Innovative Research in
Applied, Biological, and Chemical Sciences
ISSN: 3005-8449
INNOVATIVE RESEARCH JOURNALS
Review Article
IRABCS, vol. 3, issue 2, pp. 32-51, 2025
Received: November 17, 2025
Revised: December 25, 2025
Accepted: December 29, 2025
https://doi.org/10.62497/irabcs.171

Design of a Sustainable Whey–Corn Hybrid Protein Powder for Enhanced Nutrition, Functionality, and Environmental Stewardship

Muhammad Abdullah Butt[1], Muhammad Hameez Shahzad[2], Samiyah Tasleem[3], Rabiya Riaz[4], Xianjiang Ye[5], Burhan Khalid[6], Muhammad Atiq Ashraf[7], Muhammad Moeid Khan[5], Md. Shabudden Ahamed[5], Aliza Batool[8], Zhijun Xia[5], Talha Riaz[5], Muhammad Umair Arshad[1]

1 Department of Food Science, Government College University, Faisalabad, Pakistan
2 National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
3 Preston University, Karachi, Pakistan
4 Department of Chemistry, Government College Women University, Faisalabad, Pakistan
5 College of Food Science and Technology, Huazhong Agricultural University, Wuhan, China
6 College of Plant Science and Technology, Huazhong Agricultural University, Wuhan, China
7 College of Horticulture and Forestry Sciences, Huazhong Agricultural University, Wuhan, China
8 Department of Food Science and Technology, MNS University of Agriculture, Multan, Pakistan
6 E-mail any Correspondence to: Talha Riaz (talhariaz2844@gmail.com) & Muhammad Umair Arshad (umairfood1@gmail.com)

How to cite: Butt MA, Shahzad MH, Tasleem S, Riaz R, Ye X, Khalid B, Ashraf MA, Khan MM, Ahamed MS, Xia Z, Riaz T, Arshad MU. Design of a Sustainable Whey–Corn Hybrid Protein Powder for Enhanced Nutrition, Functionality, and Environmental Stewardship. IRABCS. 2025; 3(2):32–51. DOI: https://doi.org/10.62497/irabcs.171 Available from: https://irjpl.org/irabcs/article/view/171

Abstract

The escalating global protein demand and the environmental burden of conventional animal-based production necessitate the development of sustainable and nutritionally balanced alternatives. This review explores the design of a novel hybrid protein powder integrating whey protein—renowned for its superior bioavailability and leucine richness—with corn protein, a sustainable plant-based source possessing the highest leucine concentration among cereals. The innovation lies in employing physical, chemical, and enzymatic crosslinking techniques to form a unified protein matrix with enhanced solubility, stability, and bioactivity, thereby overcoming the intrinsic limitations of individual proteins. The paper critically examines the nutritional rationale underpinning this combination, emphasizing essential and branched-chain amino acids vital for muscle metabolism. It further outlines the extraction and formulation methodologies for both whey and corn proteins, detailing strategies for achieving optimal functional and sensory properties. By merging the anabolic potency of whey with the sustainability and affordability of corn, this hybrid system offers a versatile and eco-efficient ingredient suitable for beverages, bakery products, and fortified foods. Overall, this work highlights hybrid protein technology as a promising pathway toward sustainable protein innovation and future global food security.

Keywords: hybrid protein, whey protein, corn protein, protein crosslinking, sustainable food systems, functional foods, amino acid profile

1. Introduction

According to UN data, the global population is expected to surge by nearly 50% from the year 2000's levels, reaching 9.5 billion by 2050. This growth is widely acknowledged and brings with it a rising demand for food due to the increase in population. Moreover, shifts such as changing socio-demographics for example higher incomes and urbanization will lead to alterations in consumption habits and will put more pressure on the world's resources to provide not only more but also different types of foods. Consequently, not only will the quantity of food needed alter, but also the kinds of food demanded and their proportion in diets [1].

The anticipated demand for protein is especially noteworthy, with predictions indicating that the global demand for animal-based protein will double by 2050. This raises sustainability and food security concerns, primarily because animal-based foods are generally believed to emit more greenhouse gases (GHG) than plant-based foods, contributing to climate change [2]. A study conducted by concluded that dietary GHG emissions in self-selected meat-eaters are approximately twice as high as those in vegans. It is likely that reductions in meat consumption would lead to reductions in dietary GHG emissions.

Protein is an essential nutrient for building and maintaining lean muscle mass and strength, especially for bodybuilders (and other athletes also) [3]. However, not all protein sources are equal in terms of quality, quantity, and cost. Animal proteins, such as whey, are considered to be superior to plant proteins, such as pea, sorghum, corn, rice, and pulses, because they contain higher amounts of essential amino acids, especially leucine, which is the key amino acid for stimulating muscle protein synthesis. Also, it is concluded in their evidence-based study that animal proteins, due to their high quality, favorable profile of essential amino acids, and increased bioavailability compared to plant-based proteins, have a more significant impact on muscle health outcomes. These outcomes include muscle mass, muscle strength, physical performance, and the treatment of sarcopenia in adults.

However, it's important to note that while animal proteins have these advantages and higher demand, they're still environmentally less sustainable. Thus, a shift towards plant-based protein is desirable for environmental stability, ethics, affordability, food safety, consumer demand, and combating malnutrition. Plant-based proteins, rich in essential amino acids and vital macronutrients, are gaining popularity and can provide complete protein nutrition. Moreover, animal proteins are also more expensive, less sustainable, and may have adverse effects on health and the environment. The findings had revealed that the plant-based sources were significantly more resource-efficient than the animal-based sources of protein. Specifically, producing 1 kg of protein from kidney beans had required approximately 18 times less land, 10 times less water, 9 times less fuel, 12 times less fertilizer, and 10 times less pesticide compared to beef. The study had suggested that replacing beef with kidney beans in our diets could have greatly reduced our environmental footprint and prevalence of non-communicable chronic diseases. It called for societal cooperation to change the perception that red meat is a staple of a healthy and affluent diet.

The production of livestock significantly affects the environment, leading to issues such as soil erosion, water contamination, deforestation, desertification, and the production of greenhouse gases, thereby contributing to global warming (FAO, 2006, FAO, 2009). The consumption of meat and processed meat is also a public health concern, as it is linked to chronic diseases like cancer, diabetes, and cardiovascular diseases [4]. Studies also investigated the relationship between red meat consumption and mortality. The study found that consumption of meat, particularly red meat, is associated with increased risks of diabetes, cardiovascular disease, and certain cancers. The study also suggests an elevated risk of mortality associated with red meat intake. Additional health risks include the ingestion of disease-causing pathogens and chemicals like antibiotics or hormones [5]. On the other hand, a plant-based diet can lead to a reduction in body weight, fat mass, and visceral fat, as well as an improvement in insulin sensitivity.

With the global population and caloric intake projected to increase by 6 billion and 500 kcal per day per capita, respectively, between 2000 and 2100, there is a pressing need to boost protein production. This growth is expected to be accompanied by a rise in the consumption of animal products, leading to a 30% increase in global protein demand, or 10% per capita, by 2100. Without changes in dietary habits, this could put immense pressure on environmental resources [6].

Therefore, exploring alternative protein sources, such as plants, is essential to meet the growing protein demand without further damaging the environment. However, completely disregarding animal proteins, which are more bioavailable and superior to plant proteins in some aspects, would be an extreme approach that could lead to nutritional imbalances.

Hence, there is a need to develop hybrid proteins that combine the best of both animal and plant proteins. These hybrid proteins could offer a cheaper, healthier, and more eco-friendly alternative for muscle growth. This article introduces a novel concept of isolating proteins from optimal animal and plant-based sources (namely whey and corn), based on their Leucine content, an essential branched chain amino acid required for muscle strength and growth, and then blending them to create a hybrid protein powder. This powder could potentially be used for muscle strength and growth.

2. Protein Powder

The protein powder is a dietary supplement that is made by extracting protein from various sources. During the processing phase, naturally occurring carbohydrates, fats, minerals and fibers are swapped with sweeteners and flavorings. These protein supplements are frequently consumed by athletes and recreationally active adults to achieve greater gains in muscle mass and strength, and improve physical performance [7]. Proteins are crucial in food processing, providing structural, nutritional, and functional properties. They exhibit diverse properties, and some protein fractions offer health benefits. Proteins are often processed into powder form for easy transportation and extended shelf-life while preserving their properties (Figure1). Powdered proteins, derived from animal or plant extracts, find widespread use in food production, stabilization, and supplementation for various purposes, including sports nutrition [8].

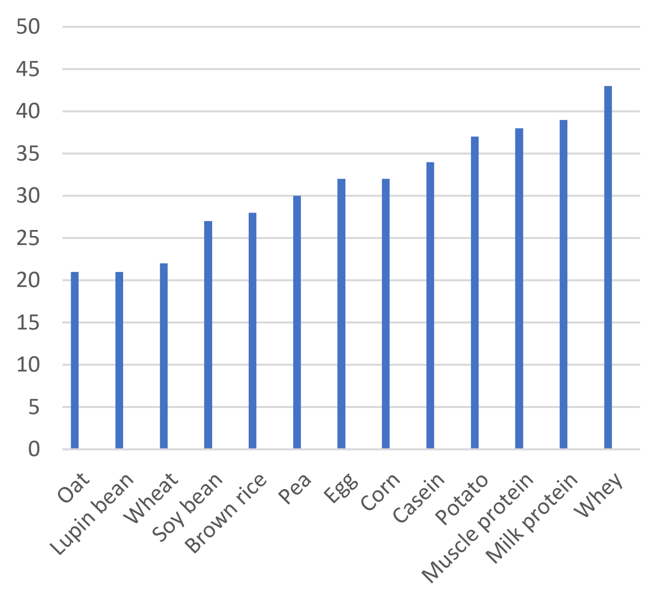

Figure 1: Comparison of the percentage of essential amino acid content of proteins from different sources [9].

2.1. Recent trends and concerns

There is a growing preference for plant-based proteins due to environmental sustainability, ethical considerations, and health reasons such as animal protein allergies and cardiovascular diseases. Livestock production's environmental impact has led to increased interest in alternative protein sources [10]. Challenges such as water scarcity, land degradation, and climate change underscore the need for sustainable protein alternatives [11].

2.2. Plant protein extraction and enrichment

In response to the increasing demand for plant-based proteins, attention has shifted to plant protein extraction, enrichment, and functionalization. Understanding and improving the physical and physicochemical attributes of protein powders are crucial for storage, handling, and processing [12].

2.3. Protein extraction

Following steps are used in the extraction of plant or even animal-based protein. The process of protein extraction begins with the selection of the source material, which can be either plant or animal tissues. Once the source material is chosen, it is crucial to ensure that it is thoroughly cleaned and dried. This preparation of the raw material is a prerequisite for the subsequent processing steps, as stated by [13]. The next step in the process is to execute lipid extraction. This involves removing fats and oils from the material, which enhances the purity of the protein extract. This step is crucial for effectively isolating proteins and improving the overall quality of the final product. The material then undergoes grinding or milling processes. These processes are utilized to reduce the particle size of the material, which facilitates subsequent homogenization and extraction. This step is crucial in the protein extraction process. The material is then homogenized to achieve uniformity. This is done by employing solvents, water, and extraction buffers such as salts, butanol, phenol, TCA/acetone, and enzymes. This step is essential for breaking down the cell structure and facilitating efficient protein extraction, as stated by [14].

