

## Black Bean: Composition, Protein Extraction and Functional Properties



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**ABSTRACT:** Black beans play an essential part in the human diet due to their high protein content and nutritional value. Black beans are high in energy, dietary fiber, protein, minerals, and vitamins, and they have well-balanced essential amino acid profiles. The production of black bean protein concentrates or isolates is of growing interest to food industry because of their functional properties and ability to improve the nutritional quality of food products. Various procedures are used to obtain protein concentrates / isolates with varying properties. Because of desired functional features such as gelling and emulsifying properties, black bean proteins have grown in popularity and could be presented as a potential supplement in a wide range of food applications. This review provides an overview of the chemical composition of black bean, current and emerging techniques for producing their protein concentrates / isolates, and major functional properties.

**KEYWORDS:** chemical composition, protein isolate, functional properties

### I. OVERVIEW OF BLACK BEANS

Black beans which their scientific name is *Vigna cylindrica* (L.) Skeels belong to the Leguminosae family (Price & Butler, 1977). They are generally found in lowlands but can grow up to 1800 m above sea level (Arora & Mauria, 1989) in tropical climate with optimal temperatures from 25°C to 35°C. They are grown mainly in India, Pakistan, Iran and Southeast Asia like Vietnam, Thai Lan, Myanmar (Arora & Mauria, 1989) . Also, they are cultivated in the USA and Australia as a fodder crop. In Vietnam, black bean is grown widely especially in Central Highlands's provinces such as Dak Lak and Gia Lai with domestic productivity estimated at 200,000 tons per year. Black grams are reported as a valuable source of protein, fiber, several B-complex vitamins and essential minerals (iron, phosphorus, calcium, magnesium and zinc) (DK, 1985). Moreover, phenolic compounds in black gram act as antioxidants to prevent the development of many diseases(López-Amorós, Hernández, & Estrella, 2006), reduce the risks of cancer, heart disease, and diabetes; inhibit plasma platelet aggregation, cyclooxygenase activity, and histamine release; as well as provide in vitro antibacterial, antiviral, anti-inflammatory, and anti-allergenic activities (Karladee & Suriyong, 2012) various foods. They can be processed into dehusked cotyledon to make fermented foods such as idli, dosa, and non-fermented foods like cooked dhal, hopper, papad and waries (spicy hollow balls)(Batra & Millner, 1974). Moreover, black grams can be added into buffalo meat burgers and beef sausages to improve the quality (Chaudhray & Ledward, 1988; Modi, Mahendrakar, Rao, & Sachindra, 2004) or into biscuits to enhance the nutritional value (Patel & Rao, 1995). Except cotyledon fraction, the other fractions of black grams like seed coat, germ, aleurone layer and plumule may contain many nutrients and be used as animal feed.

In Vietnam, there are two main types of black beans: white kernel and green kernel black beans. Green kernel black beans have smaller sizes, which are half of white kernel ones. They are also considered as an important ingredient of diet as well as the most significant food sources for people of low incomes based on the larger amount of nutrition components (protein, minerals, vitamin, and so on) and the inexpensive price. Although green and white kernel black bean belong to the same family, their nutritional values are also different. Green kernel black beans are also believed to have higher levels of antioxidants with a good source of anthocyanin content. As a result, green kernel black bean can be used to prevent many diseases (cardiovascular, cancer, diabetes and so on), help to lower blood pressure, control diabetes, maintain a good digestive system and nervous system. Moreover, they are also good for women, especially pregnant women by supporting fertility and helping in cure of anemia. Although both green and white kernel black beans bring many benefits for human, there is no scientific information about their nutritional values, the differences in their composition, especially in protein content (amino acids composition, the

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morphology, the chemical structure and so on) which is required more studies for further applications in the future. Chemical composition of green and white kernel black bean was shown in the Table 1.

