BSc THESIS

Ukasha Abdul Rehman

2024

Hungarian University of Agriculture and Life Sciences Buda Campus

Sponsored by Tempus Public Foundation

Bachelors of Food Engineering

Investigation of Amino Acids in Fortified Meals

Insider consultant: **Mednyánszky Zsuzsanna**

associate professor

IFST Department of Nutrition

Created by: **Ukasha Abdul Rehman**

Budapest

2024

Table of contents

List of tables

List of Figures

Chapter 1: Introduction

With the advancement of this era, Food is evolved too and world got attention on vegetables to due to various reasons and among them, green pea and kidney beans are most common for normal day consumption due to richness in fiber and a lot of essential nutrients. But the demerit of vegetable food is that it usually lacks with one or more essential amino acids that are necessary for human health and functions whereas almost all the animal proteins contain all the required essential amino acids (Wu, 2009). Legumes are typically low in sulfur-containing amino acids, such as methionine and cysteine, which are critical for muscle repair, detoxification, and immune function (Moughan & Rutherfurd, 2012).This deficiency prevents plant proteins from fully supporting bodily functions like protein synthesis and muscle growth (Brosnan, 2003). For those people who completely rely on plant protein (vegetarians), this limitation is a big challenge to get a complete protein in their food for them. Plant based foods, especially legumes have lower environmental footprints as compared to the animal proteins making them a crucial element in addressing global food security and environmental concerns, additionally legumes improves soil fertility, through N-fixation making agricultural practice sustainable (Graham & Vance, 2003; Smil, 1999). However, the nutritional limitations are still there and seriously needed to be addressed especially for those regions of earth where the food source of most of the population is Plant protein.

To overcome this problem, fortification has become a very important technique-addition of essential nutrients to enhance the quality of food nutrition. In this research we see fortification of plants-legume based meals can help create a complete source of protein with the Egg-white protein and whey protein from animal meals (Young & Pellett, 1994). Both of them (EWP and WP) are known because of their high amino acid score (EAAs) and Bioavailability values (BV), making them ideal for fortification (Schaafsma, 2000). This study seeks to explore the effectiveness of fortifying legume-based meals with EWPP and WPI, thereby improving the protein content and amino acid composition of these ready meals for enhanced dietary benefits.

1.1. Research Objective

I carried out my studies in connection with a research project. The goal of the project was the development of ready meals with increased protein content and optimized amino acid composition. My task was to enrich the protein content of ready meals made from legumes (green pea and kidney beans) and to examine their amino acid composition.

For this reason, my tasks were the following:

• To analyze the amino acid composition of protein powders

• To analyze the amino acid composition of different legumes

• To calculate fortification ration of legumes and protein powders to compile complete protein source

• To produce dishes on the base of the calculation

• To analyze and evaluate fortified dishes

• Protein content

• Amino acid composition

1.2. Research Questions

1. Can ready meals made from legumes be completed with the addition of animal protein?

2. Is there a difference between the amino acid composition of ready meals completed with egg-white or whey protein?

Chapter 2: Literature Review

2.1. Overview of amino acids

Amino acids are building blocks of proteins; by binding with each other- amino acids make proteins of different structure due to which functions of proteins vary, some proteins acts as catalysts , some proteins acts as a transport, gives structural support and some proteins such as G-proteins helps in transduction of signals (Branden & Tooze, 2012).

All amino acids are made of Carbon, nitrogen and oxygen and some other elements depend on the type (Marsh & Donohue, 1967). There are two types of amino acids based on the ability of humans to make them from other molecules like carbohydrate and lipids or not; are described as Essential Amino acids and non-Essential Amino acids. Essential amino acids can't be synthesized by human body itself and are needed to be taken through diet but non- essential amino acids can be synthesized by the body (Hou et al., 2015). There are 20 classified amino acids of special importance, 9 of them are Essential amino acids that include- Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Threonine, Tryptophan, and Valine; must be taken via diet (Wu, 2013). The other Eleven amino acids named as alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, proline, serine, and tyrosine, are non-essential – can be made by body in normal, healthy situation (Brosnan, 2003). But if the body becomes sick or influenced by a disease adding if it is in growth age, certain non-EEA also become essential for the body and termed as Functional amino acids due to their role in growth, reproduction and immunity (Elango et al., 2009) (Wu, 2009).

Previous researches shows, proteins are passed through these certain levels of structure to become functional which are as follows primary, secondary, tertiary, and quaternary, which is determined and dictated by the genetic code (Berg et al., 2015) depending on the functions- in Primary structure, amino acids are linked with each other through peptide bonds in a linear sequence, in secondary structure alpha helix and beta sheets are formed due to H-bonding at various positions of peptide lineage , plays important role in making building blocks of various proteins, tertiary structure is overall a three dimensional structure of polypeptides via many interactions (Nelson & Cox, 2017), and Quaternary structure is spatial arrangements of these polypeptides (Voet & Voet, 2011). These 20 discussed amino acids are the monomers of proteins big structure, all the bonding between amino acids happen due to their differences in the side chain (or R-group) attached to the central alpha-Carbon, and these chains are responsible for the functionality of amino acids such as size, charge, hydrophobicity, and reactivity—thereby influencing the amino acid's behavior within proteins. Depending on the physiochemical properties of side chains, amino acids are classified as nonpolar (hydrophobic), polar (uncharged), and charged (either acidic or basic) (Nelson & Cox, 2017).

2.2. Protein and Essential Amino Acids

Protein is the major functional and structural component of all the cells of the body. The defining characteristic of protein or amino acid is its requisite amino nitrogen group. This nitrogen group distinguishes an amino acid from for example a sugar. Proteins are macromolecules consisting of long chain of amino acid subunits. In the protein molecule, the amino acids are joined together by peptide bonds. In biological systems, the chains formed might be anything from a few amino acids (di-, tri- or oligopeptide) to thousands of units (polypeptide). The sequence of amino acids in the chain is known as the primary structure. A critical feature of proteins is the complexity of their physical structures. Polypeptide chains do not exist as long chains but they fold in a three-dimensional structure. The chains of amino acids tend to coil into helices (secondary structure). Sections of the helices may fold on each other due to hydrophobic interactions between non-polar side chains and, in some proteins, to disulfide bonds so that the overall molecule might be globular or rod-like (tertiary structure). Their exact shape depends on their function and for some proteins, their interaction with other molecules (quaternary structure) (Branden & Tooze, 2012; Scheraga, 2014; Wu, 2009).

More than 100,000 different proteins are produced by about 20,000 different protein-coding genes in the human body. Only roughly 20 amino acids are required to create all the proteins found in the human body and the majority of other forms of life, despite the fact that there are hundreds of amino acids in nature. All 20 of these amino acids are alpha-amino acids of the L isomer. They all do, with the exception of glycine, have chiral alpha carbons. And all of these amino acids, with the exception of glycine (which has a chiral center) and cysteine, are L isomers with an R-absolute configuration (S-absolute configuration, because of the sulfur containing R-group). It is important to note that pyrrolysine and selenocysteine are regarded as the 21st and 22nd amino acids, respectively. These are more modern amino acids that could be included in protein chains during ribosomal protein synthesis. Although pyrroloysine serves a purpose in life, it is not used by humans to synthesize proteins. After being translated, these 22 amino acids can potentially undergo a post-translational change to increase the variety of proteins produced (Wu, 2009).

The amounts of essential amino acids define the nutritional quality of proteins. If just one of these amino acids is deficient, the others will be broken down and expelled, impairing human and animal growth and removing nitrogen from the diet. Lysine, isoleucine, leucine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine, and methionine are the ten amino acids that are absolutely necessary. Nonetheless, combined values for these sulfur-containing amino acids are frequently provided, with cysteine frequently added as it can only be generated from methionine (which is itself necessary). Similar to this, combined values for the aromatic amino acids phenylalanine and tyrosine are frequently given as shown in Table 3 of (World Health et al., 2007). All amino acids (AA) are required for the body to grow and operate normally. They should be given in a quantity that is appropriate and in a form that is biologically accessible. This is significant since the content and availability of AA vary among different protein sources. It implies that the AA content of the various diets varies. The AA content of the various foods varies. For instance, when compared to meals of animal origin, the concentrations of lysine and sulphur AA in cereals like rice and wheat and legumes, respectively, are both much lower. Thus, each protein has a varied nutritional quality. Comparing proteins of animal and plant origin, the former are thought to be of higher quality. Protein nutritional quality is also impacted by AA digestibility. Using the Digestible Indispensable Amino Acid Score, the Food and Agriculture Organization settled on a new system of evaluating the protein quality of foods for human nutrition in 2013. (DIAAS). The usual ideal digestible AA content in the foods serves as the foundation for this methodology. The Protein Digestibility - Corrected Amino Acid Score (PDCAAS) method, in contrast to the prior approach, considers the ideal digestibility of AA rather than fecal digestibility. The ideal intake of dietary proteins should contain all 20 amino acids (essential, conditionally essential, and non-essential) in the proper amounts for bodily needs in order to support all metabolic processes, including intestinal integrity, gene expression regulation, protein synthesis, and cell signal pathway regulation(Brestenský et al., 2019).

Essential amino acids, also known as indispensable amino acids, are amino acids that humans and other vertebrates cannot synthesize from metabolic intermediates. The human body lacks the metabolic pathways necessary to produce these amino acids, thus they must be obtained from an external diet. Amino acids are categorized in nutrition as either essential or non-essential. Early research on human nutrition revealed that certain amino acids were necessary for growth or nitrogen balance even when there was an adequate supply of other amino acids, leading to the creation of these classes. There are nine essential amino acids, including phenylalanine, valine, leucine, isoleucine, tryptophan, threonine, methionine, histidine, and lysine, however variances are possible depending on an individual's metabolic status. Since it contains the first letter of each of the necessary amino acids, the mnemonic PVT TIM HaLL (short for "private Tim Hall") is frequently used to help people recall these amino acids (Le & et al., 2016). A single complete protein can supply all nine of the required amino acids in terms of nutrition. By definition, a complete protein has every one of the essential amino acids. With the exception of soy, complete proteins are typically obtained from animal-based food sources. The incomplete proteins, which are typically found in plant-based meals, also include the essential amino acids. The necessary amino acid that is found in a food protein in the smallest amount as compared to a reference food protein, such as egg whites, is referred to as the "limiting amino acid." An essential amino acid that falls short of what is necessary for humans may also be referred to as a "limiting amino acid" (Lopez & Mohiuddin, 2020).

Branched-chain amino acids (**BCAAs**; valine, leucine, and isoleucine) serve as substrates for protein synthesis and energy production, perform several signaling functions, mainly via the mammalian target of rapamycin (**mTOR**) pathway, and act as a nitrogen source for the synthesis of dispensable amino acids, particularly glutamate (GLU), glutamine (GLN), alanine (ALA), and aspartate (ASP). For many years, researchers have studied the utility of BCAAs as a dietary supplement, particularly to support anabolic pathways, in a variety of illnesses, such as cancer, liver cirrhosis, trauma, burn injury, sepsis, and renal failure. Nonetheless, there are still questions about the aetiology and metabolism of changes in BCAA levels under these conditions. In both type 1 (T1DM, insulin-dependent) and type 2 diabetes mellitus, BCAA concentrations fall specifically in hyperammonemic conditions such as liver cirrhosis and urea cycle disorders (UCD) (T2DM, insulin-independent). Due to the detrimental effects of decreasing BCAA levels on the emergence of weariness, muscular atrophy, and hepatic encephalopathy, BCAAs are advised as a nutritional supplement in liver cirrhosis. On the other hand, higher BCAA concentrations in diabetes are regarded to be a factor contributing to the development of insulin resistance and increasing the risk of a variety of complications of diabetes (Dimou et al., 2022; Holecek, 2018).

Leucine is well known for promoting muscle protein synthesis. Leucine has been shown to decrease the deterioration of muscle tissue in old rats when taken as a dietary supplement. Long-term leucine supplementation, however, disturbs muscle mass in healthy elderly men. Due to elevated quantities of leucine in the blood, both humans and animals develop insulin resistance. **Isoleucine** is beneficial for encouraging muscle recovery following exercise. The muscle tissue really breaks it down for energy. Maintaining the nitrogen balance necessary for healthy physiological function requires isoleucine. Spleen, pituitary, and thymus metabolism is also maintained by leucine. Leucine and isoleucine are thus complimentary to one another, helping to lessen muscle stiffness and tiredness while promoting muscle growth. Seafood, fish, meat, chicken, pig, and even beans and nuts are foods high in leucine. Rich sources of isoleucine are comparable. Sunflower seeds, nuts, cashew nuts, avocados, olives, lentils, eggs, and other dairy products are a few of the crucial ones(Holecek, 2018).

Tryptophan is a well-known medicinal agent and one of the essential amino acids. The female reproductive system, digestive system, and visual system all benefit from it- prevents premature ageing symptoms like baldness, sex gland decline, brittle tooth enamel, and cataracts in the eyes. The body needs tryptophan for effective absorption of vitamin A, blood coagulation, and the production of digestive juices. Additionally, this amino acid regulates nervous system diseases, calms anxiety, and works as a nutritional cure for insomnia. Another possible application for tryptophan is as a natural analgesic. Tryptophan-rich foods include seeds, nuts, soy, spinach, beef, and others. Additionally, it is a food-based medication that works best when combined with low protein food as juice or bread (Barik, 2020). Dietary tryptophan is a crucial component of protein synthesis and a precursor to biologically active substances like serotonin, melatonin, quinolinic acid, kynurenic acid, and tryptamine, as well as coenzymes crucial for electron transfer reactions (redox balance of metabolism), like nicotinamide adenine dinucleotide (NAD). NAD, which is the byproduct of tryptophan metabolism, may be created from both vitamin B3 and consumed tryptophan. With regard to dietary and nutritional sciences, interest is growing in the functions tryptophan (Trp) (Hardeland et al., 1993; Kałużna-Czaplińska & Błaszczyk, 2017). Recent studies have shown that this amino acid has a protective effect on the gut because it increases the expression of the tight junction proteins zonula occludens (ZO-1) and claudin-3 in the jejunum of experimental animals. Its essential significance is mostly revealed by its interaction with serotonin, which is crucial for the conversion of food into nutrients, Trp levels can be used by clinicians to identify a variety of metabolic conditions and the symptoms that go along with them. Due in large part to the link between Trp and the synthesis of serotonin (5-HT) and melatonin, supplementation with this amino acid is also taken into consideration for the treatment of depression and sleep disturbances. Autism spectrum disorder, obesity, anorexia and bulimia nervosa, and other disorders with peripheral symptoms are all linked to decreased serotonin secretion (Joshi et al., 2020).

