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REVIEW ARTICLE

Processing Condition and Properties of Protein Isolates from Mung Bean (*Vigna radiata*) and Quinoa (*Chenopodium quinoa* Willd.)

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ABSTRACT

The potential and application of mung bean and quinoa as protein isolates have been discovered in some studies. Mung bean and quinoa are good protein sources from legumes and pseudocereals individually. Several isolation techniques such as wet fractionation (acid-base extraction), micellization, dry fractionation, aqueous separation, or a combination of some techniques can be used to produce protein isolate. This review provided an overview of different methods and processing conditions applied during the production of mung bean and quinoa protein isolates. Extracting protein from different sources may require different conditions, which results in different proximate compositions and functional properties. Acid-base extraction is the most common method applied in mung bean and quinoa and results in high protein purity and yields. Meanwhile, micellization is an alternative method used in mung beans to produce higher protein content. Dry fractionation is also a sustainable option used in quinoa to concentrate protein fractions. Purification methods such as ultrafiltration and aqueous phase separation can be used. Different methods and processing conditions affect functional properties, including solubility, water and oil absorption, emulsification, foaming, thermal properties of isolates, affecting their suitable application.

Keywords: Protein isolates; Quinoa; Mung bean; Wet fractionation; Dry fractionation

HIGHLIGHTS

- Mung bean and quinoa are good sources of protein.
- Isolation techniques are used to extract the protein from mung bean and quinoa.
- Wet fractionation isolates protein of high purity from mung bean and quinoa.
- Micellization in salt solution isolates more protein than acid-base extraction in mung beans.
- Dry fractionation combined with aqueous separation is used in quinoa to concentrate protein fractions.

INTRODUCTION

Protein plays a vital role in human nutrition. Sufficient protein intake is needed ranging from optimal growth and health in children to optimal maintenance and health of tissues in adults (Wu, 2016). Both animals and plants are excellent protein sources, even though plants lack one or two essential amino acids. Most legumes are high in protein content (20-50%) and rich in essential amino acids, such as leucine, lysine, and phenylalanine (Kudre *et al.*, 2013). Nevertheless, some legumes can cause an allergic reaction and lack several amino acids, such as methionine. However, methionine is commonly found in pseudocereals (Elsohaimy *et al.*, 2015; Verma *et al.*, 2013). In terms of nutritional needs, consuming various plant protein

sources can complement the nutrition diet. In terms of cost, plant protein is a potential low-cost protein source for the human diet.

Numerous studies have been conducted to study the potential of plant protein isolate from various sources such as legumes, cereals, pseudocereals, mushrooms, and algae. Legumes and pseudo cereal have different compositions and characteristics. Among legumes and pseudocereals, mung bean and quinoa are good sources of protein isolate. Mung bean has a relatively high protein content of 23.84%. When processed into an isolate, mung bean exhibits some comparable characteristics with soy protein isolate (Branch & Maria, 2017). Quinoa contains relatively lower protein content compared to legumes; however, the protein content is higher among cereals such as barley (11%), rice (7.5%), and corn (13.4%). With relatively high mineral content, well-balanced amino acid, and lack of gluten, quinoa is a suitable example of pseudocereal (Föste *et al.,* 2015). Extracting protein from different sources like legumes and pseudocereals may require different processing conditions, which results in different composition and functional properties. Different compositions and functional properties may affect the application of protein isolate in the food system.

The processing method plays a significant role in determining the protein content and classification when creating the protein extract. According to the protein content on a dry basis, protein extract can be classified into flour, concentrate, and isolate. Protein isolate is the purest protein source. According to Codex Alimentarius (2019a), the protein standard for vegetable protein products is not less than 40%. However, the variability of sources, plant variety, and processing will result in different protein content ranges. For example, in the case of soybean, the standard protein content of flour is 50-65%, concentrate is 65-90%, and isolate is more than 90% (Codex Alimentarius, 2019b). Due to its high protein content and good functional properties, protein isolate has been incorporated as ingredients in many products such as meat analogs, bakeries, beverages, et cetera. The purpose of adding protein isolate in the food product is to improve the nutritional value, enhance the product's texture, and replace the animal protein. Due to varying applications, the demand for protein isolates that are relatively cheap with complete nutritional and good properties increases (Garba & Kaur, 2014).