Following the homogenization, the material is incubated with a suitable solvent to extract proteins. It's important to ensure that the solvent effectively interacts with the sample. Solvents may include water and extraction buffers. This step is critical for separating proteins from the source material, as highlighted by [15]. After the extraction, proteins are separated from the solvent through precipitation or filtration methods. Techniques like changing pH, adding salts, or using membranes aid in achieving effective protein separation. This step is crucial in the protein extraction process, as it ensures that the proteins are effectively separated from the source material, as per [16]. Physical extraction methods, such as electric pulse field, ultrasound, high pressure, microwave-assisted extraction, or subcritical water treatment, are employed to enhance protein precipitation and filtration [17].

The next phase is protein processing for powder formation. The obtained protein precipitate or filtrate is processed further to produce protein powder. Two common methods include dissolving the protein in water, adjusting dry matter content, and either spray drying or freeze drying. The product is then milled and sieved. These steps are crucial in the protein extraction process, as per [18]. In the process of protein powder formation, proteins are first dissolved in water. The dry matter content is then adjusted, and spray drying is used to produce fine protein powder particles. The resulting product is sieved to ensure a consistent and desirable particle size distribution. These steps are crucial in the protein extraction process, as per [19]. An alternative method to spray drying is to freeze dry the protein solution. This is followed by milling and sieving to obtain a fine protein powder while preserving its quality. This method yields a similarly fine and usable protein powder, as stated by [20]. After protein extraction, the crucial next step is drying to create Protein Powders (PPs). The extraction process and drying techniques significantly impact the functional properties of plant-based PPs in food formulations. Diverse methods lead to varied protein yields with distinct functional characteristics. While drying enhances storage stability, it induces partial protein denaturation, forming irreversible aggregates and altering functionality. Hydrolysis degree, molecular weight distribution, and amino acid composition influence powder properties. Proteolytic enzymes, extraction conditions, and hydrolysis impact solubility, foaming, and emulsifying capacity [21].

2.4. Factors in Powder Production

High hydrolysis may introduce bitterness due to low molecular weight hydrophobic peptides [22]. Assess particle size distribution, morphology, and moisture content post-drying. Analyze reconstitution, solubility, bioavailability, shelf stability, and consumer acceptance. Moreover, the polypeptides require analysis of their key technological properties, such as flowability, stickiness, thermal properties (specifically, glass transition temperature), and storage stability [23].

2.5. Common Drying Techniques

2.5.1 Spray Drying (SD): Spray drying (SD) is an economical method that allows for rapid dehydration and has low operational costs, making it suitable for large-scale production. However, the parameters of SD must be optimized for nutritional endpoints and functionality. The optimal conditions for SD include an inlet temperature of 160 °C, a feed rate of 6 mL/min, and a solid concentration of 35%.

Reducing protein denaturation while maintaining drying efficiency and powder quality is to lower the inlet temperature by 20°C. This can be achieved by adding ethanol to the protein extract. Bulk agents like maltodextrin or starch can be used to prevent heat damage, thereby improving solubility and antioxidant activity. Post-SD granulation may enhance solubility, particle size, and reduce dust formation. This comprehensive approach ensures the production of high-quality protein powder. (Table 1)

2.5.2. Freeze-Drying (FD): Freeze drying, also known as lyophilization, is a method that solidifies proteins with minimum structural damage. This method involves freezing, primary, and secondary drying stages. Faster reconstitution is facilitated by amorphic and disordered states. Freeze drying produces porous particles that have better solubility compared to other methods. This method ensures the production of high-quality protein powder with preserved quality and faster reconstitution.

2.6. Other Drying Methods

2.6.1. Spray-Freeze Drying: Yields light and porous structures, maintaining spherical droplet shapes [24].

2.6.2. Oven Drying: Less expensive but results in longer residence time and wrinkled-shaped particles [25].

Other Techniques (MW, supercritical anti-solvent, fluidized bed)

Effects on functional and physicochemical properties vary. Superfine grinding post-drying improves surface properties of protein particles. Understanding the intricate interplay of extraction, drying, and processing methods is crucial for producing high-quality Protein Powders with desirable functional and physicochemical attributes. Diverse techniques offer unique advantages, but careful optimization is essential to meet specific application requirements and consumer expectations [26].

2.7. Protein powders in food formulation

2.7.1. Functional properties and molecular structure: The use of protein powders in food formulations is dependent on their functional properties such as viscosity, gelation, and foaming. These properties are linked to the molecular structure and composition of the proteins. Furthermore, interactions between proteins and other ingredients can significantly influence the performance of these proteins across the food supply chain. Understanding these interactions is crucial for optimizing the use of protein powders in various food products [27].

2.7.2. Factors affecting protein functionality: The major influencers in the use of protein powders in food formulations are composition, processing methods, additives, pH values, and the food environment. These factors significantly impact the functional properties of the proteins and their interactions with other ingredients, thereby influencing their performance across the food supply chain [28]

2.7.3. Factor affecting the properties of protein powder: Solubility is influenced by factors such as particle size, surface properties, pH, temperature, solvent dielectric constant, and salt concentration. These factors are essential for liquid or semi-liquid foods as they impact characteristics such as rheological, hydrodynamic, and surface activity [29]. Foaming capacity and stability are crucial for creating cohesive films and retaining foam over time. Emulsifying activity, capacity, and stability are vital for effective emulsification and resistance to structural changes. High oil and water absorption capacities enhance texture, mouthfeel, and flavor retention. These factors collectively contribute to the overall quality of food products.

Thermal properties, including glass transition, denaturation, and heat stability, characterize temperature-induced conformational changes [30], Particle and powder properties, such as bulk density, flow, and size distribution, affect functionality and technological performance. The size varies from 10 to 900 µm, with nanosized particles being used for specific applications. These properties are critical in determining the performance and application of food products. Moisture adsorption affects molecular properties and storage stability. Water content is crucial for quality attributes like storage stability, shelf-life, taste, and nutritive value. These factors are important in maintaining the quality and longevity of food products. High-pressure homogenization enhances the water solubility and emulsifying properties of myofibrillar protein powder (PP). Drying methods, such as spray drying (SD), freeze-drying (FD), and oven drying, impact the structural characteristics, solubility, and absorption capacities. These processes are important in modifying the properties and improving the functionality of food products. In summary, the multifaceted quality attributes of Protein Powders underscore the need for precise control over factors influencing their functionality. The interplay of molecular structure, processing methods, and storage conditions significantly impacts their suitability for diverse food formulations. Understanding these nuances is paramount for optimizing protein functionality across various applications in the food industry.

3. Hybrid Protein (concept and scope)

The term hybrid protein has different meanings and scope in different fields. Some examples have been discussed below.

3.1. Molecular biology and Biochemistry

The term “hybrid protein” is often used in the field of molecular biology and biochemistry. It refers to a complex of two or more polypeptide sequences or fragments thereof which would not normally be associated. These hybrid proteins are used in study of protein targeting. Their more comprehensive definition would be, “A hybrid protein is a complex of two or more polypeptide sequences or fragments thereof which would not normally be associated but are coupled together either by fusing the genes which encode them (gene fusions) or by chemically cross-linking the purified component parts” [31].

These hybrid proteins are used in study of protein targeting.

3.2. Computational chemistry and Bioinformatics

There is a tool called PMX that can generate hybrid protein structure and topology for alchemical molecular dynamics based free energy calculations. This tool is compatible with the Gromacs molecular dynamics simulation software [32].

The alchemical molecular dynamics based free energy calculations are performed in the field of computational chemistry and bioinformatics. These calculations are used to predict the free energy change due to a mutation that could have a deleterious or a stabilizing effect on either the protein itself or its binding affinity to another protein. This can be particularly useful in drug design and understanding disease mechanisms [33].

3.3. Molecular biology and Medical biology

The two hybrid screening techniques, which include yeast two-hybrid systems and bacterial two-hybrid systems, are commonly used for the discovery of protein-protein interactions (PPIs) and protein-DNA interactions [34].

3.4. Food Science and technology

The current study, situated within the field of food science and technology, proposes the development of hybrid protein powder by first isolating proteins from plant and animal sources, and then binding their amino acids by the act of crosslinking. This powder could serve as a cost-effective, sustainable, and eco-friendly source of protein, functioning as a functional food to enhance muscle growth, development, and strength. It may be particularly beneficial for various physical activities such as aerobic sports, athletics, bodybuilding, and weightlifting.

Multiple methods exist for creating complexes of intermingled or crosslinked amino acids from two different proteins, resulting in the formation of novel hybrid proteins with distinct capabilities. Some of these methods are discussed in Section 4.

4. Comprehensive insight into protein crosslinking

The creation of one or more covalent connections between amino acid residues is known as protein cross-linking, and it causes various peptides and proteins to associate with one another and form protein networks. Generally speaking, three kinds of crosslinks can form: Blind end-links are peptides that have been altered by a crosslinker but are not connected to another peptide because one of the functional groups is still inactive. Intrapeptide crosslinks are crosslinks within the same subunit of a protein complex, while interpeptide crosslinks are crosslinks between different subunits of a protein complex or different proteins [35].

The physicochemical characteristics and functions of the newly formed assemblies are frequently different from those of the non-crosslinked versions. Food processing, textile science, tissue engineering, and medicinal research are among the many fields of study that are currently very interested in protein-protein crosslinks [36].

4.1. Types of protein crosslinking

4.1.1. Physical cross-linking: Non-covalent connections that form during processing, such as ionic, hydrogen, and hydrophobic interactions, lead to physical cross-linking. Using physical techniques like high pressure, mechanical cooling, or heating, crosslinking is accomplished in food products. These physical adjustments can be made in conjunction with other modification procedures and are safe. Furthermore, cross-linking between proteins and polyphenols is caused by covalent or non-covalent connections like hydrogen bonding and hydrophobic interactions. However, because of their strength and long-lasting interactions, conjugates made with covalent bonds are preferred by food makers due to their superior stability [37].

Cold-setting emulsion gels are frequently created via ionic cross-linking. This technique involves heating the protein solution over the globular protein's heat denaturation temperature while preventing protein aggregation. Strong electrostatic repulsion between protein molecules results from certain conditions, such as pH and ionic strength. A change in solution conditions or the addition of a cross-linking agent that lessens electrostatic repulsion causes the unfolded proteins to cross-link. The protein molecules consequently attach to one another to create a gel network, which expands the system's volume [38]. According to earlier research, protein solutions can be acidified with monovalent cations like Na^+ or with divalent cations like Mg^{2+} to create cold-setting gels. These methods increase the mechanical qualities of gels by forming physical crosslinks between protein molecules [39].

