MSc. THESIS

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Institute of Food Science and Technology

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Characterization of Plant Protein Powders: Functional and Digestive Properties

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1. introduction:

In the past years, the interest of consumers has increased significantly to go towards more healthy and sustainable options, given that the traditional approach to consuming and producing food leads to disastrous results affecting health and the planet, such as food allergies and other health issues, animal domestication-related pollution, and global warming as well as problems of food security and the need to achieve self-sufficiency. Therefore, a shift in dietary habits toward more environmentally responsible practices and observance of animal rights as well is required. The tendency to use plant proteins was a necessary issue. Various processing techniques are used to extract protein powders from plant sources such as soy, wheat, legumes, rice, pumpkin, and sunflower, among others. Protein is separated from other plant materials such as fiber, carbohydrates, and lipids to get a powder form. The flavor, texture, metabolism, and cost of plant-based protein powders, among others, may pose some difficulties. Some individuals may dislike the flavor or consistency of plant-based protein powders, while others may have financial difficulties or gastrointestinal issues. What really distinguishes plant-based proteins is that they are mostly by-products produced as waste from other industries, such as the oil industry, and used generally to feed animals in addition to crops that may have physical damage and cannot be marketed in their natural form. Plant proteins are a good choice to use not only for vegans but also for non-vegans as well. In addition to its good nutritional value and its containment of essential and non-essential amino acids vitamins, minerals, and antioxidants, Consequently, we are currently confronting an issue that is controversial. Therefore, it has become fundamental to gain a deeper comprehension of plant proteins and conduct extensive research on them to derive the maximum benefit coming from them. The technological and functional attributes are important aspects in evaluating whether they are suitable for use in food applications, These properties include solubility, oil holding capacity, pH, water activity, dry matter content, foaming capacity and stability, and emulsion properties depending on the plant source and the method of processing, different protein powders have different characteristics. Plant-based protein powders are also viable for use in culinary applications, greatly influenced by how easily they can be digested. Thus, investigating the techno-functional and digestibility characteristics may be the key to avoiding all the negatives and finding solutions to them to achieve the ideal utilization of plant protein powders and their incorporation into the commercial food industry sector whether by enrichment or addition

and usage with the greatest health and environmental benefits and the potential relying on it as a support or as a good alternative to different animal products and also for athletes proteins products.

2. Aim of the Study:

The aim of the study is to comprehensively evaluate the techno-functional properties and In vitro digestion simulation to determine protein digestibility and protein quality. Four plant protein powders rice protein powder, sunflower protein powder, pea protein powder, and pumpkin protein powder are selected and analyzed by using various analytical techniques. For the techno-functional characteristics pH measurement, color measurement, foaming capacity and foaming stability, oil holding capacity, emulsion stability, water activity, dry matter content, solubility, and rheology test are done. Determination of protein digestibility and protein quality is done also by using In vitro digestion simulation. To evaluate the suitability of plant protein powders as functional food constituents and to identify any differences in their properties and quality that may affect their potential food industry applications. Since they are the most well-known by-products, sustainability and dependability in their use are crucial, and they must be incorporated into human nutrition in the most effective and appropriate way. Through this study, we aim to find the connections between the techno-functional properties and digestibility and to contribute to the existing knowledge base on plant-based protein powders and their potential use in the development of high-quality, nutritious food products.

3. Literature Review:

3.1. Plant protein powder:

Plant proteins are recently increasing in relevance over those derived from animals. Because of their higher ethical profile, the growing concern of animal welfare associations for meat proteins, and the increased load of greenhouse gases in animal-based protein production (Kumar et al., 2022). Studies on "alternative" proteins have increased due to widespread starvation worldwide along with worries about food security and sustainable crop production (Godfray et al., 2010). Demand for meat, dairy, and fish products is continually increasing despite the harmful environmental effects of their manufacturing; By 2050, the consumption of animal-derived proteins is predicted to double (FAO,2013a). In Western nations, there has been a noticeable turning fad toward diets supplemented with more plant protein, which is influenced by more people becoming vegetarians and vegans. With the projected 9-billion-person global population by 2050, it will be possible to meet the growing need for food proteins by utilizing proteins from innovative sources, such as industrial effluents from food processing and proteins from insects, fungi, and algae (Belluco et al., 2017; Megido et al., 2014; Verbeke, 2015).

Several plant-derived protein sources have been extensively investigated and utilized as protein supplements (soybean, pea, bean, chickpea, fava bean, seeds (sunflower and pumpkin, Chia), and grains (rice, wheat, maize, and barley). Depending on bioavailability, digestibility, protein content, quality, antinutritional elements, and treatment impacts, the nutritional value of the protein source might vary considerably (Almeida et al., 2020). A complete knowledge of their physiological and functional characteristics is essential for plant proteins and their byproducts to be better utilized in human nutrition and for producing novel manufactured products with superior nutritional value (Conde et al., 2005). Plant-based proteins play significant roles in food formulations, functioning as gelling, thickening, and foaming agents as well as emulsion stabilizers, water, and fat binders. Additionally, plant-based proteins contain biological properties like antioxidant and antibacterial activity (Kumar et al., 2022).

3.1.1. Plant-based proteins powder origins:

With two primary (supplementary) functions-biological and technological-proteins are the primary component of agricultural raw resources. The nutritional and physiological properties of proteins are referred to as their bifunctionality, while their physio-chemical assets that affect the

appearance, texture, and stability of food products (such as solubility, viscosity, foaming, emulsifying, and gelling ability, and fat absorption capacity) are referred to as their techno-functionality (Panyam & Kilara, 1996; Sforza et al., 2016; Tahergorabi & Hosseini, 2017).

Humanity used traditional plant proteins like soy, beans, and pea as a source of protein. Furthermore, numerous new research is searching for new (e.g., proteins from algae and insects) (Sá et al., 2019). Plant protein plant-based powder also produced from Alternative irregular sources (e.g., agroindustry by-products from edible oil extraction) (Pojić et al., 2018).

3.1.2. Plant-based proteins powder producing:

Commercial production of plant-based protein sources typically results in the powder form to extend shelf life and maintain the final product's physicochemical, sensory quality, and nutritional qualities (Amagliani et al., 2016). The key issue is the protein powder's quality, which necessitates ideal drying processes. Delicate substances are degraded by high drying temperatures, and other drying-related parameters have an impact on the product's physicochemical and microstructural characteristics (Bicudo et al., 2014).

Spray and freeze-drying are the two most popular production processes for plant-based protein powder. Spray drying is a cost-effective method for maintaining quality because it quickly dehydrates products and requires little maintenance (Vardanega et al., 2019).

3.1.3. Plant protein composition:

Protein is essential for human health because it is found in all body organs (Kumar et al.,2022). Most nitrogen-containing substances and proteins' structural constituents in the human body are known as amino acids (Almeida et al., 2020). A protein's ability to meet nutritional criteria is determined by how many essential amino acids it contains (WHO/FAO/UNU Expert Consultation, 2007). Protein must be characterized after extraction to be analyzed and its quality assessed. By assessing their quality, protein sources' capacity to meet the body's metabolic needs can be identified (Wen et al., 2020). A protein's nutritional quality is determined by its content of essential amino acids that adhere to recognized criteria and follow the patterns needed by the human body, as well as by factors such as digestibility and bioavailability (Sá et al., 2020).

Amino acid digestion is the liberation of free amino acids from proteins and peptides. (Sousa et al., 2023). (FAO/WHO) proposed the Digestible Indispensable Amino Acid Score (DIAAS) in

2013 (FAO, 2013b). This score is based on the actual ileal digestibility of each essential amino acid.

Each amino acid has a special and crucial impact on the system's functions. The human body is unable to produce the necessary amino acids which are: histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val), hence they must be obtained through diet (Almeida et al., 2020). The body can synthesize non-essential amino acids (Boye et al., 2012).

Adults are generally advised to consume 0.8–1.0 grams of protein per kilogram of body weight (kg) each day; however, to prevent insufficiency, a minimum of 0.66 g/kg/d is recommended (Sá et al., 2020).

Table 1. The essential amino acids need recommended based on the age classification by (FAO ,2013b)

Essential/indispensable AAs	**Age group**		
AA	**Infant (0-6 month)**	**Child (6-36 month)**	**older child, adolescent, adult**
His	21	20	16
Ile	55	32	30
Leu	96	66	61
Lys	69	57	48
SAA(methionine+cysteine)	33	27	23
AAA (tyrosine+phenylalanin)	94	52	41
Thr	44	31	25
Trp	17	8.5	6.6
Val	55	43	40

3.1.4. Plant protein by products as a protein source:

The threat of reduced food supplies from existing increased and limited agricultural supplies has resulted in the utilization of food waste as an innovative food supply. Given that food waste is a widely available and affordable source of useful properly functioning substances like antioxidants, dietary fibers, proteins, carbohydrates, and colorants, it can be processed again and used to create

new, economically useful ingredients that can be produced in the food chain or outside of it (Galanakis, 2012; Lin et al., 2013; Luque & Clark, 2013).

The need to ensure food production is sustainable has led to the substitution of traditional animal proteins with innovative plant proteins derived from food byproducts (Aiking, 2011).

There are a few ways that the returned proteins from industry food waste could be placed to use such as nutrients for functional foods and nutraceuticals, biopolymer advancement substances for a wide range of food, non-food, health supplements, and biofuel uses, techno-functional foodstuffs because of their emulsifying, gelling, foaming, and water-binding features (Gupta & Nayak, 2015; Moure et al., 2006).

3.2. Protein resources in industrial food by-products of plant:

3.2.1. Sunflower Protein:

3.2.1.1. Sunflower Protein Composition:

The nutritional and practical properties of sunflower seeds have been studied extensively, and they have emerged as one of the best vegetable protein sources (Venktesh & Prakash, 1993). There are two sorts of sunflower seeds: high oil and (non-oilseed) variations. The chemical study of the two types revealed that the protein, fiber, and ash levels of their kernels are similar.

It has a significant quantity of protein (30–50%) and depending on how well the seeds are dehulled and how well the oil is extracted, the protein content of the meal may approach 66% (Ivanova et al., 2013). In sunflower seeds, the ratio of hull to kernel varies greatly (Salunkhe et al., 1992). It is reported that the seeds contain 20.78 g of protein, 51.46 g of total fat, 3.02 g of ash, 20 g of carbohydrates, and 8.6 g of fiber per 100 g, for a total energy content of 2445 KJ. Additionally, it contains a significant amount of choline (55.1 mg) and betaine (35.4 mg) (USDA, 2008).

Following oil extraction, the cake component that is left is regarded as a nutritionally dense by-product as it contains between 20% and 60% protein, 5% and 35% of fibers (from the seed peels), and 3% to 5% of the remaining fat. This portion is typically used as livestock feed (Gonzalez & Vereijken, 2007; Pedroche, 2015). Sunflower protein concentrate (SPC), which is isolated from the cake and contains more than 75% proteins, can be added to the food as a protein source (Zorzi et al., 2020).

Sunflower seeds contain a good composition of minerals contents In 100 g of seeds, there are 78 mg of calcium, 5.25 mg of iron, 325 mg of magnesium, 660 mg of phosphorus, 645 mg of potassium, 9 mg of sodium, 5 mg of zinc, 1.80 mg of copper, 1.95 mg of manganese, and 53.0 mcg of selenium (USDA, 2008).

Table 2. Proteins' amino acid content (g/100g of protein) (Ren et al., 2012)

Amino acid content	Sunflower protein isolates
Lysine	2.9
Threonine	3.7
Methionine	2.0
Valine	4.5
Isoleucine	3.8
Leucine	6.9
Phenylalanine	5.6

3.2.1.2. Sunflower Proteins Techno-functional Characteristics:

The solubility, water, or oil absorption and/or retention, viscosity, foam and emulsion generation, and stability, and the potential to form masses, fibers, and gels are some of the functional characteristics of proteins. These characteristics are essentially connected to the physical, chemical, and structural/conformational characteristics of proteins, which depend on the source material and the procedures used to isolate them. These characteristics are of huge technological significance because they influence how proteins interact in food systems throughout processing, storing, preparing, and consuming (Damodaran, 1997).

3.2.1.2.1. Sunflower Protein Solubility:

Protein solubility can be described in terms of the equilibrium between the protein and the solvent, other proteins, and the solvent. (hydrophilic) and protein-solvent (hydrophobic). The thermodynamic equilibrium in the supernatant following centrifugation determines the extent to which proteins are retained in the liquid-liquid and solid phases (Dabbour et al., 2020). Between pH 5 to 9, sunflower proteins maintain their natural form and dissociate at pH values of under 4 or

9 above (Kachrimanidou et al., 2015; Rawel et al., 2002). The phenolic connection affects the protein's solubility by affecting the net charge on its surface (Damodaran et al.,2008).

A study compared the solubility of several protein extracts, including soy, pea, hemp, brown rice, and sunflower. Proteins were the best soluble in the pH range of 10.0–12.0. SP was the third largest soluble protein, while pea protein had the highest solubility. whereas the others had close to 100% solubility, SP (sunflower protein) extract had a maximum solubility of roughly 80% (Le Priol et al., 2019). The solubility of sunflower protein is significantly influenced by the variety, agroclimatic variables, and environmental parameters during preprocessing for oil extraction, protein extraction, and isolation procedures (Kaur& Ghoshal, 2022).

3.2.1.2.2. Sunflower Protein Oil Holding Capacity:

Oil-holding capacity is the amount of oil that can be ingested per gram of protein (OHC) (Shevkani et al.,2015). The oil-holding capacity of sunflower protein isolates is 2.06 g oil/g protein (Dabbour et al., 2018). The type of available lipid, its type, dispersion, and stability, as well as the protein's structural characteristics, all affect how quickly OHC occurs. OHC is also influenced by droplet size and distribution, as well as the presence of emulsifying agents (Hall, 1996). Proteins with high hydrophobicity tend to retain oils more readily because of the interaction among lipids and proteins, which occurs as the aliphatic chains of lipids connect to the non-polar side chains of amino acids (Withana et al.,2011).