Threonine (Thr), is essential AA, one of the least present amino acids in most diets but if body is deficient of Thr, protein synthesis is limited even if other AAs are abundantly present. It helps body maintain the structure and health of gut, responsible for number of goblets cells that secrete mucus, growth rate of body, and enzymes at the surface of intestinal cells that help in digestion, A significant amount of dietary threonine is used for intestinal-mucosal protein synthesis and Mucin production is considered a "net loss" of threonine because mucins cannot be digested and reused by the body. If threonine is lacking less mucin is produced and also gut lining, infections and inflammation can also happen in the gut (Tang et al., 2021).

Histidine is essential for healthy tissue growth and repair. Its activity increases blood circulation, and histidine also helps the liver produce glycogen. Histidine thus plays a vital function in the body's metabolic processes. Enough histidine is required for infants. Less of the free form of histidine is found in the blood of rheumatoid arthritis patients. Histidine is an excellent anti-inflammatory that may be used orally, but it can also induce acidity in people who have ulcers and other gastrointestinal diseases. It treats orthopedic issues such as joint discomfort. Root and green vegetables such as beans, cauliflower, potatoes, etc., are excellent sources of histidine. Additionally, sources of histidine include grains like wheat and rice. L histidine contributes to the synthesis of blood and the preservation of the myelin coating that protects nerve cells, supporting the proper operation of the nervous system (Holeček, 2020; Moro et al., 2020).

Lysine has strong anti-virus effect. It eliminates virus infections more efficiently when used along with vitamin C, vitamin A and zinc. Lysine deficiency in the diet may cause headaches, nausea, dizziness and anemia. Cold, sores and female reproductive cycles are also influenced by lysine. The four important health benefits of lysine include protection against cold sores by blocking arginine, improve calcium absorption and retention, promote wound healing by creating collagen, and help in reducing anxiety by blocking stress response receptors. Other possible health effectiveness of lysine are control of blood pressure, diabetes, pneumonia, nephrosis and acidosis. Lysine is the natural remedy for genetical herpes as well as rickets in children (Beauman, 2005; MedShun, 2021).

Methionine is necessary for various biological functions, including protein synthesis, metabolism, and the production of important molecules like glutathione. In the livestock food, methionine is added for the fortification of feed. Synthetic production of methionine plays a crucial role in enabling low-cost animal protein production, which is directly effective for human health and ecosystem. The hiking demand of protein which rely on methionine make an overall impact on animal farming environment-land use, water consumption and greenhouse gas emission (D'Mello, 2003; Neubauer, 2021).

2.3. Concept of Food fortification

Food fortification as the name depicts is addition of nutrients in food particularly one or more essential nutrients ignoring the situation of presence or absence of that particular nutrient in the food with the aim of overall better nutritional quality (World Health, 2006). So this practice helps with the challenges of nutritional deficiencies in the population by supplementing the diets with the essential nutrients and minerals (Allen et al., 2006).

The actual practice of this concept started in early 20th century where one of the documented instance can be found when Iodine was added in the table salt to combat with the goiter, a prevalent health problem of that time (Zimmermann, 2009). In World War II-era Britain, this concept was used too when bread was fortified with iron to address iron-deficiency anemia, growing public health concern (Calvo & Whiting, 2003). In United States, fortification of milk with Vitamin-D begin in 1930s to prevent people from rickets (a condition due to vitamin-D deficiency) (Calvo & Whiting, 2003). The main idea behind food fortification is to enhance nutrition of populations especially, in developing countries especially without changing their diets but by just fortifying the staple foods like flour, salt, and, dairy products can make impact globally (Allen et al., 2006).

2.4. Importance of Amino Acids in Fortified Food

Similarly, fortification of food with essential amino acids can make a bigger impact as the body can't synthesize it by itself which are critical for body functions, muscle growth, repair, and building units of protein (Wu, 2009). Fortifying of EEAs especially, where protein consumption is very low can increase the Biological Value (BV) of proteins in the Food (Young & Pellett, 1994). This fortification helps to provide complete protein sources when added to food especially in those diets where some of the essential amino acids are missing such as cereals and Legumes. This way by making the balanced and complete diet effectively improves the health addressing macro-nutrient and micro-nutrient deficiencies (Millward, 2012). Fortification can enhance the protein quality and support muscle protein synthesis, immune function, and overall health (Schaafsma, 2000).

BV (bio-availability) of amino acids is the extent and the body's rate of AAs being absorbed and utilized determines the efficacy of fortified foods. The right proportion and sequence of amino acids can impact protein synthesis and metabolic processes. For example, methionine and cysteine are sulfur-containing amino acids that play key roles in detoxification pathways and antioxidant defense. Without adequate methionine, the body cannot efficiently produce cysteine, a precursor for glutathione, an important antioxidant (Moughan & Rutherfurd, 2012). Sequence of amino acids in proteins matters because certain sequences can enhance the bioavailability of others more importantly, for plant based proteins which are lower in certain essential amino acids and by fortifying these foods with complementary amino acids, the overall bioavailability and nutritional value of the protein can be increased (Rutherfurd et al., 2016).

Need for amino acids are different for different bodies depending on the physiological states, activity levels and life Stages (Bhutta et al., 2013).In children, higher intake of EAAs for growth and development. Insufficient lysine, threonine, or tryptophan during childhood can lead to stunted growth and cognitive impairments (Bhutta et al., 2013). And in pregnant women, fetus is needed to be fed for development so it requires more EEAs particularly, methionine because it is important for the methylation processes that are critical in DNA synthesis and cell division during pregnancy (Wu et al., 2014). Similarly, Athletes require more branched chain amino acids-BCCAs such as leucine, isoleucine, and valine to support muscle repair and growth after intense physical activity. Leucine, a key amino acid in muscle protein synthesis (Blomstrand, 2012).

2.5. Protein Quality

Protein quality, can be categorized as (i) protein to support optimal growth, (ii) amino acid balance, (iii) the degree of protein digestion and absorption, or (iv) indispensable amino acids in relation to amino acid needs, is another factor that should be taken into consideration when evaluating the nutritional value of proteins in food products (Hayes, 2020). According to the FAO of the United Nations, protein quality is correlated with the source's amino acid composition and bioavailability, which is related to digestibility which is determined by measuring the nitrogen remaining in the feaces and urine following feed trials and the digestion of chosen diets with/without protein by the animal of choice, which is typically a pig. Other methods for assessing protein quality also exist, but they typically entail expensive animal studies. The "gold-standard" proteins, such as gelatin, which are well-known to be highly digested, bioavailable, and amino acid-rich, are then compared (Hayes, 2020).

Calculations of protein quality consider variables such as biological value (BV), which is the proportion of ingested nitrogen maintained in the body, and true digestibility (D), which is the proportion of actual nitrogen absorbed from the intestine in accordance with the Mitchell technique. Proteins have a nutritional value of BV X D. The protein efficiency ratio (PER), the Protein Digestibility Corrected Amino Acid Score (PDCAAS) method, and, more recently, the FAO-approved Digestible indispensable Amino Acid Score (DIAAS) method, which is typically carried out using a rat model, are additional methods and variations for assessing protein quality (Hayes, 2020).

Protein efficiency ratio (PER)--measures a protein's capacity to support growth in young rats, and net protein utilization (NPU)--measures a protein's capacity to retain nitrogen, are two biological assays that have been used to evaluate the quality of food proteins in laboratory animals. These tests, however, undervalue the nutritional value of several plant and animal proteins for humans. For instance, since rats need more sulfur-containing amino acids than humans do, the proteins in pulses and milk casein are of lower quality in rats than in humans. It is possible to do an NPU assay in addition to a PER assay. The BV of a protein is predicted using NPU. The protein content of the animal carcasses is calculated for NPU testing. The biological value (BV) of an organism gauge how effectively it uses nitrogen. The percentage of nitrogen retained for maintenance and growth, adjusted for metabolic and endogenous nitrogen losses, is the biological value of a test protein (BV= N retained/N absorbed). Retained nitrogen is nitrogen that the body stores for growth and maintenance, whereas absorbed nitrogen is nitrogen that enters the lumen, is hydrolyzed, and is then absorbed. Yet, these tests are incredibly difficult to conduct reliability. The outcome of either a BV or an NPU assay will depend on the protein content of the animal's diet and its age. Moreover, NPU tests do not take into consideration the varying rates of dietary essential amino acid use or reutilization. An essential amino acid deficiency in a protein should result in an NPU of 0. Animals, however, have systems in place for recovering and using endogenous amino acids (Kruger, 2009).

2.6. Food Sources of Protein

Complete and Incomplete Protein

Protein is available in a variety of dietary sources. These include foods of animal and plant origins as well as the highly marketed sport supplement industry. The quality and digestibility of a protein can be used to determine how effective it is. The availability of amino acids is referred to as its quality, and how well the protein is absorbed is referred to as its digestibility. Normally, all dietary sources of animal protein are regarded as complete proteins. That is, a protein that is complete in terms of its amino acid composition. Vegetarian proteins are typically missing one or two important amino acids, making them incomplete proteins. In order to assure consumption of all required amino acids, a person who prefers to obtain their protein from vegetable sources (i.e., a vegetarian) will need to eat a range of vegetables, fruits, grains, and legumes. As a result, people can fulfill their protein needs without ingesting meat, poultry, or dairy. Ratings of protein digestibility often take into account how effectively the body can use dietary sources of protein. Vegetable protein sources typically do not rate as highly as animal proteins in terms of biological value, net protein utilization, PDCAAS, and protein efficiency ratio (Adhikari et al., 2022; Sarwar, 1997).

Animal sources of dietary protein, despite providing a complete protein and numerous vitamins and minerals, have some health professionals concern about the amount of saturated fat common in these foods compared to vegetable sources. Proteins from animal sources (i.e. eggs, milk, meat, fish and poultry) provide the highest quality rating of food sources. This is primarily due to the 'completeness 'of proteins from these sources. Although protein from these sources are also associated with high intakes of saturated fats and cholesterol, there have been a number of studies that have demonstrated positive benefits of animal proteins in various population groups. **Vegetable proteins**, when combined to provide for all of the essential amino acids, provide an excellent source for protein considering that they will likely result in a reduction in the intake of saturated fat and cholesterol. Popular sources include legumes, nuts and soy. Aside from these products, vegetable protein can also be found in a fibrous form called textured vegetable protein (TVP). TVP is produced from soy flour in which proteins are isolated. TVP is mainly a meat alternative and functions as a meat analog in vegetarian hot dogs, hamburgers, chicken patties, etc. It is also a low-calorie and low-fat source of vegetable protein. Vegetable sources of protein also provide numerous other nutrients such as phytochemicals and fiber that are also highly regarded in the diet (Lim et al., 2021; Lynch et al., 2018; Schaafsma, 2005).

Good sources of non-meat Protein

Vegetable proteins are a good source of protein since they likely result in a reduction in the consumption of saturated fat and cholesterol when combined to provide for all of the required amino acids. Soy, almonds, and legumes are common sources. In addition to these goods, textured vegetable protein, a fibrous form of vegetable protein, is also available (TVP). TVP is made from soy flour that has isolated proteins. TVP serves as a meat analogue in vegetarian hot dogs, hamburgers, chicken patties, and other foods. It is also a source of vegetable protein that is low in calories and fat. In addition to providing a variety of other nutrients, plant proteins also provide fiber and phytochemicals, which are also highly regarded in the diet (Lynch et al., 2018).

The most popular source of vegetable protein is soy. The legume family member, the soybean, was first recorded in Chinese history in the year 2838 B.C. and was valued as highly as rice, wheat, and barley as a dietary mainstay. Although soy was widely popular in other nations, it took until the 1920s for it to become well-known in the United States for its nutritional benefits. In comparison to Asian nations, Americans consume a comparatively small amount of soy protein (5g/day). Although cultural factors may have played a role, the poor protein quality rating from the PER scale may also have had an impact on people's propensities to consume protein. Soy protein was reported to be equivalent to animal protein with a score of 1.0, the maximum attainable grade, when the more precise PDCAAS scale was applied. Due to its qualities, soy is a very appealing substitute for those who prefer non animal sources of protein or who are lactose intolerant. Soy is a complete protein that has a lot of BCAAs in it. Several soy protein-related health and performance benefits, such as improving LDL-cholesterol oxidation, lowering blood pressure, and lowering plasma lipid profiles, have been reported; however, further study is still needed to support these claims (Friedman & Brandon, 2001).

The soybean can be divided into three different types: isolates, concentrates, and flour (Table 1). Further dividing soy flour into lecithinated (lecithin added), defatted (oils removed), and natural or full-fat (contains natural oils) forms. Soy flour is the least refined type of soy protein product among the three categories. It frequently appears in baked foods. Textured soy flour is a different product made from soy flour. This is mostly employed in the processing industry as a meat extender. For information on the protein content of soy flour, concentrates, and isolates, see Table 1 below.

Table 1: Types of soy protein products (FAO)

Soy Protein Form	Protein Composition
Soy flour	50%
Soy concentrate	70%
Soy isolate	90%

In the late 1960s and early 1970s, defatted soybeans were used to create soy concentrate. Concentrates are less appealing than flour because they contain less soluble carbs while still keeping the majority of the protein in the beans. Soy concentrate, which is present in yoghurt, cereals and nutrition bars has a high digestibility. The most refined form of soy protein, isolates have the highest protein content compared to flour and concentrates; however, they don't have any dietary fiber. Around the 1950s, Americans first developed isolates. They are incredibly palatable and simple to incorporate into foods like newborn formula, sports drinks, and healthy beverages(Friedman & Brandon, 2001).

