Functional properties of legumes important for food applications
– a study of pea, fava bean, and soy protein

Funktionella egenskaper hos baljväxter betydelsefulla för livsmedel – en studie om ärt-, favaböna- och sojaprotein

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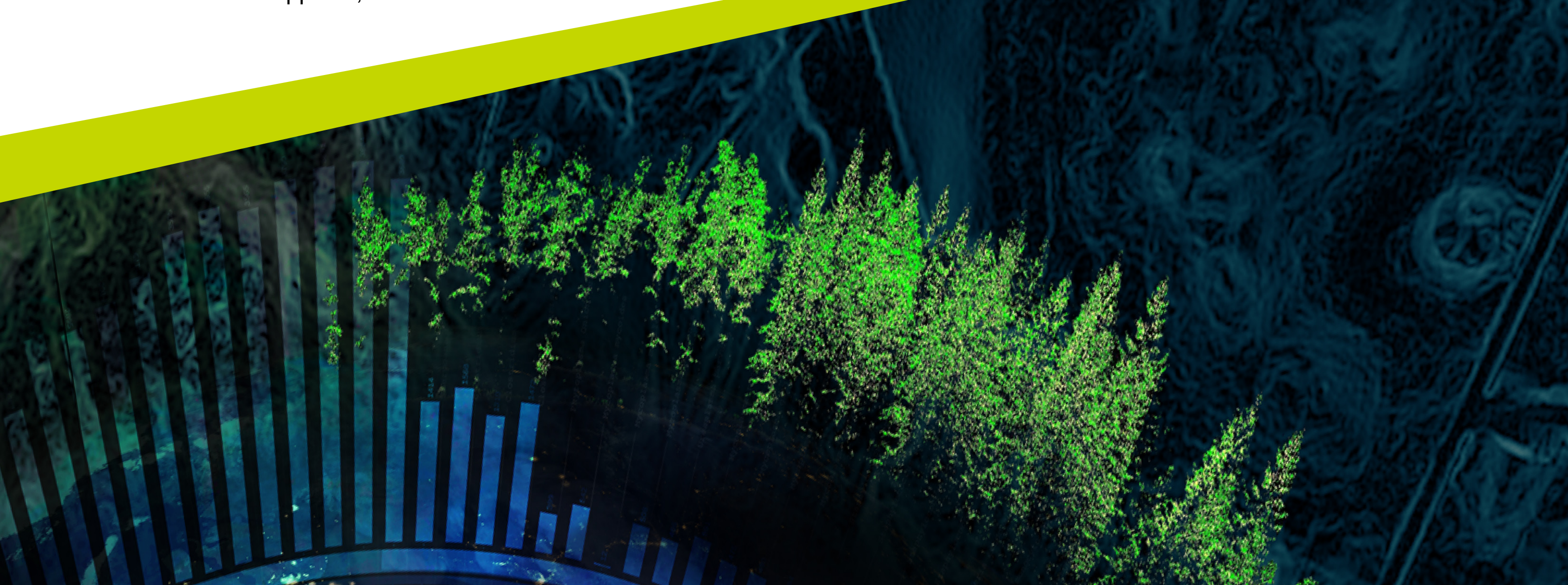

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Abstract

The market demand for foods with plant-based protein sources such as meat analogues has increased due to for example consumer awareness of environmental, ethical, and health issues. Meat analogues based on soy have dominated the market due to soybean's protein quality and eminent functional properties. However, due to sustainability issues in the food supply chain of soy, the need for new plant-based protein sources has increased. Swedish-grown legumes such as pea and fava bean are potential alternatives due to their market availability and cultivation feasibility. Plant-based protein sources such as legumes are often processed, and the proteins are extracted to produce rich-in-protein materials before being utilized in foods such as meat analogues. The aim of this literature study was to do a screening of rich-in-protein materials from pea, fava bean, and soy and to study methods to evaluate the functional properties of these that are relevant in meat analogues. This study found that rich-in-protein materials are utilized in foods both for the nutritional content as well as their functional properties. Functional properties of proteins from soy, pea, and fava beans relevant in meat analogues are solubility, water-holding capacity, fat-absorption capacity, emulsification, and gelation. Some of these functional properties can be affected by internal factors such as surface hydrophobicity, ionic charge, and surface charge of the protein molecules as well as external factors such as pH, temperature, and salt content. Emulsification properties of pea protein isolate was shown to be affected by solubility, surface charge, and surface hydrophobicity. Conditions of parameters such as pH, temperature, and salt content was shown to be of importance in protein extraction, in methods to analyze functional properties as well as in the process of producing meat analogues. To determine the influence of these parameters on the functional properties of rich-in-protein materials an empirical study is required.