3.2.1.2.3. Sunflower Protein's Emulsifying Ability:

Emulsion stability index (ESI) assesses an emulsion's ability to withstand change over time and EAI the quantity of contact area that can be stabilized per unit weight of protein (Kaur & Ghoshal, 2022). Protein isolates with pH values close to isoelectric displayed poorer emulsifying activity and less stable emulsions, although these properties improved with heat treatment. The reduced solubility and surface hydrophobicity at pH 4.5 can be implicated in the lower emulsifying activity and emulsion stability. The cause of this is thought to be the lower surface charge on protein molecules, which permitted intramolecular contact and the formation of aggregation, which also ultimately caused a loss in solubility and, consequently, a decrease in the emulsifying activity and stability of the emulsion. The proteins' limited solubility at pH 4.5 prevented them from moving quickly to the oil-water interface, which reduced their ability to emulsify and impaired the durability of their emulsions (Moure et al., 2006).

3.2.1.2.4. Sunflower Protein's Foaming Ability:

By pumping gas into the liquid phase through an aperture, whipping (pushing airflow into the liquid phase), shaking, or pouring, foams are gas bubble dispersions with continuous stages that are either liquid (often H2O) or solid (Dabbour et al., 2020). The capacity of a protein to maintain foam stability under stress is known as foam stability (Damodaran ,1997). Protein solubility is remarkably low at pH 4.5, which reduces foam production as only soluble protein components are involved in foam formation (Damodaran ,1997). The pH has a significant effect on foaming capacity. When the pH was between 3.0 and 5.0, SPI expanded more effectively (Dabbour et al., 2018). In the pH range of 7.0–9.0, sunflower meal and SPC (sunflower protein concentrate) foam stability has been reported to be higher (Huffman et al., 1975: Salgado et al., 2012).

3.2.1.3. Sunflower Protein *in vitro* Protein digestibility:

According to (Tessier et al., 2020), sunflower isolate has an ileal digestibility of 85.6 ± 2.6% of nitrogen consumed. While its amino acid profile is relatively well-balanced, sunflower protein has a lower ileal digestibility than other protein isolates tested under the same conditions.

The ability of sunflower protein concentrate to be broken down into smaller protein pieces (digestibility) when exposed to enzymes in a laboratory setting (in vitro) was studied using isoelectric precipitation. The samples with the lowest number of phenolic compounds had a slightly higher protein digestibility of 97.4% ± 0.3 ($p < 0.05$) (with a statistically significant difference). These results suggest that the presence of phenolic compounds slightly decreases the digestibility of sunflower proteins, the high digestibility values obtained in the studies still make these proteins suitable for human consumption (Salgado et al., 2012).

3.2.1.4. Nutritional and Health Benefits of Sunflower Seeds:

A serving of sunflower seeds contains 6 gr of plant protein or 12 % of the Daily Value. Vitamin E ability to eliminate dangerous chemicals known as free radicals, which can cause atherosclerosis. The best whole food source of vitamin E is sunflower seeds. An ounce of sunflower seeds contains 76% of the daily recommended amount of vitamin E. Together with vitamin E, selenium acts as

an antioxidant and guards against cell deterioration that could cause cancer and heart disease. Sunflower seeds provide 20% of the Daily Value for pantothenic acid, 11% for vitamin B6, 6% for thiamin, and 6% for niacin in a one ounce serving. A strong immune system, the ability to fight off infections, and speedy wound healing all depend on the mineral zinc which the sunflower seeds contain it. With 2 grams of fiber per one-ounce intake, sunflower seeds are a high-fiber food that will help you meet your daily requirements (Aishwarya & Anisha, 2014).

3.2.2. Pumpkin Protein:

3.2.2.1. Pumpkin Protein Composition:

It was found that the whole seed flour's protein and fat contents were 35.18% and 33.48%, respectively. The protein content rose to 51.85% after defatting using petroleum ether. This number exceeds the 43.1% of pumpkin (Cucurbita maxima) seed protein content (Horax et al., 2010; Du et al.,2012; Rezig et al., 2013)

According to research, pumpkin seed protein isolates are similar to soybean protein isolates in that they have significant amount of amino acid bioavailability (Rezig et al., 2013; Nwokolo & Sim,1987). It is reported that the protein of pumpkin seeds has a globulin structure that is similar to that of legume seeds (Yang et al., 2019; Rezig et al., 2013). This is significant because it raises the possibility that pumpkin seed protein can be used as a component of nourishing meal recipes, reducing the negative consequences of protein deficiency that vulnerable populations experience. Additionally, pumpkin seed protein isolates have potential chelating and antioxidative qualities (Yang et al., 2019; Nkosi et al., 2005; Sarkar et al., 2007)

Table 3. Pumpkin seed amino acid content (g/100 g) (Dotto & Chacha, 2020)

Amino acid content	g/100 g
Alanine	0.74-6.9
Arginine	1.70-23.10
Aspartic acid	2.05-2.70
Cystine	0.40-6.40
Glutamic acid	3.50-3.73
Glycine	1.50-6.80
Histidine	0.80-3.00
Isoleucine	0.81-4.90
Leucine	2.30-12.20
Lysine	1.50-4.00
Methionine	0.30-2.10
Phenylalanine	1.30-8.20
Proline	1.70-5.00
Serine	0.64-7.40
Threonine	0.83-3.40
Tryptophan	0.60
Tyrosine	0.83-4.30
Valine	1.36-6.70

3.2.2.2. Pumpkin Proteins' Techno- Functional Characteristics:

3.2.2.2.1. Pumpkin Protein Solubility:

Changes in pH significantly impact protein solubility, which is a crucial functional attribute. For pumpkin seed protein isolate (PPI), the pH range of 3.0 to 4.0 had the lowest levels of solubility, PPI shows higher solubility among pH 2.0 and 3.0, suggesting that these proteins might be an advantageous addition to the formulation of acidic fruit juices and beverages. The enhancement of other functional features, which rely on the initial solubilization of proteins, also depends on solubility. PPI solubility increased for pH values below 4.0 and above 5.0, with its solubility

peaking at pH 12.0 (92.18%). Various seed proteins showed similar solubility characteristics (Vinayashree & Vasu, 2021).

3.2.2.2.2. Pumpkin Protein Oil holding capacity (OHC):

PPI has 3.59–3.70 mL/g (OHC) and it is comparable to the SPI (3.78–3.98 mL/g). Protein with a high OHC may be applied in the food manufacturing such as sausages, soups, bakery products, mayonnaise, and many others (Vinayashree & Vasu ,2021).

3.2.2.2.3. Pumpkin Protein's Emulsifying Property:

For many food applications, including chopped and minced meat, coffee whiteners, cake mixes, salad dressings, mayonnaise, and frozen desserts, the protein's capacity to produce and stabilize emulsions is crucial. These food products are prepared under a variety of stresses, necessitating the need for various emulsifying and stabilizing abilities. PPI had (0.930 ± 0.015) emulsifying activities and (23.65 ± 3.15) for emulsion stabilities (Vinayashree & Vasu ,2021).

3.2.2.2.4. Pumpkin Protein's Foaming Ability:

A protein source, processing techniques, temperature, pH, protein concentration, mixing period, and foaming method are only a few of the variables that affect how proteins make foam. Most food foams are typically produced by trapping air in protein films, and the resulting foams must be stable to produce a variety of foods like whipped toppings and meringue (Kinsella & Morr, 1984). The foaming capacity of Pumpkin protein isolate (PPI) and fractions were nearly the same but differed less from soy protein isolate (60%), PPI (71.37 ± 1.93%) showed high foaming stability, proceeded by soy protein isolate (43.33 ± 4.72%). It is reported that the soy protein isolate has less foaming stability than pumpkin seed proteins (Vinayashree & Vasu ,2021).

3.2.2.3. Pumpkin protein *in vitro* protein digestibility:

Using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at 37 °C, the Alkali fraction of pumpkin protein (AF) was taken through an in vitro digestion assay. Pancreatin, is the main ingredient in SIF and it is a combination of five different enzymes (trypsin, protease, lipase, ribonuclease, and amylase). Since SGF (pepsin) has an acidic optimal pH (pH 1.2) while SIF is active at pH 7.5, solubilization of AF at varied pH values is necessary. To assess the pattern of digestibility, the protein was digested into smaller pieces after being treated with SGF and SIF individually for 60 min. The results showed that the protein is virtually entirely digested within 15

minutes, with the formation of bands with smaller molecular weights and the elimination of dense bands (Vinayashree & Vasu ,2021).

The big proteins' full digestion into smaller proteins and peptides after 15 minutes suggests that they could not exhibit any allergenic action (Fu et al., 2002).

3.2.2.1. Nutritional and Health Benefits of Pumpkin seeds:

Pumpkin seed proteins are widely used in diets, but they also have pharmacological effects like anticancer, antidiabetic, antioxidant, and hepatoprotective functions (Caili et al.,2006).

Pumpkin seeds include a lot of useful, functional elements. While the primary metabolites that maintain life are found in nutrients found in pumpkin seeds, functional components also play important roles in human health promotion and illness prevention (Pham et al., 2016; Adams et al.,2011; Rodríguez et al.,2012). Like many other seeds, pumpkin seeds are abundant in useful elements. They are abundant in provitamins, carotenoids, and vitamin E (tocopherols) (Broznić et al.,2016). Along with minor minerals like zinc, manganese, iron, calcium, sodium, and copper, pumpkin seeds are an excellent source of magnesium, potassium, and phosphorus (Amin et al.,2019; Koh et al.,2018). The mineral composition of pumpkin seeds is noteworthy. These seeds have a high potassium content (K), a low sodium content (Na), and high levels of calcium, manganese, phosphorus, and magnesium. In addition to copper, pumpkin seeds are a good source of trace metals like zinc (Zn) and iron (Fe) copper (Cu). all of which possess antioxidant properties and play a crucial role in antioxidant-dependent biocatalysts (Datta et al., 2019; Seymen et al., 2016).

Pumpkin seeds are abundant in crude protein, around 35%, and this results in a substantial and diverse supply of amino acids (Jafari et al., 2011).

3.2.3. Rice Protein :

3.2.3.1. Rice Protein Composition:

A protein's essential amino acid composition and bioavailability have a direct impact on the value of a protein as a source of nutrition. The food's chemical and physical characteristics will be affected by the composition of the amino acids (Singh & Sogi, 2018).

Rice proteins have higher biological value and higher protein efficiency quotients than those found in other cereals, and they are also more readily digested. Furthermore, compared to wheat and corn, rice proteins are a rich supply of amino acids, making them a more balanced and comprehensive option because of The higher levels of lysine and sulfur-containing amino acids. typical rice contains 18 amino acids, including 8 essential ones like isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (Jayaprakash et al., 2022). The general composition of rice protein includes the amino acids histidine (1.0–3.8%), threonine (3.15–4.43%), valine (5.0–7.31%), methionine (0.8–1.77%), phenylalanine (1.18–5.81%), isoleucine (3.60–5.35%), leucine (6.90–8.82%), and lysine (1.3–5.10%) (Eakkanaluksamee, & Anuntagool,2020). Rice protein isolate contains higher levels of methionine and phenylalanine compared to casein, and a greater amount of threonine compared to soy protein isolate. Rice protein isolate is higher in histidine, methionine, and phenylalanine than fenugreek protein isolates. As a result, combining rice protein with other plant proteins could produce a source of protein that is complete (Amagliani et al., 2016).

3.2.3.2. Rice Protein Techno-functional Properties:

3.2.3.2.1. Rice Protein solubility:

The solubility of protein increases as temperature rises, By enabling higher temperatures to extract small oligomers and a lower temperature to facilitate the release of big aggregates from rice protein, this relationship is usefully applied in alkaline extraction (Hoogenkamp et al., 2016).

Additionally, pH differences have an impact on solubility; for example, alkalinity and acidity have a major impact on the amount of protein that can be extracted from milled rice. for illustration, Commercial rice protein has a low solubility at pH 5, however, at pH 2, it is more than 55%. The solubility of rice protein hydrolysates is increased by enzymatic hydrolysis of intact protein, which lowers the molecular weight of rice protein molecules and increases the amount of ionizable groups (Lu et al., 2021).

3.2.3.2.2. Rice protein Oil holding Capacity (OHC):

Rice proteins may easily interact with water and oil because they are hydrophilic and hydrophobic characteristics (Wu et al.,2020). The OHC of brown rice protein is 2.93 mL/g and for the white rice protein is 2.56 mL/g (Jayaprakash et al., 2022).

3.2.3.2.3. Rice Protein's Emulsifying Property:

Protein surface charge, hydrophilicity, hydrophobicity, and solubility are all factors that affect rice protein's emulsifying properties. The two that prevail among all of them are hydrophobicity and aggregations. pH also has a significant effect in emulsifying properties, because alkaline and acidic pH increases the solubility of the protein, which leads to breaking disulfide bonds (Zhao et al., 2021). Proteins with lower hydrophobicity have lower emulsifying properties because they interact with oil-less. The interaction between the oil and the protein is crucial to the emulsifying property because increasing the surface hydrophobicity also improves the property (Ghanghas et al., 2020).

3.2.3.2.4. Rice Protein's Foaming Ability:

Rice protein has a hydrophobic propriety, and the hydrophobic regions on its surface interact with aqueous molecules, indicating a positive correlation between hydrophobicity and foaming capacity (Moirangthem et al., 2019). The penetration, transportation, and reorganizations of the molecule beneath the air-water surface have the greatest impact on the foaming capacity. The structure of proteins is denatured at high temperatures, and as a result, the foaming capability and foaming stability are increased. Proteins unfold and allow particles to aggregate during denaturation, which results in greater foaming qualities (Jiménez-Munoz et al.,2021).

3.2.3.3. Rice protein *in vitro* protein digestibility:

Rice protein is considered an alternative source of animal-based protein. Due to its high digestibility, rice is regarded as one of the best grains. (Jayaprakash et al., 2022).

The treatment of rice protein at 100 °C for 20 minutes had no negative effect on its essential amino acids. The effect of heat treatment on the in vitro digestibility of glutelin, globulin, and albumin was insignificant. The heat-induced interactions of glutelin, globulin, and albumin were therefore unrelated to their digestibility (Liu et al., 2020).