Legumes

The word —legume is derived from the botanical term Leguminosae. Legumes are the dry seeds of papilionaceae. Among the higher plants, they are the third largest family. They are referred to as pulses or legumes in agriculture. Soybeans, faba beans, peas, green beans, lentils, lupines, and chick peas are the most popular types of legumes. Soybeans are by far the most widely grown crop in the world, followed by faba beans and peas. Legumes offer a special benefit for soil fertility because, through a symbiotic connection with rhizobia, their roots fix nitrogen in the soil, making it available to other plants (Graham & Vance, 2003; Smil, 1999).

One dietary group for which frequent eating is strongly advised is legumes. They were a crucial source of protein and energy (starch) for people in the ancient world and the Medieval Ages.

Nowadays, meals made from animal sources, such as grains and potatoes, have taken their place. As a result, the average annual consumption of legumes in Germany, for example, is now less than 1Kg. Legumes' (pulses') reduced consumption is due to a number of factors, including their flatleuscent (bloating) effect (particularly in the case of beans), their basic, rural appearance, and the misconception that they are usually used as animal feed. The low degree of farming, which results in a lesser supply, is another factor. Last but not least, compared to items made from legumes, cereal and potato products are currently far more varied and diversified. Yet perceptions against legumes have been changing for a while now, and they are no longer always considered to be outdated. More plant-derived proteins that can be employed in human diets are urgently needed, particularly in light of the expanding global population. They are required as an alternative to diets high in fat and carbohydrates. Legumes have a protein level in the dry matter of 20–40%, which is rather high compared to cereals, which only have a protein content of 10-15%. So, it is not surprising that there is a growing trend for the production of legumes to expand from the "kitchen garden" to substantial expanses of arable ground. This is why crops used as animal fodder, like sweet lupines, faba beans, and field peas, are the focus of the nutrition and food industries. These discoveries raise the question of whether these goods satisfy the nutritional needs of humans in terms of their nutrient content, any potentially harmful compounds they may include, and even whether they may improve human health (Emmambux & Taylor, 2013; Semba et al., 2021).

Pea seeds (Pisum sativum L) are mostly used in pig feed because of specie's high nutritive value. In spite of that, methionine and cystine, are two S-containing amino acids, like tryptophan, are limiting amino acids in pea proteins. Although pea albumins contain more essential amino acids (AA) than globulins, information regarding the AA profile of the insoluble proteins and non-protein components of pea seeds is not yet accessible. Because the protein distribution in the seed is heterogeneous and the various particles do not all have the same hardness, milling might emphasize the nutritional differences between the protein fractions in particular feeds, such as cereals (Leterme et al., 1990).

In 1985, 11.6 million metric tons of dry peas (Pisum sativum) were produced worldwide. This product is a crucial source of protein for both human and animal consumption. Dry peas have been used to make protein concentrates and isolates for commercial purposes; nevertheless, the main obstacle to the sales of these products has been the challenge of competing with the well-established, adaptable soy protein materials that rule the market for food protein ingredients. Field peas have a wide range of protein contents), which are regulated by both genetic and environmental variables. It's common knowledge that Pisum sativum has 24% to 25% protein on average. The leading crop for plant-based protein is the field pea. Targeted breeding and a deeper comprehension of the level of variety and the factors impacting pea protein content, composition, and functionality may be necessary in response to increased market demand. The pea (Pisum sativum L.) is a pulse crop that is diploid (2n = 14) and has a rather big genome (4.45 Gb). Over the world, it is grown on millions of hectares (FAO, 2020). Cultivated land and global productivity have fluctuated during the past 60 years (1961–2019) (Daba & Morris, 2022; Kreplak et al., 2019).

The quantity and quality of the protein, which are influenced by genetic variables, growing environment circumstances, and processing conditions, determine the nutritional value and functional qualities of pea protein. The amount and make-up of pea protein are known to be influenced by numerous gene families, and different genes involved in starch production also indirectly affect protein content. Access to genetic diversity and knowledge of the genetic processes governing the genes regulating a trait are crucial for the success of a breeding program (Daba & Morris, 2022).

Since antiquity, soybeans have been referred to as "meat of the field" due to their high lipid and protein content. In addition to being rich in vital fatty acids and low in saturated fat, soybean oil is also a fantastic source of vitamin E. Soybean has about 15% saturated fat, whereas the levels of poly- and mono-unsaturated fats are 61 and 24%, respectively. To put it another way, soybeans are one of the few plant foods that also include omega-3 fatty acids, known as á-linolenic acids, in addition to omega-6 fatty acids. Soybean protein offers a high concentration of well-balanced amino acids. Comparable to sources of animal proteins like milk and meat, soy proteins are high in quality. Soy protein, which has a high lysine concentration, is a viable alternative to grain proteins, which are deficient in lysine. Additionally, according to recent research using rats, tumors may be selectively delayed because soybean proteins contain less methionine than casein. Beta-conglycinin (7S globulin) and glycinin (11S globulin) make up the majority of soy protein (Hawrylewicz et al., 1995).

Products made with soy protein include bioactive compounds known as phytoestrogens or isoflavones. Genistein, daidzein, and glycitein are the three main isoflavones present in soybeans. Depending on the production processes utilized, their abundance varies greatly in soy protein preparations and products that are associated to them. For those who are allergic to milk protein, soy protein offers an alternative source of protein that is highly digestible (92% to 100%), contains all essential amino acids, is a good source of lysine despite being relatively low in methionine, and is lactose free. They have very little to no saturated fat and no cholesterol. The high-quality protein, B vitamins, potassium, iron, dietary fiber, and bioactive ingredients, like as isoflavones, that can be found in these substitutes are also beneficial. There are now dairy soy products on the market that are lactose and milk protein free, such as soy milk, cheese substitutes, yoghurt, soy sour cream, soy cream cheese, and frozen soymilk desserts. Tofu, soymilk, tofu, and soy protein isolates can all be combined to create soy cheese substitutes. Today, soy cheese substitutes can be purchased pre-sliced, shredded, or in blocks in a number of classic cheese kinds, including mozzarella, cheddar, pepper jack, and jalapeo. There are also sour cream, cream cheese, and soy parmesan substitutes on the market (Jooyandeh, 2011).

Products made from soybeans are increasingly being used in the feed and food industries. Currently, India produced 9.3 million metric tons of soybeans, or around 4% of the entire global production, which totals 219.8 million metric tons. Less than 10% of this produce is actually utilized for human consumption (Gandhi, 2006). The popularity of soybeans and goods made from them is largely due to their great nutritional value, particularly in terms of protein and amino acids. Although fundamental standards and criteria for soybeans and soy meals have been created (NOPA, 1997), there are currently no recognized specifications for other soy products. The NOPA requirements only mention four chemical constituents in further detail. Today's assessments of soy products are based on a considerably wider range of tests, enabling a more precise assessment of the nutritional value of the various products. The value of soy products will increase with advancements in technological adaptations of soybean products and a greater understanding of the impact on performance and health of relatively unknown chemicals, such as isoflavones (Gandhi, 2009).

Protein powders

Egg-white powder

The chicken egg is indeed a widely consumed food item worldwide, and its various components have applications in different food products. The three main components of a whole egg are the shell, the yolk, and the white (also known as the albumen). The shell is the hard outer covering of the egg, providing protection and support to the developing embryo. It is primarily composed of calcium carbonate and a small amount of proteins. The yolk is the yellow, circular portion of the egg located within the white. It serves as the nutrient-rich food supply for the developing embryo. The yolk contains proteins, fats, vitamins (such as vitamins A, D, and E), and minerals (such as iron and phosphorus). The egg white, or albumen, surrounds the yolk and acts as a protective layer. It is a clear, viscous liquid that primarily consists of water and proteins, including ovalbumin, conalbumin, and ovomucin. The egg white provides a source of protein and is often used in various culinary applications. Apart from these main components, there is also the eggshell membrane. It is a thin film-like layer found on the inner side of the eggshell. The eggshell membrane is primarily composed of protein, including approximately 35% collagen. It also contains other substances such as hyaluronic acid, chondroitin, and small amounts of inorganic components. The eggshell membrane has gained attention for its potential applications in various fields, including food, cosmetics, and biomedical research, due to its unique composition. In the food industry, egg fractions like the egg white and yolk are commonly used in a wide range of products, including cakes, desserts, confectioneries, pies, and powdered soups. These fractions contribute to the texture, taste, and nutritional profile of the final products. Additionally, certain specialized egg products, such as powdered egg whites or yolks, are available for specific applications where separated components are required. It's worth noting that while eggs are a nutritious and versatile food source, individual dietary needs and considerations may vary. Some people may have specific allergies or dietary restrictions that require them to avoid or limit their consumption of eggs (Baláž, 2014).

Egg white proteins (EWPs) consist of various types of proteins, each with its own distinctive structure and functional properties. Some of the most abundant proteins found in egg whites include, ovalbumin, ovotransferrin, ovomucoid, ovomucin, lysozyme, globulins and avidin. Ovalbumin is the most abundant protein in egg white, accounting for approximately 54% of the total protein content. It has a globular structure and functions as a storage protein, providing amino acids to the developing embryo. Ovotransferrin, also known as conalbumin, makes up around 12% of the total protein in egg white. It has iron-binding properties and plays a role in iron transport and antimicrobial defense. Ovomucoid is a glycoprotein that constitutes roughly 11% of the total protein in egg white. It has a compact, globular structure and acts as a protease inhibitor, protecting against protein degradation. Ovomucin is a high molecular-weight glycoprotein, comprising about 3-5% of the total protein in egg white. It contributes to the viscous nature of egg white and plays a role in its gel-forming properties. Lysozyme makes up approximately 3-4% of the total protein in egg white. It has antimicrobial properties and acts as a natural defense mechanism against bacterial infections. Globulins are a group of proteins present in egg white, including ovoglobulins and ovomucoglobulins. They account for about 8-10% of the total protein content and contribute to the foaming and emulsifying properties of egg whites. Avidin is a protein that binds to biotin, a B vitamin. It represents less than 0.05% of the total protein in egg white. Avidin is known for its strong affinity for biotin and is often used in biotechnological applications for biotin detection and purification (Razi & Rashidinejad, 2022).

Understanding the structure and functionality of these proteins under different conditions, such as pH, thermal processing, high pressure, and interactions with other food ingredients, is crucial in various food industries. These conditions can affect the proteins' stability, solubility, gelation, foaming, and emulsifying properties. By studying and characterizing these proteins, food scientists can optimize their utilization as ingredients in food processing, taking advantage of their amino acid profiles, bioavailability, and functional properties to improve the quality and texture of food products (Chang & Yang, 2017).

With an amino acid score of 100 and the highest net protein consumption rate, egg protein is an astonishingly rich source of protein. Based on human studies, the information about the advantages of egg proteins for health is compiled in many reviews. Studies on the features of egg whites, which are rich in pure protein and have a variety of health benefits, have revealed that they can build muscle growth and strength, lower cholesterol, and reduce visceral fat, among other things (Matsuoka & Sugano, 2022).The majority of the elements required for human existence, with the exception of vitamin C and dietary fiber, are found in chicken eggs, making them a very nutrient-dense food. Those with dyslipidemia may be recommended to avoid them because they also contain cholesterol, but eggs are said to have little impact on blood lipid levels in healthy people. This is due to the fact that the body has mechanisms for controlling cholesterol levels, including lowering hepatic cholesterol synthesis. The available research has shown that eggs have a variety of health benefits, including improved blood lipids from phospholipids consumed from egg yolks, improved cognitive performance from choline consumed from egg yolks, and increased bone density from calcium obtained from eggshells.(Matsuoka & Sugano, 2022)

Table 2 displays the amino acid contents of casein, soy protein isolate, and dry egg white protein (EWP). EWP has the same number of branched-chain amino acids as milk protein, which is one of its characteristics. In comparison to other protein sources, it also has more amino acids including Sulphur-containing amino acids. EWP has an amino acid score of 100 and is said to have higher net protein consumption than whey protein. EWP has been shown to boost body protein in animal trials because of its high quality. Also, it was shown that body fat was decreasing, suggesting that EWP is likely to improve metabolic syndrome and therefore have a good impact on health (Kagawa, 2017).

Table 2: Amino acid compositions of casein, dried egg white, and soy protein isolates (g/100 g) (Kagawa, 2017).

Amino Acids	Casein (mg/g)	Dried Egg White (mg/g)	Soy Protein Isolate (mg/g)
Ile	5	4.4	4
leu	8.5	7.3	7
lys	7.2	6.1	5.5
met	2.7	3.2	1.1
cys	0.4	2.5	1.1
phe	4.6	5.1	4.6
tyr	5.2	3.9	3.5
thr	4	4	3.7
trp	1.1	1.3	1.2
val	6.2	5.8	4.1
his	2.7	2.1	2.4
arg	3.4	5	6.9
ala	2.7	5.3	3.6
asp	6.3	9.3	10
gul	19	12	17
gly	1.7	3.2	3.6
pro	10	3.3	4.7
ser	5.2	6	5.1

Production

Thermal treatment of egg white above the denaturation temperature causes the proteins in the egg white to undergo structural changes. Denaturation refers to the disruption and destruction of the protein's tertiary structure, which is responsible for its unique conformation and stabilized interaction network. When the protein is in its native state, it can protect its structure and maintain its functional properties. However, changes in the environment, such as high temperatures or extreme pH levels, can disrupt the normal alpha-helix and beta-sheet structures of the protein. This disruption leads to abnormal behavior, including protein aggregation, coagulation, and precipitation. To prevent or minimize denaturation during processing, pretreatment of the egg white is often necessary. This can involve the addition of salt or adjusting the pH to create conditions that are more favorable for protein stability. These pretreatment methods help protect the protein's structure and functionality during subsequent processing steps. During the drying process, such as spray drying, denaturation is particularly evident. The application of heat during drying can cause further structural changes in the proteins, leading to denaturation. This denaturation can affect the functional properties of the proteins and may result in changes in texture, solubility, and other characteristics of the dried egg product. Overall, denaturation is a significant consideration in the processing of egg white, and pretreatment methods are employed to mitigate the effects of denaturation and preserve the protein's functional properties (Preethi & Anandharamakrishnan, 2020; Vickers, 2017).