Protein isolation methods such as wet fractionation or acid-base extraction require alkaline solubilization and acid precipitation along with centrifugation steps in each step (Kusumah et al., 2020). The optimum condition of the isolation procedure depends on the proximate composition and original amount of protein content in raw materials. Other factors such as the type of alkaline or acid solution, extraction temperature, extraction time, centrifugation speed, and time will also affect the protein isolate's composition and functional properties (Kusumah et al., 2020; Toapanta et al., 2016). Ultrafiltration can be done as an alternative or additional step prior to acid precipitation (Bansal-Mutalik et al., 2017; Westfall et al., 1992). Budseekoad et al. (2018) applied enzymatic hydrolysis to produce iron-binding bioactive peptides from mung bean protein hydrolysates. Alternative methods such as salt extraction (micellization) and dry fractionation (air classification) can be used to produce protein isolate (Opazo-Navarrete et al., 2018; Rahma et al., 2000). Compared to wet fractionation, dry fractionation is more sustainable for concentrating protein-rich fractions in raw materials even with low protein content, such as cereals and pseudocereals. The dry fractionation omits the use of water and chemicals, which also minimize the waste (Opazo-Navarrete et al., 2018). Based on a rough calculation, wet fractionation is estimated to use 1-2 MJ/kg protein for mixing and 14 MJ/ kg gluten product for drying. Meanwhile, the combination of milling and air classification consumes 2 MJ/ kg to make protein concentrate (Schutyser & van der Goot, 2011).

This review is aimed to discuss the methods and processing conditions applied to produce protein isolates from mung bean (legume) and quinoa (pseudocereals). In addition, the similarity and difference in processing applied to mung bean and quinoa, as well as the implication to composition, functional properties, and application will also be explained. For this review, the information was retrieved from scientific publications related to mung bean protein isolate, quinoa protein isolate, processing of protein isolate, functional properties of protein isolate, nutritional properties of protein isolate. The publication included for

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this review were 2 sources from 1992-1997, 4 sources from 2000-2009, 11 sources from 2011-2015, and 19 sources from 2016-2021.

MUNG BEAN

Mung bean (*Vigna radiata*) is a type of grain legume that grows widely in tropical and subtropical countries. The crops are mainly cultivated in Asian countries and parts of Europe and the USA (Dahiya *et al.,* 2015). As a good protein source and low cost, mung bean is consumed in many ways, such as cooked directly as vegetables and porridge or incorporated as ingredients for dessert, bread, and cake. Mung bean is composed of 20.97-31.32% protein, 1.2-1.56% oil, 8.89-12.85% fiber, 2.96–3.39% ash, and 4.86-6.16% moisture (Anwar *et al.,* 2007). The primary storage protein fraction in mung beans is globulins (60%), albumin (25%), and other proteins (15%) (Klomklao *et al.,* 2011). Mung bean also have rich minerals such as Na, Cu, Mg, K, Ca, Zn (Anwar *et al.,* 2007).

Processing of Mung Bean Protein Isolate (MBPI)

Due to high protein content and other nutritional aspects, mung bean is a suitable protein isolate source. Different methods and processing conditions applied to the production of mung bean protein isolate are summarized in Table 1. In addition, wet fractionation (acid-base extraction) and micellization will be discussed.

A study by Kudre et al. (2013) suggested that defatting can help minimize the isolate's fat content. Saponification of fat with protein may occur during alkaline solubilization and precipitation, which will affect the final quality of the isolate. However, some studies use flour as input material (Branch & Marian, 2017; Kudre et al., 2013; Rahma et al., 2000). In general, acid-base extraction starts from the solubilization of flour in an alkaline solution. This step is essential to maximize the amount of protein that can solubilize in the solution, in which the highest solubility can be obtained at pH 12 (Brishti et al., 2020). The separation between protein-rich supernatant and other components such as fiber, carbohydrate, or fat can be done by centrifugation. Another method, such as ultrafiltration, which aims to improve the purification of proteinrich fractions, can be done to separate solubilized protein with fibers and other compounds. Ultrafiltration can be done as an alternative or additional step prior to acid precipitation (Bansal-Mutalik et al., 2017; Westfall et al., 1992). Protein-rich fraction is precipitated at an isoelectric point (minimum solubility) which is 4.6 for mung beans (Yi-Shen et al., 2018). Following that, the precipitated materials (protein-rich fraction of protein pellet) are obtained by centrifugation. Finally, the pellet is neutralized or directly dried. Drying is not necessarily the last step of QPI production; however, drying is a crucial procedure that can cause the protein to denature, affecting the protein composition and functional properties of protein isolate (Brishti et al., 2020). Additional enzymatic treatment is usually done to produce mung bean protein hydrolysate. The procedure begins with wet fractionation to obtain the mung bean protein isolate and is followed by enzymatic hydrolysis using enzymes such as alcalase, flavourzyme, trypsin, pepsin, and pancreatin (Budseekoad et al., 2018).

