

Insight into the Effect of Extraction and Spray Drying Conditions on the Nutritional and Techno-Functional Properties of Legume Protein Powder: A Review

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Highlights

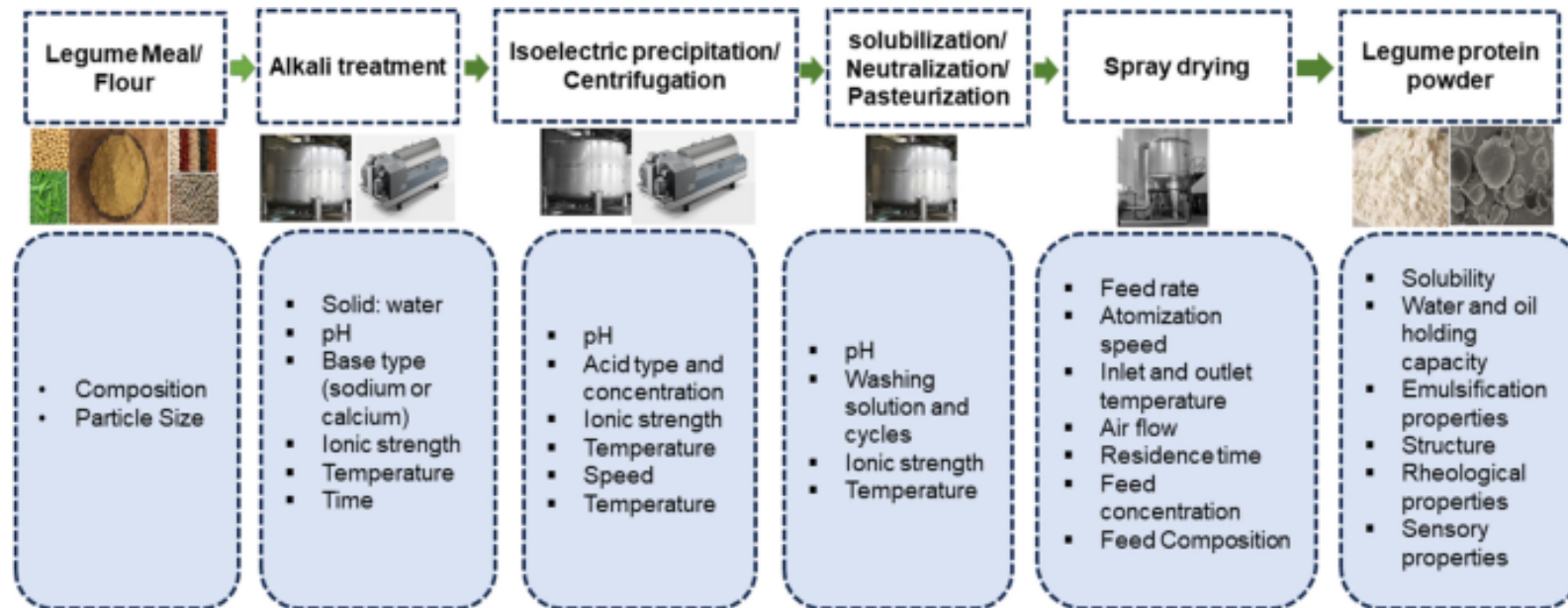
- Extraction conditions influence protein composition, structure, and functionality.
- Spray -drying stresses affect proteins' structure, properties, and functionality.
- The integration approach of isolation and drying enhances protein functionality.
- Functionality gaps exist between laboratory and commercial legume protein powder.
- Emphasis should be placed on improving physicochemical properties commercially.

Abstract

Production of legume protein powders with consistent functionality and physicochemical properties has been challenging. Additionally, the inconsistency of legume protein powders affects the potential use in various food matrices. Integrated extraction and spray drying conditions can produce legume protein powder with consistent physicochemical properties. This review gives an in-depth discussion on legume protein produced using alkali-isoelectric precipitation isolation and spray drying. Alkali-acid precipitation and spray drying affect the composition, secondary and tertiary structure, solubility, and emulsifying, foaming, and gelling properties. It was demonstrated that few studies focus on the stability of various spray-dried legume proteins during storage. The large gap between protein functionality of protein produced at different scales remains a challenge. Furthermore, off-flavor development during isolation and storage influences the potential application of legume protein. The combined effect of extraction and spray drying conditions on the properties, functionality, and storage stability should be further investigated to magnify applications of legume protein powder.

Keywords: Legume protein powder; Alkali-isoelectric precipitation; Spray drying; Physicochemical properties; Functionality

Graphical Abstract



Introduction

There has been growing interest in research and market demands of plant proteins for their potential use in food. High market demand and research interest in plant protein are attributed to health concerns, environmental sustainability, low cost, ethical, and religious attachments. Due to their functionality, nutritional values, and physicochemical properties, plant proteins have offered prolific opportunities to produce different food products. Among various sources of plant proteins, legumes have shown a high protein concentration ranging between 20 and 40%. Proteins extracted from legumes are a good source of essential amino acids. Besides, legume proteins have shown relatively high solubility, high emulsifying properties, high water and oil holding capacity, and high foaming and gelling properties. Legume proteins can also offer health benefits such as controlling cholesterol levels and reducing the risk of cardiovascular diseases. Therefore, legume proteins are crucial in human nutrition and food processing.

Extraction is one of the most significant processes during legume protein production as it determines the composition, nutritional value, organoleptic characteristics, and functional properties (Kamal et al., 2021). Conventional wet extraction methods (acid, alkaline, and salt) and novel methods such as sonication, microwave extraction, and enzyme assistance are used to isolate protein and have been extensively studied to improve the yield and purity while maintaining the physicochemical properties of resultant proteins (Bou et al., 2022). Alkali-isoelectric precipitation is a conventional wet extraction method widely applied to isolate protein from plant materials due to its high protein recovery. Alkali-isoelectric precipitation uses the advantage of enhanced solubility of legume protein at alkaline pH and precipitation at pH near the isoelectric point. Although extraction methods influence the physicochemical properties and functionality of legume proteins, spray drying is another critical step during protein production because it can control the physicochemical properties, functionality, and microstructure of the spray-dried protein.

Spray drying is commonly used to convert extracted legume proteins into powder to prolong the shelf life and facilitate handling and transport. Process parameters and raw material characteristics are crucial during spray drying to achieve high-quality plant protein powder for specific purposes. The mechanical properties of the dry skin layer at the surface of protein during evaporation determine the internal properties of protein powders which may affect the protein structure, denaturation degree, aggregate state, and storage stability (Sadek et al., 2014). Besides, high temperature can also alter the secondary and tertiary structure of protein, and change some properties such as gelling behavior, emulsifying properties, oil and water holding capacity, and solubility (Wang & Zhao, 2023). For example, spray drying of peanut protein resulted in high hydrophobicity and aggregation of globulin while albumin remained stable (Yang et al., 2022). Spray drying of plant proteins led to structural changes which influenced the thermal stability and emulsifying properties (Nie et al., 2023). In another related study, temperature, pH, and hydrophobic interaction were monitored to maintain the functional properties of pea protein isolate during spray drying (Burger et al., 2022a, b). Spray drying of protein may yield powder with distinct morphology, shape, and porosity leading to diverse functionality in food systems. Consequently, the drying step is critical to control the functionality of plant proteins and can be rationally designed to yield functionalized plant proteins for specific use.

For the past few years, advanced studies have widened the understanding of plant protein extraction and drying processes. Several reviews are available on new sources of plant proteins

(Adiamo et al., 2019; de Souza Celente et al., 2023; Kamal et al., 2021), and the effect of extraction methods on the physicochemical properties of plant proteins (Allotey et al., 2022; Momen et al., 2021; Pojić et al., 2018). Studies on novel protein extraction techniques have attracted more interest and considerable results were achieved and documented (Bou et al., 2022). Alkali extraction-isoelectric precipitation has been widely studied and utilized to isolate legume proteins effectively. However, no up-to-date review discussed the combined effect of extraction and spray drying on the properties, functionality, and composition of legume proteins. This review primarily focuses on legume proteins extracted using the alkali extraction-isoelectric precipitation technique. The present review also discusses the effect of spray drying conditions and the combined effect of extraction and spray drying conditions on the properties of legume proteins. Current gaps, challenges, and future works in the production of spray-dried legume proteins are also highlighted.

Extraction of Legume Protein

Legume proteins (soybean, pea, bean, cowpea, chickpea, lentil, and lupin) are the most popular source of plant proteins due to their low cost and abundance. Legume proteins are an important source of essential amino acids with high lysine content and limited content of sulfur amino acids (Sá et al., 2020). These proteins contribute enormously to food design because of their hydration, gelling, thickening, emulsifying properties, and oil and water holding capacity. The most considerable proteins in legumes are salt-soluble globulins which may account for 60–80%, while the water-soluble fractions known as albumins range between 10 and 20%, glutelin soluble in dilute acid or alkali with a concentration $\leq 10\%$, and alcohol soluble prolamins accounting for $\leq 6\%$. They are categorized as 2S, 7S, 11S, and 15S depending on their sedimentation coefficient (S). The molecular weights of legume proteins vary based on subunit composition and proportion (Shrestha, 2023). Protein composition and functional properties depend upon legume type, cultivar, and extraction conditions.

Legume proteins are extracted from defatted or non-defatted legume flour/meal at laboratory, pilot, and commercial scales using different techniques. Wet extraction is the generally applied method for extracting legume proteins. Alkali-isoelectric precipitation, alkali enzymes-assisted extraction, and ultrasound-assisted extraction are some common wet methods used for legume protein extraction (Miranda et al., 2022). The advantages of alkali-isoelectric precipitation over other methods include the production of highly pure proteins consisting of more than 90% protein, simplicity and easy scalability of the method, and low production cost (Allotey et al., 2022). Besides, alkali-acid precipitation is widely utilized at the industrial scale to yield highly pure protein powder for all legumes such as soybeans, peas, lentils, and beans. Figure 1 shows the schematic representation of spray-dried legume protein production using the alkali-acid precipitation technique.

