# Application of Cereal- and Legume-Derived Protein-Mineral Complexes

#### Subjects: Food Science & Technology

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Minerals play an important role in maintaining human health as the deficiency of these minerals can lead to serious health issues. To address these deficiencies, current research efforts are actively investigating the utilization of protein-mineral complexes as eco-friendly, non-hazardous, suitable mineral fortifiers, characterized by minimal toxicity, for incorporation into food products.

minerals cereal proteins legume protein-mineral complexes

### **1. Cereal and Legume Proteins**

Cereals are the edible grains or seeds that belong to the Gramineae genus of grass. Wheat and rice are of the most significance on a global scale as it accounts for more than 50% of the world's cereal production <sup>[1][2]</sup>. In both developed and developing nations, cereals serve as a staple food regardless of their cultures and beliefs. Worldwide, legumes belonging to the family Fabaceae are the second most popular food crop after cereals, thereby serving as a vital source of food for the poor living in developing and under-developing countries. Furthermore, this research discusses two key sources of protein, specifically "cereals" and "legumes".

### **1.1. Cereal Proteins**

The edible seed of several cereal grains is distinguished from one another based on their shape, size, mass, and other characteristics. There are different components of a cereal grain, namely endosperm, bran, aleurone layer, and germ <sup>[2]</sup>. The endosperm is the largest morphological component and stores nutritive compounds such as starch and proteins <sup>[3]</sup>. In cereal and pseudo-cereal grains, bran is the hard outer layer consisting of pericarp and inner seed coats rich in dietary fiber, protein, and fat. The third component is the aleurone layer which is the outermost layer of the endosperm and varies among different grains in terms of thickness. Studies have reported two types of aleurone cells in the layer: the first one is cuboidal-shaped, which covers the outer part of the endosperm, whereas the second type, also called the modified aleurone layer, functions in surrounding the embryo <sup>[4]</sup>. Finally, the germ is a by-product of cereals consisting of a majority of nutrients such as high-quality essential amino acids, lipid content, and other nutrients <sup>[5]</sup>.

In general, the chemical composition of cereals includes carbohydrates (65–75%), proteins (7–12%), lipids (2–6%), and certain minerals (1.5–2.5%). Protein is the second-largest component of cereal grains, which is of great