Micellization involves protein extraction in a salt solution. The procedure starts with the solubilization of mung bean flour to NaCl for a certain period with stirring, followed by centrifugation and filtration. An increase in ionic strength leads to higher protein recoverability. High salt protein supernatant is precipitated from salt solution using ultrafiltration and then micellization with the addition of water and left overnight in the cooling chamber. Finally, the micellized protein is separated by centrifugation, neutralized, and centrifuged before being dried using freeze-drying (Hadnađev *et al.*, 2017; Rahma *et al.*, 2000).

Method	Processing Condition		_	
	Extraction/ solubilization	Settlement/ purification	Protein	References
Wet fractionation (acid-base extraction)	2 g/L NaOH at pH 12	6 M HCl at pH 4.5	Protein content: 87.83%	Kudre <i>et al.,</i> 2013
	1 N NaOH at pH 9	1 N HCl at pH 4	Protein content: 81.53%	Branch & Maria, 2017
Micellization	0.5 M NaCl	 Ultrafiltration Micellization by adding 5x volume of water 	- Protein content: 87.9% - Yield of protein: 47.9%	Rahma <i>et al.,</i> 2000

Table 1. Processing condition of mung bean protein isolate on several studies

Composition of MBPI

The composition of isolate defines not only the nutritional quality but may also affect the functional properties of MBPI. Alkaline solubilization and acid precipitation determine the amount of protein content extracted. Acid-base extraction is able to increase the protein content from 23.84% (flour) to 81.5% (isolate). The reduction of fat content from 1.53% to 0.14% may be due to the centrifugation, extraction, and washing steps. Other components such as fiber and carbohydrates decrease from 4.95% to 0.73% and 56.43% to 8.66%, respectively. Slightly increase in ash content from 3.02% to 4.38% may be due to the addition of alkaline and acid solutions which can precipitate into salt. The moisture content of MBPI 4.56% is mainly affected by drying conditions (Branch & Maria, 2017). Kudre et al. (2013) reported that the protein content of MBPI was 87.83%, fat 1.41%, ash 4.09%, moisture 3.61%, and carbohydrates 3.06%. The difference in composition in some studies may be caused by different cultivar and processing conditions applied. The essential amino acids and sulfur amino acids contribute to 43.51% and 1.62% of total amino acids in MBPI, respectively. Predominant essential amino acids in MBPI are leucine (74 mg/g), lysine (62.4 mg/g) and phenylalanine (58 mg/g) followed by valine (46.3 mg/g), isoleucine (39.1 mg/g), and histidine (27.9%). Meanwhile, sulfur amino acids such as methionine (12.5 mg/g) and cysteine (0.5 mg/g) are present at a low level; however recent study found that protein engineering has been successfully incorporated methionine into major storage protein (8S α globulin) (Torio *et al.*, 2011).

Kudre *et al.* (2013) isolated protein from mung bean, soybean, and black bean using the wet extraction method. The study found that mung bean protein isolate (MBPI) contained lower trypsin inhibitors compared to soy protein isolate (SPI), black bean protein (BBPI), and Bambara groundnut protein isolate (BGPI). Inhibitors in legumes generally inhibit trypsin, thereby lowering the digestion and absorption of dietary protein in human consumption (Fernández-Quintela *et al.*, 1997). A study by Rahma *et al.* (2000) compared the composition of MBPI produced from acid-base extraction and salt extraction. Salt extraction with micellization results in higher protein content (87.9%) compared to acid-base extraction (81%); however, the protein yield from micellization is lower (40.9%) compared to acid-base extraction (66.5%).

Functional Properties of MBPI

Functional properties describe the behavior of MBPI as ingredients during food preparation that will affect the characteristics of the final product. Some studies have reported the functional properties of MBPI, including protein solubility, water and oil absorption capacity, foam capacity and stability, emulsification capacity and stability, and thermal properties (Branch & Maria, 2017; Brishti *et al.*, 2020; Kudre *et al.*, 2013).

Solubility is the basic but essential property of ingredients. The solubility of MBPI also depends on the pH, which the highest solubility can be obtained at pH 2, 10, 12 (Branch & Maria, 2017). Kudre *et al.*

(2013) also reported that the highest solubility of MBPI was obtained at pH 10. Brishti *et al.* (2020) observed the effect of different drying types on the solubility of MBPI. Freeze dried MBPI has porous particle morphology, promoting protein hydration and leading to higher solubility (at pH 2 and above pH 8) than spray and oven-dried MBPIs. Freeze dried MBPI has the highest solubility at pH 12 (around 105 mg/mL). Knowing this particular property is vital to understanding the suitable condition during food formulation.