During alkali-acid precipitation, the pH is first adjusted to (8–12) to degrade the protein matrix. Alkali treatment increases the surface charge and solubility of protein thereby enhancing separation from insoluble materials (Verfaillie et al., 2023). Alkali pH helps to break the cell wall plant materials which improves the elimination of lignin and other complex insoluble polysaccharide. Besides, the major proportion of legume proteins $\geq 60\%$ are soluble at alkali conditions which enhances the isolation process. On the other, the pH is shifted to an isoelectric point pH 4–5.5 during acid precipitation leading to charge neutralization between protein molecules, aggregation, and precipitation allowing the extractability of pure proteins (Abd Rahim et al., 2023). The pH of extraction media is paramount because it influences the yield,

aggregation state, solubility, and emulsifying properties of protein. It was previously reported that increasing the extraction pH from 8 to 9.5 may promote the unfolding and denaturation of protein, and formation of aggregates (Gao et al., 2020). Besides, the pH applied during acid precipitation has shown some influence on the composition and yield of protein. Legume proteins precipitated at pH 3.5 demonstrated a low protein recovery due to the interaction between extractable pectin polysaccharide with pKa near 3.5 and proteins (Verfaillie et al., 2023). At this particular pH, extractable polysaccharides may precipitate easily. Besides, the thermal degradation of 11S globulins precipitated at pH 3.5 decreased while the effect was not noted in 7S globulins. Legume proteins precipitated at pH 5.5 possess more 11S globulins than samples precipitated at pH 3.5 and 4.5.

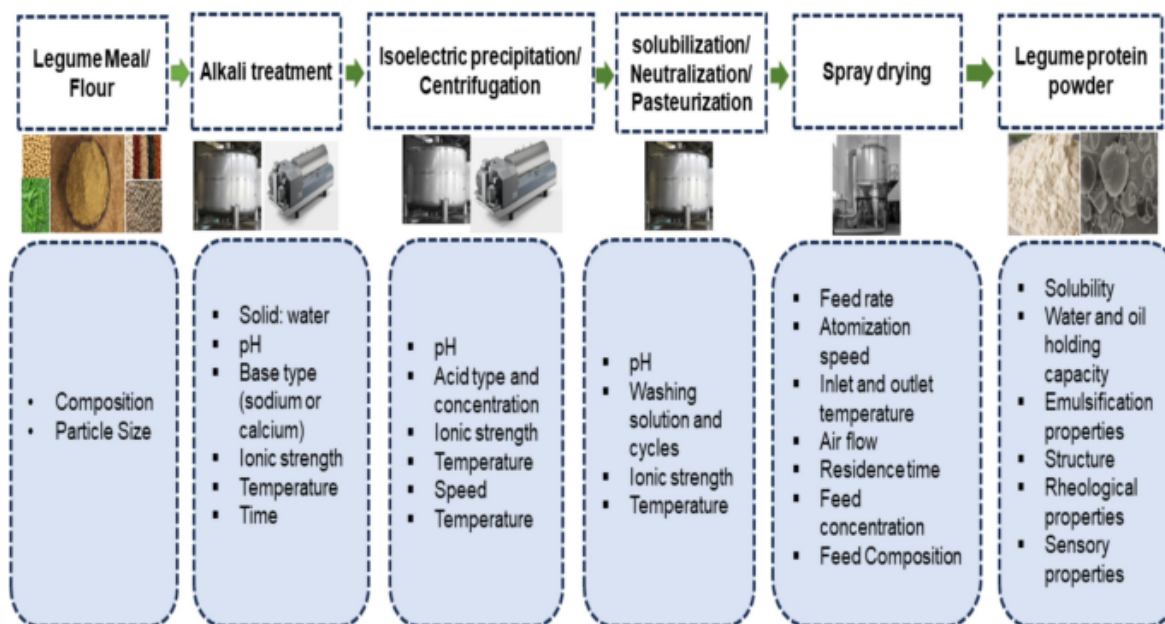


Fig. 1. Schematic representation of spray-dried legume protein production using alkali-acid precipitation technique

Apart from pH, temperature, particle size, processing time, alkali concentration, and the ratio between plant material and solvent have shown a significant influence on the protein yield, purity, and properties of legume proteins. Temperature influences the molecular interaction in the native legume proteins which under optimal conditions may cause denaturation. It is paramount to maintain the temperature at the lowest during legume protein extraction. In some cases, increasing the temperature may increase the solubility of protein and significantly increase the protein purity and yield in the extract (Preece et al., 2017). However, the temperature should be monitored to avoid loss of protein functionality. It is noteworthy that the particle size of raw material used during protein extraction affects the yield of protein. Low particle sizes have shown a high surface area which may favor the extraction yield; however, the use of smaller particle sizes favors the interaction between fibers and water increasing their water-holding capacity which can result in low legume protein yield. Extraction time influences the protein extraction yield as it was previously demonstrated that high protein yield and purity are achieved within 60–120 min (Allotey et al., 2022). Due to the energy cost implication, this interval may not be suitable for industrial scale. Also, the solid solvent ratio along with the alkali concentration may influence the yield of soybean, pea, lentil, and faba bean proteins. It

was previously demonstrated that a protein yield of 80% was achieved at 60 °C using a concentration of 4 mmol NaOH, while changing the concentration of NaOH significantly changed the protein yield of green tea leaves residue (Zhang et al., 2014). These findings are not only attributed to NaOH concentration because the solid solvent ratio and temperature have shown a significant influence on the protein yield. Choosing the appropriate alkali concentration, solid solvent ratio, pH and temperature, and ideal processing time may favor the production of high-purity protein with good physicochemical and functional properties.

Extracted legume proteins have shown versatile composition and functional properties which may be due to the difference in extraction conditions, legume type, and cultivar (Tang et al., 2021). For example, samples extracted at high alkali pH have shown a high protein while the ash concentration was linked to the formation of salt during acid precipitation. On the other hand, carbohydrates and fiber composition concentration may be associated with the refining process. Legume proteins have demonstrated a V-shaped solubility profile with the highest solubility under acid conditions (below pH 3) and alkali conditions (above pH 9) (Abd Rahim et al., 2023). The solubility of legume protein is very low at the isoelectric mostly between pH 4 and 5. The solubility depends upon the quantity of hydrophilic and hydrophobic amino acids, and in many cases, it is influenced by the number of charged amino acid chains (Dent & Maleky, 2022). Besides, the availability of hydrophilic regions of plants favors the interaction with water thereby increasing the solubility of legume protein. Controlling the denaturation degree and preventing the exposure of more hydrophobic amino acids to the surface during extraction is key to producing protein with high solubility. The solubility affects other properties such as foaming, emulsification, and gelling properties. More details on the functional properties of legume proteins will be discussed in the “[Physicochemical and Functional Properties of Spray-Dried Legume Proteins](#)” section.

Spray Drying as a Critical Step in the Production of Legume Proteins

Spray drying involves atomization of extracted legume protein into a spray followed by contact between hot air and protein which leads to moisture removal. The drying of extracted protein carries on until the recommended final moisture is attained. Dryer operating conditions and the initial properties, composition, and structure of extracted proteins influence the quality of legume protein powders. During spray drying of legume protein, atomization, flow rate, inlet and outlet temperatures significantly affect the solubility, emulsifying properties, particle size, morphology, and rheological properties of legume protein powder.

Atomization and feed rate significantly influence the distribution of extracted legume before drying which determines final powder yield, composition, and size; therefore, they must be delicately adjusted during spray drying. Rotary wheels, hydraulic nozzles, pressure nozzles, pneumatic nozzles, and ultrasonic nozzles are atomizers widely applied to induce the spray of extracted plant proteins (O'Sullivan et al., 2019). On the other hand, the feed rate may affect the mass and heat transfer during spray drying. A drastic increase in feed rate can decrease the drying yield and the mass transfer and can generate small particles due to a lower drying rate. A decrease in feed rate may result in the rapid generation of dried skin at the droplet surface which may favor case hardening. The inlet and outlet temperature are crucial drying parameters during spray drying. Too high inlet temperature can cause protein degradation, while too low temperature can lead to high moisture content and water activity which is responsible for rapid deterioration during storage (Li et al., 2023).

Effect of Spray Drying Conditions on Legume Protein

Infeed Concentration

Solid concentration and viscosity of extracted plant protein are very important as they affect the quality attributes of spray-dried legume proteins. As shown in Table 1, the infeed solid content of legume protein ranged between 10 and 25%. The solid concentration of extracted plant protein positively correlates with the viscosity and particle size of extracted legume proteins. High viscosity can destabilize the atomization process which may decrease the drying rate and favor the yield of legume protein powder with large particle size. At low infeed concentrations, there is more water to evaporate which can cause the expansion of legume protein particles. It was previously reported that an increase in infeed concentration allows the rapid drying of extracted legume protein in the drying chamber. On the other hand, a significant increase in solids increases the external porosity of spray-dried legume powders due to the reduction of agglomeration of particles during drying (Nguyen et al., 2017). Increasing the infeed concentration may yield powder with larger particle sizes and decrease the bulk density. It was noted that changing the infeed concentration can affect the color and aroma profile mostly a decrease in off-flavor intensities of legume protein powder (Park et al., 2014). In a study involving protein spray drying, an infeed concentration of 20–30% produced protein powder with a spherical shape while a 40% infeed concentration showed irregular powder. Additionally, a high infeed concentration of 40% may lead to the formation of powder with a strong out layer and low solubility attributed to protein denaturation (Siddiqui et al., 2024).

As a change in infeed concentration tends to have a significant impact on the evaporation rate, drying characteristics, and final quality of legume protein powder, it is important to carefully select the ideal concentration to preserve the functional properties of spray-dried legume proteins. Ideal infeed solids should prevent prolonged evaporation and heating as the latter can cause denaturation of protein and also can lead to economic losses. Investigation of the effect of infeed concentration, atomization, inlet, and outlet temperature on the detailed characteristics of different legume proteins could help to enhance the quality of the final legume protein powder.

Atomization, Feed Rate, Inlet, and Outlet Temperatures

According to Table 1, the feed rate ranged from 6 to 25 mL/min and the type of atomizers used were two-fluid nozzle, centrifugal nozzle, and rotary atomizer while the diameter of the atomizer varied from 1 to 3 mm. The inlet temperature and outlet temperatures used during spray drying varied from 130 to 195 °C and 60 to 97 °C, respectively (Table 1). By controlling the feed rate, atomization, inlet, and outlet temperature, the authors produced legume protein powder with different physicochemical and functional properties. Atomization and high temperature can cause the denaturation of legume proteins, and water evaporation may induce the perturbation of the hydrogen bonds between legume proteins and enclosed water molecules (George et al., 2022). Denaturation and perturbation of hydrogen bonds may alter the structure, morphology, and functionality of legume protein powder.