Freeze drying (FD) is a technique that can be used to dry egg white while minimizing denaturation. Freeze drying involves freezing the product and then subjecting it to a vacuum to remove the frozen water through sublimation, resulting in a dry powder. This method preserves the protein structure and functionality to a greater extent compared to other drying techniques. However, as you mentioned, freeze drying is a time-consuming and expensive process, which limits its industrial-scale application for drying egg fractions. The lengthy processing time and the need for specialized equipment make it less feasible for large-scale production of egg white powder. Considering the industry's perspective, there is a need to explore alternative drying approaches that can be more practical and cost-effective. Over the past few decades, research has indeed focused on investigating various methods to dry egg white while optimizing processing conditions and assessing the functionality of the resulting product. Some of the alternative drying approaches that have been explored include: alkali treatment, pulsed electric field processing, high-pressure processing, ultraviolet (UV) irradiation and high-intensity ultrasound treatment. .Exploring these alternative drying approaches and optimizing their processing conditions, researchers aim to find more efficient and cost-effective methods for producing high-quality egg white powder with preserved functionality (Preethi & Anandharamakrishnan, 2020).

Application of Egg-white in food industry

The egg as a whole is indeed considered a good source of protein and lipids. However, when we specifically consider the egg white, it primarily consists of water (about 88%) and protein (around 11%). It lacks lipids or fats. Ovomucin, a highly glycosylated protein found in egg white, contains a significant amount of carbohydrates, making up approximately 33% of its composition. This makes ovomucin a valuable source of nutrients that can provide both protein and carbohydrates. Ovalbumin is another major protein present in egg white. It has a well-balanced amino acid composition, meaning it contains a good variety and proportion of essential amino acids. This makes ovalbumin an excellent source of protein that can be utilized in various food items. Additionally, the remaining proteins found in egg white are also considered good sources of essential amino acids, further enhancing their nutritional value (Stadelman, 2001).

Lysozyme is indeed one of the major bacteriolytic proteins found in egg white. It possesses the ability to control foodborne pathogens such as Listeria monocytogenes and Clostridium botulinum, which are significant concerns in the food industry. Lysozyme is effective in preventing toxin formation by Clostridium botulinum in various food products like fish, poultry, and certain vegetables. It has been observed that lysozyme's antimicrobial properties can be enhanced through chemical and thermal treatments, which modify its structure. Furthermore, lysozyme not only inhibits microbial growth but also exhibits antiviral, anti-inflammatory, and therapeutic effects. Its versatility makes it a valuable component in the food industry. The World Health Organization and many countries allow the use of lysozyme as a food preservative. Currently, it is used in the production of various food items such as kimchi pickles, sushi, Chinese noodles, cheese, and wine. Its inclusion in these products helps to control bacterial contamination, extend shelf life, and ensure food safety (Mine, 2004).

Ovotransferrin, another protein found in egg white, is known for its robust antimicrobial activity. As a result, it can be employed to enhance the safety of various food products. Recent studies have demonstrated the effectiveness of ovotransferrin in controlling foodborne pathogens such as E. coli O157:H7 and Listeria monocytogenes. These pathogens are known to pose significant challenges in terms of food safety. Ovotransferrin and its derived peptides have shown antimicrobial properties, making them suitable agents for combating microbial growth in foods. By incorporating ovotransferrin or its peptides into food formulations, it is possible to inhibit the growth and proliferation of pathogens, thereby reducing the risk of foodborne illnesses. The use of ovotransferrin and its peptides as antimicrobial agents in food not only helps improve food safety but also extends the shelf life of products by inhibiting the growth of harmful bacteria (Zhang & Liu, 2011).

Ovomucin has demonstrated favorable inhibitory activities against various bacteria such as E. coli, Bacillus species, and Pseudomonas species. It has been reported to possess a strong antimicrobial effect against foodborne bacteria, which makes it a potential candidate for use as a food preservative in the food industry. Additionally, ovomucin exhibits good emulsifying and forming characteristics. These properties are particularly valuable in the bakery industry, where foaming and emulsifying agents play a crucial role. By incorporating ovomucin into food formulations, it can enhance the nutritional profile of the product while contributing to a desirable texture. Furthermore, specific peptides derived from egg white proteins, such as the peptide containing Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu, have shown strong free radical scavenging activity. This indicates that not only the proteins in egg white but also their peptides can be utilized in the food industry to reduce lipid oxidation in food products. Oxidation of lipids can lead to the deterioration of food quality, and utilizing these peptides can help mitigate such oxidative processes (Omana & Wu, 2010).

In summary, ovomucin and its derived peptides possess antimicrobial, emulsifying, and antioxidative properties, making them beneficial for various applications in the food industry, including as food preservatives, texture enhancers, and antioxidants (Abeyrathne & Ahn, 2013).

Whey protein powder

Whey was a significant issue for dairy plants as it was not extensively recycled. It was often treated as wastewater and disposed of along with sewage, which posed environmental risks due to the organic compounds present in whey. In cheese production, a substantial amount of whey is generated. For every ten parts of milk used, nine parts result in whey, and only one part becomes cheese. However, thanks to extensive research and studies in this field, the utilization of whey and its by-products has significantly improved. Whey can be derived from various types of milk, including cow's milk, sheep's milk, goat's milk, and even camel milk. This widens the possibilities for processing and utilizing whey. One notable development is the increased production of whey powder in the European Union. The production volume rose from 1,950,000 tons in 2011 to 2,200,000 tons in 2014. This signifies the growing recognition of whey as a valuable resource and the implementation of more sustainable practices in the dairy industry. Overall, the improved understanding and utilization of whey, along with the development of whey-derived products, have significantly reduced waste and allowed for more efficient and sustainable use of this by-product (Association, 2015).

In recent years, whey and whey preparations have become much more popular. The so-called "forgotten" is whey and whey preparations, which have been "rediscovered" and employed more frequently and productively by various food industry manufacturing facilities due to their special features. Customers who are aware of the importance of whey preparations in proper human nutrition have also gladly purchased them. The food processing industry has a long-standing inclination to swap components in the formulations of numerous goods. Products for lacto-ovo-vegetarians or foods with decreased fat and sugar might be used to observe this scenario (Królczyk et al., 2016).

This practice has been observed for several years, particularly in the development of foods with reduced fat and sugar content, as well as products catering to vegetarians and individuals with lactose intolerance. Whey and its preparations can indeed serve as substitutes in these applications. The use of whey as a substitute ingredient has been reported to have positive impacts on both consumer health and the financial aspect of many companies. By incorporating whey or whey preparations into recipes, companies can reduce the costs of raw materials, consequently lowering overall production costs. Whey preparations can be employed as partial or complete replacements for ingredients such as milk powder, eggs, fat, sucrose, and even other proteins, offering cost-effective alternatives.

There are two main types of whey: sweet whey and acid whey. Sweet whey is a by-product of ripened cheese production and has a pH range of 5.8-6.6, while acid whey is obtained from cottage cheeses and typically has a pH range of 3.6-5.1. The regulations governing the use of whey in food products are based on the Codex Alimentarius, which is a set of international food standards established by the World Health Organization and the Food and Agriculture Organization of the United Nations. In summary, the utilization of whey and its preparations as substitutes in food products offers numerous advantages, including health benefits for consumers, cost reduction for companies, and adherence to international food regulations (Food et al., 2011).

Production

Since the 1920s, there have been efforts to concentrate whey using heat-drying methods. However, the resulting product had limitations in terms of its yellow-brownish color and composition, including a high content of lactose and denatured proteins. Additionally, the high costs associated with the process limited its commercial and technological viability. Innovation came with the implementation of a new process known as spray drying, which significantly reduced thermal degradation of whey components and decreased the associated costs of concentration. However, it was not until the 1970s, with the introduction of membrane filtration techniques, that an efficient separation and concentration of whey protein fractions while preserving their integrity became possible. Membrane filtration utilizes semipermeable surfaces (membranes) with specific pore sizes, allowing the permeate (liquid portion) to flow through while blocking the retentate (concentrated fraction) based on size or molecular weight. By combining successive filtration steps, different protein fractions with varying compositions and degrees of purity can be produced. These fractions can then undergo a spray-drying process to obtain a dry product known as whey protein concentrate (WPC). The use of membrane filtration technology enables selective concentration of proteins, resulting in whey protein concentrates with specific protein compositions and functionalities. This advancement has significantly improved the efficiency and quality of whey protein concentration processes, leading to the production of WPCs with various protein profiles for different applications in the food industry.

whey protein concentrates (WPCs) have varying protein concentrations, typically ranging from 35% to 80%, along with significant amounts of lactose and minerals. This composition makes

WPCs suitable for a wide range of applications in the food industry. However, for specific purposes requiring higher purity, further purification steps can be employed. Additional purification techniques, such as ion-exchange chromatography, can be utilized to achieve higher degrees of purity by reducing lactose content and further desalination. This process results in whey protein isolate (WPI) with a protein content of at least 90%. Moreover, advanced techniques like chromatography, partial hydrolysis, selective precipitation combined with centrifugation and dialysis, can be employed to obtain pure whey protein fractions with specific compositions. Currently, whey protein fractions find extensive applications in various fields. The nutrition industry is the most significant sector, where whey proteins are widely utilized. Additionally, there is growing interest in the pharmaceutical field due to the biological activities associated with enriched whey protein ingredients. In animal feed production, concentrated whey is commonly incorporated, and it is also used as a component in growing media for bacteria, yeast, and algae. While small quantities of concentrated whey may be used in human food, its high salt and lactose contents, coupled with a lower protein composition (10%), make it less attractive for direct consumption in this context. Overall, the purification and fractionation of whey proteins have enabled their diverse applications in nutrition, pharmaceuticals, animal feed, and other industries. The varying protein concentrations and purification levels allow for the utilization of whey proteins in different contexts based on their specific compositions and functionalities (Ramos et al., 2015).

Application of Whey in food industry

Whey finds significant use in the production of infant formulas and whey drinks in the food industry. Manufacturers of baby foods widely utilize whey preparations due to their high-quality protein content and the presence of active peptides. Establishing the appropriate ratio of whey proteins to casein is a standard procedure in the formulation of whey-based supplements for infants. The ideal ratio in whey-based supplements is 60:40, which matches the ratio found in breast milk. In contrast, cow's milk has a ratio of 20:80. Whey protein concentrates (WPCs) and demineralized whey powder are commonly used in these formulations. In infant formulas, the increased amount of amino acids is crucial, particularly for the nutrition of premature infants.

Specific amino acids such as lysine, methionine, and threonine are of particular importance. These essential amino acids play a vital role in supporting the growth and development of infants. However, it's worth noting that the percentage of phenylalanine, another essential amino acid, may be lower in whey-based supplements compared to human milk. This factor is particularly important for infants with phenylketonuria, a condition that requires strict control of phenylalanine intake. Overall, whey and whey preparations are valuable ingredients in the production of infant formulas and whey-based supplements. They provide high-quality protein, essential amino acids, and active peptides that support the nutritional needs of infants, including those born prematurely (Królczyk et al., 2016; Ramos et al., 2015).

Whey preparations are utilized as media in the microencapsulation of sensitive food ingredients such as fragrances, dyes, and various types of probiotic bacteria like Bifidobacterium BB-12. When sensitive active ingredients are microencapsulated using whey protein as a protective matrix, it helps preserve the properties of these ingredients over the long term. Whey protein acts as a barrier, shielding the encapsulated components from degradation and maintaining their functionality. Moreover, the microencapsulation process results in the formation of a powdered or granulated product. This allows for controlled release of the encapsulated component, which can have various applications as a food additive. The controlled release enables precise dosing and targeted delivery of the encapsulated ingredient, enhancing its effectiveness and versatility in food products. Overall, whey preparations serve as excellent media for microencapsulation, providing protection and controlled release of sensitive food ingredients. This technology expands the possibilities for incorporating fragrances, dyes, probiotics, and other active components into food products while preserving their properties and ensuring optimal functionality (Królczyk et al., 2016). Whey proteins are utilized as edible coatings for food, providing a protective layer with desirable properties. According to various sources, whey-based coatings demonstrate good mechanical properties and serve as effective barriers against lipids, aromatics, and especially oxygen. Fruits and vegetables can be successfully coated with whey protein coatings, which help extend their shelf life by providing a protective barrier against moisture loss and microbial contamination. Whey protein coatings act as a natural and edible layer that helps maintain the quality and freshness of the coated food products. Furthermore, whey can be utilized in the production of alcoholic beverages, such as whey beer, wine, and sparkling wine known as whey "champagne." These beverages typically have a low alcohol content, usually below 1.5%. They are primarily manufactured using whey permeate, which has a low protein content, along with the addition of yeast strains like Kluyveromyces fragilis or Saccharomyces lactis. Sweeteners and flavors can be optionally added to enhance the taste profiles. Whey serves as an ideal raw material for beer production due to its high mineral content, similar to broth, and its lactose content. The presence of lactose in whey can contribute to the color and flavor of the final beer through the Maillard reaction, much like roasted malt. Overall, whey proteins find multiple applications in the food industry, including as edible coatings for fruits and vegetables and as a raw material for the production of low alcohol whey-based alcoholic beverages. These applications showcase the versatility and potential benefits of utilizing whey in various food products (Rittmanic, 2006).

2.7. Recommended protein/amino acids Intake

Based on short-term nitrogen balance studies, the Recommended Dietary Allowance of protein for a healthy adult with minimal physical activity is currently 0.8 g protein per kg body weight (BW) per day. To meet the functional needs such as promoting skeletal-muscle protein accretion and physical strength, dietary intake of 1.0, 1.3, and 1.6 g protein per kg BW per day is recommended for individuals with minimal, moderate, and intense physical activity, respectively (Lonnie et al., 2018). Long-term consumption of protein at 2 g per kg BW per day is safe for healthy adults, and the tolerable upper limit is 3.5 g per kg BW per day for well-adapted subjects. Chronic high protein intake (>2 g per kg BW per day for adults) may result in digestive, renal, and vascular abnormalities and should be avoided (Bartholomae & Johnston, 2023). High-quality proteins from animal products (such as lean meat and milk) are essential due to their complete amino acid profiles, which are vital for optimal health (Lonnie et al., 2018). These proteins contain all essential amino acids required by the body, particularly leucine, which is critical for muscle protein synthesis (King’s College, 2023).