Water or oil absorption capacity indicates the amount of water or fat absorbed by grams of protein material. The water and oil absorption capacity of mung beans is 3.33 g and 3 g, respectively, indicating the capability of MBPI to retain water and stabilize the emulsion system. This property is suitable for comminuted meat products (Branch & Maria, 2017). MBPI has a high foam capacity (89.66%), and stability is 78.33% after 30 minutes. As an ingredient, high foam capacity and stability are essential in creating stable foam in the food system (Branch & Maria, 2017) MBPIs have good thermal stability, hence suitable for food processing in high temperatures. The emulsion activity and stability index of MBPI processed with spray drying are relatively high (29.21 m²/g and 351,9 min), making it a suitable ingredient for emulsion food systems such as sausages (Brishti *et al.,* 2020).

QUINOA

Quinoa seed (*Chenopodium quinoa Willd*) is a pseudocereal that can grow in extreme environmental conditions (Collar, 2016). Quinoa grains consist of 10-15% protein content, 64% carbohydrate, and 6.1% several vitamins and minerals (USDA, 2019). Elsohaimy *et al.* (2015) reported that quinoa was composed of protein (14%), fat (6.79%), fiber (4.06%), moisture (9.68%), ash (2.97%), and carbohydrates (72.15%). Due to its high nutritional value and the absence of gluten in its composition, quinoa is considered a "super grain" consumed daily (Rana *et al.*, 2019; Valencia-Chamorro, 2015). The predominant amino acid in quinoa is glutamic (8.79g/100g protein), threonine (6.47 g/ 100 g protein), aspartic acid, glycine, arginine (around 3 g/ 100 g protein) and alanine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, tryptophan, tyrosine, valine (around 2 g/100 g protein) (Escuredo *et al.*, 2017). The variety composition of quinoa may depend on the quinoa variety, growth years, and growth conditions.

Processing of Quinoa Protein Isolate (QPI)

The potential of quinoa as a protein isolate has been discovered in several studies, ranging from the use of wet fractionation (acid-base extraction) to dry fractionation (air classification). The processing condition of wet and dry fractionation is summarized in Table 2.

Quinoa is pseudocereals that can be extracted using acid-base extraction. In principle, the process begins with removing foam from quinoa seeds before milling them into flour. This is a crucial step to eliminate the saponin, which causes a bitter taste in flour. Next, the flour is defatted with an organic solvent to remove lipid from the sample, followed by solvent evaporation. Next, the defatted flour is solubilized in an alkaline solution, stirred, and centrifuged to obtain the protein-rich supernatant. Solubilization is essential to maximize the protein dissolved in the water with higher pH of 10 can significantly improve the solubility (Elsohaimy *et al.*, 2015). During the alkalinization step, higher pH can improve the protein extractability; however, it may also affect the isolate's purity, gel formation, and solubility (Ruiz, 2016). The procedure is followed by acid precipitation and another centrifugation to obtain the protein pellet. The optimum acid precipitation for QPI occurs at pH 4.5 (Elsohaimy *et al.*, 2015). The typical last step of QPI production is drying, which can affect the application of QPI in the food system.

Starch is the main component of quinoa; thus, concentrating the protein-rich fraction from the beginning will save time and resources. Even though protein from cereals and pseudocereals can be extracted using wet fractionation, this process seems less effective since less protein is present in quinoa. Dry

fractionation is a method to concentrate protein- and starch-rich fractions. Dry fractionation has been applied to pulses (i.e., lentil, pea, bean) and cereals (i.e., wheat, barley). Considering the plant structure, raw materials containing big starch granules are more effective than small starch granules (Schutyser & van der Goot, 2011). In the beginning, quinoa seeds are pre-milled, ground, and air classified with different sieves to obtain protein-enriched flour. During air classification, wheel speed and airflow are essential to determine the protein and starch fraction (Opazo-Navarrete et al., 2018). Protein and starch fractions of quinoa are difficult to separate due to their similarity in size. Thus, rotor milling followed by sieving or air classification is applied to separate embryo (rich in protein) and perisperm (rich in starch) (Ruiz et al., 2016). To further increase the protein purity in protein-rich fractions, wet fractionation can be done (Foste et al., 2015). Alternatively, dry fractionation in combination with aqueous separation can be done to create a milder and more sustainable way of producing QPI. Aqueous phase separation involves the suspension of the proteinrich fraction in NaCl solution to increase protein solubility. This process results in 4 phase separations containing protein, starch, insoluble fiber, and soluble fiber. During dry fractionation, the milling and separation ease the dissociation of starch and protein fractions during the suspension. Following that, stirring and centrifugation are done. Due to density discrepancy, phase separation between soluble and insoluble fractions occurs, which will ease the separation of protein and carbohydrates. The liquid to layer is ultrafiltered to increase protein purity (Ruiz et al., 2016).