**Table 1: Chemical composition of green kernel and white kernel black bean**

Sample	Protein	Lipid	Ash	Total carbohydrate	Dietary fiber	References
White kernel black bean	21.06 ±0.3	1.68 ±0.26	4.2 ±0.04	59.43 ±1.22	3.06 ±0.07	(Mariscal-Moreno, Chuck-Hernández, Figueroa-Cárdenas, & Serna-Saldivar, 2021)
Green kernel black bean	24.02 ± 4.2	10.18 ±0.04	3.51 ±1.5	48.08 ±2.6	14.21 ±7.0	(LE, LE, Nguyen, & Vu, 2021)

## II. OVERVIEW OF PROTEIN

Proteins are large biomolecules and macromolecules that are made up of one or more long chains of amino acids which linked together by peptide bond (Bigman & Levy, 2020; Małeckki, Muszyński, & Sołowiej, 2021). A linear chain of amino acid residues is called a polypeptide and at least one long polypeptide can be found in a protein. Due to the complicated metabolic changes required to function two processes continuously, such as the synthesis and breakdown of the body's proteins, protein is a critical nutrient in the human diet for analyzing the body's needs. Firstly, proteins are known as the primary structural and functional components of human body cell, controlling gene expression and vital for the appropriate growth and development of the young organism. They function as biocatalysts in a variety of enzymatic systems (Sá, Moreno, & Carciofi, 2020). Proteins are also responsible for transferring oxygen (hemoglobin), iron (transferrin), and retinol (eight-stranded  $\beta$ -barrel proteins that bind extracellular retinoids, such as retinol-binding protein 4 and epididymal retinoic acid-binding protein). Actin and myosin are also muscle-contractile proteins that help in tissue repair and regeneration. Proteins are also considered as antibodies which enhance immune system. Many hormones and physiologically active substances, including as adrenaline, noradrenaline, thyroid hormones (thyroxine, triiodothyronine), histamine, and serotonin, are synthesized by using proteins as substrates. They also play a role in the production of biologically active molecules such as purine and pyrimidine bases (components of nucleotides and nucleic acids), choline (a component of phospholipids), glutathione, creatine, and a variety of other substances involved in physiological activities (Van Vranken & Weiss, 2018).

Besides the health benefits, proteins also play an essential role in the food industry. Due to hydrophilic and hydrophobic properties, proteins are applied as emulsifiers and stabilizers which can improve the texture of food. For example, emulsified meats are the one of first applications of isolated soy protein. In other to improve spread ability and prolong storage stability, protein can be modified by using acetylation process to increase emulsification properties. In mayonnaise and salad dressings, modification of egg yolk proteins improves the product quality. In addition to their ability to stabilize emulsions or foams, proteins can influence on the shelf-life and stability of foods by enhancing antioxidant activity, affecting gas exchange when used as packaging biomaterials and edible coatings and, for some proteins and peptides, their antimicrobial activity (Moreira, Pereda, Marcovich, & Roura, 2011; Wimley, 2010). For example, nisin is a potent antibacterial peptide containing a number of uncommon amino acids that is used as a food preservative in cheese (Martins, Cerqueira, Souza, Carmo Avides, & Vicente, 2010), fish, meat and beverages (Lubelski, Rink, Khusainov, Moll, & Kuipers, 2008). Another peptide,  $\epsilon$ -poly-l-lysine, exhibits antimicrobial activity which eliminates the growth of bacteria and fungi (Hamano, 2011).

There are two main types of protein which are available in the food include complete and incomplete protein. Foods that are a 'complete' protein source contain an adequate proportion of the nine essential amino acids that are needed to support biological functions in humans or other animals. Examples include proteins derived from animal foods (meats, fish, poultry, milk, eggs) and proteins derived from some plant foods (e.g., legumes, seeds, nuts and grains such as chickpeas, black-eyed peas, black beans, cashews. Some foods that are incomplete protein sources may contain all the essential amino acids, but not in the correct proportions, being deficient in one or more. For example, cereals such as maize (corn) contain lower amounts of lysine, isoleucine, methionine and threonine. Combining a cereal with a legume, such as maize with beans, soybeans with rice, or red beans with rice, provides a meal that is balanced with higher amounts of all essential amino acids.

Proteins play an important role in human nutrition which provide a good source of amino acids and nitrogen for maintaining muscles in good condition, control the immune responses, repair cells and improve their signaling (Wen et al., 2019). In addition to supplying various amino acids in the human diet, proteins are also considered as very important components in food formulations due to their desirable functionalities, including thickening and gelling ability, emulsifying, foaming, water holding and fat absorption (Cao, Bolisetty, Wolfisberg, Adamcik, & Mezzenga, 2019; Cao & Mezzenga, 2019). As a result, the demand for