Endogenous proteins are constantly being made and destroyed. Daily protein or amino acid turnover is approximately 300 g, which is about 3–4 times greater than the mean intake in the general population. The intracellular amino acid pool is made up of amino acids that come from both endogenous proteins and exogenous sources (food). Functional restrictions are linked to a quantitative breakdown of functional proteins (such as serum proteins and muscle proteins) to produce amino acids. Protein synthesis in the human body requires the intracellular availability of 20 distinct L amino acids. A total of eleven of these so-called "proteinogenic" amino acids—alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glutamic acid, glycine, proline, serine, and tyrosine can be synthesized endogenously in the human body. The indispensable [previously: essential] amino acids, also known as histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, are nine amino acids that must be consumed regularly. With the exception of lysine and threonine, metabolic requirements for essential amino acids can also be met by consuming the corresponding keto acids, which can then be transaminated in the body to produce the necessary amino acid. Histidine is currently only proven to be essential for infants; it is still unclear whether this holds true for healthy adults (Richter et al., 2019).

Food proteins range in nitrogen content from 15% to 24%. A general nitrogen percentage of 16% is used to calculate the nitrogen content. The conversion factor as a result is 6.25 g nitrogen per g protein. The basic building blocks of proteins are carbon, hydrogen, and oxygen, although they can also contain Sulphur or selenium (Marsh & Donohue, 1967). **Strictly speaking, there is no requirement for protein, but for nitrogen and the 9 indispensable amino acids**. Though protein is, indeed, the quantitatively most important source of nitrogen and amino acids in daily nutrition, reference values for daily protein intake were developed mainly for practical reasons. It can be assumed that consumption of a variety of protein sources meets the requirements for all essential amino acids since reference values are generated using mean protein requirements and a variance coefficient (Richter et al., 2019).

FAO/WHO recommended EAA composition

The amino acid composition of human milk is recommended for predicting the protein. Scoring patterns developed and published in the FAO/WHO/UNU (2007) report are recommended for age groups other than infants, and values for six-months-on are given in Table 2. Small calculation errors were found in the table given in the 2007 report for the three-to-10-year age group and these have been corrected in the present table.

• Infants (birth to 6 months), pattern of breast milk.

• Young children (6 months to 3 y), pattern for the 0.5 y old infant.

• Older children, adolescents and adults, pattern for the 3 to 10 y old child.

For regulatory purposes, two scoring patterns are recommended, the amino acid composition of human milk for infant formulas and for all other foods and population groups the pattern for young children (6 months to 3 y); refer to Table 3 in this report.

Table.3: Amino acid scoring patterns for toddlers, children, adolescents and adults (amended values from the 2007 WHO/FAO/UNU report) (World Health et al., 2007)

			His	Ile	Leu	Lys	SAA	AAA	Thr	Trp	Val
Tissue amino acid Pattern (mg/g protein)[1]			27	35	75	73	35	73	42	12	49
Maintenance amino acid pattern (mg/g protein)[2]			15	30	59	45	22	38	23	6	39
Protein requirement (g/kg/d)											
Age (Years)	**Maintenance**	**Growth[3]**	amino acid requirements (mg/kg/d)[4]								
0 to 5	0-66	0.46	22	36	73	63	31	59	35	9.5	48
1 to 2	0-66	0.2	15	27	54	44	22	40	24	6	36
3 to 10	0-66	0.07	12	22	44	35	17	30	18	4.8	29
11 to14	0-66	0.07	12	22	44	35	17	30	18	4.8	29
15 to 18	0-66	0.04	11	21	42	33	16	28	17	4.4	28
>18	0-66	0	10	20	39	30	15	25	15	4	26
			Scoring pattern mg/g protein requirement[5]								
0 to 5			20	32	66	57	27	52	31	8.5	43
1 to 2			18	31	63	52	25	46	27	7	41
3 to 10			16	30	61	48	23	41	25	6.6	40
11 to14			16	30	61	48	23	41	25	6.6	40
15 to 18			16	30	60	47	23	40	24	6.3	40
>18			15	30	59	45	22	38	23	6	39

1. Amino acid composition of whole-body protein.
2. Adult maintenance pattern.
3. Calculated as average values for the age range: growth adjusted for protein utilization of 58%.
4. Sum of amino acids contained in the dietary requirement for maintenance (maintenance protein x the adult scoring pattern) and growth (tissue deposition adjusted for a 58% dietary efficiency of utilization x the tissue pattern).
5. Amino acid requirements/protein requirements for the selected age groups. Note that these values, some of which are slightly amended from the 2007 report, are the correctly calculated values. In the published report, the value for the SAA requirement for children aged 3-10 is incorrect (18mg/kg/d) as are the SAA patterns for infant preschool and school children up to 10, (28, 26 and 24 mg/g protein).

Two main factors contribute to the likelihood that amino acid intakes from breast milk exceed the actual demand of infants. Firstly, various calculations suggest that the estimated demand for amino acids in newborns is lower than the amino acid intakes from breast milk. The FAO/WHO/UNU (2007) report indicates that the individual amino acid values in the requirement pattern at 6 months, derived from a maintenance and growth factorial model, are on average 30% lower than the intakes from breast milk. Secondly, the true ileal digestibility of amino acids in human breast milk in infants is not precisely known and may be less than 100%. Research using bottle-fed piglets as a model for human infants has shown a range of 81% to 100% for the digestibility of amino acids in human milk. This suggests that not all amino acids in breast milk may be fully absorbed and utilized by the infant's body. Despite these uncertainties, breast milk from a healthy and well-nourished mother is considered to meet the protein requirements of infants during the first six months of life. Therefore, the amino acid content of breast milk is currently recommended as the best estimate of amino acid requirements for this age group. Correcting the amounts of amino acids in human breast milk for the true ileal digestibility of amino acids in infants can provide valuable information about the amino acid pattern required by the infant. This adjustment helps account for any differences between the amino acid composition of breast milk and the actual amino acid needs of infants, allowing for a more accurate estimation of their requirements (World Health et al., 2007).

The 2007 WHO/FAO/UNU Expert Consultation extensively reviewed the available amino acid requirement values for infants, children, and adults, as well as the methodologies used to derive them. Based on their analysis, the committee endorsed the recommendation of using the amino acid content of breast milk as the best estimate for infant amino acid requirements. However, they were unable to identify reliable requirement values for any other age groups except for adults. Regarding the values for preschool children, the committee found them difficult to interpret. These values had not undergone peer review and were derived from a report that provided incomplete information about their origin. The limited details provided, such as for lysine, indicated nitrogen accretion rates that were several times higher than expected for children of that age. These values more closely resembled the needs of a 3–6-month-old infant rather than those of a preschool child, whose growth rates are significantly lower than those observed in infants. Therefore, the committee decided to adopt a factorial approach for infants and children, based on amino acid requirements for maintenance and growth. They assumed that the maintenance amino acid pattern is the same at all ages on a mg/kg body weight basis, so they adopted the adult requirement pattern. For growth, they assumed it reflects the amino acid pattern of human tissue protein. Using this approach, the committee derived amino acid requirement patterns for children aged 0.5, 1–2, 3–10, 11–14, 15–18 years, as well as for adults (Food & Agriculture Organization of the United, 2013).

Chapter 3: Materials and Methods

3.1. Sample characteristics

Protein powders:

1. Egg white protein powder (EWPP): Capriovus Kft., protein content 83 %
2. Whey Protein Isolate (WPI): Buda Family Kft., protein content 92 %

Legumes:

1. Green pea (Pisum sativum L) - frozen
2. Kidney beans (Phaseolus vulgaris L) - dry bean

Pottages:

1. Bean pottage (BP)
2. Bean pottage fortified with Egg White Protein Powder (BP+EWPP)
3. Bean pottage fortified with Whey Protein Isolate (BP+WPI)
4. Pea pottage (PP)
5. Pea pottage fortified with Egg White Protein Powder (PP+EWPP)
6. Pea pottage fortified with Whey Protein Isolate (PP+WPI)

Recipe for bean pottage:

The measured dry beans (1700 g) were soaked in water overnight. After pouring the soaking water, the vegetables were heat-treated on an electric hotplate at 90 oC for 4 hours by adding the required amount of cooking water. After the beans softened, we added the oil (100 g), milk (100 g) and flour (200 g). After thickening, it was heat treated for 15 minutes and then cooled. The composition of bean pottage can be seen in Table 4.

The prepared vegetables were pureed with a blender and then divided into 3 portions. The first portion is unfortified food. We added the calculated amount of egg white powder to the second portion, and whey protein powder to the third, so that their protein content was 12.5%. With the additions, we homogenized the vegetables again.

Table 4: Composition of bean pottage (g/100g)

	Mass g	Energy kcal	Protein g	Fat g	Carbohydrate g	Fiber g	Salt g	Sugar g
Bean pottage	100	137,31	7,54	0,37	21,85	2,43	0,02	0,05

Recipe for green Pea pottage:

The green peas (4500 g) were boiled with enough water to cover them. Salt and sugar were added to the water and boiled until the peas were tender. The flour (120 g) was fried in oil (150 g) in a pan and mixed with 1-2 tablespoons of cooking stock and milk (900 g), and then mixed with the cooked peas. After mixing, it was cooked for another 3-5 minutes until the right thickness. The composition of pea pottage can be seen in Table 5.

Table 5: Composition of pea pottage (g/100g)

	Mass g	Energy kcal	Protein g	Fat g	Carbohydrate g	Fiber g	Salt g	Sugar g
Pea pottage	100	112,12	5,06	2,89	10,98	0,11	0,01	1,47

The prepared vegetables were pureed with a blender and then divided into 3 portions. The first portion is unfortified food. We added the calculated amount of egg white powder to the second portion, and whey protein powder to the third, so that their protein content was 12.5%. With the additions, we homogenized the vegetables again.

3.2. Protein determination by Kjeldahl method

Protein content was determined by Kjeldahl method (Kjeldahl 1883), which consists of three steps.

• Digestion- to digest bonds that hold polypeptides together and convert them to simpler molecules such as water, carbon dioxide and ammonia. To perform the process, measured cheese sample was added to an empty digestion tube with addition of 25 mL of sulfuric acid (H_2SO_4) one spoon of potassium sulfate (K_2SO_4) and one spoon of copper sulfate ($CuSO_4$).

Digestion was performed at 420 0C for 30- 60 min, after that the digested tubes were cooled to 50-60 0C before addition of 250 mL of water.

• Distillation- in cooled digested samples 100 mL of 33% NaOH was added before samples were placed in the distilling unit, and the process continued until ~ 100 mL of distillate was collected.

• Titration- of the distillate was done by adding 2-3 drops of indicator and then titration with 0.1 M NaOH

Three replicates from each sample were analyzed.

3.3. Amino acid determination

To evaluate amino acids, 500–700 mg of homogenized samples were hydrolyzed in a closed hydrolyzing vessel (KUTESZ, Budapest, Hungary) at 110°C for 24 hours in a block thermostat with 10 mL 6 M L^{-1} HCl under nitrogen atmosphere (FALC Instruments, Treviglio, Italy). In a 25 ml volumetric flask, neutralization was accomplished by adding 10 ml of 4 M L^{-1} NaOH to the hydrolyzed sample and then filling the flask with buffer (pH 2.2). The neutralized samples were filtered through 0.25 µm membrane filter (Nalgene, Rochester, USA).

Amino acids were determined using an Automatic Amino Acid Analyzer AAA400 (Ingos Ltd., Prague, Czech Republic) equipped with a cation-exchange column (Ionex Ostion LCP5020 22 3 0.37 cm). Stepwise gradient elution with sodium buffer systems was used for separation. After post-column derivatization with a ninhydrin reagent, colorimetric detection was carried out at 570 and 440 nm. Three parallel samples were prepared for all analyses.

The parameters of INGOS AAA 400:

- Cation exchange column: Ionex Ostion LCP 5020, column size: 200 x 3.7 mm,
- Column temperature: 50 °C-60 °C
- Reaction temperature:120 °C
- Analysis time: 200 min
- Eluent: Li citrate buffers (Li citrate, LiCl and citric acid)
- Sample volume:100 µl

- Detection: 440 nm, 570 nm
- Eluent flow rate: 0.30 ml/min
- Ninhydrin flow rate: 0.25 ml/min
- Detection limit: 0.5 µmol/l

The assay is carried out in a strongly acidic medium, with a series of eluents of gradually weakening acidity, with step gradient elution (buffer 1: 0.18 M Li citrate, pH 2.80; buffer 2: 0.20 M Li citrate, pH 3.05; buffer 3: 0.36 M Li citrate, pH 3.35; buffer 4: 0.33 M Li citrate, pH 4.05; buffer 5: 1.20 M Li citrate, pH 4.65). Amino acids are detected spectrophotometrically on the basis of their color reaction with ninhydrin. The color reaction of proline is different from that of the other amino acids, therefore it is detected at 440 nm, while the other amino acids can be detected at 570 nm. The color reaction takes place by post-column derivatization at 120 °C. Chromatograms were evaluated using the CHROMuLAN082 program, by comparison with standard amino acid mixtures.

3.4. Amino acid score calculation

The amino acid score, also known as the chemical score, was calculated using formula in Eq. 1, expressed either as a ratio to unity (recommended) or on a percentage scale (FAO, 2018).

$$Amino\ acid\ score = \frac{mg\ of\ amino\ acid\ in\ 1\ g\ of\ test\ protein}{mg\ of\ amino\ acid\ in\ 1\ g\ of\ requirement\ pattern}$$

FAO (2018): Protein quality assessment in follow-up formula for young children and ready to use therapeutic foods. Report of the FAO Expert Working Group 6–9 November 2017, Rome, Italy: Accessed September 12, 2021 ca2487en.pdf (fao.org).

Chapter 4: Results and discussions

4.1. Protein content

Based on the experiments results all the samples had notable differences in protein content. The protein content of both egg white protein powder (EWPP) and whey protein isolate (WPI) declared by the manufacturer (83% and 91.7%, respectively), was the same as measured values by Kjeldahl methods. Kidney beans pottage 7.65%. green pea pottage had the lowest amount of protein content of 5.03%. This evaluation is consistent with earlier studies, where protein powders like whey were found to offer higher protein content and better bioavailability than plant-based sources (Ajomiwe et al., 2024) Similar findings were reported by (Ohanenye et al., 2022), showing that unfortified legume-based dishes typically contain lower protein content due to the presence of anti-nutritional factors that inhibit digestibility.