Table 2. Processing condition of quinoa protein isolate on several studies							
	Pro	cessing Conditio	n				
Method	Pre-extraction	Extraction/ solubilization	Settlement/ purification	Protein	References		
	-Washing with cold water -Defatting with chloroform and methanol	0.1 NaOH at pH 5-10	0.1 HCl at pH 3-5.5	-Protein profile: globulin, chenoprotein, and albumin	Elsohaimy <i>et al.,</i> 2015		
Wet fractionation (acid-base extraction)	Defatting with hexane	2 M NaOH at pH 8	2 N HCl at pH 2-6	-Protein content 64.78% (pH 6) to 84.32% (pH 2) -Yield of protein isolate: 3.37% (pH 2) to 6.29% (pH 4)	Toapanta <i>et al.,</i> 2016		
	Defatting with hexane (ratio flour: hexane of 1:4)	1 M NaOH at pH 10	1 M HCl at pH 4	-Protein content: 83.2% (spray drying), 86.13 (vacuum- drying), 86.2 (freeze- drying)	Shen <i>et al.,</i> 2021		
Dry fractionation	-Pre-milling with a 2 mm screen, rotor speed 4000 g with a feed rate of ~ 20 g/m -Air classification with different sieves (0.800, 0.630, and 0.315 mm) at 1500 Pa for 2.5 min	-	-	-Protein-rich fractions: 32 g protein/ g dry solid -Starch-rich fractions: 86-89 g starch/ g dry solid	Opazo- Navarrete et al., 2018		

Table 2. Processing condition of quinoa protein isolate on several studies

Milling fractionation followed by acid-base extraction	-Ultra-centrifugal mill with a mesh screen of 500μm -Roll milling followed by sieving in a rotating sifter (mesh of 200 μm)	1 N NaOH at pH 7-12	1 N HCl at pH 2-6	-Protein yield: 68% (from bran) and 52% (from whole grain flour)	Föste <i>et al.,</i> 2015
Dry fractionation followed by aqueous separation	-Air classified with wheel speed of 1000 rpm and airflow 80 m3/h -Impact milled with airflow 52m3/h and classifier wheel speed of 2500 rpm	0 – 0.5 M NaCl	Ultrafiltration with pressure 350 kPa for approximately 165 min	-Protein yield: 24- 61% -Protein purity: 47- 59%	Ruiz <i>et al.,</i> 2016

Composition of QPI

Defatted quinoa flour consists of protein (13%), fat (4.99%), moisture (9.05%), fiber (1.01%), soluble solids (2.09%) carbohydrates (69.9%). Alkaline and acid precipitation is able to increase protein content from 13% (defatted flour) to 64.78% – 84.32% (isolate) (Toapanta *et al.*, 2016). The protein content of quinoa isolate ranges from 83.2 to 86.2, which is affected by drying conditions. Spray-dried QPI has the lowest protein content due to protein loss during processing (Shen *et al.*, 2021). Aspartic, glutamic, and leucine are the most predominant amino acids in quinoa proteins (Shen *et al.*, 2021; Toapanta *et al.*, 2016). QPI contains relatively high essential amino acids, including lysine (17.13 g/100g), phenylalanine + tyrosine (9.34 g/100g), leucine (4.60 g/100g), histidine (2.76 g/100g), valine (2.03 g/100g), threonine (1.47 g/100g), methionine + cystine (1.7 g/100g) but except tryptophan (Elsohaimy *et al.*, 2015). Based on the SDS-Page result, various bands are detected in wet fractionated QPI, namely 7s globulin (60 kDa), 11s acid polypeptide (33-36 kDa), 11S basic polypeptide (20-11 kDa), and chenopodin (20-36 kDa) (Toapanta *et al.*, 2016).