3.2.3.4. Nutritional and Health Benefits of Rice Protein:

Rice proteins have attracted the attention of food scientists and nutritionists due to their excellent nutritional value, which is evident in the balanced amino acid composition and hypoallergenic, hypocholesterolemia, hypolipidemic, and anti-cancer benefits. In this context, broken rice, an inevitable residue of rice milling, defatted rice bran, and rice bran oil have all been acknowledged as appropriate materials for the extraction of proteins, especially due to their accessibility, amounts, and amino acid composition (Wang et al., 1999; Xia et al., 2012; Amagliani et al., 2017).

Rice protein is regarded as an alternate source of protein to animal-based protein because it is highly recommended for babies and the elderly due to its nutritional value, digestibility, and hypo allergenicity (Wang et al., 2018). The protein makes up about 10-12% of the dry weight of the endosperm, which is the endosperm's second-largest element after the starch (Bose et al., 2019), while the outer fiber surface of bran includes B vitamins, trace minerals, and bioactive phytochemicals like phenolics (such as lignans, alkylresorcinols, and phenolic acids), phytosterols, and carotenoids, the germ component is rich in antioxidants, vitamins B and E, phytochemicals, and lipids (Ogbuehi et al., 2016).

3.2.4. Pea Protein:

3.2.4.1. Pea Protein Composition:

Pea seeds typically include 40–50% carbohydrate, 20–25% protein, and 10–20% fiber (Dahl, Foster, and Tyler 2012; Tulbek et al. 2016). Field peas are recognized as a major source of nutrients and may be separated into a variety of components and food products that are abundant with protein, carbohydrates, and fiber (Lu et al., 2019).

The amino acid composition of pea protein is balanced and contains a lot of lysine (Schneider & Lacampagne, 2000; Nunes et al., 2006). Pea protein is richer in lysine, leucine, and phenylalanine than cereal proteins, but lower in sulfur-containing amino acids (methionine and cysteine) (Gruber et al., 2005; Pownall et al., 2010).

Table 4. Pea protein essential amino acid content (g/100 g) (Lu et al., 2019)

Amino acid content	g/100 g
Valine	2.7
Leucine	5.7
Isoleucine	2.3
Methionine	0.3
Phenylalanine	3.7
Tryptophan	0.8
Threonine	2.5
Lysine	4.7
Histidine	1.6

3.2.4.2. Pea protein Tech-functional characterization:

3.2.4.2.1. Pea protein solubility:

Pea protein isolate's subsequent functional qualities could be affected by its considerable pH dependence, which has a minimal solubility between pH 4 and 6 (Adebiyi & Aluko, 2011). According to reports, pea protein's maximal solubility ranges from 20% for commercial PPI to 90% for the laboratory-prepared version (Lu et al., 2019). This is comparable to the figures seen for products containing soybean protein. The heat-induced denaturation and possible aggregation during spray drying cause reduction in the protein solubility of commercial pea protein products (Shand et al., 2007).

3.2.4.2.2. Pea proteins Oil Holding Capacity:

The oil holding capacity (OHC) is the quantity of oil that 1 g of protein can absorb and it is affected by the method of extraction and it fluctuated from 3.5 to 5.4 (Stone et al., 2015).

3.2.4.2.3. Pea Protein Emulsification Properties:

For producing oil in water emulsions, pea protein has great emulsifying characteristics, pea protein isolate’s (PPI) emulsification capacity decreases at pH levels that are near to its isoelectric point, but it significantly increases at pH levels above pH 7 (Lu et al., 2019). It is reported that PPI's low surface charge and low solubility were the causes of its limited emulsification

capability. It has been reported that the emulsion activity was determined using a ratio of the liquid layer height to the emulsion layer height that varied from 38 to 46% (Butt& Batool ,2010). It was indicated that the range for emulsifying stability was 43 to 100% (Lu et al., 2019).

3.2.4.3.4. Pea Protein Foaming Ability:

The foam capacity of the Pea protein isolate was (316.8 %–333.8 %) at pH 3 and (366.7 % ± 2.10%) at pH 8.0. The foam stability of the pea protein isolate was 40.0±4.40g at pH=3 and 100 % at pH 8.0. The thermal treatment of isolates substantially altered their foaming properties (Barac et al., 2014)

3.2.4.3. Pea protein *in vitro* digestibility:

Pea protein dispersions were readily digested after 15 minutes of in vitro intestinal digestion simulation, and heating the dispersions caused distinct disparities in the release of free amino acids compared to the dispersions that were not heated. Heating decreased the quantity of released free amino groups per g of isolated protein. The degree of hydrolysis, particle size, and processing procedure all had an impact on the in vitro digestibility of pea protein powder, and the production conditions can be optimized to increase the in vitro digestibility of pea protein powder (Jiménez-Munoz et al., 2023). Enzymatic hydrolysis of pea protein isolate has been shown to alter their functional characteristics, with hydrolyzed proteins displaying increased solubility and decreased viscosity (Bajaj et al., 2017). The result of enzymatic hydrolysis depends on a variety of variables, including the type of enzyme used and the circumstances of the treatment. The degree of hydrolysis and the enzyme utilized both had an impact on the PPI enzymatic hydrolysates (Tamm et al., 2016).

3.2.4.4. Nutritional and Health Benefits of Pea Protein:

The seed has high amounts of protein and carbs and low levels of fat and fiber. Peas can be processed to create high protein products and have the potential to act as a protein concentrate (Savage &Deo,1989). Field pea, one of the most significant leguminous crops, is grown in 84 different nations and accounts for the greatest share (36%) of all legumes produced globally (Dahl et al., 2012).

Pea protein is distinguished from soybean or other plant proteins by its great digestion, relative lack of allergic reactions, or unfavorable health issues (Owusu-Ansah & McCurdy, 1991;

Allred et al., 2004). Commercial pea protein products, in contrast to conventional cereal proteins, are gluten-free and can help in the development of gluten-free products (Han et al., 2010; Mariotti et al., 2009). Additionally, pea protein can be utilized as a dietary supplement for physical activity and sports. Three essential branched-chain amino acids (BCAAs) with an aliphatic side chain and a branch, leucine, isoleucine, and valine, can support muscle development (Shimomura et al., 2004).

4.Materials and Methods:

Experiments have been conducted to get a thorough understanding of the digestive, functional, and distinctive properties of plant proteins. As plant proteins are mostly by-products, research is essential to explore methods of incorporating them into diets to maximize nutritional value and advance sustainability.

4.1. Material:

The experiments were conducted at The Hungarian University of Agriculture and Life Sciences (MATE), Buda campus. Budapest, Hungary. For the experiments 4 types of commercial plant protein powder were used as the following, Sunflower protein powder: Sunbloom Proteins, Hungary kft, Drégelypalánk, 50% protein content. Pea protein powder: Puris pea by Cargill BV Company, The Netherlands. (80% protein content). Rice protein powder: Rice protein (80%) by Euroduna Food Ingredients GmbH, 25355 Bramstedt, Germany. Pumpkin protein powder: Biorganik pumpkin seed protein 60% protein content.

For the digestibility experiment, enzymes and porcine bile extract were purchased from Sigma-Aldrich Company as follows, porcine pepsin (E.C. 3.4.23.1; ≥2,500 units/mg protein (E1%/280)), porcine pancreatin (E.C. 232.468.9; 8×USP;), porcine bile extract (E.C. 3.1.1.3).

4.2. Methods:

4.2.1. Technofunctional properties:

4.2.1.1. pH measurement:

A portable digital pH meter type (206-pH2, Testo SE & Co. KGaA, Titisee-Neustadt, Germany). was used to measure the pH of the protein solutions with the concentration (5 w/w %) from each sample at room temperature, three replicates have been done. Measurement was applied based on the method of (Hidas et al., 2021).

4.2.1.2. Measurement of color:

The protein samples were individually placed in transparent bags and evenly distributed properly to the measurement. The samples were then measured using a CR-400 chromameter (Konica Minolta Co., Japan) at room temperature. Five replications were carried out for each sample to

ensure precision. The CIE-LAB elements were defined and the color factors of L* ,a*, and b*, were identified within the homogeneous color space CIE-LAB. Positive values of a* indicated reddish colors while negative values indicated greenish hues. Positive values of b* corresponded to yellowish hues and negative values indicated bluish colors. The L* value ranged from 0 to 100 and indicated the lightness of the sample. The method was done based on the method of (Hidas et al., 2020).

4.2.1.3. Foaming Capacity and Stability:

Based on the method of Liu et al, (2010) with slight modification, 4 protein solutions at 5% (w/w) were prepared and stirred by using a magnetic stirrer (DLAB heated magnetic stirrer MS-H280-Pro) for 5 minutes at a maximum speed of 1500 1/min at room temperature, with 3 replicates made from each sample. The solutions were then poured into 100 ml graduated cylinders and allowed to settle for 30 minutes. Foaming capacity and foaming stability were then calculated by using equations 1. and 2.:

$$FC = \frac{V_F}{V_S} \times 100 \qquad (1.)$$

$$FS = \frac{V_{F1}}{V_F} \times 100 \qquad (2.)$$

Where:

FC: Foaming capacity (%)

FS: Foaming stability (%)

V_F: the initial volume of foam at time 0 (ml)

V_s: the sample initial volume (ml)

V_{F1}: the volume of the foam at 30 minutes (ml)

4.2.1.4. Oil Holding Capacity:

Based on the Chakraborty, (1986) method with modifying, to measure the oil holding capacity of protein powder samples, pre-weighed centrifuge tubes were prepared. Specifically, 1 g of protein powder at 5% (w/w), was added to each tube, followed by the addition of 10 g of sunflower oil. To ensure reliable results, three replicates were performed for each sample. Tubes were stirred by using a vortex laboratory mixer each one for 10 seconds every 5 minutes over a period of 30 minutes. After agitation, the tubes were centrifuged at 25°C and 1000 RCF for 15 minutes. The supernatant and pellet were then separated, and their weights were measured. The oil holding capacity was calculated using the corresponding equation 3.:

$$\mathrm{OHC} = \frac{\mathrm{Wt}_{\mathrm{wet(O)}} - \mathrm{Wt}_{\mathrm{dry(O)}}}{\mathrm{Wt}_{\mathrm{dry(O)}}} \quad (3.)$$

Where:

OHC: oil holding capacity (g /g)

$\mathrm{Wt}_{\mathrm{wet(O)}}$: the weights of the wet samples (supernatant) in OHC experiment (g/g)

$\mathrm{Wt}_{\mathrm{dry(O)}}$: the weights of the dry samples in OHC experiment (g/g)

4.2.1.5. Emulsion stability:

Based on Nikzade et al, (2012) method with modification, to conduct this measurement, protein solutions were prepared from each sample at 5% (w/w). Subsequently, 5 grams of sunflower oil were added, and three replications were performed for each sample. Homogenization of the samples was carried out using a homogenizer (IKA T25 Digital Homogenizer, 3,400- 24,000 rpm, 10 – 2000 ml, 220 V). The homogenizer probe was placed in the layer between the water-oil interface and operated at a speed of 7300 for 5 minutes. The samples were then transferred to 10 ml graduated cylinders and allowed to settle for 60 minutes. The emulsion stability was calculated using the equation 4.:

$$ES = \frac{F_1}{F_0} \times 100 \qquad (4.)$$

Where:

ES: emulsion stability (%)

F_1: the emulsion layer or serum after 60 minutes (ml)

F_0: the initial volume of the emulsion (ml)

4.2.1.6. Water Activity:

Water activity measurement was conducted using a LabMaster-aw device, where the water activity of the samples was determined at 25°C utilizing a LabMaster-aw water activity meter. The LabMaster-aw device operates on a humidity sensor that detects changes in electrical resistance, which allows for the measurement of humidity levels. By measuring the resistance of the sensor, the LabMaster-aw can determine the relative humidity of the sample and calculate its water activity. The protein powder samples were subjected to atmospheric pressure and examined using this equipment in the designated place, with each sample undergoing triplicate measurements to ensure accuracy (Mazloum et al., 2011).

4.2.1.7. Dry matter content:

It is determined based on AOAC, (1995) by measuring 1 to 2 grams with using analytical balance from each sample in pre-weighted Petri dishes. Three replicates were taken for each one. Then they were placed in the drying oven for 24 h at 105°C. They were removed and allowed to cool down in a desiccator. Samples were weighted also by using analytical balance and the dry matter content was calculated by using equation 5.:

$$DM = \frac{W_D}{W_0} \times 100 \qquad (5.)$$

Where:

DM: dry matter content (%)

W_D: weight of dry sample (g)

W_0: weight of initial sample (g)

4.2.1.8. Measurement of solubility:

Based on Meena et al, (2017) and Le et al, (2011) with slightly modifying, four protein solutions were prepared from each sample at a concentration of 5% (w/w). Homogenization of the solutions was carried out using an (IKA T25 Digital Homogenizer, 3,400- 24,000 rpm, 10 - 2000ml, 220V) for a duration of 30 seconds. The samples were subsequently stirred by a magnetic stirrer (DLAB heated magnetic stirrer MS-H280-Pro) for 30 minutes at a speed of 400 1/m, at room temperature. Subsequently, 25 ml of the sample solutions were centrifuged for 10 minutes at 25 °C and 700 RCF, and the supernatants were collected. 100 ml of the homogenized samples were placed into a laboratory refrigerator at 3 °C for 24 hours. Dry matter contents were measured in triplicates for the samples after stirring immediately, after refrigeration for 24 hours in the supernatant, and after centrifugation in the supernatant were taken and measured in pre-weighed Petri dishes. These samples were then subjected to drying oven for 24 hours at 105°C. Each sample undergoing triplicate, after which they were calculated using equations 6. and 7:

$$\mathbf{S1} = \frac{\mathrm{DM_{SA}}}{\mathrm{DM_{SB}}} \times 100 \qquad (6.)$$

$$\mathbf{S2} = \frac{\mathrm{DM_{SR}}}{\mathrm{DM_{SB}}} \times 100 \qquad (7.)$$

Where:

S1: Solubility based on the comparison between the dry matter content before and after centrifugation (%)

S2: Solubility based on the comparison between the dry matter content before and after refrigeration for 24 hours (%)

$\mathrm{DM_{SA}}$: dry matter content of the sample's supernatants after centrifuging (%)

$\mathrm{DM_{SB}}$: dry matter content of the samples before centrifuging (%)

$\mathrm{DM_{SR}}$: dry matter content of the samples after refrigerating (%)

4.2.1.9. Rheology test:

The measurement was done based on Hidas et al, (2021). Four protein solutions, each with a concentration of 5% (w/w), were prepared and subsequently subjected to homogenization using an (IKA T25 Digital Homogenizer, 3,400- 24,000 rpm, 10 - 2000ml, 220V) for a duration of 30 seconds. Following homogenization, the samples were stirred using a magnetic stirrer (DLAB heated magnetic stirrer MS-H280-Pro) for a period of 30 minutes at a speed of 400 1/min, at room temperature, each sample was replicated three times. To investigate the rheological properties of the samples, a MCR 92 rheometer (manufactured by Anton Paar, France) was employed. The rheometer was equipped with a rotating cylinder and a concentric cylinder, with the following dimensions: bob length of 40.003 mm, bob diameter of 26.651 mm, cup diameter of 28.920 mm, positioning length of 72.5 mm, and active length of 120.2 mm. The machinery was operated using the Anton Paar RheoCompass software, and measurements were taken at a stable temperature of 20°C. To calculate shear stress, the shear rate was varied logarithmically between 10 and 1000 1/s for 31 measurement points every 3 s. The flow curves were subsequently analyzed using the Herschel—Bulkley model (i.e., the shear rate-shear stress diagrams), according to the following equation. The flow curves were subsequently analyzed using the Herschel—Bulkley model (i.e., the shear rate-shear stress diagrams), according to the following equation.:

$$\tau = \tau_0 + \gamma \cdot K^n \quad (8.)$$

Where:

τ: sheer stress (Pa).