Keywords: fava bean protein, functional properties, meat analogues, pea protein, rich-in-protein materials, soy protein

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Abbreviations

ANF	Antinutritional factor
EU	European Union
FAC	Fat-absorption capacity
FbPC	Fava bean protein concentrate
FbPI	Fava bean protein isolate
FSTA	Food Science & Technology Abstracts
GHG	Greenhouse gas
OAC	Oil-absorption capacity
pI	Isoelectric point
PPC	Pea protein concentrate
PPI	Pea protein isolate
SPC	Soy protein concentrate
SPI	Soy protein isolate
TVP	Textured vegetable protein
WAC	Water-absorption capacity
WHC	Water-holding capacity

1. Introduction

In recent years, practicing sustainability has been encouraged at the same time as sustainability awareness vastly has increased. These occurrences continue day by day for many parts in the food system. A sustainable future includes sustainable diets and a protein shift which implies a reduced consumption of animal-based proteins and an increased consumption of plant-based proteins. However, an increased production and consumption of plant-based protein sources ensues some challenges. According to studies (Röös *et al.* 2020), there is an opportunity and a feasibility to increase the cultivation of protein crops in Sweden. At the same time, the market demand of plant-based foods such as meat analogues has increased due to for example consumer awareness of environmental, ethical, and health issues (Kumar 2016). Food producers play an important role in the road towards sustainability by for example connecting agriculture with consumers, but today they face a couple of challenges. Process facilities in Sweden that work with extraction and extrusion of plant-based proteins and are producing rich-in-protein materials are few. Therefore, most of the rich-in-protein materials are imported. The utilization of plant-based foods and the development of new food products with plant-based protein sources have drastically increased in recent years as well as the knowledge of the raw materials, but a lot is left to learn. The question of how food producers meet the increased demand for food products with plant-based protein sources is very accurate and very comprehensive. For the scope of this study, the question of what kind of knowledge the food industry require for a future protein shift in a more sustainable diet is more feasible.

Proteins from different sources are used in food applications for the nutritional content as well as the organoleptic and functional properties. Examples of proteins used in food products on the market today are proteins from soy, wheat, mycoprotein, which is a base to the product *Quorn*, and pea. The market of plant-based foods is growing and there is a need to find new protein sources that can be utilized in these products. Examples of Swedish grown legumes are black beans, white beans, kidney beans, yellow-, green- and grey peas, lupins, fava beans, cranberry/borlotti bean, and lentils, *etcetera*. This study will focus on the commonly used proteins of pea, fava bean, and soy. The inclusion of soy is based on the fact that it is a well-studied legume with great potential regarding both nutritional content and functional properties. However, the production of soybeans is not always considered sustainable due to for example deforestation and the amount of greenhouse gas (GHG) emissions in the food supply chain. Taking this into consideration there is a need to find other options of plant-based protein sources to process and use in food and beverages. The inclusion of pea and fava bean protein is based on the availability of them on the market today as well as the fact that it is feasible to grow them in Sweden which is beneficial both for self-sufficiency and other sustainability aspects such as reducing GHG emissions in for example transportations. Based on this, there is an opportunity to do a screening of these plant-based proteins and study their functional properties with the aim to increase the knowledge of the processing and utilization of these raw materials.

2. Method, aim, and delimitations

2.1. Method

The research method of this study has been a literature review. The literature has been found through the databases Food Science & Technology Abstracts (FSTA), Web of Science, and SLU Primo. The search words used in the databases was: plant protein; food proteins; pea protein; fava bean protein; soy protein; Soy* And protein* And process*; 7S and 11S Globulins; Soy* And protein* And propert*; Pea* And protein* And propert*; Isolate* And protein* And concentrate*; and PDCAAS And Soy protein.

2.1.1. Aim and visions

The aim of the study is to do a screening of rich-in-protein materials from pea, fava bean, and soy and to study methods to evaluate the functional properties of these that are relevant in meat analogues.

The visions that have guided the discussion in this project are to find the intended purposes for the utilization of rich-in-protein materials in meat analogues, that is to examine the sustainability aspects of them and study their functional properties that are relevant in meat analogues.