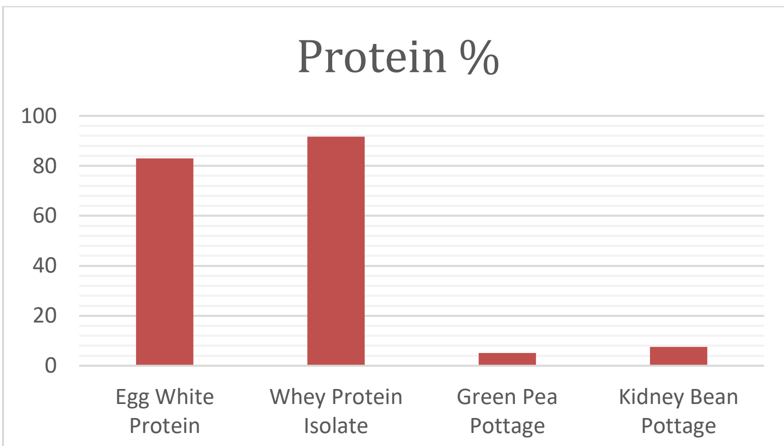

Figure 1: Protein (%) of pottages (PP & BP) and protein powders (EWP & WPI).

Kidney-Bean pottage

Table 4: Composition of fortified bean pottages

Quantity (g) to be added **to 100 g pottage**		**Protein g**
EWPP	**7.10**	5,89
Fortified pottage g	107.10	13.43
BP+EWPP	**100.00**	**12.50**
WPI	**6,26**	5,74
Fortified pottage g	106,26	13,28
BP+WPI	**100,00**	**12,50**

Table 4. represents the sample protein content of Bean Pottages per 100g was 7.54g/100g but when we added 7.10 g of Egg-white powder in it, overall protein content increased to 12.50g/100g. With the same goal for whey, when we added 6.26g of whey protein isolate to bean pottage sample, the protein content was increased from 7.54g/100g to 12.50g/100g. So, the protein content of bean pottage was increase by 65.7% with EWPP and with WPI both.

Green Pea Pottage

Table 5: Composition of fortified pea pottages

Quantity (g) to be added **to 100 g pottage**		**Protein g**
EWPP	**10,55**	8,76
Fortified pottage g	110,55	13,82
PP+EWPP	**100,00**	**12,50**
WPI	**9,40**	8,62
Fortified pottage g	109,40	13,68
PP+WPI	**100,00**	**12,50**

Table 5. represents the sample of Pea Pottages in which protein content per 100g was 5.06g but when we added 10.55 g of Egg-white powder it, overall protein content increased to 12.50g/100g. With the same goal for whey, when we added 9.40g of whey protein isolate to bean pottage sample, the protein content was increased from 5.06g/100g to 12.50g/100g. So, the protein content of Pea Pottage was increase by 147.03% with EWPP and with WPI both. Our aim was to increase the protein content to 12,5%. The target protein content of 50 g protein intake per meal calculated for 400 g meal portions was achieved.

4.2. Amino Acid Content

Table 6: Comparison of Essentiial and Non-Essential AAs content of unfortified and fortified sample.

AA composition		EWPP	WPI	BP	BP+EWPP	BP+WPI	PP	PP+EWPP	PP+WPI
			(mg AA/g sample)						
Essential (BCAA)	HIS	19.81	16.67	2.24	3.14	2.65	1,24	2.26	2.17
	LYS	53.37	82.47	5.12	8.03	9.88	2,65	12.52	12.83
	MET	31.33	19.61	0.71	2.88	1.86	0.54	2.86	2.93
	CYS	22.59	19.27	0.67	2.13	2.02	0.16	2.19	2.20
	PHE	42.42	25.41	4.1	6.48	5.17	1.78	4.32	4.27
	TYR	27.63	17.58	2.07	3.67	2.96	1.21	3.43	3.07
	THR	38.67	61.01	3.55	5.89	7.36	2.77	8.16	8.6
	TRP	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	ILE *	25.74	34.6	2.07	3.71	4.52	1.1	5.29	5.29
	LEU *	72.81	102.07	7.05	11.56	12.75	3.33	15.18	15.77
	VAL *	43.88	42.24	3.58	7.2	6.12	2.69	6.26	6.32
	Summa	**378.25**	**420.93**	**31.16**	**54.69**	**55.29**	**13.58**	**62.47**	**63.44**
Non-essential	ASP	82.04	92.08	8.6	13.74	13.32	5.83	11.5	12.3
	SER	56.25	42.44	5	8.33	7.12	3.26	7.92	4.67
	GLU	132.69	204.94	16	23.29	26.23	31.21	21.49	20.89
	PRO	18.84	61.74	2.89	5.63	7.32	2.44	5.7	6.7
	GLY	39.24	19.6	3.56	5.53	4.38	1.6	4.6	3.39
	ALA	48.15	48.86	3.15	7.01	5.63	2.88	8.01	8.08
	ARG	50.35	15.72	5.02	7.63	5.12	3.85	5.6	4.37
	Summa	**427.56**	**485.38**	**44.22**	**71.16**	**69.12**	**51.07**	**64.82**	**60.39**
Protein content (mg AA/g sample)		805.80	906.32	75.39	125.58	124.42	50.56	124.30	123.83
protein (%)		80.58	90.63	7.55	12.56	12.44	5.06	12.43	12.38

Table 8. represents that the EWPP used has a protein content of 80.58%. The protein contains 378.25 mg/g of essential amino acids. It contains the largest amount of Leucine (72.81 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 142.43 mg/g. The aromatic amino acid content (Phe+Tyr) is 70.05 mg/g. The non-essential amino acid content is 427.56 mg/g. It contains the largest amount of glutamine (132.69 mg/g). The content of aspartic acid is also high: 82.04 mg/g. The WPI used has a protein content of 90.63%. The protein contains 420.93 mg/g of essential amino acids. It contains the largest amount of Leucine (102.07 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 178.91mg/g. The aromatic amino acid content (Phe+Tyr) is 42.99 mg/g (less than EWP). The non-essential amino acid content is 485.38 mg/g. It contains the largest amount of glutamine (204.94 mg/g). The content of aspartic acid is also high: 92.08 mg/g.

The Bean Pottage (BP) used has a protein content of 7.55%. The protein contains 31.16 mg/g of essential amino acids. It contains the largest amount of Leucine (7.05 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 12.7 mg/g. The aromatic amino acid content (Phe+Tyr) is 6.17 mg/g. The non-essential amino acid content is 44.22 mg/g. It contains the largest amount of glutamine (16 mg/g). The content of aspartic acid is also high: 8.6 mg/g. After fortification of BP with EW and WPI, Protein content increased to 12.56% and 12.44%, respectively. With EWP-EAAs 54.69 mg/g that contains largest amount of leucine (11.56 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 22.47mg/g. The aromatic amino acid content (Phe+Tyr) is 10.5 mg/g. The non-essential amino acid content is 71.16 mg/g. It contains the largest amount of glutamine (23.29 mg/g). The content of aspartic acid is also high: 13.74 mg/g. BP with WPI-EAAs 55.29 mg/g that contains largest amount of leucine (12.75 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 23.39 mg/g. The aromatic amino acid content (Phe+Tyr) is 8.13 mg/g. The non-essential amino acid content is 69.12 mg/g. It contains the largest amount of glutamine (26.23 mg/g). The content of aspartic acid is also high: 13.32 mg/g.

The Pea Pottage (PP) used has a protein content of 5.06 %. The protein contains 13.58 mg/g of essential amino acids. It contains the largest amount of Leucine (3.33 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 7.12 mg/g. The aromatic amino acid content (Phe+Tyr) is 2.99 mg/g. The non-essential amino acid content is 51.07 mg/g. It contains the largest amount of glutamine (31.21 mg/g). The content of aspartic acid is also high: 5.83 mg/g. After fortification of PP with EW and WPI, Protein content increased to 12.43% and 12.38%, respectively. With EWP-EAAs 62.47 mg/g that contains largest amount of leucine (15.18 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 26.73 mg/g. The aromatic amino acid content (Phe+Tyr) is 7.75 mg/g. The non-essential amino acid content is 64.82 mg/g. It contains the largest amount of glutamine (21.49 mg/g). The content of aspartic acid is also high: 11.5 mg/g. PP with WPI-EAAs 63.44 mg/g that contains largest amount of leucine (15.77 mg/g), the total content of branched chain amino acids (Leu+Ileu+Val) is 27.38 mg/g. The aromatic amino acid content (Phe+Tyr) is 7.34 mg/g. The non-essential amino acid content is 60.36 mg/g. It contains the largest amount of glutamine (20.89 mg/g). The content of aspartic acid is also high: 12.3 mg/g.

4.3. Evaluation of the Amino acid composition of Proteins

The effect of the supplementation with protein powders on the amino acid composition of the bean and pea pottage products was investigated. The effect of the enrichment can be more clearly monitored if the composition is given in relation to the amount of amino acids in relation to the protein content of the sample. I have calculated the mg amino acid in g protein values, these data are shown in the Table 9.

After absorption, most of the amino acids are transported to the liver and converted there. BCAAs (leucine, isoleucine, valine), on the other hand, cannot be transaminated by the liver, so these amino acids provide the amino acid supply for other tissues (e.g., muscle, adipose tissue, brain). These three amino acids supply 35% of the amino acid content of muscle tissue. The amino acids that are degraded in the muscles become part of the muscles and also a source of energy for the muscle cells. Leucine enhances mTOR activity, thereby triggering protein synthesis in tissues, which can lead to muscle growth (Duan, 2018).

Leucine, isoleucine and valine reinforce each other's effects. It is important to maintain the required 2:1:1= LEU: VAL: ILE ratio for proper absorption. Therefore, we investigated the ratio and amounts of these amino acids in protein powders and fortified ready-to-eat foods. Ham et al. (2014) suggests that the amount of branched-chain essential amino acids is not the same in different sources of protein, and the raw materials and the bean pottage products were analysed. Among the supplemental protein sources, WPI contained the highest amounts of branched-chain amino acids (ILE: 38.19; LEU:112.61; VAL: 46.61 mg/g protein, ratios are LEU/ILEU:2,95; LEU/VAL: 2.41), including the highest amount of leucine. As regard the EWPP ((ILE: 31,95; LEU: 90,38; VAL: 54,44 mg/g protein, ratios are LEU/ILEU:2.82; LEU/VAL: 1.66). In case of bean pottage (B) (ILE: 27.52; LEU: 93,52; VAL: 47,44 mg/g protein, ratios are LEU/ILEU: 3,35; LEU/VAL: 1,97. According to data of pea pottage(P) (ILE: 21.76; LEU: 65.85; VAL: 53.28 mg/g protein, ratios are LEU/ILEU: 3,02; LEU/VAL: 1,23.The addition of WPI to the bean pottage improves the composition in terms of ratio (ILE: 36,34; LEU:102,51 VAL: 49,19 mg/g protein, ratios are LEU/ILEU:2.82; LEU/VAL: 2.08). However, in the bean pottage fortified with egg white protein powder (ILE: 29.55; LEU:92,08; VAL: 57,32 mg/g protein, ratios are LEU/ILEU:3.11; LEU/VAL:1.61), The addition of WPI to the Pea pottage improves the composition in terms of ratio (ILE: 42.68; LEU:127.35 VAL: 51.04 mg/g protein, ratios are LEU/ILEU:2.98; LEU/VAL:2.49). However, in the Pea pottage fortified with egg white protein powder (ILE: 42.59; LEU:122.16; VAL: 50.36 mg/g protein, ratios are LEU/ILEU:2.86; LEU/VAL:2.42).

In Pea Pottage (PP), the most abundant amino acids were glutamic acid (261.30 mg/g), followed by aspartic acid (115.41 mg/g), serine (64.43 mg/g), leucine (65.85 mg/g), arginine (76.16 mg/g) and lysine (52.42 mg/g). However, methionine 10.36 mg/g and cysteine (3.23 mg/g), sulfur-containing essential amino acids, were relatively low at, which may limit the protein quality of Pea Pottage. In addition to meth+cys, Izoleucine was one of the very low 21.76 mg/g. Proline (48.25 mg/g), was also found in lower concentrations. In Bean Pottage (BP), glutamic acid (212.28 mg/g) and aspartic acid (114.02 mg/g) were also the most abundant amino acids, similar to Pea Pottage. However, BP had higher levels of leucine (93.52 mg/g) and lysine (67.90 mg/g) compared to PP, Methionine (9.46 mg/g) was present at a lower concentration in BP than in PP, but unlike PP, cystine and Izoleucine in BP were 8.83 mg/g and 27.52 mg/g (relatively high), respectively. proline (38.30mg/g) remained low, Fortification with Egg White Protein (EWP) significantly improved the amino acid content in both pottages. In Pea Pottage + EWP, the most abundant amino acids were glutamic acid (172.91 mg/g), leucine (122.16 mg/g), aspartic acid (92.52 mg/g), and lysine (100.71 mg/g).

Table 7 : Amino Acid composition mg/g of unfortified and fortified bean pottage and pea pottage

AAs (mg/g Protein)	PP	BP	PP+EWP	PP+WPI	BP+EWP	BP+WPI
asp	115.41	114.02	92.52	99.30	109.39	107.06
thr	54.84	47.15	65.63	69.44	46.91	59.19
ser	64.43	66.35	39.58	37.71	66.29	57.19
glu	261.30	212.28	172.91	168.66	185.44	210.81
pro	48.25	38.30	45.82	54.11	44.86	58.83
gly	31.72	47.23	37.03	27.38	44.03	35.23
ala	56.99	41.80	64.46	65.22	55.84	45.23
val	53.28	47.44	50.36	51.04	57.32	49.19
cys	3.23	8.83	17.61	17.77	17.00	16.22
met	10.68	9.46	23.10	23.63	22.96	14.95
ile	21.76	27.52	42.59	42.68	29.55	36.34
leu	65.85	93.52	122.16	127.35	92.08	102.51
tyr	23.95	27.48	27.56	24.80	29.20	23.78
phe	35.18	54.39	34.73	34.51	51.56	41.59
lys	52.42	67.90	100.71	103.63	63.97	79.41
his	24.54	29.76	18.16	17.50	25.01	21.31
arg	76.16	66.57	45.07	35.26	58.60	41.15
Summa	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**

After Fortification, increase in amino acid content is represented by green color and red color represents the decrease in AAs content in protein.