τ_0: yield stress (Pa).

γ: sheer rate (1/s).

K: consistency coefficient ($Pa \cdot s^n$).

n: flow behavior index (without dimensions).

4.2.2. In vitro digestion simulation to determine protein digestibility and protein quality:

The protein digestibility of the chosen plant protein powders was evaluated after in vitro digestion simulation using the Infogest protocol (Brodkorb et al., 2019) with the method introduced by (Sousa et al., 2023). The digestive system consists of three main digestive parts, the mouth (oral), the stomach (gastric), and small intestine (intestinal). The step of measurements was carried out by the following. First, pH simulation was done at first to adjust the pH of the product in each phase to get the suitable pH environment for the digestive system where the pH in the gastric system is acidic (pH=3) and the pH of the small intestine is alkaline (pH=7). Next, in the enzymatic digestion simulation the protein samples were done in triplicates. Protein powder samples were prepared by measuring calculated weight containing 40 mg of protein and diluted to 1 g with distilled water. For the oral phase**,** 0.80 ml of simulated salivary solution (SSF), 5 µl of $CaCl_2(H_2O)_2$, and 0.195 ml of distilled water was added, mixed, and incubated for 2 minutes at 37 °C. For the gastric phase, 1.28 ml of simulated gastric solution (SGF), 1µl of $CaCl_2(H_2O)_2$, pre-measured volume of 6 M HCl (Table 4), 0.32 ml of pepsin stock solution (Porcine pepsin 65.7 mg dissolved in SGF 4.6 ml) and distilled water (Table 4) were added, and the mixture was incubated for 2 hours at 37 °C.

Table 5. HCl added volume.

Type of protein	volume (µl)	Distilled water (µl)
Rice	25	374
Pumpkin	20	379
Pea	25	374
Sunflower	50	349

For the Intestinal phase1.7 ml of simulated intestinal solution (SIF), 8 µl (0.001 ml) of $CaCl_2(H_2O)_2$, 0.50 ml of bile solution (prepared by Porcine bile extract 11.4 mg dissolved in 7.1 ml SIF), 1 ml of pancreatin stock solution (porcine pancreatin of 706.2 mg dissolved in 14.3 ml SIF) and pre-measured volumes of 1 M NaOH and water (Table 6) were added and incubated for 2 minutes at 37 °C.

Table 6. NaOH added volume

Type of protein	volume (µl)	Distilled water (µl)
Rice	10	782
Pumpkin	0	792
Pea	0	792
Sunflower	20	772

For amino acid determination:

Protein powders:

For amino acid measurement of the protein powders, 10 mg of each protein powder was dissolved in 6 M HCl (with 1% phenol content) and hydrolyzed using a Milestone Ethos One microwave oven (see below).

Digests:

Methanol (CH_3OH) with the amount of 32 ml was added to reach 80 v/v % to separate the undigested part, first using the vortex for 30 seconds, then it is incubated for 1 hour at -20°C, then samples were centrifuged at a speed of 6000 RPM for 20 minutes at temperature 4°C. After that, the supernatants were separated for amino acid determination as following. For amino acid measurement of the digests, 1000 µL from the supernatant was transferred into a 1.5 mL microtube and was evaporated using a rotary vacuum centrifuge, and the remaining material was dissolved in 6 M HCl (with 1% phenol) and hydrolyzed using a Milestone Ethos One microwave oven (see below).

Hydrolysis:

All samples were digested using a Milestone Ethos microwave oven with two types of hydrolysis methods which were necessary to analyze all amino acids. The heat profile of hydrolysis was: i) general method: 10 °C/min to 180 °C, 20 min incubation, and cooling, and ii) method for tryptophan determination: 10 °C/min to 180 °C and subsequent cooling. The hydrolyzed samples were taken up in 5 mL (powders) or 2.5 mL (digests) of borate buffer (pH = 8.51), filtered (22 µm HPLC filter), and derivatized. For derivatization 10 µL sample was added to 70 µL borate buffer

then 20 µL Waters AccQTag reagent (AQC; 6-aminoquinoly-N-hydroxysuccinimidyl carbamate) was added and mixed. After 1 min rest at room temperature, the mixture was incubated for 10 min at 55 °C then filtered (22 µm HPLC filter). For separation Waters Acquit UPLC H-Class instrument was used equipped with AccQ UPLC BEH C18 2.1x100 mm, 1.7 mm column (column temperature: 43 °C; injected volume: 10 µL; flow rate: 0.7 mL/min). For detection, a PDA detector of 260 nm was used. Quality and quantity evaluation was made with amino acid standards.

For Digestible Indispensable Amino Score (DIAAS) of protein powder samples calculation:

Digestible Indispensable Amino Acid Score (DIAAS) ratios were calculated by using the essential amino acid (EAA) composition and ileal digestibility of four plant protein powders (rice, pea, pumpkin, sunflower) in accordance with the three FAO-defined reference pattern scores for infants (0-6 months), children (6-36 months), and older children, adolescents, and adults (FAO, 2013). .

The DIAAS value of a protein is determined by the amount of the most restricting digestible indispensable amino acid (DIAA). The in vitro DIAAS is calculated based on the (9.):

$$\text{In vitro DIAAS} = \text{MIN}\left[\frac{\frac{mg\ BAA_i/g\ product}{mg\ AA_i/g\ product} \times mg\ AA_i\ /g\ product\ protein}{mg\ AA_i\ /g\ reference\ protein}\right] \qquad (9.)$$

where:

AA_i: is the amount of each amino acid (mg).

BAA_i: is the bio accessible amount of each amino acid (mg)

Reference protein composition is given by FAO for three age groups (FAO,2013b)

5. Results and Discussion:

5.1. Techno functional properties results:

5.1.1. Results of the pH measurement:

The pH values of the four protein samples are depicted in Figure .1, which reveals acidity and alkalinity among the samples. The rice protein exhibits the lowest pH level, averaging around 6.34±0.025, which is consistent with Thomas et al, (2014) reports that rice protein powder's pH level varies between 6.07 and 6.45. The highest alkali protein level is pea as it is shown with an average of 7.14 ± 0.025 in comparison with Ebert et al, (2020) the PH levels were between (6.72 to 7.61). The pH levels of protein powders derived from oil manufacturing, such as pumpkin and sunflower, are between pH 6.34 and 6.89, based on Ebert et al, (2020) research. Our results indicate that the average pH level of sunflower protein powder is 7.08± 0.03, while that of pumpkin protein powder is 6.87±0.025. Comparing our samples to the literature, we conclude that the pH levels of our samples are consistent with those reported in the literature. We observed that pea protein powder has the highest pH level, followed by sunflower and pumpkin protein powders, with rice protein powder having the lowest pH level. Plant protein powders' native pH is a key factor in deciding whether they are appropriate as food components for example in low pH products (such as soft drinks), low to slightly acidic (such as bread, dairy, and meat items), or neutral pH such as vegetable juices, tofu-based products (Ebert et al., 2020).

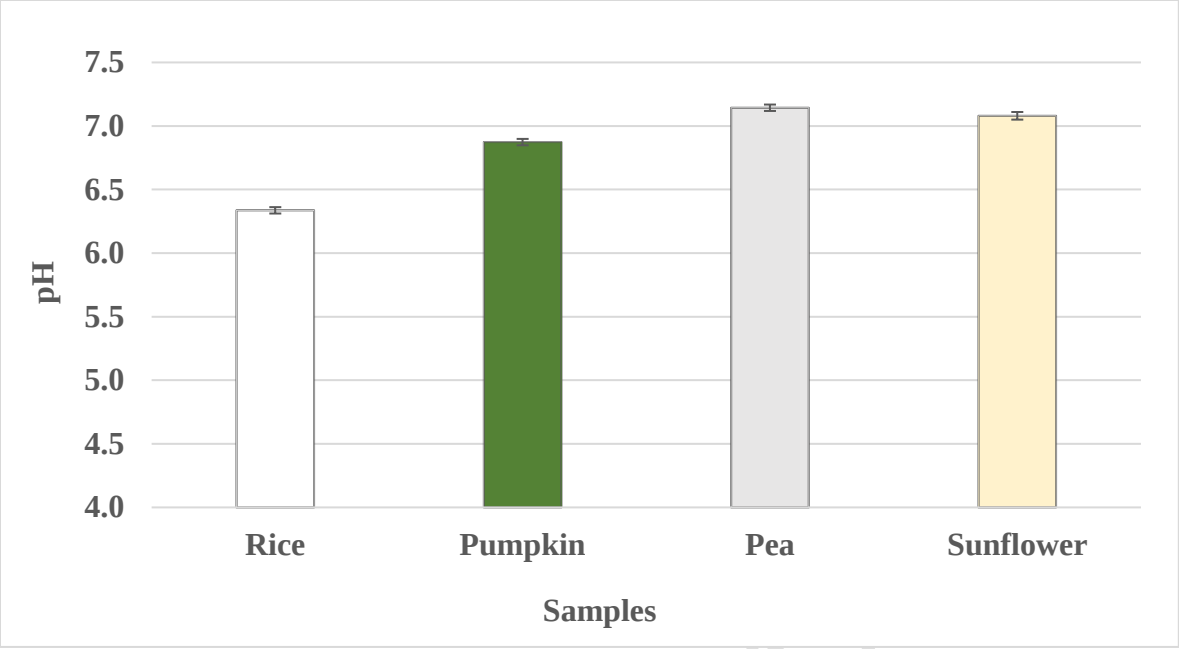

Figure 1. pH values of the solutions (5 w/w%) of the different plant-based protein powders

5.1.2. Result of the color masurement :

5.1.2.1. L* values:

Figure 2., displays the L* values of the samples, and it is shows that the Rice protein powder has an average value of 92.39 ± 0.51, whereas Ebert et al, (2020) report varying L* values based on the difference in commercial trade names and protein content (purity), ranging from 89.5 ± 0.1 to 79.2 ± 0.1. Our sample exhibits higher L* values, indicating lighter color. Similarly, the Sunflower protein powder has an average L* value of 84.38 ± 0.3296, which is closer to the L* values reported by (Ebert et al., 2020) ranging from 83.3 ± 0.1 to 60.3 ± 0.9, with our sample being slightly lighter. Figure 2 depicts that our Pumpkin protein powder has an L* average of 72.466 ± 0.212, and this value aligns with the results reported by Ebert et al, (2020), which found L* values ranging from 78.9 ± 0.2 to 61.9 ± 0.2 for studied pumpkin protein powders. In contrast, the Pea protein powder result exhibits an L* value of 88.818 ± 0.173, which contradicts the L* values reported by Ebert et al, (2020), ranging from 86.9 ± 0.1 to 83.3 ± 0.1 for the analyzed samples. L* value indicates the lightness of the sample. We can conclude from Figure 3 below that the highest L* values and the lightest are Rice protein followed by Pea protein powder then sunflower and pumpkin powder respectively.

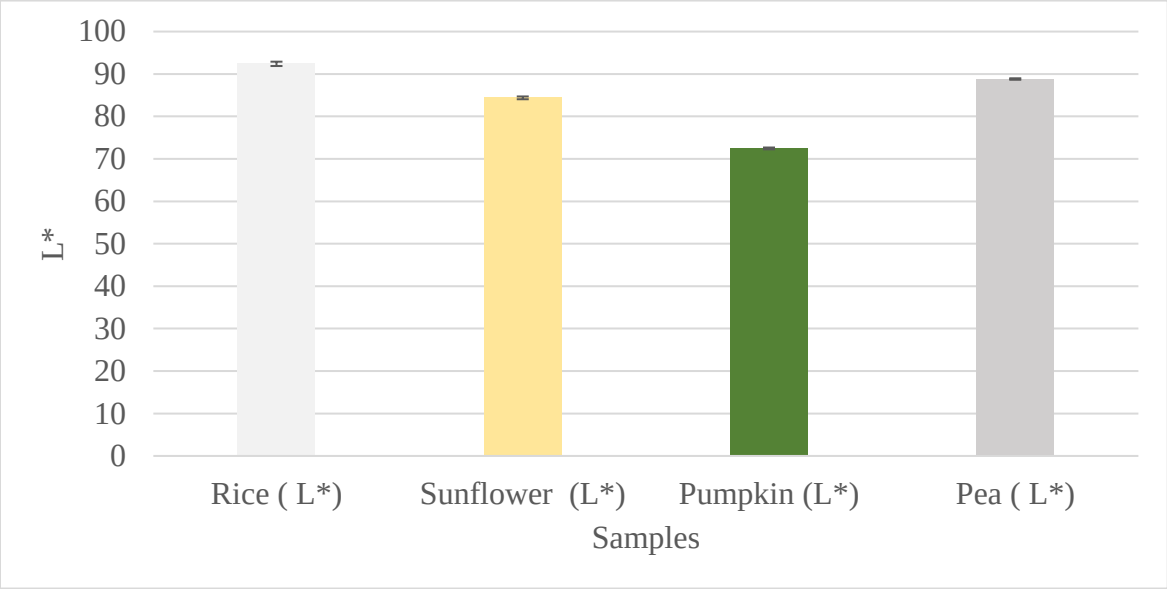

Figure 2. Color measurement L*

5.1.2.2. a* values:

Figure 3., demonstrates the a* values of the samples, and it is evident that the Rice protein powder has an average value of 0.07±0.0531, whereas Ebert et al, (2020) report varying a* values for different samples, ranging from 3.6 ± 0.1 to 0.0 ± 0.1. Our sample exhibits a* value compatible with the reference. Similarly, the Sunflower protein powder has an average a* value of 0.208 ± 0.0327, which is in line with the a* values reported in Ebert et al, (2020), ranging from 2.9 ± 0.1 to 0.4 ± 0.1. For our Pumpkin protein powder, Figure 3., displays an a* average of -4.16±0.0477, corresponding with the results reported by Ebert et al, (2020), which found a* values ranging from 0.8 ± 0.1 to (− 4.1 ± 0.1) for studied pumpkin protein powders. In contrast, our Pea protein powder samples exhibit an a* value of 0.04± 0.0245, which contradicts the a* values reported by Ebert et al, (2020) ranging from 3.8 ± 0.1 to 2.2 ± 0.1 for the analyzed samples. Positive values of a* indicated reddish colors while negative values indicated greenish hues, It can be clearly observed from the Figure 3., below the pumpkin protein powder is the greenish sample and the most reddish sample is sunflower protein powder and rice protein powder and pea protein powder has the lowest reddish color respectively.