Table 1 Spray drying legume protein extracted using various conditions

Legume protein	Extraction conditions			Spray drying conditions			Observations and protein yield	References	
	Flour: water	Alkali Conditions and protein yield	Acid Conditions and protein yield	Inlet and outlet Temp °C	Infeed Content % and pH	Drying scale			Atomizer Type and nozzle diameter, feed rate
Pea protein	1:15	pH 9 25 °C and 90 °C for 60 min 1250×g The protein yield was not determined	pH 4.5 1250×g The protein yield was not determined	165 °C, 180 °C, and 195 °C; 80 °C, 87 °C, and 97 °C	12% pH 5, 7, and 9	Laboratory spray dryer (pilot plant spray dryer)	Two fluid nozzle atomizers Diameter of the nozzle 2.5 mm Centrifugal nozzle	The temperature did not have a significant effect on particle size distribution but affected the hydrophobicity Spray drying pH demonstrated a significant impact on the size distribution pH 9 showed a small and uni- modal size of approx. 20 µm with high solubility and high emulsifying properties pH 7 had a size of approxi- mately 40 µm with a unimodal distribution pH 5 wide peak ranging between 10 and 1000 µm Laboratory spray dryer small particle and unimodal distribu- tion, high solubility in water Pilot plant spray dryer average particle size 40 µm The protein yield was not determined	Burger, et al. (2022a, b)
Mung bean pro- tein isolate	1:10	(pH 9) for 90 min stirring speed 2000 rpm at 30 °C 8586 g The protein yield was not deter- mined	pH 4.5 Centrifugation at 8586 g The protein yield was not deter- mined	185 °C and 90 °C	25% pH 7	Pilot scale spray dryer	Rotary atomizer Flow rate 6 mL/ min	The spray drying temperature affected the lightness of the protein powder The drying temperature used in this study did not cause the dissociation of protein subunits at the quaternary structure level Hydrophobic amino acids were more resistant during spray drying Hydrophilic amino acids were affected by spray drying Drying conditions affected the solubility, water absorption, and oil absorption capacity The protein yield was not determined	Brishti, et al. (2020)

Table 1 (continued)

Legume protein	Extraction conditions			Spray drying conditions				Observations and protein yield	References
	Flour: water	Alkali Conditions and protein yield	Acid Conditions and protein yield	Inlet and outlet Temp °C	Infeed Content % and pH	Drying scale	Atomizer Type and nozzle diameter, feed rate		
Soy protein isolate	1:20	pH 7, 8, and 9 shaking at 150 rpm for 60 min at 25 °C Centrifugation 10,000 g or 15,000 g for 10 min Protein % yield at pH7 (59–63%) pH 8 (60–64.8%) pH 9 (60–64.7)	pH 3.5, 4.5, or 5.5 Centrifugation 10,000 g or 15,000 g for 10 min Protein % yield at pH 3.5 (53–55%) pH 4.5 (52–56%) pH 5.5 (50.8–52%)	157 °C and 74 °C	10% and pH 7	Laboratory mini spray dryer	Flow rate 9 mL/min Two fluid nozzles Nozzle diameter 3 mm	Protein powder produced from samples centrifugated at 10,000 g showed aggregates The powder produced from samples centrifugated at 15,000 g showed less aggregate and high solubility The particle size also depended on the centrifugation degree and the state of colloidal suspension Increasing the centrifugation speed decreased the protein content Protein Yield was not determined	Verfaillie, et al. (2023)
Peanut protein isolate	1:10	Alkali treatment at pH 8 at 25 °C Centrifugation at 10,000 g for 15 min The protein yield was not determined	Acid precipitation pH 4.5 Centrifugation at 10,000 g for 10 min The protein yield was not determined	180 °C and 85 °C	10% and pH 7	Laboratory mini spray dryer	Flow rate 6.5 mL/min	The drying temperature decreased the oil holding capacity High temperature caused shrinkage of droplets Collapsed and uneven surface morphology High temperature decreases the protein disulfide bonds Temperature applied during drying affected the alpha helix content The protein yield was not determined	Gong, et al. (2016)
Pea protein	1:10 Flour particle size 100 µm	pH 8 for 120 min Centrifugation at 9000 g for 30 min The protein yield was not determined	pH 4.5 The protein yield was not determined	185 °C and 60–70 °C	> 2% and pH 8	Laboratory mini spray dryer	Flow rate 25 mL/min Diameter nozzle: 1 mm	The high temperature increased the losses of proteins High temperature decreased the solubility Spray drying increases resistance to dilatational deformation of interfacial layers The protein yield was not determined	Yang et al. (2022)

Table 1 (continued)

Legume protein	Extraction conditions			Spray drying conditions				Observations and protein yield	References
	Flour: water	Alkali Conditions and protein yield	Acid Conditions and protein yield	Inlet and outlet Temp °C	Infeed Content % and pH	Drying scale	Atomizer Type and nozzle diameter, feed rate		
Black bean protein concentrate	1:20	pH 8 at 60 °C for 60 min Centrifugation at 4000 g for 30 min at 25 °C Protein % yield pH 8 (41%)	pH 4.5 Protein % yield pH 4.5 (50.3–60.5%)	155 °C and 70 °C	Not provided	Laboratory mini spray dryer	Flow rate 12 mL/min	Sample pretreatments (high temperature pressure cooking and extrusion) showed a significant impact on emulsifying properties, emulsion capacity, and protein yield Protein % yield 56.7%	Alfaro-Diaz et al. (2021)
Cowpea protein isolate	Not provided	Alkali treatment pH 8 Centrifugation at 10,000 g for 20 min The protein yield was not determined	at pH 4.3 Centrifugation at 10,000 g for 15 min The protein yield was not determined	180 °C and 85 °C	Infeed not provided pH 7	Laboratory mini spray drying	Flow rate 6.5 mL/min	Spray drying conditions influenced color parameters, solubility, bulk density, and porosity of the powders Spray drying conditions affected the emulsion capacity and foaming capacity The protein yield was not determined	Rudra et al. (2016)
Pea protein isolate	1:15	pH 9 and centrifugation (centrifugation speed not provided) The protein yield was not determined	pH 4.5 The protein yield was not determined	130 °C and 70 °C	20% and pH 7	Laboratory mini spray dryer	Flow rate 10 mL/min	The temperature did not alter the secondary structure of protein and powder porosity Drying conditions did not alter water holding capacity, emulsion stability, and solubility The protein yield was not determined	Cui et al. (2021)
Yellow pea protein isolate	1:15	pH 8.5, 9, 9.5, and 10 for 1 h at 23 °C Centrifugation at 5524 g for 20 min at 4 °C The protein yield was not determined	pH 4.5 Centrifugation at 5524 g for 10 min The protein yield was not determined	130 °C and 80 °C	20% and pH 7	Laboratory mini spray dryer	Two-fluid nozzle The feed rate of 0.3 L/h Aspirator flow of 40 L/h	The exterior morphology of powders was irregular and sphere-shaped. Hollow particles were also observed The protein yield was not determined	Cui et al. (2020)

Table 1 (continued)

Legume protein	Extraction conditions			Spray drying conditions				Observations and protein yield	References
	Flour: water	Alkali Conditions and protein yield	Acid Conditions and protein yield	Inlet and outlet Temp °C	Infeed Content % and pH	Drying scale	Atomizer Type and nozzle diameter, feed rate		
Pea protein isolate	1:15	pH 8.5 Centrifugation at 6000 g for 20 min The protein yield was not determined	pH 4.5 Centrifugation at 6000 g for 10 min The protein yield was not determined	160 °C and 100 °C	20% and pH 7	Laboratory mini spray dryer	feed rate of 0.15 l/h Aspirator flow of 40 l/h	The exterior of the powder was smooth and regular The size was uniform; however, formulation with maltodextrin produced powder with excellent properties compared to gum Arabic The use of gum Arabic and maltodextrin decreased the extent of beany flavor The protein yield was not determined	Lan et al. (2019)

Direct exposure of plant protein to mechanical stress and temperature above 180 °C can cause the denaturation of legume proteins (Saha et al., 2019). A low feed rate and high atomization speed/pressure can create a high surface area and may increase the temperature of the spray up to 10–15 °C. This increases the susceptibility of legume protein to thermal degradation which was confirmed by a significant decrease of amino acids in mung bean (*Vigna radiata*) protein isolate powder (Brishti et al., 2020). There is a relationship between atomization speed, feeding rate, and temperature on the insolubility index of protein where increasing atomization speed and temperature increased the insolubility index of walnut milk protein powder from 0.62 to 3.5 mL (Saha et al., 2019). Additionally, high atomization pressure may induce a slight change in three-dimensional structure leading to exposure of buried hydrophobic amino acids and poor foam stability of rehydrated legume protein powder. In some scenarios, extreme atomization conditions may significantly decrease α -sheet and β -sheet (Dao et al., 2022). The alteration of legume protein structure affects the emulsifying properties and solubility. An increase in the atomization speed at a medium feed rate can yield high-density small particles with a smooth surface while a high-speed rate and high atomization may result in large coarse particles with a rough surface (Pinto et al., 2021). Figure 2A and B show the effect of inlet and outlet temperatures' pH on the morphology of protein powder. Comparing atomizer performance, it was noted that fluid nozzle can generate particle size ranging between 8 and 800 μm while pneumatic nozzles, spray wheels, and rotating cups may generate particle size varying from 3 to 250 μm , 2 to 550 μm , and 25 to 950 μm , respectively. It should be noted that the average particle size is linked to the viscosity and feed rate of any specific atomizer. Legume proteins have demonstrated different compositions, molecular weights, amino acids, viscosity, rheological properties, and solubility. Therefore, the atomization process, nozzle type and design, feed rate, and inlet and outlet temperature should be optimized for different legume protein types to preserve their functional properties. Figure 3A, B, and C display emulsions prepared using pea protein spray dried at different inlet temperatures. Maltodextrin, starch, and trehalose are utilized to prevent heat damage; however, they should be thoroughly applied as they significantly decrease the concentration of legume protein in the final powder. Besides, the atomizer should yield sprays of the same size which may easily cause uniform drying and low moisture content of final powder. The size of atomized legume proteins should be carefully controlled to prevent legume protein denaturation.