importance due to its functionality and the availability of different amino acids with varying molecular weights <sup>[6]</sup>. A systemic study on the plant protein was carried out by "Thomas Osborne", known for his work towards the classification of plant proteins based on their solubility in different solvents, and 'cereal grain' was the first to be studied for their protein classification  $\square$ . Moreover, they are categorized into four types depending on their solubility, namely albumins (soluble in water), globulins (soluble in a salt solution), prolamins (soluble in ethanol aqueous solution), and glutenins (dilute acid or alkaline solution). Different types of cereal grains such as wheat, rice, maize, barley, and oats consist of a variety of proteins that are discussed in more detail <sup>[3]</sup>. For instance wheat consists of a storage protein called gluten (80-85%) which divides into two subgroups, gliadins (alcohol soluble) and glutenins (alcohol insoluble). Gliadins and glutenins are composed of distinct structures, each with different molecular weights and amino acid contents [9]. In gliadins,  $\alpha$ ,  $\beta$ , and y categories of monomeric proteins are present with a molecular weight of 30-45,000 Da and contain the amino acids glutamine (30-40%), proline (15-20%), cysteine (2-3%), and lysine (<1.0%), whereas  $\omega$  gliadins are also the monomeric type of proteins but have a different molecular weight, i.e., 40–75,000 Da containing the amino acids glutamine (40–50%), proline (20– 30%), phenylalanine (8–9%), and lysine (0–0.5%). On the other hand, glutenins are polymeric proteins with high molecular weight (65-90,000%) subunits, low molecular weight (30-45,000%) subunits, and exceptionally high levels of glutamine and proline <sup>[10]</sup>. Along with this, it also contains metabolic proteins such as albumin, globulin, and amphiphilic proteins. Albumins and globulin are suggested to have better amino acid content due to high levels of methionine and lysine. The rice protein, which is hypoallergenic and highly acceptable for consumption, consists of protein content that varies with its types of milling fractions such as paddy (5.6–7.7%), hulled rice (2.0–2.8%), brown rice (7.1–8.3%), rice bran (11.3–14.9%), and milled rice (6.3–7.1%). Solubility and molecular weight are the two criteria by which rice proteins are divided into four groups. The albumins protein is water soluble and is present in the endosperm (3.8–8.8%) and the bran layer (35%) of rice with its molecular weight in a range of 14–16 kDa. Globulin is a salt-soluble protein that is present in endosperm up to 9.6–10.8% and about 15–26% in the bran layer of rice. These are further classified into four types  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\dot{\omega}$  having a different range of molecular weight. The amino acid content for globulin is higher in cysteine and methionine but lower in lysine content. Prolamins are soluble in alcohol and comprise protein up to 2.6-3.3% in the endosperm and 4% in the bran layer. Additionally, studies showed three subunits of the polypeptide with varying molecular weights, i.e., 10, 13, and 16 kDa were also identified. In terms of amino acids, it contains a high level of glutamine, glycine, alanine, and arginine (Eakkanaluksamee and Anuntagool, 2020). Lastly, glutelins which are readily soluble in acidic (pH < 3.0) and alkaline conditions (pH > 10.0) and function as major storage proteins in rice are present in the endosperm up to 66–78% and up to 11–27% in the bran layer. They are composed of two polypeptide subunits, namely acidic and basic, with molecular weights of 30-39 and 19-25 kDa <sup>[11]</sup>. Maize, which is known as the queen of cereals, generally consists of poor-quality protein. The protein content of maize is up to 10% and they are classified into four categories based on their solubility: albumins (3%), globulins (3%), glutelins (34%), and prolamins (>60%) [12]. Prolamins are called zeins and are further classified into four types, having a wide range of molecular weights,  $\alpha$ (19 & 22 kDa),  $\beta$  (15 kDa), y (50, 27 & 16 kDa), and  $\delta$  (18 & 10 kDa). These proteins contain different types of amino acids. For example,  $\beta$ -zein is characterized by high levels of cysteine and methionine,  $\delta$ -zein is rich in methionine, y-zein contains cysteine, while  $\alpha$ -zein lacks all essential amino acids. Barley (Hordeum vulgare), a cereal grain, consists of four different proteins: albumins, globulins, glutelins, and prolamin. Prolamin is divided into another class of protein called hordeins containing 35–45% protein and is further classified into different fractions with each having a specialty. B-hordeins, serving as the major storage protein, coexist with C-hordeins, comprising approximately 10–20% protein content. D-hordeins exhibit a substantial molecular weight exceeding 100 kDa, while  $\gamma$ -hordeins are characterized by their abundance in sulfur-rich amino acids <sup>[13]</sup>. Oat is a highly protein-rich cereal and its content is distributed throughout its structure: endosperm (12%), bran (18–26%), and germ (29–38%). They are further classified into four categories: globulins, prolamins, albumins, and glutelins. Globulins, consisting of 70–80% of protein, are major storage proteins and are soluble in the salt solution. Additionally, it consists of a 12S subtype which has been reported to consist of 74% of  $\beta$ -sheets and its molecular weight ranges up to 53–58 kDa. The other two subtypes are 7S and 3S, which are also storage proteins with different molecular weights ranging from 50–70 kDa and 48–52 kDa. Oat prolamins, called avenins, consist of high levels of glutamic and glutamine amino acids. Avenins have a molecular weight of 17–34 kDa and a protein content of up to 4–14%. Albumins, a metabolic protein, contains a rich source of lysine amino acid. It has a molecular weight in the range of 14–17, 20–27, and 36–47 kDa with 1–12% of protein content. Glutelins, which are soluble in dilute acidic and alkaline conditions, contain a protein content of less than 10%.