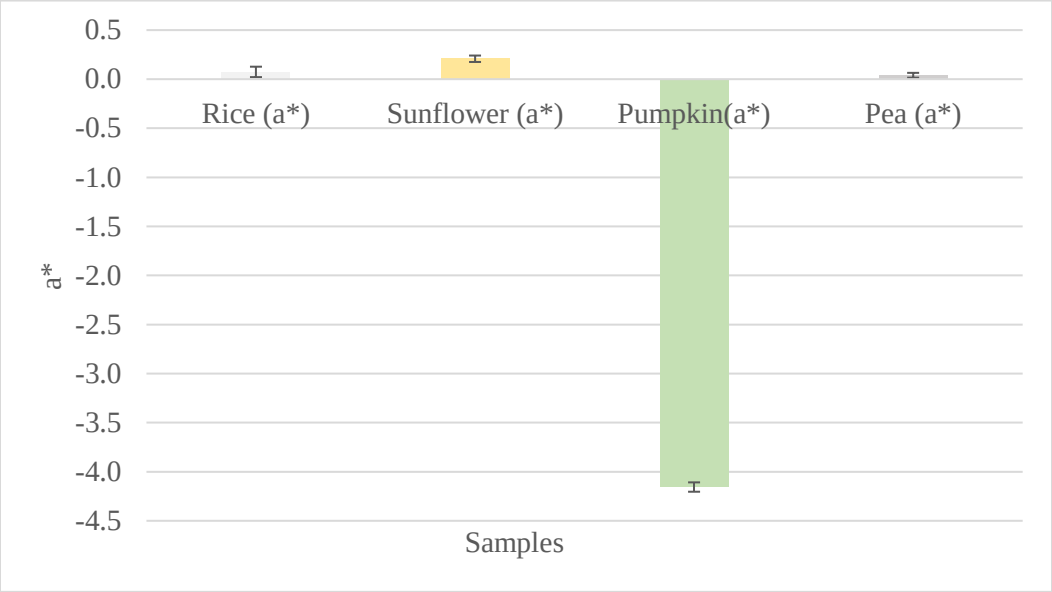

Figure 3. Color measurement a*

5.1.2.3. b* values:

The b* values of the samples were observed in Figure 4., the rice protein powder had an average value of 8.1 ± 0.0889, while Ebert et al, (2020) reported that the b* values varied between 21.8 ± 0.1 to 13.6 ± 0.2 among different samples. Thus, it can be inferred that our sample's b* values are not consistent with the reference. The average b* value of sunflower protein powder was 9.016± 0.0915, which is in line with the values reported by Ebert et al, (2020) that ranged from 15.5 ± 0.3 to 8.7 ± 0.2. Figure 5. shows that the average b* value of pumpkin protein was 15.954 ± 0.117, which differs from the values reported by Ebert et al, (2020) ranging from 30.9 ± 0.3 to 23.8 ± 0.5. Pea protein powder had an average b* value of 15.554 ± 0.0577, which is not consistent with the values reported by Ebert et al, (2020) ranging from 25.9 ± 0.1 to 19.2 ± 0.1 for the studied samples. Positive values of b* corresponded to yellowish hues and negative values indicated bluish colors. Pumpkin protein powder has the highest b* values that's means it has the yellowest color among the samples while pea protein powder, sunflower, and rice protein powders have the lowest yellow color respectively.

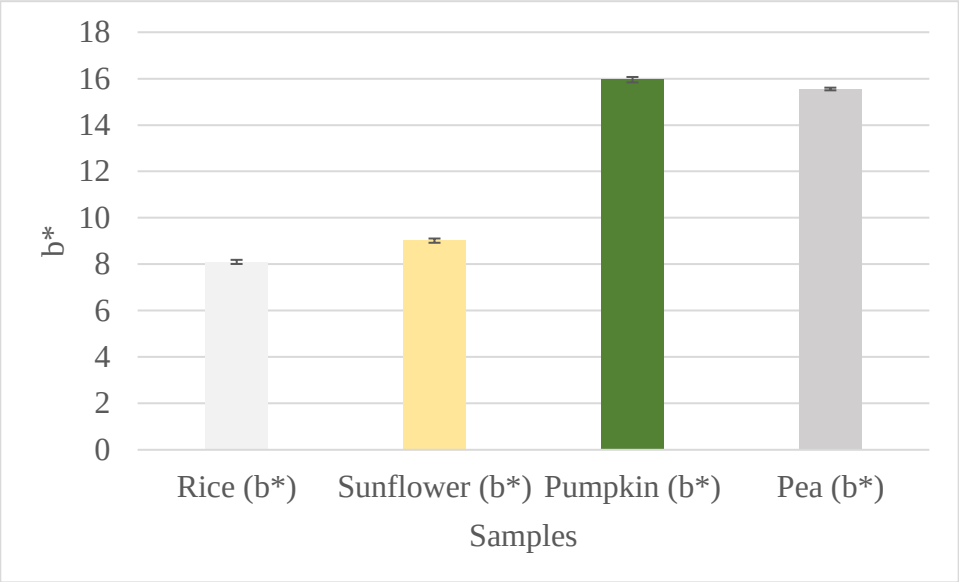

Figure 4. Color measurement b*

5.1.3. Results of the foaming capacity and foaming stability measurements :

5.1.3.1. Results of foaming capacity (FC):

Figure 5., illustrates the foaming capacity values of the protein samples. Pea protein powder exhibits the highest foaming capacity, with an average of 235.61% ± 12.22. Rice protein powder follows with an average FC value of 225% ± 9.45, and sunflower protein powder has an average of 194.87% ± 4.44. Notably, pumpkin protein powder demonstrates a significantly lower foaming capacity, with an average value of 2.38%, which is almost negligible. The results indicate that the FC values of the analyzed pea, rice, and sunflower protein samples were comparable to the FC value of soy protein reported by Moure et al, (2006) was 253.46, while the FC values of our sample's pea and rice protein powders fell within a comparable range but slightly lower and then the sunflower protein powder followed by them, these findings suggest that the results of our sample in case of the pea, rice, sunflower protein powders exhibit satisfactory FC values in comparison with soy protein. In contrast with the others, pumpkin protein powder has a significantly low FC values as the figure (5) These results appear to contradict the results reported by (Vinayashree and Vasu, 2020), who posited that the foaming capacity of pumpkin protein isolate and fractions were comparable, albeit marginally lower than those of soy protein isolate.

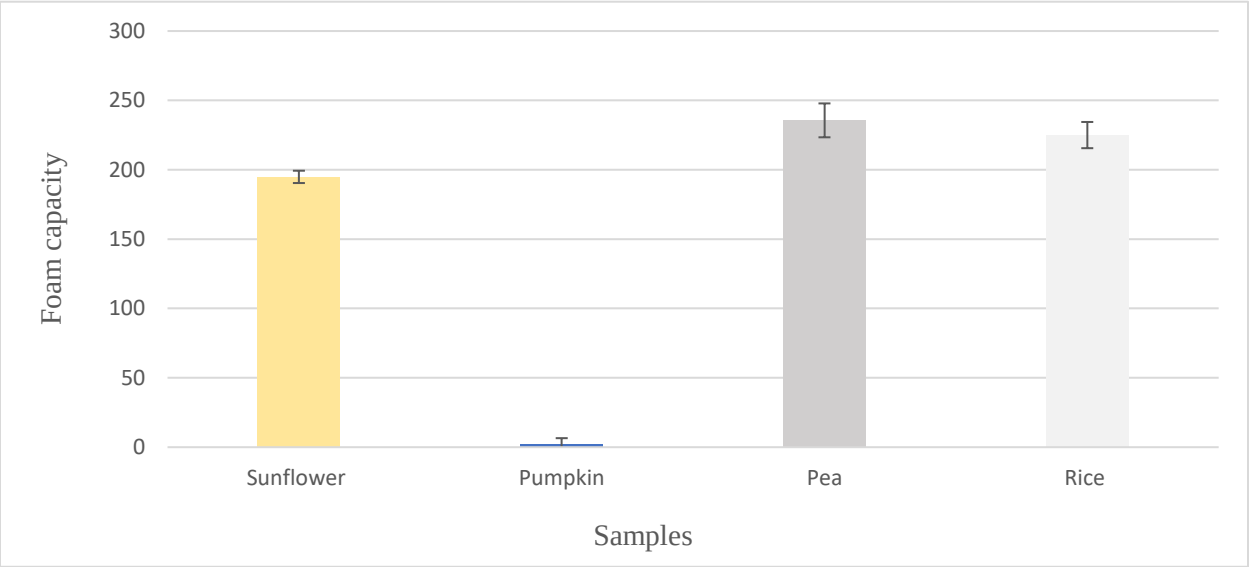

Figure 5. Foaming Capacity of the solutions (5 w/w%) of the different plant-based protein powders

5.1.3.2. Results of the foaming stability (FS):

The (FS) results as it is shown in Figure 6., sunflower protein powder has the highest FS value on average 80.28 % ± 1.60 followed by Pea protein powder and Rice where they have 64.53 % ± 2.82, 53.02 % ± 3.95 respectively. Pumpkin protein powder as figure 7 shows has 0 foam stability. In comparison with soy protein powder based on Moure et al, (2006) Foaming stability of soy protein powder has 41.80 %. Our samples show higher values of sunflower, pea, and rice protein powder respectively. It is reported by Vinayashree and Vasu, (2020) that the Soy Protein isolate has less foaming stability than pumpkin seed that is in contrast with our results in this study.

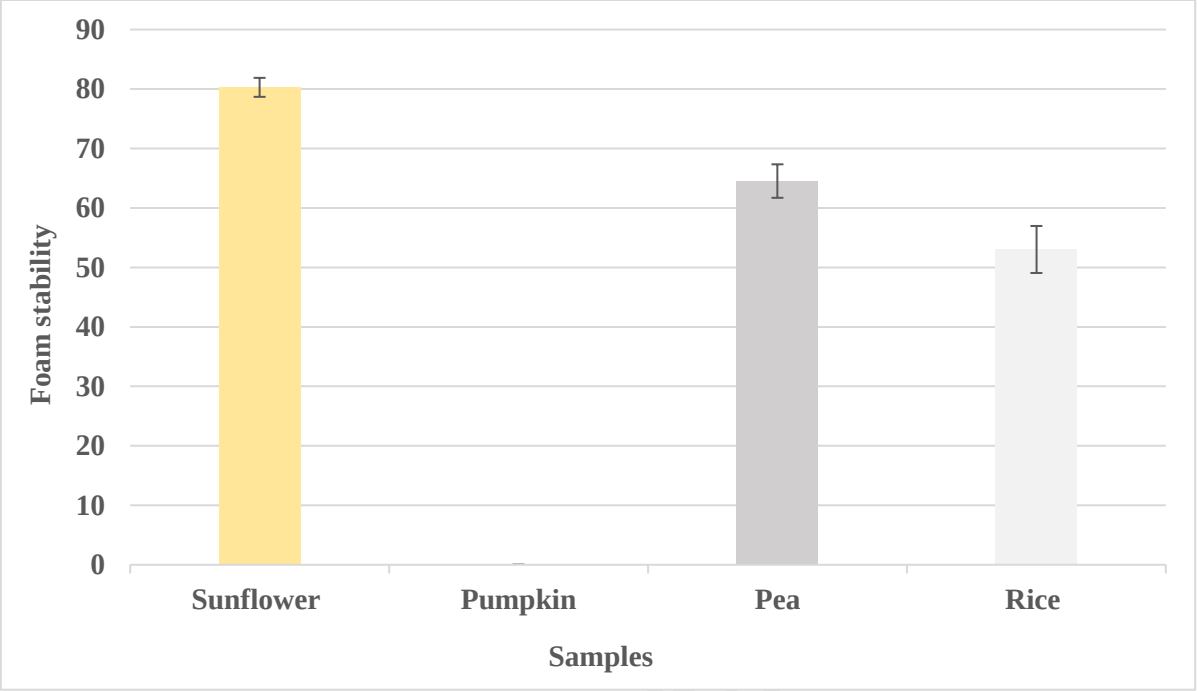

Figure 6. Foaming stability of the solutions (5 w/w%) of the different plant-based protein powders

In the field of food science, the functional properties of protein powders, such as their foaming capacity and foaming stability, are of utmost importance, as they play a significant role in determining the quality and characteristics of various food products. These properties are closely linked to the protein's ability to lower the surface tension between water and air, and their correlation is directly influenced by the structural properties of the protein (Jia et al., 2019).

The production of different types of food products that require aeration and stabilization, such as meringues, mousses, and ice cream, necessitates the use of protein powders with optimal foaming capacity and stability. These protein-stabilized food foams showcase the importance of these properties in food science (Zhang et al., 2021).