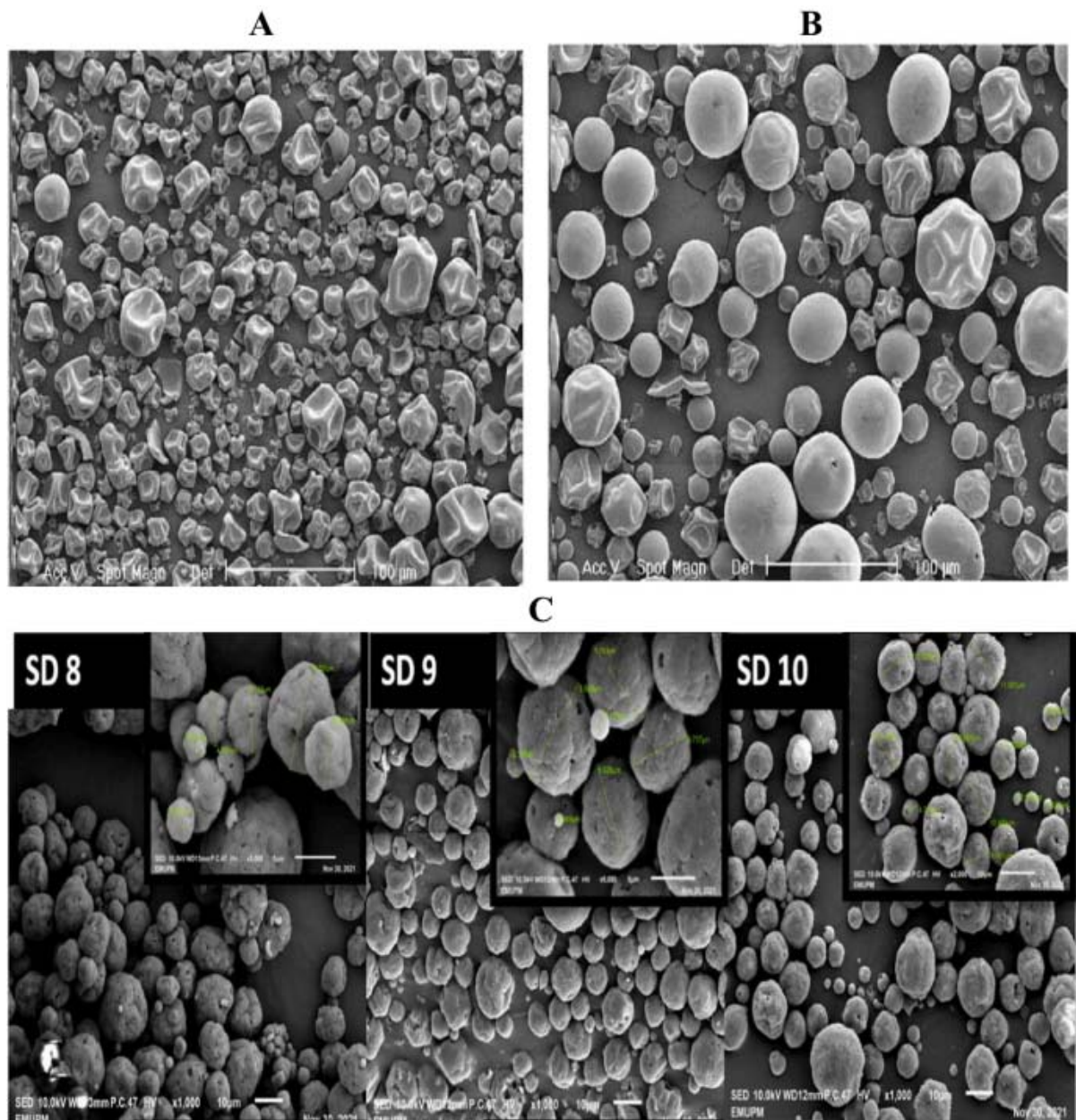


Fig. 2. Effect of inlet and outlet temperature and extraction pH on the morphology of spray-dried protein. **A** Inlet and out temperature 145 °C/85 °C, **B** inlet and out temperature 205 °C/105 °C, **C** SD8: pH 8, SD9: pH 9, and SD10: pH 10. (Sources Kim et al., 2009; Abd Rahim et al., 2023)

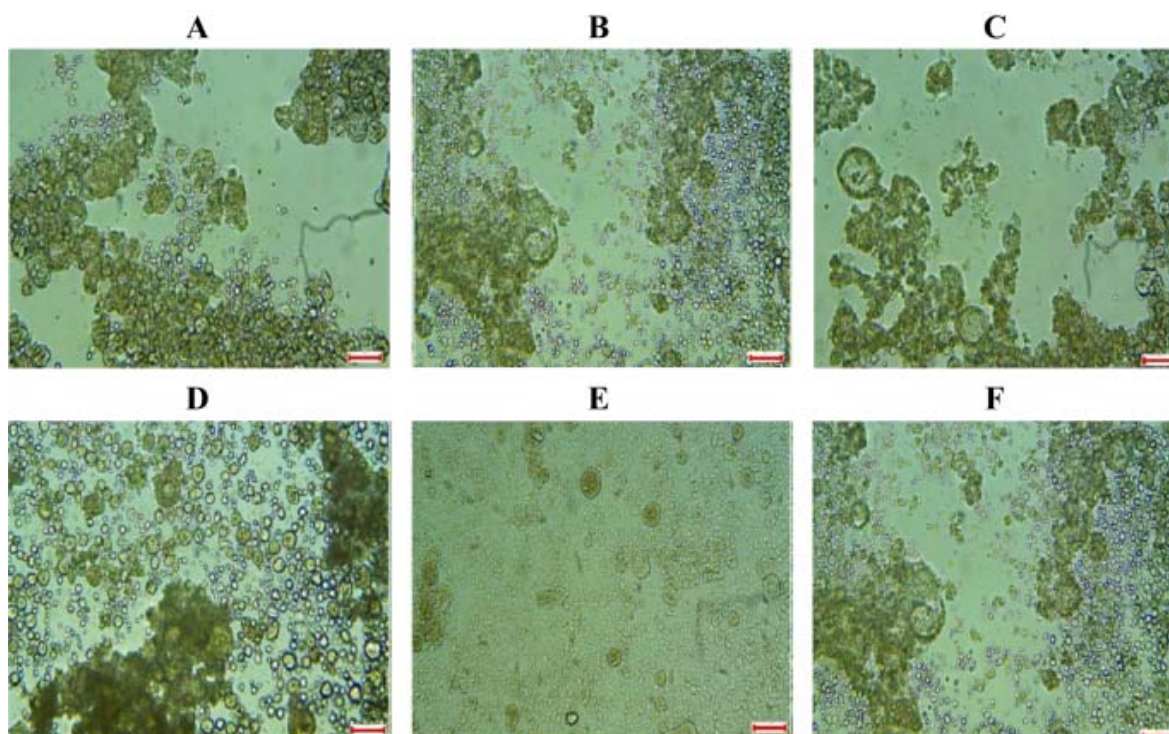


Fig. 3. Emulsion prepared using spray-dried pea protein extracted using alkali isoelectric precipitation method. **A** Inlet and outlet temperature 165 °C/80 °C, **B** inlet and outlet temperature 180 °C/87 °C, **C** inlet and outlet temperature 195 °C/97 °C, **D** pH 5, **E** pH 9, **F** pH 7. The scale bar represents 50 μm

Spray Drying Scale

Legume protein powder may be produced using a laboratory scale (0.01–0.5 kg/h), pilot plant (0.2–20 kg/h), and large commercial (10–1000 kg/h) spray dryer. Considering the drying space among different scales, numerous variations can occur such as evaporation capacity, throughput, and particle physical and chemical properties (Poozesh & Bilgili, 2019).

As shown in Table 1, most published studies use laboratory spray dryers while only very few works applied pilot or commercial spray dryers. Therefore, the results obtained may not reflect the real situation at pilot and commercial scales. For example, a study revealed that laboratory spray dryer produces particles with an average size of approximately 10 μm while pilot spray dryers and commercial spray dryers yield particles with an average size of 30 μm and 50 μm, respectively (Burger et al., 2022a, b). The minuscule dimension of the drying chamber of the laboratory spray dryer limits the residence time; thus, the average particle size of the infeed materials must be kept smaller to achieve a high degree of drying. Although a similar geometry was observed between the pilot spray dryer and commercial spray dryer, the residence time is twice as long in the commercial spray dryer as in the pilot scale. The residence time is linked with drying chamber dimension and airflow and can significantly affect the color, solubility, and emulsifying properties of legume protein powder (Bordón et al., 2021). The changes associated with the residence time may be attributed to extended exposure of legume powders to high temperatures. The particle temperature increases until it gets to the exit of the spray dryer; therefore, the longer time may lead to a higher deterioration degree.

However, spray drying at a laboratory scale offers more control over the “pilot or commercial” spray drying processes which may result in the high protection of legume proteins and powder with desired physicochemical properties. There are challenges associated with lab-scale drying such as the ability to produce a very small quantity of legume protein which may limit the availability of high-quality proteins. Drying legume protein with an average particle size $\geq 15\text{--}30\ \mu\text{m}$ using a laboratory spray dryer may result in powder with high moisture which may affect the particle size and induce crystallization during storage (Pinto et al., 2021). Additionally, lab-scale spray drying has shown a relatively low powder yield compared to commercial and pilot spray dryers. Low powder yield may be attributed to the low thermal efficiency and adhesion of small particle powder during spray drying. A previous study reported a spray drying yield of 60–80% and high thermal efficiency during pilot spray drying while the yield was below 50% at the laboratory scale (Bordón et al., 2021). Pilot and commercial spray dryers are used to spray dry a vast amount of legume protein powder within a wide range of particle sizes. Before spray drying, legume proteins undergo different extraction processes, separation, and purification which later have a significant impact on the properties of spray-dried legume proteins. Depending on the composition and sensitivity of legume protein to high temperatures, it is necessary to optimize spray drying to avoid degradation during drying and storage.

Combined Effect of Extraction and Spray Drying Conditions on Legume Protein

The extraction conditions have an impact on the fractionation, composition, and properties which in turn may affect the shear response, evaporation rate, and particle formation during the spray drying process. Figure 4 displays a summary of the effect of processing steps on legume protein powder.