### **1.2. Legume Proteins**

In legume seeds, the structures of the protein are reported to have a high content of β-sheets and a comparatively low content of  $\alpha$ -helix. Legumes have two major components, namely the seedcoat and the embryo. The structure and composition of seed coats vary among different species of legumes [14]. It functions in transferring information related to the external environment while also protecting the embryo. Moreover, the embryo contains two cotyledons, namely radicle and plumule, and it functions in enclosing the embryo axis while also providing a source of food storage for its development. The chemical composition of legumes includes proteins (20-45%), along with essential amino acids, dietary fiber (5-37%), and complex carbohydrates (up to 60%). In addition to this, it also contains certain minerals and vitamins and is generally low in fat content with no cholesterol levels, making it a nutritious rich crop <sup>[15]</sup>. According to the new classification system, legumes are classified into three sub-families which include Papilionoideae, Caesalpinioideae, and Mimosoideae. The major variety of edible legumes is covered under Papilionoideae such as peanut, chickpea, soybean, common bean, clover, mung bean, lentils, lupins, faba beans, vetches, and peas [16]. According to the studies, different species of legumes have a wide range of proteins based on their amino acid composition and molecular weight. Chickpeas consist of 17-22% protein which is divided further into globulins, albumins, glutelins, prolamins, and some residual proteins [16][17]. Globulins are the main storage proteins consisting of 56 g per 100 g of protein. They comprise two types of protein, which are 11S legumin and another 7S vicilin, having a molecular weight range of 320-400 kDa and 145-190 kDa, respectively  $\frac{117}{2}$ . Albumins are richer than globulins as they contain more essential amino acids such as tryptophan, lysine, and threonine, and also these fractions include more enzymatic and metabolic proteins. Glutelins are structurally similar to globulins as they belong to the 11-12S family of globulins, whereas prolamins are reported to be found in trace amounts irrespective of the chickpea variety. The soybean protein is utilized by consumers in various forms and it generally contains about 70% of total protein <sup>[18]</sup>. Studies have revealed that the soybean protein is called a complete food as it consists of all nine essential amino acids and is highly nutritious <sup>[19]</sup>. They are further divided into two types of storage proteins which are 11S (globulins, glycinin) and 7S ( $\beta$ -conglycinins). The 11S (globulins, glycinin) protein is a hexameric complex having a molecular weight in the range of 320–275 kDa, whereas 7S ( $\beta$ conglycinins) further divides into three sub-units ( $\beta$ ,  $\alpha$ , and  $\alpha$ ') having 50, 60, and 71 kDa size of molecular weights [14]. The amino acid composition of soybean contains histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine <sup>[20]</sup>. Mung bean contains approximately 85% of total proteins which contribute majorly to the storage of proteins in the mung bean <sup>[21]</sup>. It includes three types of proteins based on their solubility, albumins (soluble in water), globulins (soluble in dilute saline), and prolamins (soluble in alcoholwater mixtures). Different sub-types, namely 7S (basic type), 8S (vicilin type), and 11S (legumin type) of globulin, are also present <sup>[21][22]</sup>. Molecular weight also varies among sub-types, such as 8S having a range of 26–60 kDa, 7S having 28 and 16 kDa sizes, and 11S having 40 and 24 kDa sizes. Globulins and albumins consist of 60% and 25% of protein from the total content of mung bean protein. Moreover, mung bean is rich in certain essential and aromatic amino acids such as leucine, isoleucine, valine, and glutamic acid although they are deficient in sulfurcontaining amino acids such as methionine and cysteine along with tryptophan <sup>[22]</sup>. Peas contain 20–25% protein and pea germplasm, which is a part of pea seed, has also been reported to have a protein content of approximately 30%, although it differs based on genotypes and environmental factors <sup>[23]</sup>. Moreover, it can be classified into four types: globulin, albumin, prolamin, and glutelin. Globulins are soluble in the salt solution and contain 55-65% of total protein and are further divided into sub-types, namely 11S legumin and 7S vicilin. Legumin (11S) is a hexameric protein whereas vicilin (7S) is a trimeric protein with both having a molecular weight in the range of 320–400 kDa and 150–180 kDa, respectively  $\frac{17}{2}$ . The amino acid varies in both the sub-types as it has more sulfur-containing amino acids in the legumin type per unit of protein. Albumins have 18-25% of total protein and are classified into two small molecular weight albumins, namely PA1a and PA1b, with a range of 6 kDa and 4 kDa in size, respectively, and they have also shown a high content of cysteine amino acid. Further, prolamin and glutelin are also a group of storage proteins, but they present only small amounts of protein in pea seeds. Also, the pea protein is identified as a protein having a well-balanced amino-acid profile since it contains high levels of lysine, leucine, and phenylalanine  $\frac{24}{2}$ .