5.1.4. Results of the oil holding capacity (OHC) measurement :

The results of the oil holding capacity (OHC) analysis for various protein powders are presented in Figure 7., the OHC values for rice protein powder were found to be on average 2.14 g /g ± 0.44 ≅ 2.33 ml/g, which is comparable to the value of 2.56 ml/g reported by Jayaprakash et al, (2022).

Similarly, the OHC value for pumpkin protein powder was found to be an average of 2.28 g/g ± 0.24 ≅ (2.48 ml/g.), which is slightly lower than the range of 3.59-3.70 mL/g reported by Vinayashree and Vasu, (2021). In contrast, the OHC value for sunflower protein powder was found to be an average of 1.77 g/g ± 0.14, which is slightly lower than the value of 2.06 g/g reported by Dabbour et al, (2018).

The OHC values for pea protein powder were found to be on average 3.02 g/g ± 0.59, which is comparable to the range of 3.5-5.4 reported by Stone et al, (2015) based on the extraction method. Overall, Figure 7., indicates that pea protein powder has the highest OHC values, followed by pumpkin and rice protein powders respectively, and then sunflower protein powder. When compared to soy protein, which has values of 2.81±0.4 according to Foh et al, (2011), our sample of pea protein powder has a higher value, while pumpkin and rice protein powders have marginally smaller values than soy protein. The OHC value for sunflower protein powder is also slightly lower than soy protein.

Based on the findings, it can be concluded that all the protein powders analyzed in this study have high OHC values, which make them suitable for use in food manufacturing. Proteins with high oil-holding capacity can be used to produce various food products such as ground meat, meat substitutes, soups, sausages, bakery products, dressings for salads, and mayonnaise (Vinayashree & Vasu, 2021).

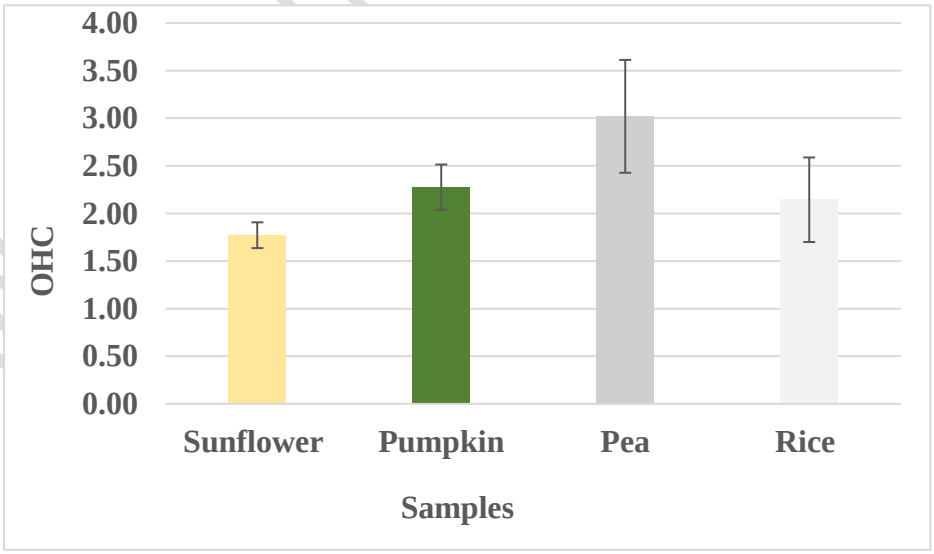

Figure 7. Oil holding capacity of the solutions (5 w/w%) of the different plant-based protein powders.

5.1.5. Results of the emulsion stability (ES) measurement:

The results of the emulsion stability (ES) for the protein powder samples are presented in Figure 8., It is observed that the rice protein powder exhibited the highest ES, with an average value of 97.92 ± 0.22, followed by sunflower protein powder with an average value of 97.28 ± 1.05, and pea protein powder with an average value of 91.75 ± 3.57. On the other hand, the pumpkin protein powder had the lowest ES value, with an average of 81.77 ± 0.87. In comparison to soy protein powder, which has an ES value of 82.40 ± 2.94 based on the study by Brishti et al, (2017), it is evident that the ES of our samples is higher except for pumpkin protein powder which is slightly lower. Protein emulsifying characteristics are frequently assessed using emulsion stability (Amagliani et al., 2016). It can be concluded that a high emulsion stabilization ability is exhibited by our samples, which is significant for utilization across various food industries, and based on (Vinayashree & Vasu, 2020) for many food applications, including chopped and minced meat, coffee whiteners, cake mixes, salad dressings, mayonnaise, and frozen desserts, the protein's ability to create and stabilize the emulsions is crucial. During preparation, these food products are subjected to variable degrees of stress, necessitating the need for various emulsifying and stabilizing capacities.

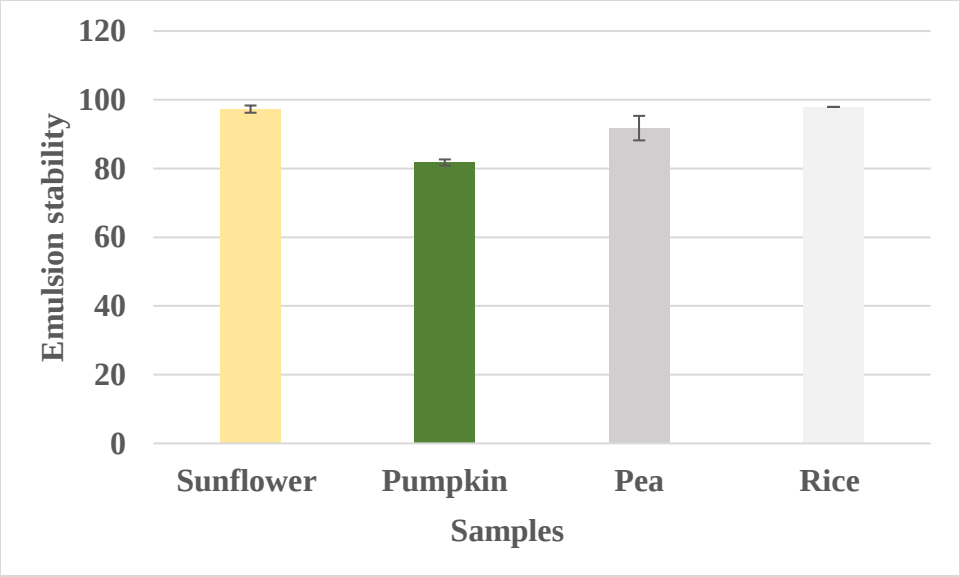

Figure 8. Emulsion stability of the solutions (5 w/w%) of the different plant-based protein powders

5.1.6. Results of water ativity measurement:

Figure 9., shows the water activity levels of the samples. It refers that the rice protein powder has an average water activity of 0.272 ± 0.0026, which agrees with the results of Amagliani et al, (2016), who found a range of 0.18-0.46. The pea protein powder has an average water activity of 0.366 ± 0.0090, agrees with Mehle et al, (2020) who observed a water activity of roughly 0.3 for pea protein powder. The average water activity of the sunflower protein powder is 0.251 ± 0.0045, which is in line with a study by (Erdem & Kaya, 2021) that mentioned sunflower protein powders have water activity levels higher than 0.2. The pumpkin protein powder has an average water activity of 0.348 ± 0.0012 while Pongjanta et al, (2006) found that pumpkin protein powder had a water activity of about 0.24,

Pea protein powder has the highest water activity, followed by rice protein powder and pumpkin protein powder, according to Figure 9, while sunflower protein powder has the lowest water activity. The food's microbiological, chemical, and physical stability are significantly influenced by the water activity, which reveals how much water is bound inside the meal and how easily it can participate in certain reactions. Food products with a water activity of less than 0.6 do not undergo microbiological (bacteria, yeasts, or molds) growth (Amagliani et al., 2016). We can say that our samples are microbiology stable.

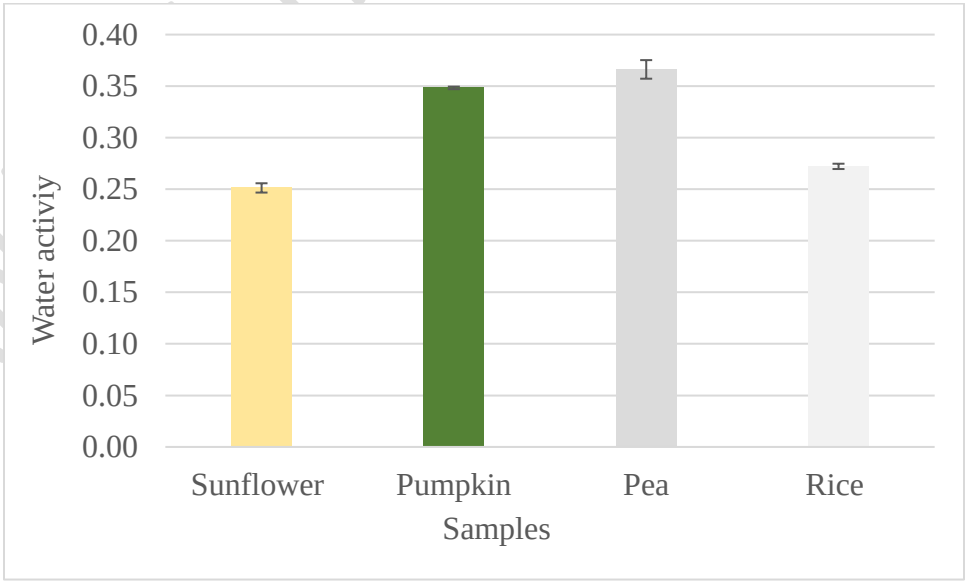

Figure 9. Water activity of the solutions (5 w/w%) of the different plant-based protein powders

5.1.7. Results of the dry matter content measurement:

We can figure out based on Figure 10., below that the pea protein powder has the highest dry matter content value with an average of 93.08 % ± 0.77 followed by sunflower protein powder with a value of an average of 92.94 ± 0.16. Rice protein powder has a (DM) value average of 92.49 % ± 0.16. Pumpkin protein powder has the lowest DM value with on average 90.30 ± 0.43%.

In comparison with the specifications, we can observe that the sunflower protein powder and rice protein powder are consistent with their specification which says the max moisture of sunflower protein powder is 6% and the rice protein powder 8%. Based on our previous water activity and dry matter content measurement results, we can conclude that our protein samples have low moisture, and water activity, our previous results insure the microbial stability for the studied samples.

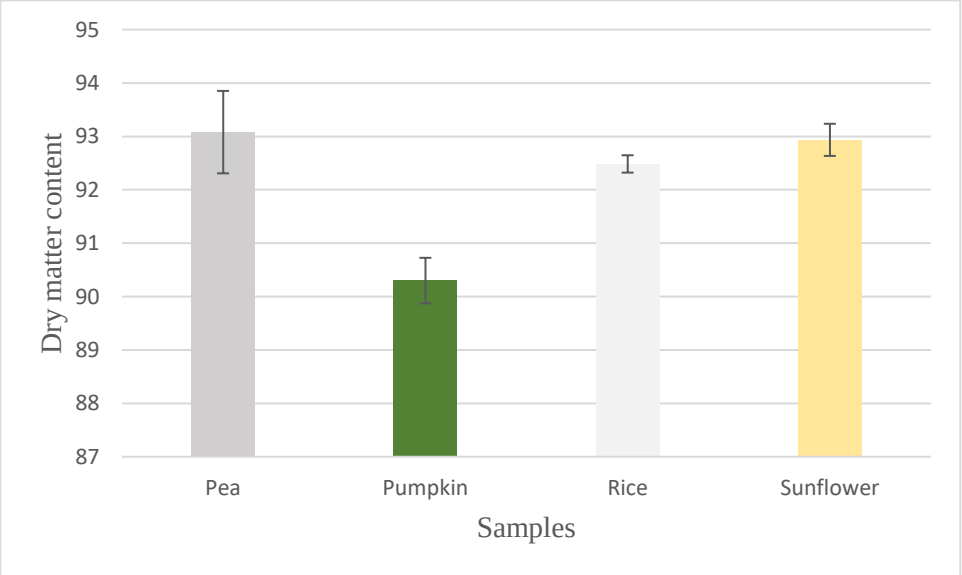

Figure 10. Dry matter content

5.1.8. Results of the solubility measurement:

5.1.8.1. Results of the solubility based on the dry matter content:

We can observe based on Figure 11., below that pumpkin protein power has a high solubility with an average 47.33 %, then the sunflower protein powder with an average 41.36 %. In terms of solubility, Pumpkin Protein Isolate showed minimum solubility between pH 4.0 and 5.0, increased

solubility between pH 4.0 and 5.0 and reached its maximum solubility at pH 12.0 (92.18%) Vinayashree et al, (2020). It is reported that between pH 4.0 and 6.5, sunflower protein isolates had the lowest solubility (20–30%), and pH 10 had the maximum solubility (80–95%), between pH 5 to pH 9, sunflower proteins maintain their natural form however, at pH 4 or above, they dissociate Kaur et al, (2022). Pea protein powder and rice protein powder have the lowest solubility values in the conditions of the experiment with an average of 9.11 % and 1.910 % respectively. Based on Ebert et al, (2020) Comparisons across and within genera revealed that, regardless of the provider and composition, some plant sources' solubilities changed significantly, while others did not. For instance, the solubilities of pea powder ranged from 8.5% to 42.4% and rice protein varied with the lowest 3.9% implying that some powders were nearly insoluble, whereas others were fully soluble, these results are nearly consistent with our results. Based on Jayaprakash et al, (2022) Protein extraction from milled rice can be considerably boosted with alkalinity and acidity, pH changes also change solubility, for instance, commercial rice protein's lowest solubility at pH 5 is displayed, however, at pH 2, it is greater than 55%. Pea protein Isolate has a minimal solubility between pH 4 and 6 and it is very pH dependent (Lu et al., 2019). Some Species of Pea proteins show a high solubility at PH 3 and another species show a high solubility at pH 8 (Barac et al., 2010). The parameters that determine solubility are split into two important parameters, internal (such as the type of amino acids present on the protein surface) and outer (such as temperature, additives, ionic strength, and pH) (Yousefi et al., 2022).