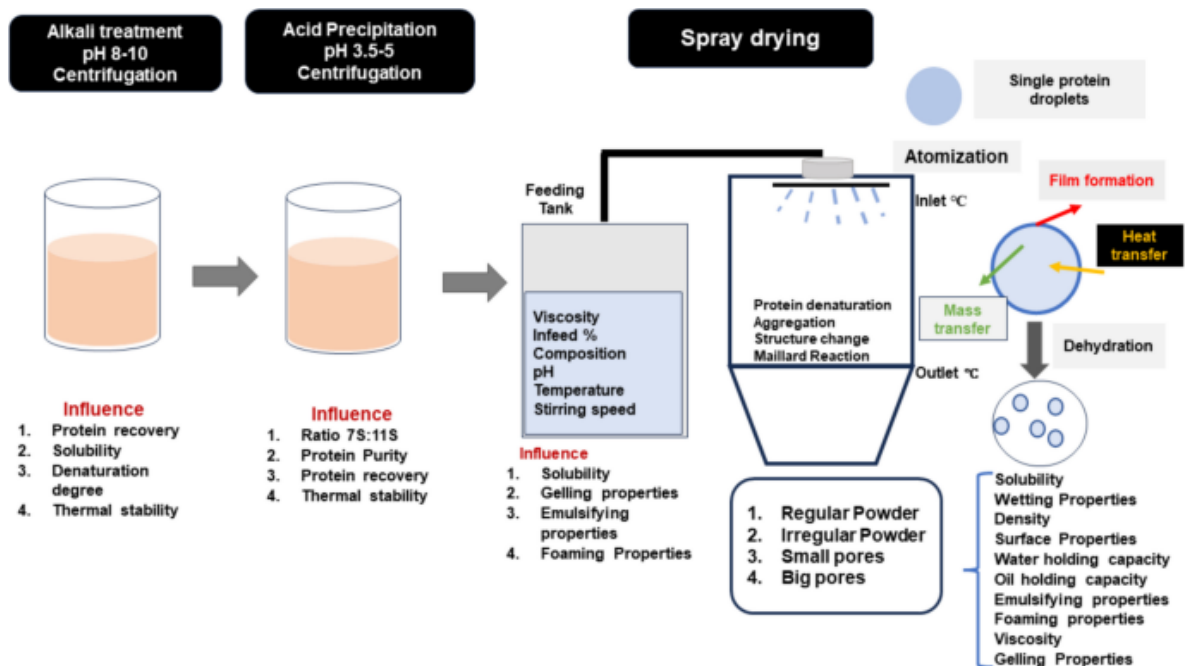


Fig. 4. Summary of the effect of processing steps on legume protein powder

As shown in Table 1, the alkaline extraction-acid precipitation method is the major method widely utilized for extraction as it allows a high yield of protein from legumes. The pH during alkali treatment of legume protein ranged from pH 7 to 9.5, while the pH for acid precipitation ranged from pH 3.5 to 5.5 (Table 1). The temperature used during legume protein extraction varied from 25 to 90 °C, while the solid water ratio ranged from 1:20 to 1:10. It is evident that the combination of extraction conditions and spray drying have a direct influence on protein recovery, physicochemical and functional properties.

Effect of pH and Temperature

Drying protein extracted at various pH revealed a significant variation in particle size distribution, where the sample extracted at pH 8 produced small particles with unimodal distribution while samples extracted at pH 9.5 produced larger particles with a multimodal distribution (Abd Rahim et al., 2023). These findings can be attributed to the further aggregation and formation of dense network structures of less denatured legume protein extracted at pH 9 during spray drying. Figure 2C shows the effect of various alkali pH on the morphology of protein powder. Mild alkaline extraction of legume protein enhances the thermal stability, decreases the browning effects, and yields spherical particles. On the other hand, the zeta potential of legume protein powder increases as the extraction pH increases which may be due to the availability of amino acid groups and the protein conformation change (Julakanti et al., 2023).

Spray drying pH has also shown a considerable effect on the solubility and emulsifying properties of legume proteins. Under similar drying conditions, spray drying pea protein at pH 5, 7, and 9 produced powder with a solubility of 23%, 56%, and 66%, respectively (Burger et al., 2022a, b). The particle size of pea protein spray dried at pH 5, 7, and 9 was 71.5 µm, 30.5 µm, and 24.3 µm, respectively. The large particle size at pH 5 may be attributed to the low electrostatic repulsion at pH near the isoelectric point. Additionally, the hydrophobic interaction at pH 5 demonstrated an important role in the aggregation of legume protein particles during spray drying. On the other hand, changing the pH of the extracted legume protein from pH 7 to pH 9 or from pH 7 to pH 5 before spray drying significantly increased the hydrophobicity. These findings suggested that the pH affects the conformation of legume proteins and the number of hydrophobic regions at the protein surface which may influence the functionality of resultant powders (Julakanti et al., 2023). Figure 3D, E, and F represent emulsions prepared using pea protein spray dried at different pH.

Spray-dried soy protein produced at extraction temperatures of 25 °C, 40 °C, and 60 °C showed different denaturation enthalpies (Preece et al., 2017). It was also revealed that spray-dried powder of legume protein extracted at 60 °C has a significantly lower denaturation enthalpy. These findings may be attributed to the combination of alkali pH and temperature that enhances protein susceptibility to denaturation. The solubility of the samples extracted at 25 °C was relatively low approximately 91% at pH 7, while the solubility of samples extracted at 40 °C, and 60 °C was 95.3% and 93.7%, respectively. The low solubility of powders extracted at 25 °C was attributed to the high percentage of native protein during spray drying. Samples extracted at 60 °C revealed a high hydrophobicity due to the high hydrophobic regions exposed during the extraction process. Powder resulting from legume protein extracted at high temperatures (40 and 60 °C) showed the highest foaming capacity emulsification capacity and emulsification stability which may be due to the availability of sufficient hydrophobic patches.

Effect of Centrifugation and Separation

Centrifugation and decanting are applied to separate particles with different sizes and densities during legume protein extraction. This step is paramount as it influences the protein powder yield, protein fractions, and particle size.

As shown in Table 1, the centrifugation speed used during legume protein extraction ranges from $3200 \times g$ to $15,000 \times g$ using the lab scale while the centrifugation speed was $1250 \times g$ at the pilot scale. The results revealed that increasing the centrifugation speed resulted in legume protein powder with fewer aggregates although this may significantly lower the protein content in the spray-dried powders (Burger et al., 2022a, b). The significant decrease in legume protein powder yield may be attributed to the loss of soluble aggregate. High centrifugal speed yields powder with low average particle size, low polydispersity index (PDI), and high charge density. This processing step also affects the protein fraction and molecular weight distribution of final legume protein powder. Centrifugation at high speed can cause a significant change in soy protein from larger aggregates to native globulin (Verfaillie et al., 2023). Considering large-scale separation using a decanter centrifuge, the design of the reactor mostly inner diameter, the residence time, and the infeed concentration are key factors in obtaining high-quality legume proteins suitable for food application (Haller & Kulozik, 2019). It was demonstrated that a slight increase in plant material flour can result in a 13% increase in finished product yield (Preece et al., 2017). Contrary to these results, it was reported that decreasing the particle size from 223.4 to 89.5 μm can significantly increase the protein recovery (Preece et al., 2017). On the other hand, decanter centrifuges are effective when the plant materials are not too fine. Previous findings on casein fractionation using a decanter centrifuge showed that an increase in the centrifugal speed from 2000 to 4000 g drastically increased the protein yield because the increase in angular velocity positively correlates with separation efficiency (Schubert et al., 2018). The separation of legume protein is also affected by the viscosity and the difference in density among particles. It was pointed out that the separation or decantation step should be carefully designed to achieve a small particle size and sufficient zeta potential. The latter is crucial to the stability of legume protein powder suspensions.

Composition and Properties of Spray-Dried Legume Proteins

Spray-dried legume proteins have prominent nutritional and functional properties causing their potential use in various food products. The composition of legume protein powder plays an important role in food application, functional properties, and storage stability. Besides, legume type and cultivar type, extraction method and drying method, and conditions can have a significant effect on the functional performance.

Chemical Composition of Spray-Dried Legume Proteins

The composition of spray-dried legume protein is presented in Table 2. The protein content of all samples ranged from 60 to 90%. The variation in protein content may be attributed to the grinding before extraction, isolation process, and drying losses. Factors such as pH, salt type, and concentration used during the extraction process have a big impact on the protein content. The solubilization effect and losses of protein fractions under various pH and ionic strengths lead to different protein content (Shrestha et al., 2021). Given the importance of protein composition during various food applications, extraction processes and spray drying can be adjusted to yield legume protein powder for specific applications.

Table 2 Composition and properties of spray-dried legume proteins

Protein	Composition						Solubility At pH 7 %	Particle size (µm)	Water holding capacity g of H ₂ O/1 g protein (g/g)	Oil holding capacity g of oil/ 1 g of protein (g/g)	Emulsifying activity index (m ² /g)	Emulsion stability index (minutes)	Reference
	Protein %	Fat %	Moisture %	Ash %	Fibers %	Carbohydrates %							
Mung bean protein	75	1.23	3.59	0.48	0.17	18.9	45	17.77	3.03	4.00	29.21	351.90	Brishti et al. (2020)
Cowpea protein isolate	88	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	58	Not mentioned	4.64	2.52	101.83	3656.1	Rudra et al. (2016)
Pea Protein Isolate	85	2.12	5.11	4.69	Not mentioned	8.5	83	Not mentioned	2.40	3.29	31–39	Not mentioned	Cui et al. (2021)
Black bean protein concentrate	60–80	Not mentioned	Not mentioned	Not mentioned	Not mentioned	Not mentioned	72–86	Not mentioned	1.46	1.32–1.54	Not mentioned	Not mentioned	Alfaro-Diaz et al. (2021)
Soy protein isolate	78.9	Not mentioned	3	4.2	Not mentioned	Not mentioned	60–70	5–50	3.9	2.3	44	1400	Blaise et al. (2017)
Pea protein isolates	90	4.7	6–9	5.3–8.5	Not mentioned	Not mentioned	40–62	7.5–18.5	Not mentioned	Not mentioned	Not mentioned	Not mentioned	García Arteaga et al. (2021)
Lupin protein isolate	81–94	3.7	Not mentioned	2.6–3	7–11	0.5	65	Not mentioned	1.8	1.2	Not mentioned	Not mentioned	Devkota et al. (2023)
Common bean protein concentrate	80	2.67	3.71	1.77	Not mentioned	15.83	72.5	9.82	1.9	1.3	18.87	21	de Paiva Gouvêa et al. (2023)

The moisture content of all legume protein powder varied between 3 and 9% (Table 2). A low moisture content below 5% is important for storage stability. The variation in moisture content was due to the composition of extracted legume protein and drying conditions. High inlet temperature causes the rapid evaporation of water and promotes the formation of crust which prevents further moisture loss resulting in protein powder with high moisture content while low inlet temperature enhances the evaporation of water before skin formation leading to powder with low moisture content (Phosanam et al., 2021). Outlet temperature helps to adjust the final moisture content whereas high outlet temperature leads to low moisture content. By controlling the heat and mass transfer using various feeding rates, we can influence the evaporation rate and produce powder with low moisture content. On the other hand, an increase in protein concentration positively correlates with high final moisture content due to the strong ability of protein to bind water. It was demonstrated that the glass transition of legume protein powder increases with a decrease in moisture content (Ogolla et al., 2019). Also, increasing the moisture content to some extent tends to increase powder cohesiveness. Furthermore, the moisture content of spray-dried legume proteins can influence the stickiness, oxidation of lipids, caking, and browning. Feeding rate and inlet temperature may be controlled to yield protein powder within low moisture levels allowing high storage stability. As legume protein powders are hygroscopic, they should be stored in a completely closed environment to avoid uptake or loss of moisture.