Ruiz et al. (2016) reported that air classification was able to improve the protein fraction from 14.5% (flour) to 23.9% (fine fraction). After further processing with aqueous fractionation, including ultrafiltration, the protein purity is 59%, with a protein yield of 61%. Even though the protein content in dry fractionation is lower than in wet fractionation, dry fractionation is reported to have lower energy and water consumption and can retain the native state of the protein. Furthermore, high purity is not always necessarily essential for food applications; some applications still require other components such as fiber and carbohydrates (Opazo-Navarrete *et al.*, 2018; Schutyser & van der Goot, 2011).

Functional Properties of QPI

Modification of processing conditions in protein isolation not only affects the components but may also affect the functional properties of protein isolate. Therefore, several studies investigate the functional properties of QPI, such as solubility, water and oil absorption, foaming capacity and stability, and emulsion capacity and stability (Abugoch *et al.*, 2008; Elsohaimy *et al.*, 2015; Shen *et al.*, 2021).

Protein solubility determines the suitable condition during the formulation of food. A study from (Abugoch *et al.*, 2008) found that different pHs used during alkaline solubilization (pH 9 and pH 11) can significantly affect the solubility of protein isolate in solution. QPI extracted at pH 9 has better solubility in pH 5-6 at 77% and increases up to 85% above pH 7. At pH 11, more proteins are denatured and exposed to hydrophobic groups. This phenomenon results in lower solubility of QPI at pH 5-7 at only 22% and above pH

7 at 41%. Ruiz (2016) mentioned that suspension of QPI extracted at pH 8 and 9 formed semi-solid gel when heated, while QPI extracted at pH 10 and 11 were unable to form gel during heating. The formation of gel occurs due to higher hydration and swelling capacity in QPI pH 8 and 9. Therefore, QPI that is extracted below pH 9 and above 10 is suitable for semi-solid gelled foods and liquid food, respectively.

Drying affects the functional properties of isolates. A study by Shen *et al.* (2021) compared the vacuum, spray, and freeze-dried QPI. Freeze dried QPI has high surface hydrophobicity, contributing to the higher emulsification capacity (56.63% at pH 7) and stability (51.90% at pH 7) as well as oil absorption capacity (3.19 g oil/protein at pH 7). Oil absorption capacity is related to the amount of hydrophobic amino acid residues in the protein and hydrophobic amino acid content. Surface hydrophobicity is related to the binding of emulsifiers and oil droplets due to the domination of hydrophobic amino acid residues and hydrophobic amino acid content of exposed hydrophobic amino acid residues and hydrophobic amino acid content is correlated with oil absorption capacity. Compared to vacuum and freeze-dried QPI, spray-dried QPI has better WAC properties (2.76 g water/ protein at pH 7), indicating as better ingredients for the food system with solubility and gelation as important attributes since they can affect the texture of the product (Shen *et al.*, 2021).

Based on dry fractionation, protein-rich fraction shows lower water retention capacity compared to starch-rich fraction due to the capability of water to absorb water and form a gel upon heating. However, protein-rich fractions show high water retention capacity at low temperatures (below 60°C). Furthermore, both fractions' solubility increases in the temperature range of 20-40°C. Based on properties, the protein-rich fraction can be used as ingredients for gluten-free products (Opazo-Navarrete *et al.,* 2018).

CONCLUSION

Proteins isolated from mung bean and quinoa are potential ingredients used for food formulation due to protein content and good functional properties. Wet fractionation can be applied for mung bean and quinoa, resulting in high protein purity. In general, alkaline solubilization affects the amount of protein solubilized in solution; ideally, higher pH leads to high solubility but might impair the functional properties of isolates. The isoelectric points for both mung bean and quinoa are around 4. Micellization in salt solution results in higher protein content compared to acid-base extraction in mung beans. Dry fractionation combined with aqueous separation is a sustainable option used in quinoa to concentrate protein fractions. Purification methods such as ultrafiltration and aqueous phase separation can be used further to improve the purity and yield of protein fractions. There is still room for further exploration to find optimum conditions and more sustainable ways to isolate protein and mung bean.