The Maillard reaction, commonly referred to as non-enzymatic glycation, can cause proteins to cross-link. The flavours, colours, textures, and scents connected to food browning are altered by this chemical reaction [40]. A condensation event between amine groups in protein molecules and carbonyl groups in reducing sugar molecules during the earliest phases of the Maillard reaction produces several Amadori products. Furthermore, it has been reported that a range of polymerised compounds can be produced during the intermediate and advanced stages through the creation of reactive intermediates like sugar-derived dicarbonyl compounds. Protein molecules become less soluble as a result of the Maillard reaction, which also causes covalent connections to form between them. This alters the gel's ability to retain water and its rheological characteristics [41].

Another processing-induced protein modification that can happen in food protein systems is protein cross-linking through the dehydroalanine (DHA) pathway; however, unlike the Maillard reaction, this activity does not require the presence of sugar. Alkaline conditions and heat treatment cause it. First, a β-elimination process forms cysteine, serine (including glycoserine and phosphoserine), or cysteine on DHA [42]. Protein cross-links lysinoalanine (LAL), lanthionine (LAN), and histidinoalanine can be created when the alkene DHA further reacts via Michael addition with either lysine, cysteine, or histidine residues in proteins and peptides. Physical crosslinking is frequently accomplished via heat treatment.

4.1.2. Chemical crosslinking: A cross-linking agent must be added for chemical cross-linking to occur. Strong binding sites are typically produced by this kind of cross-linking using covalent bonds [43]. Covalent links between proteins can be created using a broad range of chemical substances. Bifunctional chemical substances are known as chemical crosslinking molecules [44].

Reactivity complementary to a particular functional group in amino acid residues, such as primary amines, carboxyl groups, or sulfhydryl groups, is referred to as a cross-linking agent. These include monofunctional crosslinkers like formaldehyde, which only have one reactive group, and bifunctional chemical crosslinkers like glutaraldehyde, which carry two reactive groups to target protein locations. Certain chemical agents are selective for amino acid residues, whereas others can target a wide variety of functional groups. From simple protein-protein interaction research to histochemical and microscopic applications, chemical crosslinkers have several uses [45]. Due to their ability to react with primary amines and create both intra- and inter-crosslinking in polymer chains, aldehydes are generally utilised extensively. Research that employs genipin as a cross-linking agent can be categorised under this heading. Genipin is a naturally occurring chemical that causes lysine and arginine to react, giving the resultant material a distinctive bluish hue [46].

Genipin, tannic acid, nordihydroguaiaretic acid, procyanidins, and citric acid are examples of natural chemical crosslinkers. The N-terminal/epsilon amino group of lysine side chains in proteins is where genipin reacts with primary amines. Additionally, it coats the protein with chitosan nanoparticles after cross-linking ovalbumin [47].

4.1.3 Enzymatic cross-linking: Enzymatic cross-linking agents, as opposed to chemical ones, have been used in food processing to alter the structure of foods in order to increase their shelf life and alter its texture, look, and flavour. Enzymes are an appealing technique that has drawn a lot of attention due to their selectivity and typically moderate response circumstances. Compared to chemical crosslinkers, enzymes are seen to be more "natural" [48].

According to the enzymatic mechanisms, two types of cross-linking reactions can be identified: enzyme-mediated covalent linkage via reactive species that are enzymatically generated by oxidoreductases such as laccase, tyrosinase, and peroxidase, which then react with proteins to form protein networks, and direct covalent linkage catalysed by transferases such as transglutaminase via protein enzyme-thioester intermediates [49].

Protein and polysaccharide molecules can be cross-linked by enzymatic technology, and the resulting protein-polysaccharide conjugate has higher solubility and frequently better functional qualities like better gelling and stronger emulsifying ability [50].

Research on transglutaminase enzymes (EC 2.3.2.13, TG), also known as glutaminyl peptide-amine γ-glutamyl transferases, has demonstrated their role in protein cross-linking. TG enzymes are members of the acyltransferase family, which aids in a certain chemical reaction. In this process, glutamine and lysine, two amino acids that are components of proteins or peptides, form a covalent connection. An intramolecular or intermolecular interaction forms this connection, which is known as an ε-(γ-glutamine)-lysine isopeptide bond. In this enzymatic reaction, glutamine served as the acyl donor and lysine as the acyl acceptor in an acyl transfer reaction. Because of its side chain accessibility, which is dependent on the protein's structural conformation, it was found that the TG enzyme might catalyse the crosslinking of lysine and glutamine [51].

Because laccase-mediated cross-linking can drastically alter dietary qualities, it has been the subject of much research in the past. It is evident that laccases greatly increase emulsion stability in an alkaline environment. Cross-linking between laccase and tiny reactive phenolic chemicals found in the globular protein also affects system characteristics including colour and solubility [52].

Monophenol-odiphenol oxygen oxidoreductase (EC 1.14.18.1), another name for tyrosinase, is typically found in microbes, plants, animals, and even people. Food items can benefit from tyrosinase-mediated cross-linking in a number of ways, including enhanced antioxidant and antibacterial activity, increased stability of emulsions or nanoparticles, pepsin resistance, improved water retention, and increased mechanical strength of gels [53]. Furthermore, there is no environmental contamination as a result of this cross-linking. Tyrosinases can effectively cross-link low-complexity dietary proteins, but they are unable to catalyse reactions with complex or folded proteins. Consequently, a low molecular weight substance is introduced as a mediator in these processes, such as phenol or caffeic acid.

The oxidoreductase group of enzymes, which includes peroxidases (EC 1.11.1.x), is present in plant, animal, and microbial cells [54]. A peroxidase-catalyzed reaction that depends on the availability of the co-substrate H_2O_2 is one of the main benefits that make horseradish peroxidase (HRP) preferred in the food business. Compared to the dissolved oxygen concentration in this reaction, the H_2O_2 concentration is easier to control. By altering the H_2O_2 concentration and time, this aids in regulating the cross-linking. Furthermore, the cross-linking of HRP by horseradish peroxidase improves the stability of the emulsions, the antibacterial and antioxidant properties, and the complex's thermal sensitivity [55].

4.2. Applications of protein crosslinking

The food industry frequently uses protein cross-linking to alter the texture, stability, and sensory qualities of food items. Understanding the cross-linking agent's structure and mechanism of action is crucial for its use in practical applications including food processing and illness treatment [56].
The meat processing industry has seen a significant increase in the commercial significance of value-added meat in recent years. Crosslinking techniques are being researched as a result of consumer desire for a high-quality, reasonably priced product. By giving the low-value flesh more value, these techniques aid in the effective utilisation of the carcass. This method, which entails glueing meat parts together, is referred to as restructure [57]. Commercially, a variety of crosslinking agents are used to enhance the texture of meat and meat products. In chicken myofibrillar protein, cross-linking facilitated by recombinant microbial transglutaminase (MTG) produced from the Pichia pastoris GS115 strain demonstrated enhanced adhesion, chewiness, and hardness [58]. MTG can cross-link actin, myofibrillar protein myosin heavy chain, β-conglycinin subunits, and acidic soy protein component subunits. Adding TG to caiman steaks could enhance their textural qualities and cooking yield without changing their flavour or colour. In order to lower salt content without sacrificing sensory qualities, they suggested using TG and MgCl2 together [59].

Enzymatic cross-linking is used to produce yoghurt, which eliminates the need for stabilisers and non-fat solids or lowers the solids' concentration without altering the product's texture. The use of TG with milk gels produced by chemical acidification and yoghurt starting culture has been studied by numerous researchers [60]. Using Zea mays TG generated from Pichia pastoris GS115, enzyme-mediated cross-linking produced yoghurt with a stronger acid gel, higher consistency, cohesiveness, viscosity index, and perceived viscosity [61]. Yoghurt stability in terms of pH, viscosity, and water-holding capacity was improved by enhancing kappa-casein and alpha-lactalbumin crosslinking with TG treatment. According to reports, the effect became more noticeable as the TG content increased.

Bread made with red rice flour and cassava flour, TG, and chitosan at concentrations of 0, 1, and 2%, respectively, exhibited a lighter brown colour because to low specific volumes and an incomplete Maillard reaction. Furthermore, TG created isopeptide linkages, particularly in the gluten portion, which resulted in the development of protein aggregates that enhanced the structural integrity of bread [62]. The manufacture of flavoured bread with a longer shelf life was made possible by the positive synergistic action of TG and yeast [63].

Because laccase-mediated protein cross-linking greatly boosted the dough's elastic and viscous modulus, laccase was used to reduce thiols and total phenols by up to 28% and 93%, respectively. The impact of Trametes maximum CU1 laccase on bread's physicochemical quality was also assessed, and it was effectively applied to increase bread's height, hardness, and weight loss [64]. Since disulphide linkages have been seen in the egg yolk and egg white noodles, natural cross-linking in noodles has also been documented. This leads to protein cross-linking and enhanced functionality [65].

Additionally, laccase encourages the creation of protein networks during the cheese and bread-making process, which raises the yield, increases the antioxidant activity, decreases the hardness and chewability, and increases the elastic and viscous modulus [66]. Tyrosinase enhances droplet size distribution, rheological behaviour, emulsion stabilisation, and protein network formation [67]. Similar to this, peroxidase helps create a protein network, which raises the storage modulus and gel strength [68].

The study found that a variety of enzymes and constituents can change the cross-linking and bonding of the gluten protein, hence influencing its characteristics and network development. This results in safer products by altering the way the gluten functions [69]. Studies show that using transglutaminase, laccase, tyrosinase, or peroxidase to crosslink potato protein alters the protein's structural, thermal, and gel characteristics to varied degrees. The secondary structural alterations in enzyme-treated proteins are validated by FTIR spectra [70]. Higher stability, increased β-sheet, antiparallel β-sheet, and β-helix content, and a considerable decrease in random coiling are all results of the enzymatic changes. The crystalline peaks of potato protein are unaffected by enzyme treatment [71]. Overall, protein cross-linking provides flexible solutions for modifying protein-based materials and creating innovative products with enhanced properties for various industrial applications.

5. Selection of Animal & Plant Protein based on Nutritional value (BCAAs) for the development of hybrid protein

Now that the concept of hybrid protein formulation in the field of food science and technology—particularly through amino acid crosslinking—and its benefits have been comprehensively understood, it is important to recognize that proteins for novel hybrid formation cannot be selected at random. Instead, several factors must be considered, one of the most critical being the nutritional properties of the selected protein. This includes choosing proteins with a high content of essential amino acids and a strong Protein Digestibility Corrected Amino Acid Score (PDCAAS). Among these, the three branched-chain amino acids (BCAAs) are especially valuable for promoting muscle growth, development, and strength.

5.1. Essential amino acids (EAA)

Among the amino acids essential amino acids are of prime importance for human body. The efficacy of essential amino acid supplementation has been studied as a strategy to increase dietary protein intake and improve muscle mass, strength, and function in older adults. A sufficient daily protein intake which ensures presence of these amino acids is widely recognized to be fundamental for the successful management of sarcopenia, particularly in older undernourished adults [72].