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protein consumption is more and more increased and this becomes a key challenge in the developing country due to the decrease in food resources and the limitation of acceptance. It is estimated that there is nearly 25% of the world population lack suitable protein supply (Azam, Khan, Ahmad, Khan, & Ali, 2014). Based on their desirable properties including their high yield and essential amino acids content with right balance signaling, animal-based proteins is considered as the most common protein used in the world (Wen et al., 2019). Despite of these advantages, developing renewable and sustainable replacement for animal-based proteins has gained more attention because of their adverse effects on the environment, high cost, limited availability and limited acceptance by some consumers such as vegetarians and vegans. Moreover, the consumption of animal proteins may have detrimental consequences for human health such as high blood pressure and obesity (Hu, 2005; Richter, Skulas-Ray, Champagne, & Kris-Etherton, 2015). Legumes, with the high protein content, have the potential to succeed in the plant protein ingredient market because they are not cause allergens such as soy, peanut, whey and wheat, milk and eggs do. Furthermore, legume protein is considered as the low-cost, environmental-friendly and animal-friendly products which can subsequently be used as functional ingredients.

### **III. PROTEIN IN BLACK BEANS**

Black beans are known as the good source of protein, in which their protein content are higher than that of soybean and even that of meat, eggs, or milk. The protein content of black beans is around 20–25%, which makes these grains an attractive source of proteins for extraction and modification. Black beans not only contain optimal source of high-quality protein but also provide a favorable balance of amino acids such as leucine, lysine and phenylalanine (Duranti, 2006). Protein in black bean has a wide range of beneficial effects on the human nutrition and health such as lowering of plasma cholesterol, prevention of cancer, diabetes and obesity, and protection against bowel and kidney disease (Duranti, 2006).

In the present day, malnutrition has been becoming the serious problem in the developing country due to the high price of animal. Thus, black bean is known as an inexpensive alternative for the animal-based protein with the similar nutrition value. Because of the high amount of protein content, black bean can be applied to produce protein concentrates (at least 70% of protein) and protein isolates (more than 90% of protein). Protein isolates or concentrates obtained from black beans have become important in the food industry. In formulations containing oil, proteins can be applied as emulsifiers to form and stabilize an emulsion; the black bean proteins must be capable of migrating to the oil/water interface and forming an interfacial layer. The black bean protein stabilized fat globules must also be capable of repelling each other, in order to avoid flocculation or coalescence (McClements, Bai, & Chung, 2017). Moreover, protein from black beans is recommended to be used as a suitable ingredient for infant food formulations as well as for gels, puddings, ice cream, baked goods, among others or for protein supplementation. However, in Asia, crude protein extracted from black beans are not commonly applied in the food industry, instead that they are thrown away as a by-product in starch process (Khaket, Dhanda, Jodha, & Singh, 2015). Because of these characteristics, the obtainment of crude black bean protein has raised the interest of many researchers due to the raise of nutritional value into food through a low cost when incorporated into food.

### **IV. METHOD FOR EXTRACTION OF PROTEIN**

#### **4.1 Traditional methods for protein extraction**

Several methods used for plant-based protein extraction consist of acid, alkali, and ionic strength modulation in solution. The main principle of their methods is based on cell disruption and protein solubilization. Since proteins are contained in protein bodies inside cell walls cell disruption such as homogenization and colloid milling is required before they can be totally solubilized and extracted. After cell components are disintegrated, protein solubilization will be employed to separate proteins in the sample selectively from different substances that may interfere in the proteomic assay. This process can be finished by using organic solvents such as acetone, TCA or aqueous solutions as altering pH level, salt concentration, and ionic strength. Aqueous alkaline extraction is considered as the most common method for protein extraction. In this method, sodium hydroxide is used to dissolve the soluble proteins and then break hydrogen, amide and disulfide bonds for improvement in extraction yield (Fabian & Ju, 2011). Compared to other methods, alkaline extraction is believed to be easy to conduct, have the low cost and moderately high yield (Damerval, De Vienne, Zivy, & Thiellement, 1986). For example, protein content extracted from alkali resulted in a higher amount which is approximately 50% of total content in soy—whereas acid extraction reached a maximum of 20% for yellow pea protein; salt extraction gave 15% protein extraction yield at pH 4.5 and 90% at pH 11 from *Rosa rubiginosa*. Due to less cell wall breakdown and protein solubility at the isoelectric point, acid-based extraction is less promising (Pojić, Mišan, & Tiwari, 2018). Furthermore, in the research of (Chakraborty, Sosulski, & Bose, 1979), protein isolate from legume extracted by alkaline extraction (pH 8.5) was over than 80% including 90.5% protein for chickpea protein isolate, 90.1% for pea protein isolate, 91.2% for Great Northern bean isolate, 89.3% for lentil protein isolate, 91.2% for lima bean isolate and 88.3% for