REFERENCES

- Abugoch, J.L.E., 2009. Quinoa (Chenopodium quinoa Willd.): composition, chemistry, nutritional, and functional properties. Adv. Food Nutr. Res. 58, 1–31. https://doi.org/10.1016/S1043-4526(09)58001-1
- Abugoch, L. E., Romero, N., Tapia, C. A., Silva, J., & Rivera, M. (2008). Study of some physicochemical and functional properties of quinoa (Chenopodium quinoa Willd) protein isolates. *Journal of Agricultural and Food chemistry*, *56*(12), 4745-4750. https://doi.org/10.1021/jf703689u
- Anwar, F., Latif, S., Przybylski, R., Sultana, B., & Ashraf, M. (2007). Chemical composition and antioxidant activity of seeds of different cultivars of mungbean. *Journal of food science*, *72*(7), S503-S510. https://doi.org/10.1111/j.1750-3841.2007.00462.x

- Bansal-Mutalik, R., Bhide S., Gibson, B., Hall, C., Jakubasch, M., Kleiner J., Lanquar, V., Mahadevan, S., Niekowal, T., Proulx, J., Roche, B., Xu, M., Flatt, J., Park, N., 2017. Mung bean protein isolates. WO 2017/143298. WIPO: PCT
- Branch, S., & Maria, S. (2017). Evaluation of the functional properties of mung bean protein isolate for development of textured vegetable protein. *International Food Research Journal*, 24(4), 1595-1605.
- Brishti, F. H., Chay, S. Y., Muhammad, K., Ismail-Fitry, M. R., Zarei, M., Karthikeyan, S., & Saari, N. (2020). Effects of drying techniques on the physicochemical, functional, thermal, structural and rheological properties of mung bean (Vigna radiata) protein isolate powder. *Food Research International*, 138, 109783. https://doi.org/10.1016/j.foodres.2020.109783
- Budseekoad, S., Yupanqui, C. T., Sirinupong, N., Alashi, A. M., Aluko, R. E., & Youravong, W. (2018). Structural and functional characterization of calcium and iron-binding peptides from mung bean protein hydrolysate. *Journal of Functional Foods*, *49*, 333-341. https://doi.org/10.1016/j.jff.2018.07.041
- Codex Alimentarius International Food Standards. 2019a. CXS 174-1989. General Standard For Vegetable Protein Products (VPP).
- Codex Alimentarius International Food Standards. 2019b. CXS 175-1989. General Standard For Soy Protein Products.
- Collar, C. (2016). Quinoa. Encyclopedia of Food and Health, 573-579.
- Dahiya, P.K.; Linnemann, A.R.; Van Boekel, MAJS; Khetarpaul, N.; Grewal, R.B.; Nout, M.J.R. Mung bean: Technological and nutritional potential. Crit. Rev. Food Sci. Nutr. 2015, 55, 670–688. [CrossRef] [PubMed]. https://doi.org/10.1080/10408398.2012.671202
- Elsohaimy, S. A., Refaay, T. M., & Zaytoun, M. A. M. (2015). Physicochemical and functional properties of quinoa protein isolate. *Annals of Agricultural Sciences*, *60*(2), 297-305. https://doi.org/10.1016/j.aoas.2015.10.007
- Escuredo, O., Martín, M. I. G., Moncada, G. W., Fischer, S., & Hierro, J. M. H. (2014). Amino acid profile of the quinoa (Chenopodium quinoa Willd.) using near infrared spectroscopy and chemometric techniques. *Journal of Cereal Science*, *60*(1), 67-74. https://doi.org/10.1016/j.jcs.2014.01.016
- Fernández-Quintela, A., Macarulla, M. T., Del Barrio, A. S., & Martínez, J. A. (1997). Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. *Plant Foods for Human Nutrition*, 51(4), 331-341. https://doi.org/10.1023/A:1007936930354
- Föste, M., Elgeti, D., Brunner, A. K., Jekle, M., & Becker, T. (2015). Isolation of quinoa protein by milling fractionation and solvent extraction. *Food and Bioproducts Processing*, 96, 20-26. https://doi.org/10.1016/j.fbp.2015.06.003
- Garba, U., & Kaur, S. (2014). Protein isolates: Production, functional properties and application. *International journal of current research and review*, *6*(3), 35.