The rise in essential amino acid (EAA) concentrations after protein ingestion influences muscle protein synthesis rates. The EAA content and amino acid (AA) composition of the protein source affect this response. Plant-based proteins often have lower EAA contents and lack sufficient leucine, lysine, and/or methionine, which may result in a lower anabolic capacity compared to animal-based proteins. A comparison of EAA contents and AA composition of various plant and animal-based proteins, as well as human skeletal muscle protein, revealed significant differences. For instance, plant-based proteins like oat, lupin, and wheat had lower EAA contents (21-22%) than animal-based proteins (whey 43%, milk 39%, casein 34%, and egg 32%) and muscle protein (38%). AA profiles also varied among plant-based proteins, with leucine contents ranging from 5.1% (hemp) to 13.5% (corn), compared to 9.0% (milk), 7.0% (egg), and 7.6% (muscle protein).

Methionine and lysine were typically lower in plant-based proteins compared with animal-based proteins and muscle proteins. In conclusion, there are large differences in EAA contents and AA composition between various plant-based protein isolates. However, combinations of various plant-based protein isolates or blends of animal and plant-based proteins can provide protein characteristics that closely reflect the typical characteristics of animal-based proteins [73]. The important EAAs are the BCAAs, which include leucine, isoleucine, and valine. At molecular level, these three amino acids are branched in structure instead of being linear. They are critical for skeletal, muscle and whole-body anabolism and energy homeostasis. They also serve as signalling molecules, for example, being able to activate mammalian/mechanistic target of rapamycin complex 1 (Mtorc1), which has implications for macronutrient metabolism. The proteins containing these three amino acids are used for building muscle mass.

It is also reported that the branched-chain amino acids (BCAAs) play a crucial role in skeletal muscle and whole-body anabolism, energy homeostasis, and macronutrient metabolism. Beyond their function as protein synthesis substrates, BCAAs act as signalling molecules, activating mTORC1, a key kinase complex essential for promoting and maintaining muscle mass. BCAAs also have roles in suppressing proteolysis, promoting glucose transport, regulating body weight, and contributing to cholesterol synthesis.

5.2. L-Leucine

L-Leucine is a very important essential amino acid. Among the branched chain amino acids L-Leucine is the one which actually plays the role in the synthesis of protein [74]. It, upon facilitation with the activation of the mammalian/mechanistic target of rapamycin complex 1 (mTORC1 - a serine/threonine kinase complex), exerts a significant influence in the augmentation and preservation of muscle mass. It is imperative to underscore that the initiation of mTORC1 activation is intrinsically precipitated by the dietary incorporation of leucine and other branched-chain amino acids [75]. The study simplifies the role of leucine as follows: Leucine can activate crucial signalling proteins that are part of the protein kinase B-mammalian target of rapamycin (mTOR) pathway. This pathway is responsible for initiating translation, a critical process in protein synthesis. The remaining two amino acids (Isoleucine and Valine) do not have an actual role in this process [76].

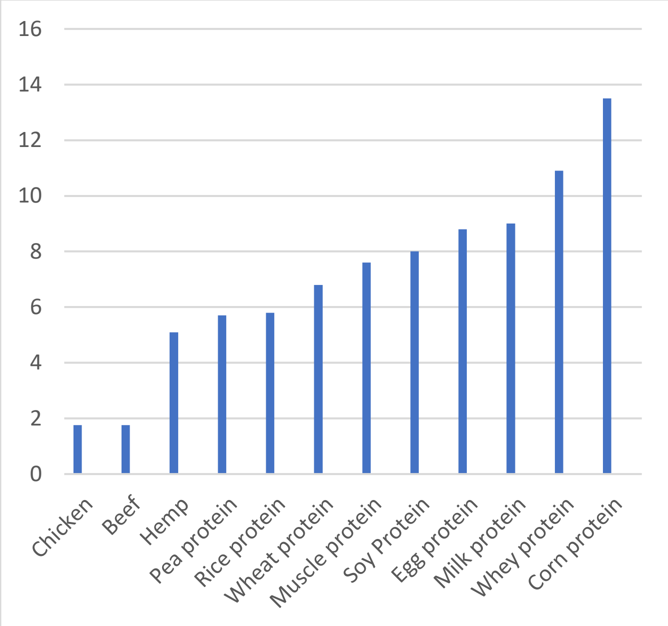

Figure 2: Percentage of Leucine in Different food groups on the Weight/Weight basis [77]

The major functions that L-Leucine plays in the body are the following.

1. Muscle building
2. Muscle strength
3. Athletic performance (endurance/agility)
4. Blood sugar control
5. Wound healing
6. Cognitive functioning

It is important to note that the amount of leucine can influence muscle protein synthesis, with approximately 3 to 4g of leucine per serving needed to promote maximal protein synthesis [78]. Overconsumption of Leucine or other branched-chain amino acids (BCAAs) is also not good, as it can lead to adverse effects. This is due to the elevation of circulating levels of BCAAs and their ketoacids, coupled with impaired catabolism of these amino acids. These factors are implicated in the onset of insulin resistance and its associated conditions, including type 2 diabetes, cardiovascular disease, and certain types of cancer [79].

5.3. Sources of Leucine

Wheat protein, soy protein, egg protein and whey protein contain 6.8%, 8.0%, 8.8% and 10.9% leucine, respectively. According to the study of [80], hemp contains 5.1% leucine, while muscle protein, milk protein and corn protein contain 7.6%, 9.0%, and 13.5% leucine, respectively. According to a study, a 3-ounce (85-gram) chicken breast and a 3-ounce beef contain 1.5 grams of leucine each. This means both of these meats contain 1.76% leucine. According to the study of [81], rice protein and pea protein contain 5.8% and 5.7% leucine, respectively. From Figure 2, it is clear that among animal proteins, whey protein has the highest content of leucine, and among plant proteins, corn protein has the highest concentration of this essential branched-chain amino acid. Thus, these two proteins can be combined to create a hybrid protein.

6. Corn Protein

6.1. Introduction to corn protein

Corn proteins, predominantly consisting of zeins, make up over 60% of the total proteins in whole grain and 70% in the endosperm. These zeins, categorised into α, β, and γ divisions, are prolamins and storage proteins that reside within spherical structures known as protein bodies [82].

On the other hand, corn protein derived from the germ part of the corn structure, which comprises about 11% of the total weight of normal dent corn, contains 18-22% crude protein (dry basis). This accounts for about 29% of the total protein in whole corn. More than 60% of these proteins are the water-soluble albumins and saline-soluble globulins, which have a highly desirable amino acid balance, providing a better nutritional value to the germ protein than endosperm protein. Interestingly, these albumins and globulins are also known to have functional properties superior to those of the alcohol-soluble prolamins and dilute alkali-soluble glutelins, which are the predominant storage proteins in the endosperm. This highlights the diverse and complex nature of corn proteins and their potential nutritional and functional benefits [83]

6.2. Functional Properties of corn protein

Corn protein has several functional properties that make it valuable ingredient in food products [84]. These properties have been discussed in detail in Table 1.

Table 1: Functional Properties of Corn Proteins.

Property	Description
Protein Solubility	Corn protein exhibits good solubility, which is an important property for its use in food systems. For instance, purple corn protein concentrate (PCPC25 and PCPC35) presented a protein solubility of 59.43% at pH 8.0.
Water and Oil absorption	Corn protein can absorb water and oil. This property is beneficial in food formulations as it can improve the texture and mouthfeel of the product. PCPC25 showed a water absorption capacity of 27.77% and an oil absorption capacity of 24.94%.
Antioxidant activity	Corn protein also exhibits antioxidant activity, which can contribute to the shelf-life and nutritional value of food products. PCPC25 and PCPC35 showed high values of antioxidant activity.
Emulsifying Capacity	Corn protein has been found to have good emulsifying properties, making it useful in products like salad dressings and mayonnaise.
Foaming Capacity and Stability	Corn protein can form and stabilize foams, which is important in products like meringues and whipped toppings.
Heat Coagulability and Gelation	Corn protein can coagulate and form gels upon heating, which is a crucial property in products like custards and certain types of sausages.
Multifaceted health benefits	Corn peptides have demonstrated a variety of functions both in vitro and in vivo, including antihypertensive, hepatoprotective, anti-obesity, antimicrobial, antioxidative, mineral-binding, and alcohol metabolism acceleration properties. As of now, 22 bioactive corn peptide sequences have been recognized.

7. Whey Protein

7.1. Introduction to whey protein

Whey protein, often referred to as WP, denotes the group of proteins that are water-soluble and derived from dairy milk. Supplements of whey protein, specifically those with a protein concentration of over 80% (known as WPC 80) or over 90% (referred to as WPI 90), here WPC stands for whey protein concentrates and WPI stands for whey protein isolates. They have gained popularity. They are particularly favored by athletes and individuals who are keen on building muscle mass [85].

Whey protein has various functional properties that make it useful for food applications, such as solubility, gelation, water and fat binding, emulsification, foaming, and antioxidant activity. Whey protein can be modified by different processing methods, such as hydrolysis, cross-linking, glycation, and nanoencapsulation, to enhance its bioactivity and functionality. Moreover, they can be used as an active food ingredient in the production of functional foods, such as beverages, dairy products, meat products, bakery products, and edible and active packaging [86]. Further details are discussed in Table 2.

7.2. Functional Properties of Whey Protein

Table 2: Functional Properties of Whey Proteins.

Property	Description	Reference
Solubility	Whey proteins have excellent solubility but increasing temperature can	[87]