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mung bean isolate. (Paredes-López, Ordorica-Falomir, & Olivares-Vázquez, 1991) used similar conditions for alkaline extraction method has been found a protein isolate from chickpea is about 84.8% protein However, the extraction of proteins with alkaline extraction have the disadvantages of a low protein recovery (Sawada, Venancio, Toda, & Rodrigues, 2014), large processing times and contamination by solvents. Although alkaline extraction is a more environment friendly than organic solvent extraction, it may affect the quality of the protein and the extraction rate is also low. Besides, extremely alkaline solution may still cause some possible safety or nutritional problems of protein, which preferably has to be avoided (Hamano, 2011).

### 4.2 Modern methods for protein extraction

Many modern and innovative methods have been carried out to enhance the extraction yields of protein from plant-based substrates and reduce the limitation of traditional methods such as microwave heating, extrusion, supercritical fluid extrusion, ultrasound, pulsed electric field, gamma irradiation, and high-pressure processing of the substrate (Karki et al., 2009). The general principle of these eco-innovative technologies is damage cell walls to facilitate liquid diffusion into cell and protein dissolution and release into the liquid medium, which significant increase the extraction yield. For example, microwaves at 725 W for 8 min increased protein extraction by 77% from defatted peanut flour slurry (Ochoa-Rivas, Nava-Valdez, Serna-Saldívar, & Chuck-Hernández, 2017); ultrasound at 2.56 W/mL for 2 min increased protein 34% from defatted soy flakes (Karki et al., 2009); 100 MPa pressure on soy slurry yielded up to 82% protein; and 20 kV/cm of electric field increased proteins yield up to 80% from rapeseed leaves (Pojić et al., 2018).

## V. FUNCTIONAL PROPERTIES OF BLACK BEAN PROTEIN

Functional characteristics of protein play an important role in foodstuff processing, food product formulation as well key interest to manufacturers of pharmaceutical and cosmetic products. Proteins can have surface properties such as the ability to form or stabilize emulsions (interfacial oil/water interface), the ability to create or stabilize foams (interfacial air/water interface), or solubility (combining the connections between water and proteins). In addition, proteins have hydrodynamic properties based on intermolecular interactions, including gelation, texture, and molding sensory properties (taste and smell) (Foegeding & Davis, 2011). The functional properties of proteins depend directly on the physicochemical properties of protein such as their molecules structure, size, shape, susceptibility to denaturation, flexibility, amino acid composition, hydrophilicity and hydrophobicity, the charge distribution in the molecule, the nature and number of micro domain structures, the ability of the entire molecule or its constituent domains to adapt to changing environmental conditions, and the nature of the interrelationships between different proteins and other food components (Yada, 2017). The functional properties of proteins are also affected by important environmental factors in the protein's location, such as pH, temperature, pressure, and ionic strength (Wijaya, Patel, Setiowati, & Van der Meeren, 2017). Proteins form complex systems with other food ingredients that affect the formation of their functional properties, and additionally, technological processes play a significant role in shaping proteins 's functional properties (Lam & Nickerson, 2013). In most proteins, many hydrophilic functional groups are located on the surface of the molecules. However, the hydrophobic groups are not entirely located inside them. In globular proteins, 40–50% of the molecule's surface may be occupied by hydrophobic amino acid residues (Gruner et al., 2016). Their specific distribution in the polypeptide chains affects the surface formation of protein molecules, the ability to create oligomers and micellar structures, and functional properties (Udgaonkar, 2013).

## VI. CONCLUSIONS

Black bean protein are food resources that offer various health benefits. Black bean protein have many good qualities and can be considered most suitable for the preparation of protein isolates because of their high protein content, low cost and wide acceptability. Various methods are used for protein extraction and every technique has its own advantages. The functionality of proteins is closely related to their physical and chemical properties, such as molecular weight, amino acid composition and sequence, structure, surface electrostatic charge, and effective hydrophobicity. Because of the functional properties of protein isolates, their application to food products is increasing.

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