enhanced proteomics analysis. *Journal of separation science* 31(11): 2032-2039.
- Mandal V, Mohan Y, Hemalatha S (2007) Microwave assisted extraction- an innovative and promising extraction tool for medicinal plant research. *Pharmacognosy reviews* 1(1): 7-18.
- Vergara Barberán M, Lerma García M, Herrero Martínez J, Simó Alfonso EF (2015) Use of an enzyme-assisted method to improve protein extraction from olive leaves. *Food chemistry* 169: 28-33.
- Roselló Soto E, Barba FJ, Parniakov O, Galanakis CM, Lebovka N, et al. (2015) High voltage electrical discharges, pulsed electric field, and ultrasound assisted extraction of protein and phenolic compounds from olive kernel. *Food and Bioprocess Technology* 8(4): 885-894.
- Thangaraj P (2016) Quantification of secondary metabolites. In *Pharmacological Assays of Plant-Based Natural Products* pp. 49-55.
- Pichersky E, Gang DR (2000) Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. *Trends in plant science* 5(10): 439-445.
- Crozier A, Clifford MN, Ashihara H (2008) Plant secondary metabolites: occurrence, structure and role in the human diet. *John Wiley & Sons*.
- Sosulski F (1979) Organoleptic and nutritional effects of phenolic compounds on oilseed protein products: a review. *Journal of the American Oil Chemists' Society* 56(8): 711-715.
- Méchin V, Damerval C, Zivy M (2007) Total protein extraction with TCA-acetone. In *Plant Proteomics Humana Press, USA*, p. 1-8.
- Hurkman WJ, Tanaka CK (1986) Solubilization of plant membrane proteins for analysis by two-dimensional gel electrophoresis. *Plant physiology* 81(3): 802-806.



This work is licensed under Creative Commons Attribution 4.0 License

To Submit Your Article Click Here:

[Submit Article](#)

DOI: [10.32474/CIACR.2018.02.000130](https://doi.org/10.32474/CIACR.2018.02.000130)



## Current Investigations in Agriculture and Current Research

### Assets of Publishing with us

- Global archiving of articles
- Immediate, unrestricted online access
- Rigorous Peer Review Process
- Authors Retain Copyrights
- Unique DOI for all articles