	decrease the solubility of whey proteins, particularly at their isoelectric points (pH 4.5) and at near neutral pH (6.8) This property of whey protein is required in frozen desserts, confectionary, bakery, whipped toppings, processed meats, and coffee whitener industries	
Water binding	In sauces and soups, water-binding and acid solubility capabilities are improved because of addition of whey proteins during the operation of thermal processing. This enhancement contributes to the consistency and flavor profile of these food items, making them more appealing to consumers.	[88]
Aeration	In confectionery and bakery products such as bread, biscuits, and cakes, whey protein can enhance the aeration in product structure. This improvement contributes to the texture and mouthfeel of these baked goods, making them more enjoyable to consumers.	
Texture providing	Whey protein provides texture to various foods. Dairy products such as yogurt, ice cream, and milk drink can have skim milk powder replaced by demineralized lactose-free whey powder. This replacement provides a desirable body and mouthfeel of milk products. This substitution can enhance the sensory attributes of these dairy products while catering to those with lactose intolerance.	[89, 90]
Gel Formation (gelation)	In meat, seafood, comminuted meat, and fish products, the properties of gelation, and hydrophilicity are improved with the use of whey protein. This enhancement contributes to the texture, stability, and overall quality of these food products, making them more appealing to consumers. Whey proteins forms firm gels when heated above 70°C. The gelation of WOC is also pH-dependent. At alkaline pH, firm gels are formed, primarily due to the aggregation of β-lactoglobulin, a protein that aggregates under both acidic and alkaline conditions. α-lactalbumin, another protein in WPC, does not aggregate at pH levels above 7 but forms aggregates under acidic conditions. The denaturation of α-lactalbumin at neutral pH increases the viscosity, while the denaturation of β-lactoglobulin results in gel formation. The absence of NaCl is conducive to the formation of firm WPC gels, indicating that NaCl may disrupt protein-protein interactions necessary for gel formation. They have better gelling properties as compared to casein. This property of whey protein finds application in confectionary, bakery, and processed meats	[91, 92]
Emulsification	A concentration of whey protein above 2% is required for stable emulsion formation. Optimal creaming stability, viscosity, and protein adsorption occur at pH 5. Adsorption of α-lactalbumin increases and β-lactoglobulin decreases as pH decreases to 5 or 3. Sugar ester, monostearate, and lecithin reduce creaming stability. The emulsion is heat-stable without salt but gels when heated with CaCl2. Among whey proteins β-lactoglobulin, immunoglobulin, and lactoferrin adsorb selectively at pH 7. Electrostatic nature and protein conformation are crucial for adsorption. Whey proteins have limited gelling properties as compared to casein proteins. This property of whey protein has its applications in frozen desserts, confectionary, processed meats, and coffee whitener industries In meat, seafood, comminuted meat, and fish products, whey protein enhances the properties of emulsification, and hydrophilicity. This improvement aids in the texture, stability, and overall quality of these food items, increasing their appeal to consumers.	[93]
Foaming	The foamability of WPI at pH 5 is increased, but the foam stability only increases at pH 7. Heat treatment increases the foaming properties of WPI at pH 7 This property of whey protein is required in frozen desserts, confectionary, bakery, and whipped toppings whitener industries	[94]
Adhesion	Due to their compact globular structures and low molecular weights, native whey proteins do not possess strong adhesive properties. However, when subjected to heat, these globular structures can unfold, leading to the formation of polymers or aggregates through intermolecular disulphide bonds via a thiol–disulphide interchange. This heat-induced transformation allows whey protein polymers to exhibit improved adhesive properties	[95]

	This property of whey protein has its applications in bakery and processed meat industries	
Flavoring and coloring agent	Sugar confectionery products such as lollipops, toffees, candies, caramels, chocolate, and cotton candy have their color and flavor increased by interacting with amino acids present and lactose present in the whey proteins during heat processing under Maillard reactions. This process enhances the sensory appeal of these confectionery items, making them more enjoyable to consumers.	[96]
Flavor binding	Among the milk proteins the whey protein has exhibited the capability to bind different lipophilic compounds including the flavors. This property has its application in the processed meat industry	[97, 98]
Fat and Water Binding	Whey proteins being hygroscopic can bind water and fat present in the food. This capability of whey proteins can have applications in various edibles. For example in a study Whey protein isolates (WPI) were used for the creation of bread. The results showed that WPI because of their good water and fat binding property improved dough stability and enhanced the farinographic quality. However, dough development time was also prolonged.	[99]
Bioactive properties	Whey proteins are rich in bioactive peptides, possessing antioxidant, antihypertensive, and antimicrobial activities. These properties confer several health benefits when ingested	[100]
Nutritional and Nutraceutical properties	Whey proteins act as nutraceuticals and provide many health benefits due to the presence of significant bioactive proteins such as lactoperoxidase, lysozyme, lactoferrin, and numerous growth factors. These components collectively contribute to the health-promoting properties of whey products. Whey protein is used in infant formula to simulate human milk, providing nutritional benefits. This modification helps to closely mimic the nutritional profile of human milk, offering a suitable alternative for infants who cannot be breastfed. This is crucial in ensuring the healthy growth and development of infants. With whey protein, the bioavailability of salts like calcium and zinc is improved, and essential vitamins are provided in diets. This is particularly beneficial in dietetic foods for the elderly, helping to meet their specific nutritional needs and support their overall health. This is a key consideration in the formulation of dietary products for older adults. Whey proteins are used in dietetic foods for weight management. A satiety-enhancing dietetic diet with a high protein, low-fat content, and an ideal amino acid composition is developed. This formulation aids in weight management by promoting feelings of fullness and providing essential nutrients, making it a beneficial choice for those seeking to manage their weight effectively.	[101, 102]

7.3. Tolerance of Whey and Corn protein (on the basis of Self-reported GI complaints)

Tolerance of whey protein and corn protein was checked by checking the stool type and frequency of stool of participants. Their feelings of bloating, belching, flatulence, nausea, diarrhea and constipation was also checked. All the participants had stable stool type and frequency and they fitted in a healthy stool pattern. Barebones of the study is that the consumption of 40 gram of either protein (Whey or corn) for the duration of one week has not any impact on gastrointestinal track and these proteins are totally tolerable for human body. (Table 3)

Table 3: Comparison of sweet whey and acid whey.

Components	Sweet Whey	Acid Whey
Protein (g/l)	6-10	6-8
Lactose (g/l)	46-52	44-46
Minerals (g/l)	2.5-4.7	4.3-7.2
pH	>5.6	<5.6

7.4. Bioavailability of Whey and Corn protein

The quality of dietary protein is defined in part by its capacity to provide amino acids (AA) and especially the essential amino acids (EAA). A study demonstrated that total amino acid (TAA) uptake for corn protein was 61% compared to that of whey protein. For total essential amino acids (TEAA), this was 51% for corn protein when compared to whey protein.

8. Reasons for adding corn protein

From the above discussion, it is clear that whey protein has greater bioavailability as compared to the Corn protein. The question that normally arises in the mind of an average man is that if whey protein is qualitatively better than corn protein, then why are we moving towards the addition of corn protein into it? Well, there are three reasons.

8.1. Challenges with whey protein

Whey protein extraction has several dilemmas and

predicaments. These challenges such as high cost, low extraction yield and negative environmental impacts have been discussed below.
The whey protein is extremely expensive and commonly unaffordable for a layman. For example
The Protein Works Whey Protein 80 - 500 g (1.1 lbs) - Chocolate Silk: Rs. 5,4991
The Protein Works Whey Protein 80 - 1 kg (2.2 lbs) - Chocolate Silk: Rs. 9,2991
The Protein Works Whey Protein 80 - 2 kg (4.4 lbs) - Chocolate Silk: Rs. 16,4991
Nitro Tech Lean Muscle Builder 4lbs Milk Chocolate - New Series Whey Peptides: Rs. 21,9991
Mass Tech Mass Gainer Protein Powder, Build Muscle Size & Strength with High-Density Clean Calories, Chocolate, 6Lbs: Rs. 17,9991 (Dazraz)
Only miniscule number of proteins are extracted from a large quantity of whey. For example, it is stated that only 0.7% proteins (beta-lactoglobulin (BLG), alpha-lactalbumin (ALA), bovine serum albumin (BSA) and immunoglobulins) are extracted from whey on the basis of Weight/Volume from the total volume of Whey. Whey is obtained from the milk produced by the livestock. The overburden on livestock is causing very big menaces such as soil erosion, water contamination, deforestation, desertification, and the production of greenhouse gases, thereby contributing to global warming [103]. Hence the reliance on the animal food sources needs to be decreased.

8.2. Benefits of Corn protein

There are many benefits of corn protein such as cost-effectiveness, high protein content, and environmental sustainability. The study of [104] discovered that corn in contrast to the whey contains about 10-15% proteins depending upon the type of variety.
8.3. Global Market Potential of corn protein
According to a report by Fortune business insights, global zein market (the major protein in corn) is about to be increased to 4760 million US dollars in year 2030. And the compound annual growth rate (CAGR) of zein protein will remain 7.3% during the year 2030. The report further says that the top producers of zein protein will be North America, Europe and Asia Pacific (which includes our Pakistan also). While discussing about Asia Pacific the report said that because of the rising awareness towards health benefits offered by plant-based ingredients and food products the demand for zein like products will be significantly boosted in the Asian Pacific Market [105]. Thus, Pakistan needs to exploit the potential of global market of zein protein and need to do its mass production and mass utilization in order to increase country's revealed comparative advantage (RCA) of the protein (Table 4).

Table 4: The typical composition (%) of major whey products.

	Protein	**Lactose**	**Fat**	**Ash**	**Moisture**
Whey powder	11.0-14.5	63.0-75.0	1.0-1.5	8.2-8.8	3.5-5.0
Demineralized whey	11.0-15.0	70.0-80.0	0.5-1.8	1.0-7.0	3.0-4.0
WPC 34	34.0-36.0	48.0-52.0	3.0-4.5	6.5-8.0	3.0-4.5
WPC 60	60.0-62.0	25.0-30.0	1.0-7.0	4.0-6.0	3.0-5.0
WPC 80	80.0-82.0	4.0-8.0	4.0-8.0	3.0-4.0	3.5-4.5
WPI	90.0-92.0	0.5-1.0	0.5-1.0	2.0-3.0	4.5
Permeated solids (food grade)	3.0-8.0	65.0-85.0	<1.5	8.0-20.0	3.0-5.0

9. Extraction Methods

The detailed extraction methods of whey, whey protein (isolates and concentrates) and corn protein isolates have been discussed in this section.

9. 1. Extraction method of Whey protein

The steps for the extraction of whey protein are discussed below.

9.1.1. Whey manufacturing: Whey, also known as milk serum, is a clear solution that is strained from milk curd coagulated by rennet or acid. It contains small molecules not involved in milk curdling and can be strained out. The primary components of whey solids are lactose, protein (mainly whey protein), and minerals. Whey liquid contains over 50% of whole milk solids, including the majority of minerals, and nearly all whey proteins and lactose. Depending on the method of coagulation, milk can result in different types of whey, generally categorized into sweet whey and acid whey [106]. It has a pH higher than 5.6 and is usually from cheese manufacturing (rennet coagulated) [107]. It has a pH below 5.6 and is from coagulation by fermentation or by adding acid [108]. Whey protein can be concentrated up to 80% of total solids via ultra-filtration technology. The most common WPC include WPC34, WPC60, and WPC80, which contain 34%, 60%, and 80% of protein, respectively [109]. With an additional step of micro-filtration, the protein content of WPC80 can be further concentrated to 90% by removing extra fat, which is called WPI. WPC80 and WPI can also be instantized for sport and adult protein supplements [110].

The procedure for lab-scale microfiltration and ultrafiltration of whey involves several steps. The first step is to prepare the whey solution. Choose the appropriate membrane for filtration. Microfiltration membranes have pores on the order of 0.1 – 10 µm in size. Ultrafiltration membranes have smaller pores, on the order of 0.005 – 0.1 µm in size [111]. Set up the filtration system, which typically includes a feed tank, a pump to pressurize the system, the membrane module, and collection tanks for the permeate and retentate [112]. The operating conditions such as temperature and pressure need to be set. For instance, one study performed ultrafiltration at temperatures ranging from 5 to 40°C and pressures from 1 to 3 bar (1 bar = 14.5038 psi) [113]. Start the filtration process. The whey is pumped through the membrane module. The smaller molecules (like water, lactose, and minerals) pass through the membrane and are collected as permeate. The larger molecules (like proteins) are retained by the membrane and are collected as retentate [114]. The extracts are freeze-dried and stored at room conditions until further use. For whey protein specifically, the frozen samples are freeze-dried at −51 °C at a pressure <0.133 mBar for approximately 96 hours. The most potent in vitro antioxidative whey protein hydrolysate (WPH) is generated using Alcalase® under free-fall pH conditions, followed by spray drying (SD), which has oxygen radical absorbance capacity and Trolox equivalent (TE) antioxidant capacity values of 1132 and 686 µmol TE/g, respectively. These results demonstrate that both the hydrolysis and the drying process impact the biofunctional (antioxidant) activity of WPHs [115]. Analyze the results. This includes measuring the concentration of proteins in the retentate and the permeate and assessing the functional properties of the concentrated proteins [116].