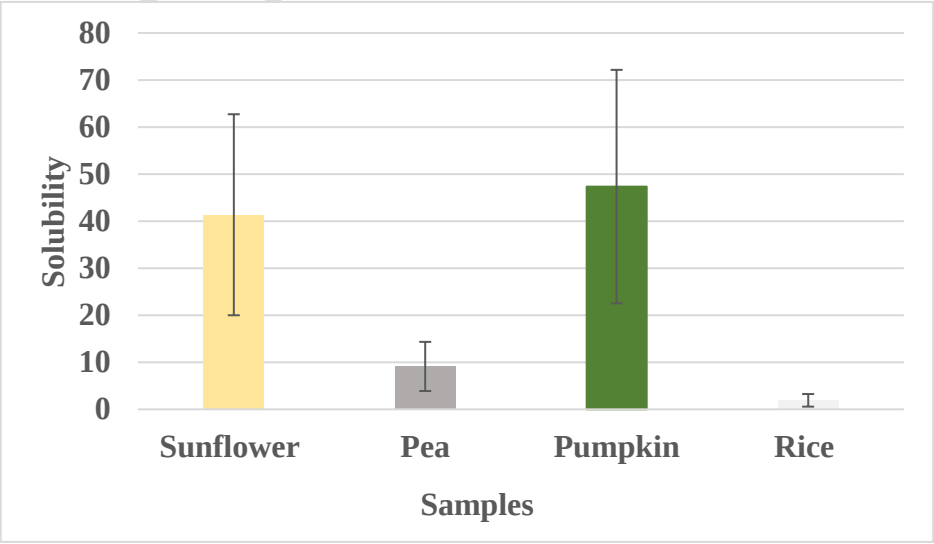

Figure 11. Solubility values based on the dry matter content

5.1.8.2. Results of the Solubility based on the comparison between the dry matter content before and after refrigeration for 24 hours:

We can figure out from Figure 12., below that pumpkin protein powder has an average solubility value of 22.47 % and in comparison, with our result above it was 47.33 %, so refrigerating without centrifuging negatively affected our pumpkin sample and decrease it. In the case of sunflower, the figure also shows the value on average 20.86 % and comparing with our results we can conclude that it has decreased. Pea protein powder also shows a lower value with an average of 0.94%. In contrast, rice protein shows a higher solubility value with an average of 5.23%.

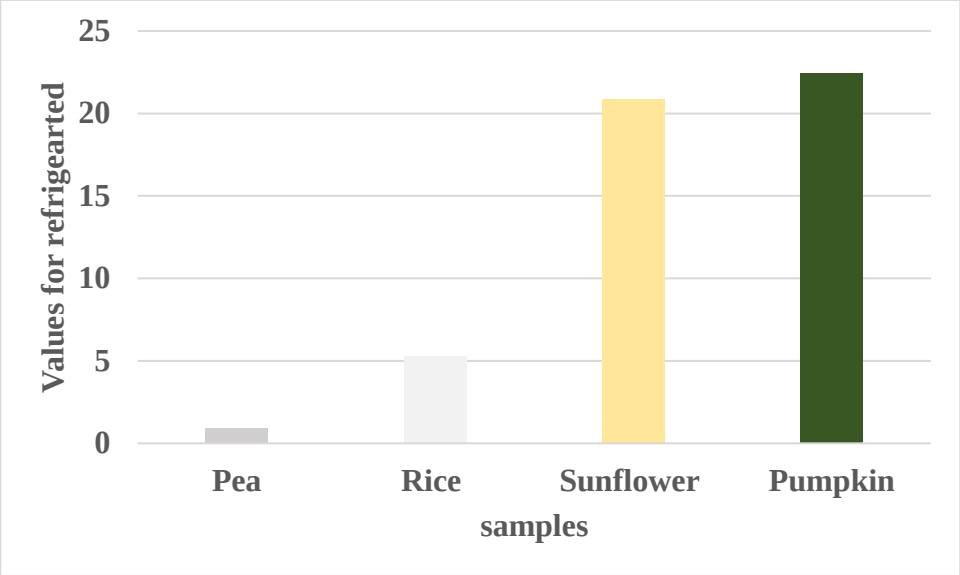

Figure 12. Solubility based on the comparison between the dry matter content before and after refrigeration for 24 hours

5.1.9. Results of the rheology measurement:

Our protein powder samples were investigated by their rheological properties, The flow curves were analyzed using the Herschel—Bulkley model, and our samples were studied at temperature 20 °C. We can conclude from the figure 10 below based on the curves between shear rates and shear stress there is a non-linear relationship. The shear stress increased with the increasing of the shear rate. From Figure 10., Sunflower protein powder has the highest shear stress data as it is observed where the shear stress is on average 6.16 (Pa) when the shear rate is 1000 1/s followed

by pea protein powder with the shear stress on average 5.52 (Pa) at the shear rate 1000 1/s, then pumpkin protein powder which shows a shear stress value on average 4.96 (Pa) when the shear rate is 1000 1/s. we can observe that the rice protein powder has the lowest shear stress with a value of the average of 4.81 (Pa) when the shear rate is at 1000 1/s.

Where the flow behavior index shows results below for the analyzed samples, we can observe from Figure 13., the dilatant behavior of protein powder samples at temperature 20°C was determined between the shear rate 10 to 1000 (1/s) and shear stress (Pa) and we can figure out from our samples that the dilatant behavior implies that the sample's viscosity rises as the shear rate increases. The sample gets increasingly flow-resistant as the shear rate rises, requiring more force to keep the shear rate constant. Dilatant fluids is a non-Newtonian flow behavior, and it is defined by an increase in the material's volume or thickness as the shear rate rises. Highly concentrated suspensions, such as paper coatings or slurries, and thick combinations of water and cornstarch are a few examples of dilatant fluids (Jankowska et al., 2023). It's crucial to comprehend how plant protein powders flow to process and use in both food and non-food applications as efficiently as possible.

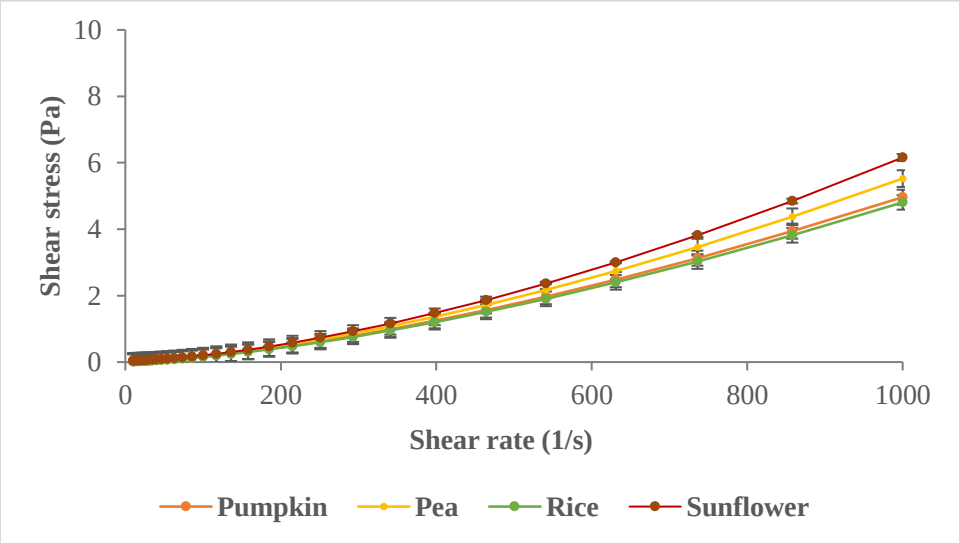

Figure 13. Flow curve of the solutions (5 w/w%) of the different plant-based protein powders

Table 7. Samples, (τ)0, K, n, results

	(τ)0	st.dev	K	st.dev	n	st.dev
Pumpkin	1.00E-2	1.46E-3	1.47E-4	8.38E-06	1.51	1.09E-2
Sunflower	3.67E-2	8.50E-3	1.17E-4	1.32E-05	1.57	1.83E-2
Rice	1.15E-2	6.06E-4	1.37E-4	9.00E-06	1.51	9.89E-3
Pea	1.61E-2	9.39E-3	1.46E-4	3.29E-05	1.53	4.05E-2

5.2. Results of *in vitro* digestion simulation:

For amino acid measurement of the protein powders, 10 mg of each protein powder was dissolved in 6 M HCl (with 1% phenol content) and hydrolyzed using a Milestone Ethos One microwave oven and we can observe our results as it is shown in Figure 14., our results are consistent with the original amount of the studied protein powders where the pea and rice have 80 % protein followed by Pumpkin and sunflower with 60% and 50% respectively. Sunflower protein powder has the lowest amino acid composition, with an average of 50465 (mg/100 g) ± 968.7, whereas pea protein powder has the highest at 77193 (mg/100 g) ± 1401.486, then rice protein powder at 76619.5 (mg/100 g) ± 3676.248, and pumpkin at 69108 (mg/100 g) ± 10931.87. It corresponds to the quantities mentioned by the producer, as we mentioned earlier.

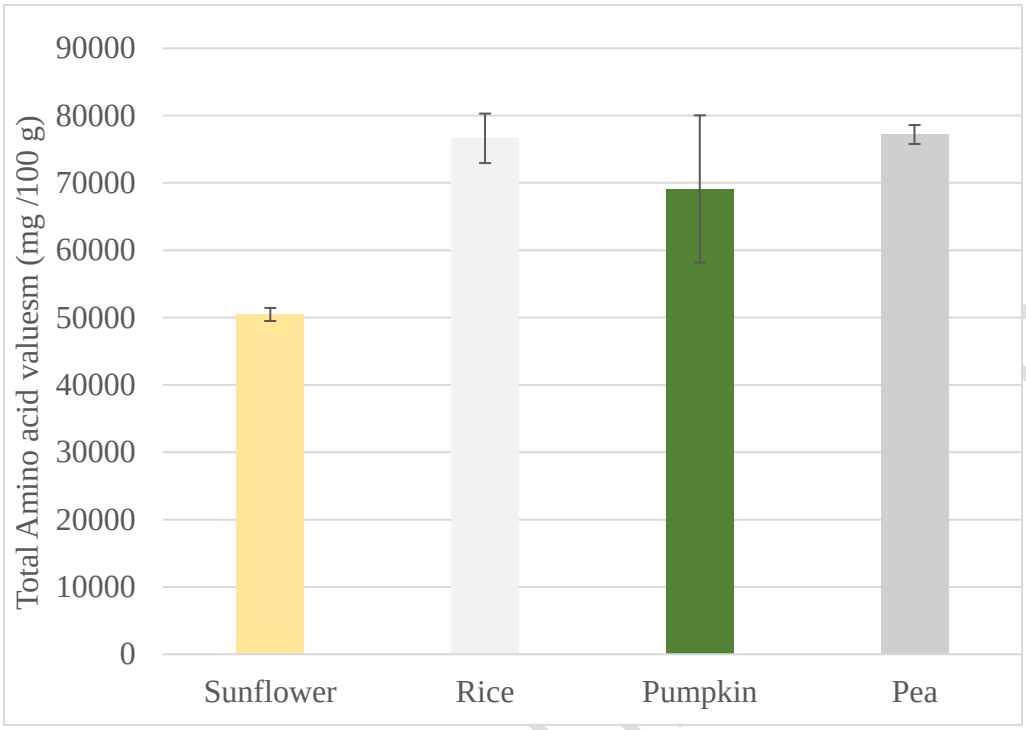

Figure 14. The total amount amino acid composition of the protein powders

Figure 15., shows the results that for the sunflower protein powder, in case of essential amino acids it has Lysine with amount of 2214 (mg/100 g) ± 41.01 (2.214 g/100g), and Threonine with an average amount of 2194 (mg/100 g) ± 39.6 (2.194 g/100g) and Methionine with 1164 (mg/100 g) ± 15.56 (1.164 g/100 g) and Valine 2982 (mg/100 g) ± 79.9 (2.982 g/100 g) and Isoleucine 2383 (mg/100 g) ± 46.67 (2.383 g/100 g) and Leucine with a 3708 (mg/100 g) ± 57.98 (3.708 g/100g) and Phenylalanine with an amount of 2657(mg/100 g) ± 53.74 (2.657 g/100g). In comparison with Ren et al, (2012) mentioned that sunflower protein contains Lysine 2.9 (g/100g), Threonine 3.7(g/100g), Methionine 2.0 (g/100g), Valine 4.5 (g/100g), Isoleucine 3.8 (g/100g), Leucine 6.9 (g/100g), Phenylalanine 5.6 (g/100g), we can figure out that our results contradict Ren et al, (2012) as our samples showed lower results than what was mentioned.

In the case of pumpkin protein powder, the Figure below shows it has an Arginine with an amount of 12026 (mg/100 g) ± 1989.8 (12.026 g/100g g) and histidine with an average of 1766 (mg/100 g) ± 295.571 (1.766 g/100g), isoleucine with 2726.5 (mg/100 g) ± 444.77 (2.726 g/100g), leucine with 5304.5 (mg/100 g) ± 854.892 (5.304 g/100g), lysine 2751.5 (mg/100 g) ± 454.67 (2.752

g/100g), methionine with 1482.5 (mg/100 g) ± 214.253 (1.483 g/100g), phenylalanine 3765 (mg/100 g) ± 615.183 (3.765 g/100g), threonine 2216.5 (mg/100 g) ± 355.675 (2.217 g/100g), tryptophan 325(mg/100 g) ± 5.657 (0.325 g/100g), valine with an average amount of 3713(mg/100 g) ± 591.141(3.713 g/100g). Our results are consistent with Dotto and Chacha, (2020) that said pumpkin seed amino acid content of Arginine 1.70-23.10 (g/100g) and Histidine 0.80-3.00 (g/100g), Isoleucine 0.81-4.90 (g/100g), Leucine 2.30-12.20 (g/100g), Lysine 1.50-4.00 (g/100g), Methionine 0.30-2.10 (g/100g), Phenylalanine 1.30-8.20(g/100g) , Threonine 0.83-3.40 (g/100g), Tryptophan 0.6 (g/100g), Valine 1.36-6.70 (g/100g) except tryptophan was slightly lower.