As shown in Table 2, the amount of fat in legume protein varied from 1.23 to 9%. The difference in polarities of solvent and method used during defatting affect the fat content. Legume protein powders with high-fat content have poor wettability and dispersibility in aqueous media. Findings have previously reported that increasing the fat content in protein powder from 1 to 9% leads to a slower and longer dissolution process while a further significant increase may cause floating (Fournaise et al., 2021). The interaction of fats and protein during protein isolation and spray drying favors the formation of hydrophobic β -sheets which enhances the hydrophobic nature and poor solubility of protein powder in water (García Arteaga et al., 2021). Spray-dried plant protein powder tends to assemble because of the sticky properties of fats. The surface should be monitored to avoid oxidation, poor flowability, and stickiness. High atomization speed increases the surface ratio and migration of fats to the surface; also, high inlet and outlet temperatures induce cracking, fissuring, and melting of fats leading to high surface fats (Habtegebriel et al., 2021). Adjusting the inlet and outlet temperatures and atomization speed can lead to proper encapsulation of fats and enhanced storage stability of legume protein powder with elevated fat content.

As shown in Table 2, the carbohydrate profile of spray-dried legume protein ranged from 8.5 to 18.9%. Isolation processes, legume varieties, and origin affect the final concentration of carbohydrates in protein powders. There are two types of legume protein, highly refined isolates with a protein composition of over 80–90% and concentrates with a protein range between 50 and 70% (Ma et al., 2022). Protein concentrates have a higher concentration of carbohydrates than protein isolate. It was demonstrated that alkali extraction increases the protein yield while acid ameliorates the yield of carbohydrates (Gerliani et al., 2020). Additionally, enzyme-assisted extraction enhances the unbinding of protein and carbohydrate which may significantly affect the carbohydrate content in final powder. Proteins are labile to heat and the presence of high concentration of carbohydrate during spray drying may increase the thermal stability of protein. Depending on the conformation of legume protein at the air interface during spray drying, carbohydrates may dominate the droplet development, shape, and smoothness of the final powder. In some cases, the presence of carbohydrates increases the porosity which results in higher inward water diffusion and good rehydration behavior of

legume protein powder (Han et al., 2022). However, spray drying of legume protein with a high concentration of carbohydrates can lead to a Maillard reaction that affects the color and aroma of dried powder. The extent of the Maillard reaction is influenced by inlet temperature, feed concentration, and residence time.

The mineral content of legume protein powders ranged between 0.48 and 8.5% (Table 2). The data revealed that most samples contained an ash content of $\geq 3.5\%$. The high concentration of ash may be due to the application of strong acid and alkali during pH adjustment that may contribute to salt formation leading to a high ash content in powder (Gao et al., 2020). Sodium or calcium hydroxide and acids are widely used during plant protein extraction. A calcium concentration of 10 mg/g of proteins influences the surface hydrophobicity of protein powder and significantly decreases the solubility of legume protein powder (Peng et al., 2020). High calcium induces a strong electrostatic attraction between the polypeptide and hydrophobic interaction; the combination effect of this phenomenon with high temperature during spray drying significantly decreases the solubility (Manassero et al., 2018). Finding alternative bases for calcium hydroxide and washing protein with water in the absence of calcium could be ways of reducing mineral composition and increasing the solubility of legume proteins.

Physicochemical and Functional Properties of Spray-Dried Legume Proteins

Solubility, particle size, water and oil holding capacity, emulsion capacity, and stability are current methods widely used to characterize protein functionality. Table 2 shows the functional properties of spray-dried legume protein powders.

The solubility is used to determine the functional performance of spray-dried legume proteins because it reflects protein-protein and protein-solvent interaction under various conditions. It is very important to verify whether a certain legume protein can be used in food, beverage, and pharmaceutical products as denaturation and aggregation affect the solubility of protein. Solubility correlates with the pH where the solubility of protein at the isoelectric point is very low. As shown in Table 2, the solubility of legume proteins at pH 7 ranged from 40 to 86%. Variation in legume protein solubility can be attributed to the difference in protein surface charges because of the exposure or formation of charged residue (Mune Mune & Sogi, 2015). Besides, exposure of buried hydrophobic protein residue after spray drying can lead to the production of insoluble aggregate which in turn lowers the solubility of legume proteins. In addition, the concentration and type of salt and solvent used during extraction can affect the solubility of legume proteins. On the other hand, the content and ratio between water-soluble (albumin), salt-soluble (globulin), and organic solvent (prolamins) proteins affect the solubility and functionality (Ma et al., 2022).

As shown in Table 2, the particle size of legume protein ranged from 5 to 50 μm which is in agreement with previous studies demonstrating that spray drying produces particle size below 250 μm . The particle size variation may be attributed to legume protein composition, feeding rate, and atomization speed during spray drying. Powder particle size affects the flow and its reconstitution behavior. A relatively large particle size enhances the flowability due to a low specific surface area. On the other hand, a significant decrease in particle size increases the contact area between particles which results in high cohesive force and a low flow index (Barone et al., 2019).

Water holding capacity designates the amount of water that can be absorbed by 1 g of protein material. As shown in Table 2, the water holding capacity varied from 1.46 to 4.64 g/g. The

water absorption capacity is influenced by the availability of water acceptors in the protein hydrophilic group. The water molecules bind to charged groups, backbone peptide groups, amide groups, hydroxyl groups, and nonpolar residues of amino acids (Blaise et al., 2017). Besides, the water holding capacity is affected by the physical configuration of the protein network, microstructure, powder pore size, and amino acid composition. Low salt concentration increases the water holding capacity because of salt ions binding water to proteins. Carbohydrates possess hydrophilic parts including polar and side-charged chains; their concentration in legume protein samples can improve the water-holding capacity (Cortez-Trejo et al., 2021). On the other hand, oil holding capacity indicates the amount of oil that can be absorbed by 1 g of protein. As displayed in Table 2, the oil-holding capacity of legume protein powder ranged from 1.2 to 4 g/g. The interaction between lipids and proteins results from the binding between the aliphatic chain of lipids and the non-polar side chain of amino acids; consequently, legume proteins with high hydrophobicity tend to hold more lipids. The high hydrophobicity of legume protein is attributed to the exposure of the hydrophobic group during the extraction and spray drying processes. The oil-holding capacity of legume proteins is associated with protein composition mostly hydrophobic amino acids and distribution, and the pore structure (Stone et al., 2015).

The emulsifying activity index refers to the ability of legume protein to form an emulsion, while the emulsifying stability index gives a measure of the stability of diluted emulsion over a determined time (Ma et al., 2022). As shown in Table 2, the emulsion activity index and emulsion stability index of legume protein powder varied from 18.87 to 102 m²/g and from 21.26 to 3656 min, respectively. The findings showed differences in the emulsifying activity index and emulsifying stability index among various proteins which may be attributed to the source and cultivar of legume. Besides, extraction conditions and time can affect the emulsifying activity index and emulsifying stability index. However, the surface charge, solubility, hydrophobicity, and balance between hydrophobic and hydrophilic interaction significantly alter the emulsifying activity index of legume proteins. Considering these factors, it was demonstrated that legume protein produced by isoelectric precipitation possessed a higher emulsifying activity index than its counterpart produced using salt extraction (Ma et al., 2022). On the other hand, emulsion stability is influenced by protein concentration, molar mass and oil globule in suspension, size distribution and spatial arrangement, and the thickness of the interfacial film (Ferreira et al., 2021).

Gaps and Challenges

Gaps

In the current literature review, it was observed that most studies focus on the effect of infeed composition, properties, and spray drying conditions on the functional characteristics of legume protein powders. Very few studies address the challenges associated with the storage stability of legume protein powders. Also, works investigating and modeling the effect of processing conditions, composition, handling, and storage conditions with emphasis on spray-dried legume protein powder are scarce. For example, the effect of 1-year storage at different temperatures on the *in vitro* digestibility of soy protein isolate was examined (Guo et al., 2020). Another related study investigated the storage stability of soy protein powders spray dried at pH values of 5–8 (Guo et al., 2020). The effect of preheating soy protein isolates at 80 or 90 °C before spray drying was also reported (Guo et al., 2015). Taking into account the effect of legume source, cultivar, and extraction conditions on the composition, properties, and functionality of protein powder, these studies are not sufficient to draw relevant conclusions

and recommendations. The protein content and fat content of 24 commercial legume proteins varied from 56 to 92% and 5 to 13%, respectively, while carbohydrates range from 1 to 25% and ash content varies from 1 to 9% (Jakobson et al., 2023). The composition and particle size characteristics of legume protein powders can favor the susceptibility to physical and chemical degradation during handling, transport, and storage. Particles with low average particle size diameter are susceptible to adhesion, high concentrations of carbohydrates decrease the flow properties, and high-fat content favors lipid oxidation. In light of the challenges linked to stability, it is paramount to maintain the moisture content, water activity, relative humidity, minimize oxygen concentration, and exposure to light. The changes that occur during bulk storage, transport, and distribution adversely influence the physicochemical properties, functional properties, and sensory properties.

Challenges and Future Works

Challenges

The solubility of legume protein powder affects the gelling properties, application, processing, stability in various matrix, and sensory characteristics of food products (Grossmann & McClements, 2023). A significant difference in the solubility of legume protein powder produced using different production scales was observed which may be a major problem affecting the potential application in various food formulations. For example, commercial pea protein isolates have demonstrated a solubility ranging from 81 to 84 g/kg at pH 7 while laboratory spray samples showed a solubility range of 92.7–103.5 g/kg (Burger et al., 2022a, b). Besides, the solubility of spray-dried commercial pea protein, soy protein, faba bean, chickpea, and mung bean at pH 7 was between 40 and 60% (Jakobson, et al., 2023). Low solubility may be due to a relatively high denaturation degree and aggregation rate. During spray drying, high temperature causes the exposure of hydrophobic amino acids at the protein-air interface. Later, the hydrophobic amino acid at the protein-air interface forms a skin layer around particles which may prevent easy diffusion of water resulting in a protein with a low nitrogen solubility index (John et al., 2021). Also, the use of calcium hydroxide or calcium salt during extraction enhances legume protein hydrophobic interaction and aggregation; the combined effect of calcium and high temperature during commercial spray drying results in legume protein with low solubility (Peng et al., 2020).

Development of beany off-flavor and bitter taste are major challenges affecting the application of legume protein isolate and concentrate powder. Beany off-flavor results from the enzymatic and non-enzymatic oxidation of lipids, while bitterness is mostly due to the breakdown of protein into low molecular peptides composed mostly of hydrophobic amino acids (Gao et al., 2020).