- Gong, K.-J., Shi, A.-M., Liu, H.-Z., Liu, L., Hu, H., Adhikari, B., & Wang, Q. (2016). Emulsifying properties and structure changes of spray and freeze-dried peanut protein isolate. Journal of Food Engineering, 170, 33–40. https://doi.org/10.1016/j. jfoodeng.2015.09.011.
- Hadnađev, M. S., Hadnađev-Dapčević, T., Pojić, M. M., Šarić, B. M., Mišan, A. Č., Jovanov, P. T., & Sakač, M.
 B. (2017). Progress in vegetable proteins isolation techniques: A review. *Food and Feed research*, 44(1), 11-21.
- Klomklao, S., Benjakul, S., Kishimura, H., & Chaijan, M. (2011). Extraction, purification and properties of trypsin inhibitor from Thai mung bean (Vigna radiata (L.) R. Wilczek). *Food chemistry*, *129*(4), 1348-1354. https://doi.org/10.1016/j.foodchem.2011.05.029
- Kudre, T. G., Benjakul, S., & Kishimura, H. (2013). Comparative study on chemical compositions and properties of protein isolates from mung bean, black bean and bambara groundnut. *Journal of the Science of Food and Agriculture*, *93*(10), 2429-2436. https://doi.org/10.1002/jsfa.6052
- Kusumah, S. H., Andoyo, R., & Rialita, T. (2020, February). Protein isolation techniques of beans using different methods: A review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 443, No. 1, p. 012053). IOP Publishing.
- Opazo-Navarrete, M., Freire, D. T., Boom, R. M., Janssen, A. E., & Schutyser, M. A. (2018). Dry fractionation of quinoa sweet varieties Atlas and Riobamba for sustainable production of protein and starch fractions. *Journal of Food Composition and Analysis*, 74, 95-101. https://doi.org/10.1016/j.jfca.2018.09.009
- Rahma, E. H., Dudek, S., Mothes, R., Görnitz, E., & Schwenke, K. D. (2000). Physicochemical characterisation of mung bean (Phaseolus aureus) protein isolates. *Journal of the Science of Food and Agriculture*, *80*(4), 477-483. https://doi.org/10.1002/(SICI)1097-0010(200003)80:4%3C477::AID-JSFA553%3E3.0.CO;2-0
- Rana, G. K., Singh, N. K., Deshmukh, K. K., & Mishra, S. P. (2019). Quinoa: New Light on An Old Superfood: A Review. *Agricultural Reviews*, 40(4), 319-323. http://dx.doi.org/10.18805/ag.R-1927
- Ruiz, G. A. (2016). Exploring novel food proteins and processing technologies: a case study on quinoa protein and high pressure—high temperature processing.
- Ruiz, G. A., Arts, A., Minor, M., & Schutyser, M. (2016). A hybrid dry and aqueous fractionation method to obtain protein-rich fractions from quinoa (Chenopodium quinoa Willd). *Food and Bioprocess Technology*, 9(9), 1502-1510. https://doi.org/10.1007/s11947-016-1731-0
- Schutyser, M. A. I., & van der Goot, A. J. (2011). The potential of dry fractionation processes for sustainable plant protein production. *Trends in Food Science & Technologies*, 22, 154–164. https://doi.org/10.1016/j.tifs.2010.11.006
- Shen, Y., Tang, X., & Li, Y. (2021). Drying methods affect physicochemical and functional properties of quinoa protein isolate. *Food Chemistry*, *339*, 127823. https://doi.org/10.1016/j.foodchem.2020.127823
- Toapanta, A., Carpio, C., Vilcacundo, R., & Carrillo, W. (2016). Analysis of protein isolate from quinoa (Chenopodium quinoa Willd). Asian J. Pharm. Clin. Res, 9, 332-334.

Torio, M. A. O., Adachi, M., Garcia, R. N., Prak, K., Maruyama, N., Utsumi, S., & Tecson-Mendoza, E. M. (2011). Effects of engineered methionine in the 8Sα globulin of mungbean on its physicochemical and functional properties and potential nutritional quality. *Food research international*, 44(9), 2984-2990. https://doi.org/10.1016/j.foodres.2011.07.010

USDA. Quinoa, uncooked. (2019). Retrieved from USDA.

- Valencia-Chamorro, S. A. (2015). Quinoa: Overview. Encyclopedia of Food Grains: Second Edition, 1–4, 341–348.
- Verma, A. K., Kumar, S., Das, M., & Dwivedi, P. D. (2013). A comprehensive review of legume allergy. *Clinical reviews in allergy & immunology*, 45(1), 30-46. https://doi.org/10.1007/s12016-012-8310-6
- Westfall, P.H., Nardelli, C. A., Schimpf, K. J., 1992. Isolation of proteins by ultrafiltration. US5658714A. United State. https://patents.google.com/patent/US5658714A/en
- Wu, G. (2016). Dietary protein intake and human health. *Food & function*, 7(3), 1251-1265. https://doi.org/10.1039/C5FO01530H
- Yi-Shen, Z., Shuai, S., & FitzGerald, R. (2018). Mung bean proteins and peptides: Nutritional, functional and bioactive properties. *Food & nutrition research*, *62*. https://dx.doi.org/10.29219%2Ffnr.v62.1290