For pea protein powder, the Figure below shows it has a histidine with an average of 2073 (mg/100 g) ± 38.184 (2.073 g/100g), isoleucine with 3923 (mg/100 g) ± 84.853 (3.923 g/100g), leucine with 7128.5 (mg/100 g) ± 140.714 (7.129 g/100g), lysine 6337 (mg/100 g) ± 137.179 (6.337 g/100g), methionine with 842 (mg/100 g) ± 137.179 (0.842 g/100g), phenylalanine 4608 (mg/100 g) ± 63.639 (4.608 g/100g), threonine 3236 (mg/100 g) ± 60.811 (3.236 g/100g), tryptophan 417.5 ± 9.192 (0.4175 g/100g), valine with an average amount of 4447 (mg/100 g) ± 84.852 (4.447 g/100g).In comparison with Lu et al, (2019) that said pea protein powder contains Valine 2.7 (g/100g) , Leucine 5.7 (g/100g) , Isoleucine 2.3 (g/100g) , Methionine 0.3 (g/100g), Phenylalanine 3.7 (g/100g), Tryptophan 0.8 (g/100g) , Threonine 2.5 (g/100g) , Lysine 4.7 (g/100g) , Histidine 1.6(g/100g) , we find that our results show higher amounts than Lu et al, (2019) except the tryptophan was slightly lower.

In the case of rice protein powder, the results shows it has a histidine with an average of 1939 (mg/100 g) ± 86.267 (1.939 g/100g), isoleucine with 3534.5 (mg/100 g) ± 157.684 (3.535 g/100g), leucine with 7043.5 (mg/100 g) ± 330.218 (7.044 g/100g), lysine 2649(mg/100 g) ± 115.965 (2.649 g/100g), methionine with 2220.5 (mg/100 g) ± 102.530 (2.221 g/100g), phenylalanine 4533 (mg/100 g) ± 199.404 (4.533 g/100g), threonine 3037.5 (mg/100 g) ± 150.613 (3.038 g/100g), valine with an average amount of 5150 (mg/100 g) ± 234.759 (5.15 g/100g). The general composition of rice protein of the essential amino acids based on Eakkanaluksamee and Anuntagool, (2020), histidine 1.0–3.8 (g/100g), threonine 3.15–4.43(g/100g), valine 5.0–7.31(g/100g), methionine 0.8–1.77(g/100g), phenylalanine 1.18–5.81(g/100g), isoleucine 3.60–5.35(g/100g), leucine 6.90–8.82(g/100g), and lysine 1.3–5.10(g/100g). In comparison with Eakkanaluksamee and Anuntagool, (2020) histidine, valine, phenylalanine, lysine, and leucine

results are consistent with the reference and for methionine, our result is higher and lower in the case of isoleucine and threonine.

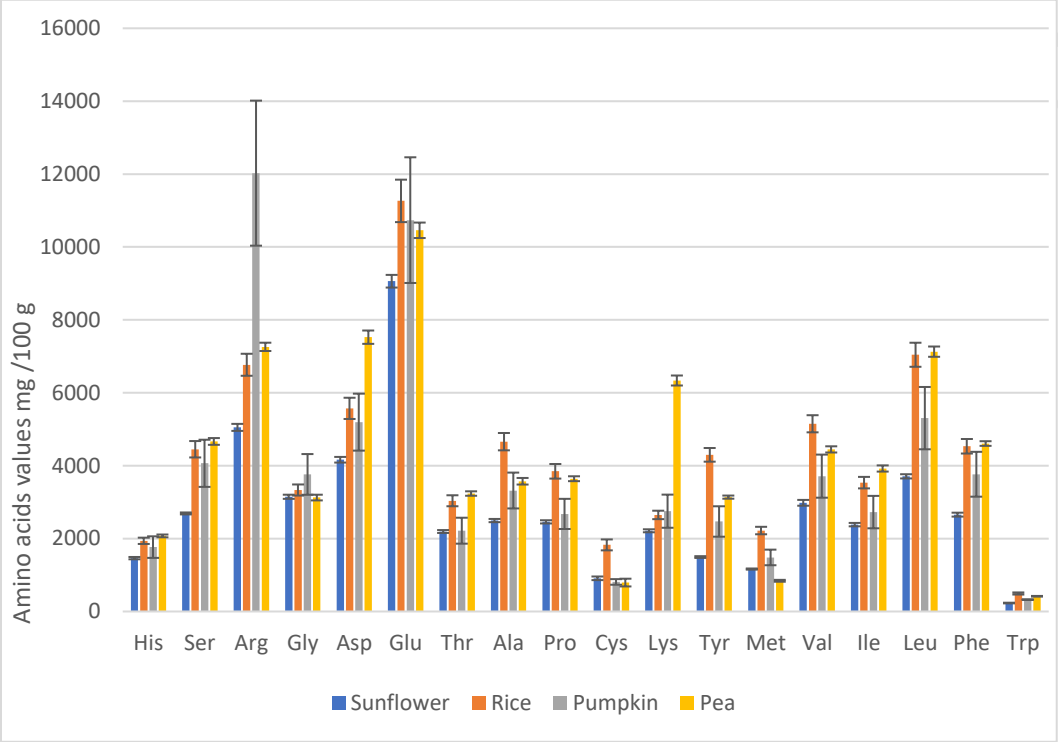

Figure 15. The amount amino acid composition of the protein powders samples

We can see from the Tables 8.,9., bellow the results for the amino acid composition (essential amino acids and non-essential amino acids)

Table 8. The amino acid amounts in sunflower and rice protein powders

Protein powder samples	**Sunflower**		**Rice**	
Amino acid	**Average (mg /100 g)**	**SD**	**Average (mg /100 g)**	**SD**
His	1464	31.82	1939	86.27
Ser	2690	26.87	4451	224.86
Arg	5051	96.17	6768.5	301.93

Gly	3147	56.57	3332.5	152.03
Asp	4163	77.78	5573	291.33
Glu	9061	173.95	11265	582.66
Thr	2194	39.6	3037.5	150.61
Ala	2495	44.55	4659	237.59
Pro	2459	43.84	3845.5	200.11
Cys	910	49.5	1827	149.91
Lys	2214	41.01	2649	115.97
Tyr	1493	24.04	4297.5	187.38
Met	1164	15.56	2220.5	102.53
Val	2982	79.9	5150	234.76
Ile	2383	46.67	3534.5	157.68
Leu	3708	57.98	7043.5	330.22
Phe	2657	53.74	4533	199.4
Trp	232	9.19	493.5	28.99
Sum	50465	969	76619.5	3676

Table 9. The amino acid amounts in pumpkin and pea protein powders

protein powder samples	**Pumpkin**		**Pea**	
Amino acid	**Average (mg /100 g)**	**SD**	**Average (mg /100 g)**	**SD**
His	1766	295.57	2073	38.18
Ser	4065.5	647	4665.5	92.63
Arg	12026	1989.8	7260.5	113.84
Gly	3760	558.61	3125.5	78.49
Asp	5194.5	781.35	7525.5	183.14
Glu	10737	1723.93	10458	212.13
Thr	2216.5	355.67	3236	60.81
Ala	3318.5	491.44	3573	90.51
Pro	2676.5	413.66	3642.5	67.18
Cys	810.5	77.07	793	106.07
Lys	2751.5	454.67	6337	137.18
Tyr	2469	417.19	3137.5	43.13
Met	1482.5	214.25	842	25.46
Val	3713	591.14	4447	84.85

Ile	2726.5	444.77	3923	84.85
Leu	5304.5	854.89	7128.5	140.71
Phe	3765	615.18	4608	63.64
Trp	325	5.66	417.5	9.19
Sum	69108	10931.87	77193	1401.49

Results For Digestible Indispensable Amino Score (DIAAS) of protein powder samples calculation:

Based on the scoring pattern from 0.5 to 3 years old and according to the DIAAS value, the FAO report also suggests classifying proteins into three groups based on quality: 75 (no quality claim), 75–99 (high-quality protein), and 100 (excellent quality protein) (FAO, 2013b). According to the previous, protein samples were classified in Table 10., and it shows that sunflower has the highest DIAAS values with 92 % followed by rice protein powder with 73%. Pea and pumpkin protein powders have the lowest DIAAS values with 46 and 45 respectively. Depending on the quality classification by FAO, (2013b) mentioned above, sunflower protein powder has high-quality protein, and rice, pea, and pumpkin protein powders are classified as no quality claim proteins (DIAAS values lower than 75%). Herreman et al, (2020) reported rice protein has a 47% DIAAS value, and also mentioned that pea protein has a DIAAS value of 70 % which contrasts with our results as it is shown in Table 11. We can conclude that our studied protein powder samples have all essential and non-essential amino acids with a high-quality protein classification in the case of sunflower protein powder, Although our samples contain all amino acids (essential and non-essential), they are in quantities less than the daily needs and cannot be completely relied upon for nutrition, so it is preferable to use them in fortifying and enriching food.

Table .10 Digestible indispensable amino acid values of studied protein powders according to the FAO, 2013b reference pattern score

AA	His	Ile	Leu	Lys	SAA (methionine +cysteine)	AAA (tyrosine+ phenylalanine)
Sunflower	1.81	1.51	1.09	0.92	2.51	1.89
Rice	1.56	1.32	1.40	0.73	1.73	2.85
Pumpkin	1.04	0.59	0.70	0.45	1.78	1.31
Pea	1.53	1.29	1.15	1.51	1.46	1.93

Table .11 Digestible indispensable amino acid values of studied protein powders according to the FAO, 2013 reference pattern score and limiting AA

AA	Thr	Trp	Val	DIAAS %	limiting AA
Sunflower	1.73	6.30	1.46	92	Lys
Rice	1.75	0.98	1.89	73	Lys
Pumpkin	0.77	1.06	0.80	45	Lys
Pea	1.42	0.46	1.15	46	Trp

5.3. Summary of the results:

Our study has revealed a good correlation between the studied properties and the results, when compared with other findings in the literature, demonstrating that the functional properties of proteins are highly affected by many factors such as pH, temperature, moistures content, analytical method, protein type and the conditions of the experiment. In the case of protein solubility, it has been linked to various functional properties in several studies. Yousefi et al, (2022), noted that internal characteristics (such as the type of amino acids present on the protein surface) affect solubility and that enzymatic hydrolyzation increases solubility. Arteaga et al, (2020) claimed that several proteolytic enzymes might be used to increase the solubility of isolated Pea proteins. Significantly better at pH= 4.5 (from 2 to 71%) because of changing electrostatic force, releasing small molecules and hydrophilic amino acids, and altering protein structure. The solubility of the Rice bran protein can be increased by employing Trypsin, as Zang et al, (2018) have stated. The release of soluble peptides from insoluble clumps or precipitates and an increase in ionizable groups contribute to improved heat stability. This leads us to the fact that enzymatic digestion can greatly affect the functional properties of proteins.

6. Conclusion and suggestions:

Protein powders made from rice, sunflower, pea, and pumpkin may be a useful source of protein that is sustainable and can reduce waste. However, the technological-functional properties, digestion, and other aspects that affect the viability of plant-based protein powder for use in food applications. The purpose of this study is to investigate the acceptability of the chosen vegan protein powder as a functional food ingredient by evaluating its technological-functional characteristics and in-vitro digestion. From the techno-functional measurements, in the case of the pH measurement findings varied between slight acidity and alkalinity. Foaming capacity stability findings suggest that the results of our sample in the case of the pea, rice, and sunflower protein powders exhibit satisfactory FC values in comparison with soy protein and pumpkin showing low numbers, and in the case of foaming stability our samples have good results whereas pumpkin shows no stability. Oil holding capacity results show high numbers and it is an important propriety in case of desirable texture. It can be concluded that a high emulsion stabilization ability is exhibited by our samples, and it is critical for a lot of food products such as mayonnaise. Samples have low water activity and high dry matter content, so they are microbiologically stable. In the case of solubility, a lot of parameters determine solubility, and they are in two categories, internal (such as the type of amino acids present on the protein surface) and outer (such as temperature, additives, ionic strength, and pH) and in the case of studied measurement circumstances samples shows low solubility especially pea and rice protein powder. In the case of rheology our samples show that the dilatant behavior implies that the sample's viscosity rises as the shear rate increases. The sample gets increasingly flow-resistant as the shear rate rises, requiring more force to keep the shear rate constant. For the in vitro digestibility results we can conclude that our samples contain all the essential and non- essential amino acids and the DIAAS value for the sunflower protein powder shows high quality. We can give some recommendations:

- In case of Sunflower protein powder due to its good emulsion stability, foaming stability, solubility values, and high DIAAS value, it can be utilized as a high-quality protein supplement, emulsifier, foaming agent, gluten-free component, and nutritional fortifier in a variety of food products. Athletes, bodybuilders, and anyone trying to up their protein intake for fortifying plant-based foods like non-dairy milk, vegan cheese, and meat substitutes may notably benefit from it. whipped cream, mousses, and meringues.

-In the case of pea protein powder can be used in nutritional supplements such as bars and snacks, and as a gluten-free ingredient in a variety of culinary products and as a meat substitute. as an emulsifier, It has good emulsion stability, high OHC, and FC values, but may not be the best option for use as protein shakes or food beverages because of the low solubility values.

- Rice protein powder can be used in a variety of food products as an emulsifier, ingredient for meat substitutes, foaming agent, nutritional supplement, and gluten-free ingredient. It has the best emulsion stability, good OHC, and FC findings, and good foaming stability. However, it might have low solubility values, which could restrict its use in some food products.

- pumpkin protein powder can be utilized due to its high solubility values, as a dietary supplement or a component in baked goods. However, its poor FC and FS performance, poor DIAAS value, and low emulsion stability may restrict its use in some food preparations.

- we can conclude that our protein powder samples have all essential and non-essential amino acids in the case of sunflower shows a high-quality protein. However, our samples amino acids quantities are below daily requirements, they cannot be fully relied upon for nutrition. It is therefore preferable to use them to fortify and enrich food.

- Our study provided satisfactory results in the study of plant-based protein powder, and we can consider it an important base that can be relied upon in the development of food and increase the contribution to sustainability, the use of by-products, and the reduction of waste.

- We suggest that further studies be conducted on the effect of enzymatic digestion on protein functional properties

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