9.2. Extraction method of Corn protein

The procedure for extraction of protein from corn is discussed below.

9.2.1. Preparation of Corn: The first step is to prepare the corn for extraction. This can be done by milling the corn to obtain flour [117].

9.2.2. Protein Extraction: Add an alkaline protease to the milled corn to prepare a corn digest. Then, add 6% (v/v) Bacillus subtilis to the corn digest, and ferment at 37°C for 18 hours to fully hydrolyze the corn protein [118].

9.2.3. Sterilization: Sterilize the mixture to prepare the fermentation broth [119].

9.2.4. Protein Separation: Protein extraction and separation processes can be classified into two main categories: dry fractionation and wet extraction processes. Wet extraction processes are the most common methods used to produce plant protein ingredients and include conventional processes such as alkaline extraction–isoelectric precipitation (AE-IP), salt extraction–dialysis (SED), and micellar precipitation (MP) [120].

9.2.5 Protein Purification: After extraction, the protein needs to be purified. This can be done using various techniques such as chromatography or centrifugation. These steps are the bare bones and simplified information. The detailed depiction of the steps of the extraction of protein from corn is given in Figure 3.

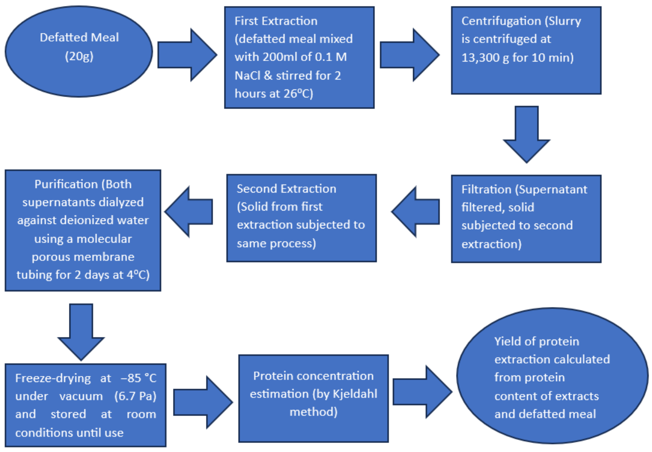

Figure 3: Corn protein manufacturing. [121, 122]

10. Preparation of Hybrid protein powder

To create a hybrid protein powder by combining whey protein (concentrate or isolate) and corn protein isolate, one needs to follow the following four steps:
10.1 Choose the type and ratio of whey protein and corn protein isolate

Whey protein can be either isolate or concentrate, and corn protein isolate is a novel food ingredient that is produced from corn gluten slurry by washing it with ethanol and removing the non-protein components. Isolate whey protein has less fat and lactose than concentrate, but it is also more expensive. Corn protein isolate has a minimum protein content of 85% and a high branched-chain amino acid content, particularly leucine. One can experiment with different ratios of whey and corn protein isolate to find the one that suits the desired taste and nutritional needs. i.e., 75% whey and 25% corn, 50% whey and 50% corn or 75% corn and 25% whey, etc.

10.1. Flavor enhancement

Addition of some flavorings and sweeteners to enhance the taste of hybrid protein powder is necessary as it enhances tangibility, palatability and customer acceptance and future demand. Natural ingredients like cocoa powder, vanilla extract, cinnamon, honey, stevia, or fruits could also be used. The developers can also add some supplements like greens powder, chlorella, maca, or flax seeds to boost the nutritional value of the hybrid protein powder.

10.2. Homogenization of Ingredients

Blend all the ingredients together in a high-speed blender or food processor until they are well combined and form a fine powder. Sifting of the powder might be needed to remove any lumps or clumps. At lab scale production, one can use a coffee grinder or a mortar and pestle to grind the ingredients into a powder.

10.3 Packaging and storage

Store the hybrid protein powder in a hermetically sealed airtight container in a cool and dry place.

Customers could use hybrid protein powder by mixing it with water, milk, or plant-based milk and enjoy it as a shake or smoothie. They could also use it to make protein bars, muffins, pancakes, or other baked goods.

11. Intended users of hybrid protein powder

There are many people belonging to different classes who can be benefited by the usage of this protein powder. The most important ones are discussed below.

11.1. Bodybuilders

To build muscle, most people consume a daily protein intake of 1.4 to 2.0 grams per kilogram (kg) of their body weight. However, it's often challenging to meet this requirement through diet alone [123]. Therefore, hybrid protein powder can be an effective solution to meet their protein needs.

Emerging research suggests that higher protein intakes, exceeding 3.0 g/kg/day, may positively impact body composition in resistance-trained individuals, specifically by promoting fat loss. The optimal protein intake per serving for athletes to maximize muscle protein synthesis (MPS) varies and depends on factors such as age and recent resistance exercise stimuli. The general recommendation is 0.25 g of high-quality protein per kg of body weight, or an absolute dose of 20 to 40 g. Acute protein doses should aim to contain 700 to 3000 mg of leucine and/or a higher relative leucine content, along with a balanced array of the essential amino acids (EAAs) [124].

11.2. Other Athletes and people who regularly exercise

Dietary guidelines for athletes recommend a protein intake of 1.2 to 1.7 g/kg/day for both endurance and resistance-trained athletes. Protein serves not only as a substrate providing the building blocks for protein synthesis, but also as a key trigger for exercise-induced phenotypic changes. Leucine, in particular, plays a significant role and may be crucial in enhancing protein-mediated recovery and adaptation. Athletes aiming to gain muscle mass and strength are likely to consume higher amounts of dietary protein than those training for endurance [125]. Athletes from various sports, not just bodybuilders, often require a higher protein intake. They commonly use protein powders and shakes to support muscle growth, recovery, and performance.

11.3. Older adults

The US Dietary Reference Intakes (DRI) recommends a daily dietary protein intake of 0.8 g/kg for all individuals aged 19 years and older. This recommended dietary allowance (RDA) is considered adequate for nearly all individuals [126].

However, to help prevent or slow down age-related muscle loss, known as sarcopenia, individuals may need to increase their protein intake. For those with a weak stomach who find it difficult to digest red meat (beef or mutton) and lentils, hybrid protein powder can be a beneficial alternative. It is palatable, nutritious, and easily digestible, making it an excellent protein source.

11.4. Vegetarians and vegans

They may have difficulty meeting their protein needs from plant-based sources alone, and who may benefit from protein powders and supplements that provide complete protein and essential amino acids.

11.5. People who are dieting or using meal replacements

These types of people often want to adopt ketogenic diet (rich in proteins and fat and low in carbohydrates). They may use hybrid protein powders in the form of shake as a way to control their calorie intake, increase their satiety, and preserve their lean body mass.

12. Conclusion

In light of the projected global population growth and the subsequent increase in protein demand by 2050, it is imperative to address the sustainability concerns associated with conventional animal-based protein sources. The environmental impact, high cost, and potential health risks associated with excessive consumption of animal proteins necessitate a shift towards more sustainable alternatives. This article proposes a novel concept of creating a hybrid protein powder by combining whey protein (known for its superior leucine content) and corn protein isolate (derived from corn germ) to achieve a more balanced, cost-effective, and environmentally friendly protein source. The hybrid protein powder has the potential to offer a solution that caters to the increasing global protein demand while addressing environmental and health concerns associated with traditional protein sources.

Acknowledgements

The authors are thankful to the Department of Food Science, Government College University, Faisalabad, Pakistan, for support.

Authors' contributions

Muhammad Abdullah Butt and Rabiya Riaz contributed to literature collection, organization, and drafting of the initial manuscript. Xianjiang Ye provided critical guidance on the scientific framework and conceptual alignment of the review. Burhan Khalid and Muhammad Atiq Ashraf assisted with literature synthesis and manuscript editing. Muhammad Moeid Khan and Md. Shabudden Ahamed supported data interpretation and thematic structuring of the review. Talha Riaz conceived the review concept, led the writing, analysis, and integration of all sections, and coordinated the overall preparation of the manuscript. Muhammad Umair Arshad provided supervision, critical evaluation, and final approval of the manuscript.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability

No new data were created or analyzed in this study. Data sharing does not apply to this article.

Declarations

Ethics approval and consent to participate: The authors declare that they have no human and/ or animal studies in this manuscript.