The addition of EWP resulted in a notable increase in leucine and lysine. Methionine (23.10 mg/g) content also improved significantly, cysteine (17.61 mg/g), while improved, remained among the least abundant amino acids. Isoleucine was significantly, improved to 42.59 mg/g. In Bean

Pottage + EWP, trends were observed, with glutamic acid (185.44 mg/g), leucine (92.08 mg/g), aspartic acid (109.39 mg/g), and lysine (63.97 mg/g) being the most abundant amino acids. Similar to PP+EWP, fortification affected BP amino acid content for these amino acids by lowering than unfortified BP. Methionine (22.96 mg/g) content improved significantly, contributing to a better sulfur amino acid balance. However, cysteine (17.00 mg/g) remained low, but improved significantly and proline (44.86 mg/g) continued to be one of the least abundant amino acids. Fortification with Whey Protein Isolate (WPI) also resulted in significant improvements in amino acid content. In Pea Pottage + WPI, the most abundant amino acids were glutamic acid (168.66 mg/g), leucine (127.35 mg/g), aspartic acid (99.30 mg/g), and lysine (103.63 mg/g). WPI fortification provided a higher leucine content compared to EWP, making this combination particularly rich in this muscle-building amino acid. Methionine (23.63 mg/g) levels increased significantly, providing a more complete sulfur-containing amino acid profile. Cysteine (17.77 mg/g) remained among the least abundant amino acids, although its content improved with WPI fortification. In Bean Pottage + WPI, glutamic acid (210.81 mg/g), leucine (102.51 mg/g), aspartic acid (107.06 mg/g), and lysine (79.41 mg/g) were the most abundant amino acids, similar to the EWP-fortified version. Methionine (14.95 mg/g) content increased but not significantly as PP+EWP. Cysteine (16.22 mg/g) remained one of the least abundant amino acids, as did proline (58.83 mg/g), despite fortification with WPI. Overall, in case of PP and BP fortification with Egg-White protein and Whey Protein Isolate, some of the already abundant AAs such as arginine and glutamic acids comparatively with the unfortified form were decreased and some of the essential AAs which were in least amount like isoleucine, methionine etc. were significantly increase in comparison to their unfortified versions.

4.3. Essential Amino Acids content & Score

I have calculated the amino acid scores (AAS) of our samples by the optimal essential amino acid content of reference material as defined for adults by FAO/WHO (2013). Amino acids that reach a value of 1 or above are able to satisfy the protein requirements by the body (means they are complete proteins).

However, if one essential amino acid is missing from the dietary intake, the participation of other amino acids in protein synthesis is limited. An essential amino acid of the given protein is said to be limiting if it is present in the lowest percentage relative to the corresponding amino acid in the amino acid composition of the FAO/WHO reference protein (hen egg white protein).

The AAS values of the samples can be found in Table 10.

Table 8:Comparison of Essential amino acids mg/g protein of fortified and unfortified pottages with the reference protein.

EAAs Content mg/g Protein	**His**	**Ile**	**Leu**	**Lys**	**Meth+Cys**	**Phe+Tyr**	**Tre**	**Try**	**Val**
FAO Reference Protein	15.00	30.00	59.00	45.00	22.00	38.00	23.00	6.00	39.00
Bean pottage	29.76	27.52	93.52	67.90	18.29	81.87	47.15		47.44
AAS	1.98	0.92	1.59	1.51	0.83	2.15	2.05		1.22
Bean pottage+Egg white protein	25.01	29.55	92.08	63.97	41.18	80.76	46.91		57.32
AAS	1.67	0.99	1.56	1.42	1.87	2.13	2.04		1.47
Bean pottage + Whey Protein	21.31	36.34	102.51	79.41	31.17	65.38	59.19		49.19
AAS	1.42	1.21	1.74	1.76	1.42	1.72	2.57		1.26
Pea pottage	24.54	21.76	65.85	52.42	13.92	59.13	54.84		53.28
AAS	1.64	0.73	1.12	1.16	0.63	1.56	2.38		1.37
Pea pottage+Egg white protein	18.16	42.59	122.16	100.71	40.71	62.29	65.63		50.36
AAS	1.21	1.42	2.07	2.24	1.85	1.64	2.85		1.29
Pea pottage + Whey Protein	17.50	42.68	127.35	103.63	41.40	59.31	69.44		51.04
AAS	1.17	1.42	2.16	2.30	1.88	1.56	3.02		1.31

In the case of the protein powders, both protein supplements have essential amino acid values above 1, so as complete proteins they can be used to supplement certain plant protein sources. In leguminous plants, for example, Sulphur-containing amino acids (methionine, cysteine) are the limiting amino acids, the deficiency of which should be properly compensated by egg white protein powder $AAS_{MET+CYS}$: 3,04 and whey protein powder $AAS_{MET+CYS}$:1.95, as they have an exceptionally high $AAS_{MET+CYS}$ value. Indeed, the $AAS_{MET+CYS}$ value of bean pottage: $AAS_{MET+CYS}$ 0.83 improved in B+EWPP and B+WPI products $AAS_{MET+CYS}$: 1.87; 1.42, respectively. And the $AAS_{MET+CYS}$ value of Pea pottage: $AAS_{MET+CYS}$ 0.63 improved in B+EWPP and B+WPI products $AAS_{MET+CYS}$: 1.85; 1.88, respectively. The AAS value for isoleucine (0.92) in the case of bean pottage is also below 1. The AAS value calculated for isoleucine in the egg white protein powder is 1,25. This is 37,5 mg ILE/g protein compared to the required 30 mg ILE/g protein, which is not sufficient to complete the amino acid of legume, since the ILE content of B+EWPP product was only 29.55 mg ILE/g protein, the AAS_{ILE} value remained below 1 (0.99). Whey protein contains 38,19 mg ILE/g protein, with an AASILE value of 1.27. This excess already successfully completes the isoleucine content of the bean pottage (36,34 mg ILE/g protein), where the AAS_{ILE} value is now 1.21.

The AAS value for isoleucine (0.73) in the case of Pea pottage is also below 1. Even though the ILE content of EWP powder is not sufficient to complete the AAS_{ILE} of Bean pottage but in Pea pottage, ILE content of P+EWPP product was 42.59 mg ILE/g protein, the AAS_{ILE} value of above 1 (1.42) successfully completing the ILE content of Pea pottage. Whey protein contains 38,19 mg ILE/g protein, with an AAS_{ILE} value of 1.27. This excess already successfully completes the isoleucine content of the Pea pottage (42.68 mg ILE/g protein), where the AAS_{ILE} value is now 1.42.

As regard the above-mentioned results, we can conclude that the maximum protein utilization of 83% can be expected for bean pottage (based on the lowest AAS_{CYS} 0.83), and for B+EWPP product this rate increased to 99% (AAS_{ILE} 0.99). In case of B+WPI, there is no any obstruction against the utilization of absorbed amino acids, with a minimum AAS_{ILE} of 1.21, the amino acids ingested and absorbed in the diet, when fortified with endogenous amino acids, allow the synthesis of additional protein molecules. Similarly, we can conclude that the maximum protein utilization of 63% can be expected for Pea pottage (based on the lowest AAS_{CYS} 0.63), and for P+EWPP product this rate increased to 73% (AAS_{ILE} 0.73). In case of P+WPI, there is no any obstruction against the utilization of absorbed amino acids, with a minimum AAS_{ILE} of 1.42, the amino acids ingested and absorbed in the diet, when fortified with endogenous amino acids, allow the synthesis of additional protein molecules.

4.4. Discussion

Fortification of ready meals like kidney beans pottage and green pea pottage successfully increased the protein content, amino acids content and amino acid scores more or equal to the recommended value of FAO. However, the values of amino acid scores are near to FAO values in case of EWP (egg-white protein) and higher than FAO value when fortified with protein. So, it can be stated EWP and WP fortification can be used to achieve the complete Plants based protein meal. But specifically, EWP is more beneficial if the goal is to achieve complete protein meal but WPI fortification is more beneficial if the goal is to get excess overall protein content. Specifically, in case of pea pottage, both EWP and WPI, had nearly same results for limiting amino acids (isoleucine, methionine+cystine) but in BP, EWP didn't stand that good because it didn't increase the content of isoleucine much, the attained value was almost same as FAO value. WPI stand quite good with the Bean pottage fortification attaining all the goals.

References

Abeyrathne, E. N. S. L. H. Y., & Ahn, D. U. (2013). Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. *Poultry Science*, *92*(12), 3292-3299.

Adhikari, S., Schop, M., de Boer, I. J., & Huppertz, T. (2022). Protein quality in perspective: a review of protein quality metrics and their applications. *Nutrients*, *14*(5), 947.

Ajomiwe, N., Boland, M., Phongthai, S., Bagiyal, M., Singh, J., & Kaur, L. (2024). Protein nutrition: Understanding structure, digestibility, and bioavailability for optimal health. *Foods*, *13*(11), 1771. https://doi.org/10.3390/foods13111771

Allen, L., de Benoist, B., Dary, O., & Hurrell, R. (2006). *Guidelines on food fortification with micronutrients*. World Health Organization. https://www.who.int/publications/i/item/9241594012

Association, E. E. W. P. (2015). Key Whey Figures. Available at: http://ewpa.euromilk.org/aboutewpa/facts-fi gures.html.

Baláž, M. (2014). Eggshell membrane biomaterial as a platform for applications in materials science. *Acta biomaterialia*, *10*(9), 3827-3843.

Barik, S. (2020). The Uniqueness of Tryptophan in Biology: Properties, Metabolism, Interactions and Localization in Proteins. *Int J Mol Sci*, *21*(22). https://doi.org/10.3390/ijms21228776

Bartholomae, E., & Johnston, C. S. (2023). Nitrogen balance at the recommended dietary allowance for protein in minimally active male vegans. *Nutrients*, *15*(14), 3159. https://doi.org/10.3390/nu15143159

Beauman, J. G. (2005). Genital herpes: A review. *American Family Physician*, *72*(8), 1527-1534.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2015). *Biochemistry*. W.H. Freeman and Company.

Bhutta, Z. A., Das, J. K., Rizvi, A., Gaffey, M. F., Walker, N., Horton, S., & Black, R. E. (2013). Evidence-based interventions for improvement of maternal and child nutrition: What can be done and at what cost? *The Lancet*, *382*(9890), 452-477. https://doi.org/10.1016/S0140-6736(13)60996-4

Blomstrand, E. (2012). A role for branched-chain amino acids in reducing central fatigue. *Journal of Nutrition*, *136*(2), 544S-547S. https://doi.org/10.1093/jn/136.2.544S

Branden, C. I., & Tooze, J. (2012). Introduction to Protein Structure. https://doi.org/10.1201/9781136969898

Brestenský, M., Nitrayová, S., Patráš, P., & Nitray, J. (2019). Dietary Requirements for Proteins and Amino Acids in Human Nutrition. *Current Nutrition & Food Science*, *15*(7), 638-645. https://doi.org/10.2174/1573401314666180507123506

Brosnan, J. T. (2003). Interorgan amino acid transport and its regulation. *The Journal of Nutrition*, *133*(6), 2068S-2072S. https://doi.org/10.1093/jn/133.6.2068S

Calvo, M. S., & Whiting, S. J. (2003). Prevalence of vitamin D insufficiency in Canada and the United States: Importance to health status and efficacy of current food fortification and dietary supplement use. *Nutrition Reviews*, *61*(3), 107-113. https://doi.org/10.1301/nr.2003.marr.107-113

Chang, C. L. X. L. J. N. F. Z. M. Z. B., & Yang, Y. (2017). Effect of enzymatic hydrolysis on characteristics and synergistic efficiency of pectin on emulsifying properties of egg white protein. *Food Hydrocolloids*, *65*, 87-95.

D'Mello, J. P. F. (2003). *The role of methionine on metabolism, oxidative stress, and diseases*

Amino acids in animal nutrition. CABI Publishing. https://doi.org/10.1007/s00726-010-0502-x

Daba, S. D., & Morris, C. F. (2022). Pea proteins: Variation, composition, genetics, and functional properties. *Cereal chemistry*, *99*(1), 8-20.

Dimou, A., Tsimihodimos, V., & Bairaktari, E. (2022). The critical role of the branched-chain amino acids (BCAAs) catabolism-regulating enzymes, branched-chain aminotransferase (BCAT) and branched-chain α-keto acid dehydrogenase (BCKD), in human pathophysiology. *International Journal of Molecular Sciences*, *23*(7), 4022. https://doi.org/10.3390/ijms23074022

Dzikunoo, J. A. G. S., & Saalia, F. K. (2015). Effect of methods of extraction on physicochemical properties of soy proteins (tofu). *American Journal of Food Science and Nutrition Research*, *2*(5), 138-144.

Duan Y., Tan B., Li J., Liao P., Huang B., Li F., Xiao H., Liu Y., Yin Y. (2018): Optimal branched-chain amino acid ration improves cell proliferation and protein metabolism of porcine enterocytesin in vivo and in vitro. Nutrition (2018): 173-181 DOI: 10.1016/j.nut.2018.03.057.

Eadmusik, S. P. P., & Nitithamyong, A. (2013). Production of hard tofu from calcium fortified soybean milk and its chemical and sensory properties. *Asia-Pacific Journal of Science and Technology*, *18*(3), 371-379.

Elango, R., Ball, R. O., & Pencharz, P. B. (2009). Amino acid requirements in humans: With a special emphasis on the metabolic availability of amino acids. *Amino Acids*, *37*(1), 19-27. https://doi.org/10.1007/s00726-009-0234-y

Emmambux, M. N., & Taylor, J. R. (2013). Morphology, physical, chemical, and functional properties of starches from cereals, legumes, and tubers cultivated in Africa: A review. *Starch-Stärke*, *65*(9-10), 715-729.