The bitterness of legume protein is also linked to the average hydrophobicity of the protein and the arrangement of hydrophobic amino acids in the peptide. In a study involving 20 spray-dried commercial legume proteins, bitterness and metallic taste were detected in 20 samples while all samples selected for volatile compound analysis revealed a significant content of compounds responsible for beany off-flavor (Liu et al., 2023). Commercial legume protein isolate powders including pea protein, soy protein, faba bean, chickpea, and mung bean were subjected to sensory evaluation and the results showed that bitterness and astringence were the most dominant characteristics of all samples (Jakobson et al., 2023). In another related study, high concentrations of aldehydes, esters, ketones, alcohols, alkanes, ketones, lactones, phenols, phenones, alkenes, and hydrocarbons responsible for beany grassy and rancid flavors were

identified in various legume seeds and flour (Rajhi et al., 2021). These findings indicate that the raw materials for legume protein powder production have a big influence on the flavor profile. The flavor profile of legume protein is different between raw material, various processing steps, and protein powders, where some flavor compounds fade away while new volatile and flavor compounds are formed (Gao et al., 2020).

Future Works

The main purpose of spray drying is to remove water from extracted legume proteins. Hence, poor functional properties especially low solubility, off-flavor, and bitterness remain the biggest challenges after spray drying. To overcome these challenges, a rational integration of alkali-isoelectric precipitation and spray drying and novel spray drying technology can be used to improve the functional properties and reduce off-flavor and bitterness development. The rational combination and novel technology should consider the economic aspects and large-scale application. The legume protein powder produced should possess ameliorated functional properties, low off-flavor, and low bitterness that meet consumer and market demands. Future research may focus on the use of controlled high-pressure homogenization and steam cooking before alkali-acid precipitation and spray drying. These two treatments may degrade legume protein into small fractions thereby promoting high solubility, high water-holding capacity, and high foaming stability. On the other hand, co-spray drying legume proteins with emulsifying salt, salt ions, disaccharides, and cyclodextrins induces the conformation change, surface charge, and hydrophobicity. Such changes improve the solubility, emulsifying properties, gelling properties, and foaming properties. These techniques independently or combined may improve the functional properties of legume protein and also reduce off-flavor and bitterness in legume protein powder. For example, combined salt ions and microfluidic treatment before spray drying improved the functional properties of soy protein powder (Zheng et al., 2021).

Conclusion

This review provides a general outline of spray-dried legume protein isolated using an alkali-acid precipitation technique. Small changes in isolation and spray drying conditions may result in significant variations of structure and composition which may affect the functionality, stability, and applications. An integrated approach of alkali-acid precipitation isolation and spray drying to relate to physicochemical properties and functionality is necessary to monitor the structure and composition of legume protein. Therefore, a comprehensive knowledge of the combined effect of alkali-acid and spray drying on secondary and tertiary protein structure, solubility, emulsifying and foaming properties, water and oil holding capacity, and rheological and sensory properties is crucial. Most studies reported in this review were conducted at a laboratory scale; therefore, it is paramount to conduct substantial experiments to understand the integrated effect of extraction and spray drying to improve the quality of legume protein powder at a large scale. Particular interest should be placed on developing consistent functionalized spray-dried legume protein with improved functionality, and physicochemical and sensory properties at a commercial scale.

Contributions

Bertrand Muhoza: conceptualization, writing—review and editing, methodology, investigation, original draft. Eugenie Kayitesi: writing and editing. Yves Harimana: writing and editing. Angelo Uriho: review and editing. Liu Qian: review and editing.

Competing Interests

The authors declare no competing interests.

Data Availability

No datasets were generated or analysed during the current study.

References

- Abd Rahim, F. N., Wan Ibadullah, W. Z., Saari, N., Brishti, F. H., Mustapha, N. A., Ahmad, N., & Arulrajah, B. (2023). The effect of alkaline extraction and drying techniques on the physicochemical, structural properties and functionality of rice bran protein concentrates. *International Journal of Biological Macromolecules*, *242*, 124908.
- Adiamo, O. Q., Netzel, M. E., Hoffman, L. C., & Sultanbawa, Y. (2019). Acacia seed proteins: Low or high quality? A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *19*, 21–43.
- Alfaro-Diaz, A., Urías-Silvas, J. E., Loarca-Piña, G., Gaytan-Martínez, M., Prado-Ramirez, R., & Mojica, L. (2021). Techno-functional properties of thermally treated black bean protein concentrate generated through ultrafiltration process. *LWT*, *136*, 110296.
- Allotey, D. K., Kwofie, E. M., Adewale, P., Lam, E., & Ngadi, M. (2022). A meta-analysis of pulse-protein extraction technologies: Impact on recovery and purity. *Journal of Food Engineering*, *327*, 111048.
- Barone, G., O'Regan, J., & O'Mahony, J. A. (2019). Influence of composition and microstructure on bulk handling and rehydration properties of whey protein concentrate powder ingredients enriched in α -lactalbumin. *Journal of Food Engineering*, *255*, 41–49.
- Blaise, K., Nicolas, N. Y., Clémence, B., Richard, K., & Vuong, Q. V. (2017). Influence of spray-drying temperature on physicochemical and functional properties of protein isolates of three leguminous plants (*Canavalia ensiformis*, *Vigna unguiculata*, and *Glycine max*) from Cameroon. *Cogent Chemistry*, *3*, 1388140.
- Bordón, M. G., Alasino, N. P. X., Villanueva-Lazo, Á., Carrera-Sánchez, C., Pedroche-Jiménez, J., del Carmen Millán-Linares, M., Ribotta, P. D., & Martínez, M. L. (2021). Scale-up and optimization of the spray drying conditions for the development of functional microparticles based on chia oil. *Food and Bioproducts Processing*, *130*, 48–67.
- Bou, R., Navarro-Vozmediano, P., Dominguez, R., Lopez-Gomez, M., Pinent, M., Ribas-Agusti, A., Benedito, J. J., Lorenzo, J. M., Terra, X., Garcia-Perez, J. V., Pateiro, M., Herrera-Cervera, J. A., & Jorba-Martin, R. (2022). Application of emerging technologies to obtain legume protein isolates with improved techno-functional properties and health effects. *Comprehensive Reviews in Food Science and Food Safety*, *21*, 2200–2232.
- 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.

Burger, T. G., Singh, I., Mayfield, C., Baumert, J. L., & Zhang, Y. (2022a). Comparison of physicochemical and emulsifying properties of commercial pea protein powders. *Journal of the Science of Food and Agriculture*, *102*, 2506–2514.

Burger, T. G., Singh, I., Mayfield, C., Baumert, J. L., & Zhang, Y. (2022b). The impact of spray drying conditions on the physicochemical and emulsification properties of pea protein isolate. *LWT*, *153*, 112495.

Cortez-Trejo, M. C., Gaytán-Martínez, M., Reyes-Vega, M. L., & Mendoza, S. (2021). Protein-gum-based gels: Effect of gum addition on microstructure, rheological properties, and water retention capacity. *Trends in Food Science & Technology*, *116*, 303–317.

Cui, L., Bandillo, N., Wang, Y., Ohm, J.-B., Chen, B., & Rao, J. (2020). Functionality and structure of yellow pea protein isolate as affected by cultivars and extraction pH. *Food Hydrocolloids*, *108*, 106008.

Cui, L., Kimmel, J., Zhou, L., Chen, B., & Rao, J. (2021). Improving the functionality of pea protein isolate through co-spray drying with emulsifying salt or disaccharide. *Food Hydrocolloids*, *113*, 106534.

Dao, H. M., Sahakijpijarn, S., Chrostowski, R. R., Moon, C., Mangolini, F., Cui, Z., & Williams, R. O., 3rd. (2022). Aggregation of lactoferrin caused by droplet atomization process via a two-fluid nozzle: The detrimental effect of air-water interfaces. *Molecular Pharmaceutics*, *19*(7), 2662–2675.

de Paiva Gouvêa, L., Caldeira, R., de Lima Azevedo, T., Galdeano, M. C., Felberg, I., Lima, J. R., & Grassi Mellinger, C. (2023). Physical and techno-functional properties of a common bean protein concentrate compared to commercial legume ingredients for the plant-based market. *Food Hydrocolloids*, *137*, 108351.

de Souza Celente, G., Sui, Y., & Acharya, P. (2023). Seaweed as an alternative protein source: Prospective protein extraction technologies. *Innovative Food Science & Emerging Technologies*, *86*, 103374.

Dent, T., & Maleky, F. (2022). Pulse protein processing: The effect of processing choices and enzymatic hydrolysis on ingredient functionality. *Critical Review in Food Science and Nutrition*, *63*(23), 1–12.

Devkota, L., Kyriakopoulou, K., Bergia, R., & Dhital, S. (2023). Structural and thermal characterization of protein isolates from Australian lupin varieties as affected by processing conditions. *Foods*, *12*(5), 908.

Ferreira, K. C., Bento, J. A. C., Caliari, M., Bassinello, P. Z., & Berrios, J. D. J. (2021). Dry bean proteins: Extraction methods, functionality, and application in products for human consumption. *Cereal Chemistry*, *99*, 67–77.

- Fournaise, T., Petit, J., & Gaiani, C. (2021). Main powder physicochemical characteristics influencing their reconstitution behavior. *Powder Technology*, *383*, 65–73.
- Gao, Z., Shen, P., Lan, Y., Cui, L., Ohm, J. B., Chen, B., & Rao, J. (2020). Effect of alkaline extraction pH on structure properties, solubility, and beany flavor of yellow pea protein isolate. *Food Research International*, *131*, 109045.
- García Arteaga, V., Kraus, S., Schott, M., Muranyi, I., Schweiggert-Weisz, U., & Eisner, P. (2021). Screening of twelve pea (*Pisum sativum* L.) Cultivars and their isolates focusing on the protein characterization, functionality, and sensory profiles. *Foods*, *10*, 758.
- George, S., Thomas, A., Kumar, M. V. P., Kamdod, A. S., Rajput, A., Jayasree Joshi, T., & Abdullah, S. (2022). Impact of processing parameters on the quality attributes of spray-dried powders: A review. *European Food Research and Technology*, *249*, 241–257.
- Gerliani, N., Hammami, R., & Aïder, M. (2020). Extraction of protein and carbohydrates from soybean meal using acidic and alkaline solutions produced by electro-activation. *Food Science & Nutrition*, *8*, 1125–1138.
- 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.
- Grossmann, L., & McClements, D. J. (2023). Current insights into protein solubility: A review of its importance for alternative proteins. *Food Hydrocolloids*, *137*, 108416.
- Guo, F. X., Xiong, Y. L., Qin, F., Jian, H. J., Huang, X. L., & Chen, J. (2015). Examination of the causes of instability of soy protein isolate during storage through probing of the heat-induced aggregation. *Journal of the American Oil Chemists' Society*, *92*, 1075–1084.
- Guo, F., Lin, L., He, Z., & Zheng, Z. P. (2020). Storage stability of soy protein isolate powders containing soluble protein aggregates formed at varying pH. *Food Science & Nutrition*, *8*, 5275–5283.
- Habtegebriel, H., Edward, D., Wawire, M., Seifu, E., & Gaukel, V. (2021). Surface fat and insolubility of whole camel milk powders as affected by spray drying operating parameters. *Food and Bioproducts Processing*, *128*, 121–132.
- Haller, N., & Kulozik, U. (2019). Separation of whey protein aggregates by means of continuous centrifugation. *Food and Bioprocess Technology*, *12*(6), 1052–1067.
- Han, J., Fitzpatrick, J., Cronin, K., Maidannyk, V., & Miao, S. (2022). Breakage behaviour and functionality of spray-dried agglomerated model infant milk formula: Effect of proteins and carbohydrates content. *Food Chemistry*, *391*, 133179.
- Jakobson, K., Kaleda, A., Adra, K., Tammik, M. L., Vaikma, H., Krisciunaite, T., & Vilu, R. (2023). Techno-functional and sensory characterization of commercial plant protein powders. *Foods*, *12*(14), 2805.