Competing interests: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Ahsan H. Amino acid arginine and adducts: autoimmune activity. J Immunoassay Immunochem. 2018;39(6):577–594.
2. Riaz T, Zeng X, Ye X, Yu T, Xia Z, Ahmad T, et al. Eco-efficient valorization of agri-food and industrial waste into high-functionality microbial proteins: bacteria, yeast, and algae. Eur Food Res Technol. 2026;252:54. doi: 10.1007/s00217-025-05015-2.
3. Alane D, Raut N, Kamble DB, Bhotmange M. Studies on preparation and storage stability of whey-based mango herbal beverage. Int J Chem Stud. 2017;5(3):237–241.
4. Ali R, Wang M, Zafar MH, Zhang H. Research progress in the role and mechanism of leucine in regulating animal growth and development. Front Physiol. 2023;14:1252089.
5. Khalid B, Din ZU, Saini A, Islam MA, Ashraf MA, Saeed HZ, et al. The environmental paradox of nanobioinoculants: balancing agricultural benefits against ecosystem risks. Innov Res Appl Biol Chem Sci. 2025;3(2):4–19. doi: 10.62497/irabcs.161.
6. Amiza MA, Ow YW, Faazaz AL. Physicochemical properties of silver catfish (Pangasius sp.) frame hydrolysate. Int Food Res J. 2013;20(3):1255–1262.
7. Raza M, Hussain Z, Abbas F, Ashraf MA, Imene HH, Riaz T. Advanced strategies for detection and diagnosis of potato viruses: harnessing molecular innovations and digital tools for precision agriculture. Hosts Viruses. 2025;12:39–46. doi: 10.17582/journal.hv/2025/12.39.46.
8. Bastos DHM, Gugliucci A. Contemporary and controversial aspects of the Maillard reaction products. Curr Opin Food Sci. 2015;1:13–20.
9. Umar R, Arif K, Malik S, Munawar ST, Sibt-e-Abbas M, Riaz R, et al. Food-derived garlic polysaccharides as emerging functional ingredients: structure, microbiota–immunity interactions, and health implications. Carpathian J Food Sci Technol. 2025;17(4):106–127. doi: 10.34302/crpjfst/2025.17.4.10.
10. Saleem H, Naz A, Sandhu AS, Fatima M, Tahir O, Zafar MJ, et al. Pharmacological and therapeutic values of turmeric. In: Khan A, Mohsin M, Khan AM, Aziz S, editors. Complementary and alternative medicine: Chinese/traditional medicine. Lahore: Unique Scientific Publishers; 2024. p. 77–84. doi: 10.47278/book.CAM/2024.383.
11. Boldridge WC, Ljubetič A, Kim H, Lubock N, Szilágyi D, Lee J, et al. A multiplexed bacterial two-hybrid system for rapid characterization of protein–protein interactions and iterative protein design. Nat Commun. 2023;14(1):4636.
12. Boye JI, Alli I, Ismail AA, Gibbs BF, Konishi Y. Factors affecting molecular characteristics of whey protein gelation. Int Dairy J. 1995;5(4):337–353.
13. Amin SE, Qazi ZA, Karim A, Masood S, Soomro MB, Soomro F, et al. Identification of lab-grown meat and its nutritional impacts on human health. Vet Res. 2021;14(3):34–39.
14. Buchert J, Ercili Cura D, Ma H, Gasparetti C, Monogioudi E, Faccio G, et al. Crosslinking food proteins for improved functionality. Annu Rev Food Sci Technol. 2010;1(1):113–138.
15. Canto AC, Lima BRC, Suman SP, Lazaro CA, Monteiro MLG, Conte-Junior CA, et al. Physicochemical and sensory attributes of low-sodium restructured caiman steaks containing microbial transglutaminase. Meat Sci. 2014;96(2):623–631.
16. Chen X, Zhou R, Xu X, Zhou G, Liu D. Structural modification by high-pressure homogenization for improved functional properties of freeze-dried myofibrillar protein powder. Food Res Int. 2017;100:193–200.
17. Farzand A, Arshad MA, Maqbool R, Akram HMB, Ahmad A, Mushtaq MZ, et al. Allelopathic correspondence in soil ecosystem between microbes and plants. J Plantarum. 2025;7(2):75–91.
18. Kanwal R, Hafeez-ul-Haq M, Waseem A, Riaz T, Rehman ZU, Fazal A, et al. Fungitoxic properties of essential oils to treat tinea. In: Zafar MA, Abbas RZ, Imran M, Tahir S, Qamar W, editors. Complementary and alternative medicine: essential oils. Lahore: Unique Scientific Publishers; 2024. p. 81–89. doi: 10.47278/book.CAM/2024.192.
19. de Wit JN. Nutritional and functional characteristics of whey proteins in food products. J Dairy Sci. 1998;81(3):597–608.
20. Dhayal SK, Gruppen H, de Vries R, Wierenga PA. Controlled formation of protein nanoparticles by enzymatic crosslinking of α-lactalbumin with horseradish peroxidase. Food Hydrocoll. 2014;36:53–59.
21. Duke M, Vasiljevic T. Whey ultrafiltration. In: Drioli E, Giorno L, editors. Encyclopedia of membranes. Berlin: Springer; 2015. doi: 10.1007/978-3-642-40872-4_2053-1.
22. El-Sayed MM, Chase HA. Trends in whey protein fractionation. Biotechnol Lett. 2011;33:1501–1511.
23. Ermis E, Tekiner IH, Lee CC, Ucak S, Yetim H. An overview of protein powders and their use in food formulations. J Food Process Eng. 2023;46(5):e14326.
24. Riaz T, Zeng X, Ye X, Yu T, Xia Z, Khan MM, et al. The biophysics of nanocellulose packaging: linking nanoscale structure to food preservation and safety. Food Biophys. 2025;20:196. doi: 10.1007/s11483-025-10082-9.
25. Esser D, Wehrens R, Lenaerts K, Engel J, van den Dool RT, Bastiaan-Net S, et al. Evaluating and comparing tolerance, nutritional quality and bio-functional activity of bovine-plasma, corn and whey proteins: outcomes of a randomized double-blind controlled trial. Curr Res Food Sci. 2023;7:100588.

26. Fairhead M, Thöny-Meyer L. Crosslinking and immobilisation of different proteins with recombinant Verrucomicrobium spinosum tyrosinase. J Biotechnol. 2010;150(4):546–551.
27. Food and Agriculture Organization of the United Nations. Livestock's long shadow: environmental issues and options. Rome: FAO; 2006.
28. Food and Agriculture Organization of the United Nations. The state of food and agriculture 2009: livestock in the balance. Rome: FAO; 2009.
29. Food and Agriculture Organization of the United Nations. World agriculture: towards 2030/2050 – 2012 revision. ESA Working Paper No. 12-03. Rome: FAO; 2012.
30. Fenelon MA, Hickey RM, Buggy A, McCarthy N, Murphy EG. Whey proteins in infant formula. In: McSweeney PLH, O'Mahony JA, editors. Whey proteins. London: Elsevier; 2019. p. 439–494.
31. Fernández-d'Arlas B. Tough and functional cross-linked bioplastics from sheep wool keratin. Sci Rep. 2019;9(1):1–12.
32. Floret C, Monnet AF, Micard V, Walrand S, Michon C. Replacement of animal proteins in food: how to take advantage of nutritional and gelling properties of alternative protein sources. Crit Rev Food Sci Nutr. 2023;63(7):920–946.
33. Fortune Business Insights. Zein protein market size, share and global trend 2023–2030. 2023.
34. Shaheen M, Saeed HZ, Babar AS, Khalid B, Fatima M, Jameel S, et al. Flavonoids: chemistry, extraction, and their multifaceted role in health and product development. RADS J Food Biosci. 2024;3(2):108–118.
35. Gapsys V, Michielssens S, Seeliger D, De Groot BL. pmx: automated protein structure and topology generation for alchemical perturbations. J Comput Chem. 2015;36(5):348–354.
36. Gharibzahedi SMT, Chronakis IS. Crosslinking of milk proteins by microbial transglutaminase: utilization in functional yogurt products. Food Chem. 2018;245:620–632.
37. Glazer B. Building a high-throughput yeast two-hybrid assay to screen for novel chronic hepatitis B drugs. Methods Mol Biol. 2023;2561:115–132.
38. Gorissen SH, Crombag JJ, Senden JM, Waterval WH, Bierau J, Verdijk LB, et al. Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids. 2018;50:1685–1695.
39. Gui Y, Li J, Zhu Y, Guo L. Roles of four enzyme crosslinks on structural, thermal, and gel properties of potato proteins. LWT. 2020;123:109116.
40. Guo M, Wang G. Whey protein polymerisation and its applications in environmentally safe adhesives. Int J Dairy Technol. 2016;69(4):481–488.
41. Guo M, Wang G. History of whey production and whey protein manufacturing. In: Guo M, editor. Whey protein production, chemistry, functionality, and applications. Oxford: Wiley; 2019. p. 1–12.
42. Gusmão TAS, Gusmão RP, Moura HV, Silva HA, Cavalcanti-Mata MERM, Duarte MEM. Production of prebiotic gluten-free bread with red rice flour and microbial transglutaminase. J Food Sci Technol. 2019;56(6):2949–2958.
43. Ha HK, Rankin SA, Lee MR, Lee WJ. Development and characterization of whey protein-based nano-delivery systems: a review. Molecules. 2019;24(18):3254.
44. Hadidi M, Jafarzadeh S, Forough M, Garavand F, Alizadeh S, Salehabadi A, et al. Plant protein-based food packaging films: recent advances. Trends Food Sci Technol. 2022;120:1–16.
45. Khalid B, Malik S, Asghar A, Khan N, Jan A, Ashraf F, et al. Interdisciplinary approaches to mitigate crop stress for sustainable agriculture. Agric Res Rep. 2025;3(1):17–28.
46. Hedman TP, Saito H, Vo C, Chuang SY. Exogenous cross-linking increases the stability of spinal motion segments. Spine. 2006;31:E480–E485.
47. Henchion M, Hayes M, Mullen AM, Fenelon M, Tiwari B. Future protein supply and demand. Foods. 2017;6(7):53.
48. Hochwallner H, Schulmeister U, Swoboda I, Spitzauer S, Valenta R. Cow's milk allergy. Methods. 2014;66(1):22–33.
49. Hojilla-Evangelista MP. Extraction and functional properties of non-zein proteins in corn germ. J Am Oil Chem Soc. 2012;89:167–174.
50. Hojilla-Evangelista MP. Improved solubility of corn germ protein. J Am Oil Chem Soc. 2014;91:1623–1631.
51. Iltchenco S, Preci D, Bonifacino C, Fraguas EF, Steffens C, Panizzolo LA, et al. Whey protein concentration by ultrafiltration. Cienc Rural. 2018;48:e20170904.
52. Ince C, Condict L, Ashton J, Stockmann R, Kasapis S. Molecular characterisation of β-lactoglobulin–hexanal interactions. Food Hydrocoll. 2024;146:109260.
53. Isaschar-Ovdat S, Fishman A. Crosslinking of food proteins mediated by oxidative enzymes. Trends Food Sci Technol. 2018;72:134–143.
54. Aslam T, Shahzadi K, Fatima N, Asghar A, Riaz R, Ashraf MA, et al. UV-visible spectrophotometric method for exemestane. Handard Medicus. 2025;68(2):61–73.
55. Ispoglou T, Witard OC, Duckworth LC, Lees MJ. Essential amino acid supplementation in older adults. Proc Nutr Soc. 2021;80(2):230–242.
56. Jäger R, Kerksick CM, Campbell BI, Cribb PJ, Wells SD, Skwiat TM, et al. ISSN position stand: protein and exercise. J Int Soc Sports Nutr. 2017;14:20.
57. Jansens KJA, Telen L, Bruyninckx K, Vo Hong N, Gebremeskel AF, Brijs K, et al. Mechanical properties of glassy cross-linked proteins. Eur Polym J. 2017;88:231–245.
58. Jao D, Xue Y, Medina J, Hu X. Protein-based drug-delivery materials. Materials. 2017;10:517.