Food, & Agriculture Organization of the United, N. (2013). *Dietary protein quality evaluation in human nutrition: Report of an FAO Expert Consultation*. FAO. https://www.fao.org/3/i3124e/i3124e00.htm

Food, Agriculture Organization of the United, N., & World Health, O. (2011). *Codex Alimentarius: Milk and milk products*. FAO & WHO. https://www.fao.org/publications/codex-alimentarius

Friedman, M., & Brandon, D. L. (2001). Nutritional and Health Benefits of Soy Proteins. *Journal of Agricultural and Food Chemistry*, *49*(3), 1069-1086. https://doi.org/10.1021/jf0009246

Gandhi, A. P. (2009). Quality of soybean and its food products. *International Food Research Journal*, *16*(1), 11-19.

Graham, P. H., & Vance, C. P. (2003). Legumes: Importance and constraints to greater use. *Plant Physiology*, *131*(3), 872-877. https://doi.org/10.1104/pp.017004

Guzeler, N., & Yildirim, C. (2016). The utilization and processing of soybean and soybean products. *Journal of Agricultural Faculty of Uludag University*, *30*, 546-553.

Ham D, Caldow M, Lynch G, Koopman RN(2014): Leucine as a treatment for muscle wasting: A critical review. Clinical Nutrition 33 (2014) 937-945- dx.doi.org/10.1016/j.clnu.2014.09.016

Hardeland, R., Reiter, R. J., Poeggeler, B., & Tan, D. X. (1993). The significance of the metabolism of the neurohormone melatonin: Antioxidative protection and formation of bioactive substances. *Neuroscience & Biobehavioral Reviews*, *17*(3), 347-357. https://doi.org/10.1016/S0149-7634(05)80016-8

Hartanti, A. T. R. G., & Hidayat, I. (2015). Rhizopus species from fresh tempeh collected from several regions in Indonesia. *HAYATI Journal of Biosciences*, *22*(3), 136-142.

Hawrylewicz, E., Zapata, J. J., & BlairWilliam, H. (1995). Soy and experimental cancer: animal studies. *The Journal of Nutrition*, *125*, 698S-708S.

Hayes, M. (2020). Measuring Protein Content in Food: An Overview of Methods. *Foods*, *9*(10). https://doi.org/10.3390/foods9101340

Holecek, M. (2018). Branched-chain amino acids in health and disease: Metabolism, alterations in blood plasma, and as supplements. *Nutrition & Metabolism*, *15*(1), 33. https://doi.org/10.1186/s12986-018-0271-1

Holeček, M. (2020). Histidine in Health and Disease: Metabolism, Physiological Importance, and Use as a Supplement. *Nutrients*, *12*(3), 848. https://doi.org/10.3390/nu12030848

Hou, Y., Yin, Y., & Wu, G. (2015). Dietary essentiality of “nutritionally non-essential amino acids” for animals and humans. *Experimental Biology and Medicine*, *240*(8), 997-1007.

Jooyandeh, H. (2011). Soy products as healthy and functional foods. *Middle-East Journal of Scientific Research*, *7*(1), 71-80.

Joshi, J. H., Kalainathan, S., Kanchan, D. K., Joshi, M. J., & Parikh, K. D. (2020). Effect of L-threonine on growth and properties of ammonium dihydrogen phosphate crystal. *Arabian Journal of Chemistry*, *13*(1), 1532-1550. https://doi.org/10.1016/j.arabjc.2017.12.005

Kagawa, A. (2017). *Standard Tables of Food Composition in Japan 2017 (Seventh revised version)*. Kagawa Education Institute of Nutrition.

Kałużna-Czaplińska, J., & Błaszczyk, A. (2017). The significance of tryptophan in the regulation of redox balance and immunity. *Metabolic Brain Disease*, *32*(5), 1287-1301. https://doi.org/10.1007/s11011-017-0045-x

King’s College, L. (2023). *Protein nutrition, healthy ageing and climate change: how do we combine the three?* https://www.kcl.ac.uk

Kreplak, J., Madoui, M.-A., Cápal, P., Novák, P., Labadie, K., Aubert, G., Bayer, P. E., Gali, K. K., Syme, R. A., & Main, D. (2019). A reference genome for pea provides insight into legume genome evolution. *Nature genetics*, *51*(9), 1411-1422.

Królczyk, J. B., Dawidziuk, T., Janiszewska-Turak, E., & Sołowiej, B. (2016). Use of Whey and Whey Preparations in the Food Industry—A Review. *Polish Journal of Food and Nutrition Sciences*, *66*(3), 157-165. https://doi.org/10.1515/pjfns-2015-0052

Kruger, C. L. (2009). The biological value of proteins: Nitrogen retention and utilization. *Journal of Nutritional Science and Vitaminology*, *55*(4), 287-295.

Le, & et al. (2016). Amino acid metabolism and essential amino acids. *Journal of Nutrition*, *45*(2), 123-130.

Leterme, P., Monmart, T., & Baudart, E. (1990). Amino acid composition of pea (Pisum sativum) proteins and protein profile of pea flour. *Journal of the Science of Food and Agriculture*, *53*(1), 107-110.

Lim, M. T., Pan, B. J., Toh, D. W. K., Sutanto, C. N., & Kim, J. E. (2021). Animal protein versus plant protein in supporting lean mass and muscle strength: A systematic review and meta-analysis of randomized controlled trials. *Nutrients*, *13*(2), 661. https://doi.org/10.3390/nu13020661

Lonnie, M., Hooker, E., Brunstrom, J. M., Corfe, B. M., Green, M. A., Watson, A. W., Williams, E. A., Stevenson, E. J., Penson, S., & Johnstone, A. M. (2018). Protein for Life: Review of Optimal Protein Intake, Sustainable Dietary Sources, and the Effect on Appetite in Ageing Adults. *Nutrients*, *10*(3), 360. https://doi.org/10.3390/nu10030360

Lopez, M. J., & Mohiuddin, S. S. (2020). Biochemistry, essential amino acids. In *StatPearls [Internet]*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557845/

Lynch, H., Johnston, C., & Wharton, C. (2018). Plant-based diets: Considerations for environmental impact, protein quality, and exercise performance. *Nutrients*, *10*(12), 1841. https://doi.org/10.3390/nu10121841

Marsh, R. E., & Donohue, J. (1967). Crystal structure studies of amino acids and peptides. *Advances in protein chemistry*, *22*, 235-256.

Matsuoka, R., & Sugano, M. (2022). Health Functions of Egg Protein. *Foods*, *11*(15). https://doi.org/10.3390/foods11152309

MedShun. (2021). *How L-Lysine Can Help Alleviate Cold Or Flu Symptoms*. https://www.medshun.com

Millward, D. J. (2012). Amino acid scoring patterns for protein quality assessment. *British Journal of Nutrition*, *108*(S2), S31-S43. https://doi.org/10.1017/S0007114512002462

Mine, Y. M. F. L. S. (2004). Antimicrobial peptides released by enzymatic hydrolysis of hen egg white lysozyme. *Journal of Agricultural and Food Chemistry*, *52*, 1088-1094.

Moro, J., Tomé, D., Schmidely, P., Demersay, T.-C., & Azzout-Marniche, D. (2020). Histidine: A Systematic Review on Metabolism and Physiological Effects in Human and Different Animal Species. *Nutrients*, *12*(5), 1414. https://doi.org/10.3390/nu12051414

Moughan, P. J., & Rutherfurd, S. M. (2012). Gut luminal endogenous proteins as a source of amino acids for the host: Implications for amino acid requirements in neonates. *British Journal of Nutrition*, *108*(S2), S329-S334. https://doi.org/10.1017/S0007114512002474

Nelson, D. L., & Cox, M. M. (2017). *Lehninger Principles of Biochemistry*. W.H. Freeman and Company.

Neubauer, J. (2021). A planetary health perspective on synthetic methionine. *The Lancet Planetary Health*, *5*(8), e379-e386. https://doi.org/10.1016/s2542-5196(21)00138-8

Ohanenye, I. C., Ekezie, F.-G. C., Sarteshnizi, R. A., Boachie, R. T., Emenike, C. U., Sun, X., Nwachukwu, I. D., & Udenigwe, C. C. (2022). Legume seed protein digestibility as influenced by traditional and emerging physical processing technologies. *Foods*, *11*(15), 2299. https://doi.org/10.3390/foods11152299

Omana, D. A. W. J., & Wu, J. (2010). Co-extraction of egg white proteins using ion-exchange chromatography from ovomucin-removed egg whites. *Journal of Chromatography B*, *878*(21), 1771-1776.

Preethi, R. S. D. M. J. A., & Anandharamakrishnan, C. (2020). Conductive hydro drying as an alternative method for egg white powder production. *Drying Technology*, *39*(3), 324-336.

Ramos, Ó. L., Pereira, R. N. C., Rodrigues, R. M. M., Teixeira, J., Vicente, A., & Malcata, F. (2015). Whey and whey powders: Production and uses.

Razi, S. M. F. H. A. S., & Rashidinejad, A. (2022). An overview of the functional properties of egg white proteins and their application in the food industry. *Food Hydrocolloids*. https://doi.org/108183

Richter, M., Baerlocher, K., Bauer, J. M., Elmadfa, I., Heseker, H., Leschik-Bonnet, E., Stangl, G., Volkert, D., Stehle, P., & on behalf of the German Nutrition, S. (2019). Revised Reference Values for the Intake of Protein. *Ann Nutr Metab*, *74*(3), 242-250. https://doi.org/10.1159/000499374

Rittmanic, S. (2006). US whey proteins in ready-to-drink beverages. *US Dairy Export Council*, 7-8.

Rutherfurd, S. M., Montoya, C. A., Zou, M. L., Moughan, P. J., & Drummond, K. J. (2016). Food protein quality evaluation using the Digestible Indispensable Amino Acid Score (DIAAS). *Nutrition Research Reviews*, *29*(1), 37-47. https://doi.org/10.1093/nutrit/nuw022

Sarwar, G. (1997). The Protein Digestibility–Corrected Amino Acid Score Method Overestimates Quality of Proteins Containing Antinutritional Factors and of Poorly Digestible Proteins Supplemented with Limiting Amino Acids in Rats1. *The Journal of Nutrition*, *127*(5), 758-764. https://doi.org/https://doi.org/10.1093/jn/127.5.758

Schaafsma, G. (2000). The Protein Digestibility–Corrected Amino Acid Score (PDCAAS)—A concept for describing protein quality in foods and food ingredients: A critical review. *Journal of Nutrition*, *130*(7), 1865S-1867S. https://doi.org/10.1093/jn/130.7.1865S

Schaafsma, G. (2005). The Protein Digestibility-Corrected Amino Acid Score (PDCAAS)--a concept for describing protein quality in foods and food ingredients: A critical review. *Journal of AOAC International*, *88*(3), 988-994.

Scheraga, H. A. (2014). *Protein structure*. Academic Press.

Semba, R. D., Ramsing, R., Rahman, N., Kraemer, K., & Bloem, M. W. (2021). Legumes as a sustainable source of protein in human diets. *Global Food Security*, *28*, 100520.

Smil, V. (1999). Nitrogen in crop production: An account of global flows. *Global Biogeochemical Cycles*, *13*(2), 647-662. https://doi.org/10.1029/1999GB900015

Stadelman, W. J. C. O. J. (2001). *Egg Science and Technology (4th ed.)*. Avi Publishing Company.

Starzyńska-Janiszewska, A. S. B., & Wikiera, A. (2015). Proteolysis in tempeh-type products obtained with Rhizopus and Aspergillus strains from grass pea (Lathyrus sativus) seeds. *Acta scientiarum polonorum Technologia alimentaria*, *14*(2), 125-132.

Tang, Q., Tan, P., Ma, N., & Ma, X. (2021). Physiological functions of threonine in animals: beyond nutrition metabolism. *Nutrients*, *13*(8), 2592.

Vickers, N. J. (2017). Animal communication: when I'm calling you, will you answer too? *Current Biology*, *27*(14), R713-R715.

Voet, D., & Voet, J. G. (2011). *Biochemistry*. Wiley.

World Health, O. (2006). *Guidelines on food fortification with micronutrients*. https://www.who.int/publications/i/item/9241594012

World Health, O., Food, Agriculture Organization of the United, N., & United Nations, U. (2007). *Protein and amino acid requirements in human nutrition*. World Health Organization. https://apps.who.int/iris/handle/10665/43411

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. *Amino Acids*, *37*(1), 1-17. https://doi.org/10.1007/s00726-009-0269-0

Wu, G. (2013). Functional amino acids in nutrition and health. *Amino Acids*, *45*(3), 407-411. https://doi.org/10.1007/s00726-013-1500-6

Wu, G., Bazer, F. W., Dai, Z., Li, D., Wang, J., & Wu, Z. (2014). Amino acid nutrition in animals: Protein synthesis and beyond. *Annual Review of Animal Biosciences*, *2*, 387-417. https://doi.org/10.1146/annurev-animal-022513-114113

Young, V. R., & Pellett, P. L. (1994). Plant proteins in relation to human protein and amino acid nutrition. *The American Journal of Clinical Nutrition*, *59*(5), 1203S-1212S. https://doi.org/10.1093/ajcn/59.5.1203S

Zhang, T. Z. J. Y. H. Y. Y. Z. P., & Liu, J. (2011). Purification technology and antimicrobial activity analysis of antimicrobial peptides from ovalbumin. *Chemical Research in Chinese Universities*, *27*, 361-365.

Zimmermann, M. B. (2009). Iodine deficiency. *Endocrine Reviews*, *30*(4), 376-408. https://doi.org/10.1210/er.2009-0011

DECLARATION

Ukasha Abdul Rehman (student Neptun code: H2W0UA) as a consultant, I declare that I have reviewed the thesis and that I have informed the student of the requirements, legal and ethical rules for the correct handling of literary sources.

I recommend / do not recommend[1] the final thesis / dissertation / portfolio to be defended in the final examination.

The thesis contains a state or official secret: yes no*[2]

Date: 2024 year 10 month 23 day

insider consultant

[1] The appropriate one should be underlined.

[2] The appropriate one should be underlined.

Author's declaration

Undersigned Ukasha Abdul Rehman (Neptun code: H2W0UA)

Specialization: Baking and Pasta Technologies

I declare that the BSc thesis titled "Investigation of Amino Acids in fortified meals" is my own work. Those parts, which were taken from other authors are clearly specified and the references are listed.

If my declaration is false, I understand that the Final Exam Committee will exclude me from the final exam and I have to prepare a new thesis work.

Budapest, 17th of Oct 2024

____

Student's signature