Therefore, plant-based proteins have a greater scope in replacing animal-based proteins since they have a huge potential in providing a sustainable approach. Moreover, the choice of a protein source is equally essential since its structure and side chains decide the efficient binding of minerals, thereby leading to the preparation of protein-mineral complexes. Also, new applications can be focused on by using these high-potential legume- or cereal-based proteins to overcome the problem of toxicity by binding them with inorganic minerals.

## 2. Fortification of Food Products

Protein-mineral complexes derived from cereals and legumes have found extensive applications in fortifying food products, particularly in addressing global malnutrition issues. These complexes can be enriched with essential minerals like iron, zinc, calcium, and vitamins. When incorporated into various food items such as cereals, bread, pasta, and dairy alternatives, they serve as an effective means to combat nutrient deficiencies, especially in regions where access to diverse and nutritious foods is limited <sup>[25][26][27]</sup>. For instance, iron-fortified cereal products have been pivotal in alleviating iron-deficiency anemia, a prevalent health concern worldwide. Additionally, these

complexes can be tailored to release nutrients gradually during digestion, ensuring optimal absorption and bioavailability <sup>[28]</sup>.

### 3. Enhancing Nutritional Value

The different applications of cereal- and legume-based protein-mineral complexes are related to several improved functional effects such as the increase in antioxidant properties, enhanced bone mineral density in vivo, and decreased deficiency of iron in vitro, and future recommendations should focus on the utilization of these complexes as a food ingredient for both dietary and nutraceutical supplementation of mineral for future research. Moreover, the hydrolyzed red lentil protein + iron complexes in vitro decreased the expression levels (DMT— Divalent metal transporter 1, TFR—Transferrin receptor, and ANKRD—Ankyrin repeat domain 37 mRNA) which were induced due to iron deficiency anemia. The decrease in the anemic condition in Caco-2 cells was due to the protein-iron complexes treatment which was supplied to this cell culture system <sup>[29]</sup>. Also, some protein-mineral complexes served antioxidant functionality due to their strong mineral binding and chelating effects. The major benefit of antioxidant functionality is it helps in protecting the body against oxidative stress and reduces the risk of chronic diseases. Antioxidants are compounds that neutralize the free radicles in the body, which are harmful molecules that damage the cells and tissues. Thus, increasing the functionality of antioxidants' cereal- and legume-based protein-mineral complexes may help prevent or mitigate the damage caused by the radicle.

For instance, the mung bean protein + iron complexes showed antioxidant function in vitro, and the hydrolyzed chickpea protein + copper complexes also demonstrated a similar functionality <sup>[30]</sup>. Moreover, protein-mineral complexes have shown promising effects in providing health benefits to humans. Peanut protein + calcium complexes when supplied to male mice in vivo improved the bone structure, promoted calcium absorption, and also enhanced the bone mineral density with no adverse side effects <sup>[31]</sup>. Therefore, providing a source of calcium that is easily absorbed by the body, peanut protein + calcium complexes may help to prevent or mitigate the effects of calcium deficiency.

A lot of studies on cereal- and legume-based protein-mineral complexes suggested that with a wide range of positive effects on human health in eradicating deficiencies, these can be used as an approach for the future in developing novel dietary and nutraceutical supplements at the industrial level <sup>[32]</sup>.

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