- John, H., Chandra, P., Giri, S. K., & Sinha, L. K. (2021). Effect of processing methods on 11S/7S protein and nitrogen solubility index of soy protein isolate. *Journal of The Institution of Engineers (India): Series A*, *102*, 989–994.
- Julakanti, S., Charles, A. P. R., Zhao, J., Bullock, F., Syed, R., Myles, Y., & Wu, Y. (2023). Hempseed protein (*Cannabis sativa* L.): Influence of extraction pH and ball milling on physicochemical and functional properties. *Food Hydrocolloids*, *143*, 108835.
- Kamal, H., Le, C. F., Salter, A. M., & Ali, A. (2021). Extraction of protein from food waste: An overview of current status and opportunities. *Comprehensive Review in Food Science and Food Safety*, *20*, 2455–2475.
- Kim, E. H. J., Chen, X. D., & Pearce, D. (2009). Surface composition of industrial spray-dried milk powders. 3. Changes in the surface composition during long-term storage. *Journal of Food Engineering*, *94*(2), 182–191.
- Lan, Y., Xu, M., Ohm, J. B., Chen, B., & Rao, J. (2019). Solid dispersion-based spray-drying improves solubility and mitigates beany flavour of pea protein isolate. *Food Chemistry*, *278*, 665–673.
- Li, J., Sun, J., Gu, L., Su, Y., Yang, Y., Chang, C., & Han, Q. (2023). Foaming properties of dried egg white at different outlet temperatures. *Journal of Food Engineering*, *343*, 111379.
- Liu, Y., Cadwallader, D. C., & Drake, M. (2023). Identification of predominant aroma components of dried pea protein concentrates and isolates. *Food Chemistry*, *406*, 134998.
- Ma, K. K., Greis, M., Lu, J., Nolden, A. A., McClements, D. J., & Kinchla, A. J. (2022). Functional performance of plant proteins. *Foods*, *11*(4), 594.
- Manassero, C. A., David-Briand, E., Vaudagna, S. R., Anton, M., & Speroni, F. (2018). Calcium addition, pH, and high hydrostatic pressure effects on soybean protein isolates—Part 1: Colloidal stability improvement. *Food and Bioprocess Technology*, *11*(6), 1125–1138.
- Miranda, C. G., Speranza, P., Kurozawa, L. E., & Kawazoe Sato, A. C. (2022). Lentil protein: Impact of different extraction methods on structural and functional properties. *Heliyon*, *8*, e11775.
- Momen, S., Alavi, F., & Aider, M. (2021). Alkali-mediated treatments for extraction and functional modification of proteins: Critical and application review. *Trends in Food Science & Technology*, *110*, 778–797.
- Mune Mune, M. A., & Sogi, D. S. (2015). Functional properties of protein concentrates of cowpea and Bambara bean involving different drying techniques. *Journal of Food Processing and Preservation*, *39*, 2304–2313.
- Nguyen, D. Q., Nguyen, T. H., Mounir, S., & Allaf, K. (2017). Effect of feed concentration and inlet air temperature on the properties of soymilk powder obtained by spray drying. *Drying Technology*, *36*, 817–829.

- Nie, H. N., Dong, H., Chen, Y. L., Hao, M. M., Chen, J. N., Tang, Z. C., Liu, Q. Z., Li, J. K., Xu, X. B., & Xue, Y. L. (2023). Effects of spray drying and freeze drying on the structure and emulsifying properties of yam soluble protein: A study by experiment and molecular dynamics simulation. *Food Chemistry*, *409*, 135238.
- Ogolla, J. A., Kulig, B., Bădulescu, L., Okoth, M. W., Esper, G., Breitenbach, J., Hensel, O., & Sturm, B. (2019). Influence of inlet drying air temperature and milk flow rate on the physical, optical and thermal properties of spray-dried camel milk powders. *Food and Bioprocess Technology*, *12*, 751–768.
- O’Sullivan, J. J., Norwood, E.-A., O’Mahony, J. A., & Kelly, A. L. (2019). Atomisation technologies used in spray drying in the dairy industry: A review. *Journal of Food Engineering*, *243*, 57–69.
- Park, C. W., Bastian, E., Farkas, B., & Drake, M. (2014). The effect of feed solids concentration and inlet temperature on the flavor of spray-dried whey protein concentrate. *Journal of Food Science*, *79*, C19–C24.
- Peng, Y., Dewi, D. P. A. P., Kyriakopoulou, K., & van der Goot, A. J. (2020). Effect of calcium hydroxide and fractionation process on the functional properties of soy protein concentrate. *Innovative Food Science & Emerging Technologies*, *66*, 102501.
- Phosanam, A., Chandrapala, J., Zisu, B., & Adhikari, B. (2021). Storage stability of powdered dairy ingredients: A review. *Drying Technology*, *39*, 1529–1553.
- Pinto, J. T., Faulhammer, E., Dieplinger, J., Dekner, M., Makert, C., Nieder, M., & Paudel, A. (2021). Progress in spray-drying of protein pharmaceuticals: Literature analysis of trends in formulation and process attributes. *Drying Technology*, *39*, 1415–1446.
- Pojić, M., Mišan, A., & Tiwari, B. (2018). Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends in Food Science & Technology*, *75*, 93–104.
- Poozesh, S., & Bilgili, E. (2019). Scale-up of pharmaceutical spray drying using scale-up rules: A review. *International Journal of Pharmaceutics*, *562*, 271–292.
- Preece, K. E., Hooshyar, N., & Zuidam, N. J. (2017). Whole soybean protein extraction processes: A review. *Innovative Food Science & Emerging Technologies*, *43*, 163–172.
- Rajhi, I., Baccouri, B., Rajhi, F., Mhadhbi, H., & Flamini, G. (2021). Monitoring the volatile compounds status of whole seeds and flours of legume cultivars. *Food Bioscience*, *41*, 101105.
- Rudra, S. G., Sethi, S., Jha, S. K., & Kumar, R. (2016). Physico-chemical and functional properties of cowpea protein isolate as affected by the dehydration technique. *Legume Research*, *39*(3), 370–378.
- Sá, A. G. A., Moreno, Y. M. F., & Carciofi, B. A. M. (2020). Plant proteins as high-quality nutritional source for human diet. *Trends in Food Science & Technology*, *97*, 170–184.

- Sadek, C., Schuck, P., Fallourd, Y., Pradeau, N., Le Floch-Fouéré, C., & Jeantet, R. (2014). Drying of a single droplet to investigate process–structure–function relationships: A review. *Dairy Science & Technology*, *95*, 771–794.
- Saha, D., Nanda, S. K., & Yadav, D. N. (2019). Optimization of spray drying process parameters for the production of groundnut milk powder. *Powder Technology*, *355*, 417–424.
- Schubert, T., Meric, A., Boom, R., Hinrichs, J., & Atamer, Z. (2018). Application of a decanter centrifuge for casein fractionation on pilot scale: Effect of operational parameters on total solid, purity and yield in solid discharge. *International Dairy Journal*, *84*, 6–14.
- Shrestha, S., van't Hag, L., Haritos, V. S., & Dhital, S. (2021). Lupin proteins: Structure, isolation, and application. *Trends in Food Science, Technology*, *116*, 928–939.
- Shrestha, S., van' t Hag, L., Haritos, V., & Dhital, S. (2023). Comparative study on molecular and higher-order structures of legume seed protein isolates: Lentil, mungbean, and yellow pea. *Food Chemistry*, *411*, 135464.
- Siddiqui, S. A., İl, U., Jain, S., Elsheikh, W., Ali Redha, A., Kurt, A., & Toker, O. S. (2024). Impact of drying on techno-functional and nutritional properties of food proteins and carbohydrates - A comprehensive review. *Drying Technology*, *42*(4), 592–611.
- Stone, A. K., Avarmenko, N. A., Warkentin, T. D., & Nickerson, M. T. (2015). Functional properties of protein isolates from different pea cultivars. *Food Science and Biotechnology*, *24*, 827–833.
- Tang, X., Shen, Y., Zhang, Y., Schilling, M. W., & Li, Y. (2021). Parallel comparison of functional and physicochemical properties of common pulse proteins. *LWT*, *146*, 111594.
- Verfaillie, D., Janssen, F., Van Royen, G., & Wouters, A. G. B. (2023). A systematic study of the impact of the isoelectric precipitation process on the physical properties and protein composition of soy protein isolates. *Food Research International*, *163*, 112177.
- Wang, X., & Zhao, Z. (2023). A mini-review about direct steam heating and its application in dairy and plant protein processing. *Food Chemistry*, *408*, 135233.
- Yang, J., Mocking-Bode, H. C. M., van den Hoek, I. A. F., Theunissen, M., Voudouris, P., Meinders, M. B. J., & Sagis, L. M. C. (2022). The impact of heating and freeze or spray drying on the interface and foam stabilising properties of pea protein extracts: Explained by aggregation and protein composition. *Food Hydrocolloids*, *133*, 107913.
- Zhang, C., Sanders, J. P. M., & Bruins, M. E. (2014). Critical parameters in cost-effective alkaline extraction for high protein yield from leaves. *Biomass and Bioenergy*, *67*, 466–472.
- Zheng, L., He, M., Zhang, X., Regenstein, J. M., Wang, Z., Ma, Z., Kong, Y., Wu, C., Teng, F., & Li, Y. (2021). Gel properties and structural characteristics of soy protein isolate treated with different salt ions before spray drying combined with dynamic high-pressure microfluidization. *Food and Bioprocesses Processing*, *125*, 68–78.