59. Jayachandran B, Parvin TN, Alam MM, Chanda K. Chemical crosslinking strategies for proteins. Molecules. 2022;27(23):8124.
60. Jiménez-Rosado M, Bouroudian E, Perez-Puyana V, Guerrero A, Romero A. Soy protein-based bioplastics. J Clean Prod. 2020;262:121517.
61. Joung JK, Ramm EI, Pabo CO. A bacterial two-hybrid selection system. Proc Natl Acad Sci USA. 2000;97(13):7382–7387.
62. Khalid B, Javed MU, Ikram M, Ateeq M, Ashraf MA, Riaz T, et al. Precision rewiring of stress-responsive gene networks via CRISPR. J Crop Health. 2026;78(2):1–14.
63. Karthikeyan S, Saari N. Drying effects on mung bean protein isolate. Food Res Int. 2020;138:109783.
64. Kinsella JE. Advances in food and nutrition research. San Diego: Academic Press; 1990.
65. Kleekayai T, O'Neill A, Clarke S, Holmes N, O'Sullivan B, FitzGerald RJ. Whey protein hydrolysate characteristics. Antioxidants. 2022;11(2):399.
66. Krolczyk J, Dawidziuk T, Janiszewska-Turak E, Sołowiej B. Use of whey in the food industry. Pol J Food Nutr Sci. 2016;66(3):157–165.
67. Lambrecht MA, Rombouts I, Nivelle MA, Delcour JA. Protein networks in egg noodles. J Food Sci. 2017;82(1):24–35.
68. Langyan S, Yadava P, Belwal T, Kaul T. Sustaining protein nutrition through plant-based foods. Front Sustain Food Syst. 2022;6:1037086.
69. Langyan S, Yadava P, Khan FN, Dar ZA, Singh R, Kumar A. Sustaining protein nutrition. Front Nutr. 2022;8:772573.
70. Ashraf MA, Khan AS, Shireen F, Nawaz S, Ayyub S, Mohibullah S, et al. Peach diseases in a changing climate. Microb Pathog. 2025;209:108110.
71. Li H, Cui Y, Zhang L, Liu H, Yu J. Recombinant Zea mays transglutaminase. Food Sci Biotechnol. 2017;26(3):723–730.
72. Li N, Girard AL. Whey protein–proanthocyanidin interactions. Food Hydrocoll. 2023;134:108100.
73. Li X, Li S, Liang X, McClements DJ, Liu X, Liu F. Applications of oxidases in food systems. Trends Food Sci Technol. 2020;103:78–93.
74. Latif MF, Aleem MT, Bakhsh M, Sohail A, Riaz T, Bilal A. Pomegranate seed oil in cookies. Int J Biol Res. 2019;2(1):246–256.
75. Liu F, Ma C, Gao Y, McClements DJ. Food-grade covalent complexes. Compr Rev Food Sci Food Saf. 2017;16(1):76–95.
76. Lu ZX, He JF, Zhang YC, Bing DJ. Pea protein composition and applications. Crit Rev Food Sci Nutr. 2020;60(15):2593–2605.
77. Maes E, Dyer JM, McKerchar HJ, Deb-Choudhury S, Clerens S. Protein cross-linking and human health. Expert Rev Proteomics. 2017;14(10):917–929.
78. Manhivi VE, Amonsou EO, Kudanga T. Laccase-mediated crosslinking of amadumbe flour. Food Chem. 2018;264:157–163.
79. Ashraf MA, Ateeq M, Zhu K, Asim M, Mohibullah S, Riaz T, et al. Phytohormone networks under hypoxia. Plant Cell Environ. 2025;48(1):1–16.
80. Latif MF, Naqvi SMT, Shahzadi N, Riaz T, Sohail A. Defatted wheat germ cookies. Nat Sci. 2019;17(8):110–116.
81. Minj S, Anand S. Whey proteins and derivatives. Dairy. 2020;1(3):233–258.
82. Miwa N. Microbial transglutaminase in food innovation. Anal Biochem. 2020;597:113638.
83. Mondor M, Hernández-Álvarez AJ. Processing technologies for plant protein isolates. In: Plant protein foods. Cham: Springer; 2022. p. 61–108.
84. Muthukumarappan K, Swamy GJ. Food microstructure and stability. In: Food microstructure. Cambridge: Woodhead; 2018. p. 161–191.
85. Myers DJ, Hojilla-Evangelista MP, Johnson LA. Corn protein functional properties. J Am Oil Chem Soc. 1994;71:1201–1204.
86. Anwar H, Javed A, Shaheen M, Saeed HZ, Hussain M, Wajid M, et al. Polyphenols and gut microbiota. Pak J Sci. 2025;77(2):284–298.
87. Nielsen SD, Knudsen LJ, Bækgaard LT, Rauh V, Larsen LB. Lactose influence on Maillard reaction. Foods. 2022;11(7):897.
88. Niño-Medina G, Gutiérrez-Soto G, Urías-Orona V, Hernández-Luna CE. Laccase effects on bread quality. Cogent Food Agric. 2017;3(1):1328762.
89. Norton LE, Wilson GJ, Layman DK, Moulton CJ, Garlick PJ. Leucine content and muscle synthesis. Nutr Metab. 2012;9:1–9.
90. Ye X, Yang R, Riaz T, Chen J. Porphyra haitanensis protein stability. Int J Biol Macromol. 2025;293:139291.
91. O'Flynn TD, Hogan SA, Daly DFM, O'Mahony JA, McCarthy NA. Soy protein isolate properties. Molecules. 2021;26(10):3015.
92. Ogilvie O, Roberts S, Sutton K, Larsen N, Gerrard J, Domigan L. Microbial transglutaminase in bread. Food Chem. 2021;340:127903.
93. Paliya BS, Sharma VK, Sharma M, Diwan D, Nguyen QD, Aminabhavi TM, et al. Protein-polysaccharide nanoconjugates. Food Chem. 2023;428:136709.

94. Shahriar SMS, Akther S, Hossain MS, Dipti S, Zakir HM, Salam SMA, et al. Germination and roasting effects. Carpathian J Food Sci Technol. 2025;17(2):203–220.
95. Pasiakos SM, McLellan TM, Lieberman HR. Protein supplements and performance. Sports Med. 2015;45:111–131.
96. Paul A, Gaiani C, Cvetkovska L, Paris C, Alexander M, Ray C, et al. Whey protein powder storage. J Food Eng. 2022;326:111050.
97. Shah AA, Mahmood MA, Farooq K, Qayyum Z, Amjad N, Nasib MU, et al. Herbal antioxidants in clinical practice. J Food Technol. 2021;19(3):32–37.
98. Perez-Puyana VM, Jiménez-Rosado M, Martínez I, Romero A. Pea protein bioplastics cross-linking. ACS Appl Polym Mater. 2024;6(3):1450–1462.
99. Petrotchenko EV, Serpa JJ, Borchers CH. Cross-linking in structural proteomics. In: Proteomics for biological discovery. Hoboken: Wiley; 2019. p. 175–196.
100. Phillips SM, Van Loon LJC. Dietary protein for athletes. Sports Med. 2020;50(1):243–260.
101. Saeed HZ, Shaheen M, Khalid B, Umar A, Anwar H, Riaz T, et al. Food biofortification advances. RADS J Biol Res Appl Sci. 2024;15(2):99–113.
102. Pořízka J, Slavíková Z, Bidmonová K, Vymětalová M, Diviš P. Bread fortified with whey protein. Foods. 2023;12(13):2635.
103. Pugsley AP. Hybrid proteins and targeting signals. In: Membrane biogenesis. New York: Plenum Press; 1988. p. 399–418.
104. Reid-McCann RJ, Brennan SF, McKinley MC, McEvoy CT. Animal vs plant protein and sarcopenia. Syst Rev. 2022;11(1):1–9.
105. Ribeiro AS, Nunes JP, Schoenfeld BJ. Protein intake in bodybuilders. Sports Med. 2019;49:1481–1485.
106. Sabaté J, Sranacharoenpong K, Harwatt H, Wien M, Soret S. Environmental cost of protein choices. Public Health Nutr. 2015;18(11):2067–2073.
107. Santhi D, Kalaikannan A, Malairaj P, Arun Prabhu S. Microbial transglutaminase in meat foods. Crit Rev Food Sci Nutr. 2017;57(10):2071–2076.
108. Atiq M, Khan AS, Azam M, Ayyub S, Kaleem MM, Nawaz S, et al. Postharvest management technologies. Eur Food Res Technol. 2025.
109. Sarabandi K, Sadeghi Mahoonak A, Hamishekar H, Ghorbani M, Jafari SM. Casein hydrolysate microencapsulation. J Food Eng. 2018;237:86–95.
110. Scarborough P, Appleby PN, Mizdrak A, Briggs AD, Travis RC, Bradbury KE, et al. Dietary greenhouse gas emissions. Clim Change. 2014;125(2):179–192.
111. Raza M, Ashraf MA, Ateeq M, Rashid S, Riaz T, Khalid B, et al. Environmental variables and fruit growth. J Surv Fish Sci. 2024;11(4):314–321.
112. Shi J, Tauriainen E, Martonen E, Finckenberg P, Ahlroos-Lehmus A, Tuomainen A, et al. Whey protein isolate and obesity. Int Dairy J. 2011;21(8):513–522.
113. Shinde G, Kumar R, Chauhan SK, Subramanian V, Nadanasabapathi S. Whey proteins in food industry. Asian J Dairy Food Res. 2018;37:283–290.
114. Manzoor E, Ghani A, Khan MR, Sultana M, Ishaque A, Nasir E, et al. Guava leaf antioxidants. Ann Plant Sci. 2019;8(5):3553–3562.
115. Stark M, Lukaszuk J, Prawitz A, Salacinski A. Protein timing and hypertrophy. J Int Soc Sports Nutr. 2012;9:1–8.
116. Akbar BA, Arshad MA, Khalid B, Baloch R, Ahmad A, Rouf S, et al. Chloroplast engineering for pest control. J Pure Appl Agric. 2025;10(1):1–20.
117. Teixeira LSM, Feijen J, van Blitterswijk CA, Dijkstra PJ, Karperien M. Enzyme-catalyzed crosslinkable hydrogels. Biomaterials. 2012;33(5):1281–1290.
118. Amin SE, Qazi ZA, Karim A, Masood S, Soomro MB, Soomro F, et al. Traceability in halal food industry. Pak J Soc Sci. 2021;18(5):85–91.
119. Shaheen C, Ahmad IA, Aslam R, Naz S, Mushtaq S, Ahmed S, et al. Therapeutic uses of fenugreek. J Res Appl Sci Biotechnol. 2024;3(5):39–50.
120. Ishaq H, Nisar K, Riaz R, Murtaza MS, Shahbaz M, Munir T, et al. Medicinal uses of Delphinium brunonianum. Int J Adv Eng Manag. 2024;6(10):507–516.
121. Ahamed MS, Asghar A, Hayat N, Muqaddas, Bashir L, Saif S, et al. Optimizing bioactive compound extraction. South Asian J Life Sci. 2025;13:58–65.
122. Nawaz S, Khan N, Malik S, Muzamil MN, Asghar A, Rashid S, et al. Lettuce genomics and BSA/BSR. South Asian J Life Sci. 2025;13:50–57.
123. Ye X, Yang R, Yang Z, Huang B, Riaz T, Zhao C, et al. ACE-inhibitory peptides from Porphyra haitanensis. Food Biosci. 2025;106460.
124. Khalid B, Javed MU, Ashraf MA, Saeed HZ, Shaheen M, Riaz T, et al. Microbial biostimulants against viral diseases. Hosts Viruses. 2025;12:93–110.
125. Maqbool R, Anwar I, Khalid B, Ashraf MA, Riaz T, Nadeem MA. Allelopathic effects of Lepidium sativum. J Plant Biota. 2025;4(1):16–21.
126. Khalid B, Javed MU, Riaz T, Ashraf MA, Saeed HZ, Shaheen M, et al. Refining plant-virus interactions. Hosts Viruses. 2025;12:47–61.

Disclaimer: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article or claim that may be made by its manufacturer is not guaranteed or endorsed by the publisher