2.1.2. Delimitations

This study has focused on plant proteins from pea, fava bean, and soy in the food applications meat analogues. The functional properties included in this study are solubility, water-holding capacity (WHC), fat-absorption capacity (FAC), emulsification and gelation. The internal and external factors that have been included in this study are surface charge, hydrophobicity, ionic charge, pH, and temperature. The environmental and health aspect of producing meat substitutes from pea, fava bean and soy protein has been included in this study whereas the economical aspect has not been included.

3. Background

This chapter will describe protein molecules and proteins in foods as rich-in-protein material. It will continue with a description of each of the functional properties of proteins connected to the food applications meat analogues.

3.1. Proteins

Proteins are essential nutrients in human diets due to various biological functions, such as transport, storage, structure, and growth. Proteins also have a vital role in the immune system (Abrahamsson *et al.* 2014). Proteins in foods have often been connected to animal-based protein sources such as fish, beef, pork, and poultry. However, in recent years the topic of plant-based protein sources such as soybean, pea, fava bean, quinoa, lupin, lentils, and chickpeas has increased. Regardless of the source, proteins are consumed for the means to be digested into amino acids and to be rebuilt to specific protein molecules that possess specific biological functions important for our health.

Proteins contain amino acids in different compositions and numbers. Amino acids contain an amino group (NH_2), a carboxyl acid group (COOH) and a side chain (R) as illustrated in **Figure 1**. A protein is a chain of amino acid units connecting the COOH and NH_2 group through peptide bonds. There are 20 unique amino acids and a protein can contain several hundred thousand units (Phillips & Williams 2011). Nine of these 20 amino acids are essential for humans, i.e., vital to be included in the diet for the ability to build all the proteins required to perform the biological functions in the body. The essential amino acids need to be included in the diet due to the inability for the body to produce them. The so called non-essential amino acids, on the other hand, can be produced in the body with sufficient amount of nitrogen and carbohydrates present (Coultate 2001). The order of amino acids in proteins defines the primary structure. The primary structure and the three other structures, the secondary, tertiary, and quaternary, determine the shape, the biological functions, and the properties of a protein (Abrahamsson *et al.* 2014). For the protein to maintain the ability to perform these functions the structures need to be intact, i.e., the native state of a protein molecule is required. Changes to the structures can be a result of several different reasons, an example is protein denaturation. When protein denaturation occurs, the protein molecule has been converted from its native state into its denatured state and the protein loses its biological functions. When it comes to proteins in foods the state of a protein molecule is not significantly important. The purpose of proteins in the diet is the content of essential amino acids and these are not affected by the state of the protein molecule.

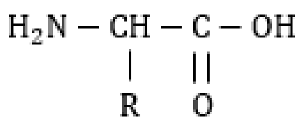

Figure 1. *The structure of an amino acid. (Adapted from Phillips & Williams 2011, p. 2).*

3.2. Rich-in-protein materials

Besides the importance of consuming proteins due to nutritional aspects, proteins are used in foods for their functional properties. Before being utilized in foods, plant-based protein sources are often processed, and the protein extraction results in rich-in-protein materials with functional properties. Examples of rich-in-protein materials are protein isolates, protein concentrates, protein hydrolysates, and textured protein. When it comes to rich-in-protein materials in foods, the state of the protein – native or denatured – can have both positive and negative effects on its functional properties, for further explanation see *3.4 Functional properties of proteins*.

3.2.1. Protein isolates and protein concentrates

A protein isolate contains about 90 percent protein and a protein concentrate contains about 70 percent protein. Apart from protein, isolates and concentrates also contain moisture, lipids, minerals, and carbohydrates (Karaca *et al*. 2011). The extraction methods to produce these rich-in-protein materials of pulses mentioned in Kiosseoglou and Paraskevopoulou (2011) are wet protein extraction and air classification. Air classification uses the size and density difference of protein and starch molecules for the separation of pulse flour. According to the authors air classification is mainly suitable for the production of protein concentrates and wet protein extraction is mainly suitable for protein isolates. The wet protein extraction method includes three parts. In the first part, separation of the proteins occurs in an acidic or alkaline aqueous solution. In the second step the proteins are recovered either by ultrafiltration or by isoelectric precipitation, where the proteins' isoelectric point (pI) is exploited. In the final step the focus is to dehydrate the protein mixture either by freeze-drying or spray-drying (Kiosseoglou & Paraskevopoulou 2011; Boye *et al.* 2010). The pI defines the pH value where the protein molecule has a net zero charge. Depending on the two different methods used in the second step of the wet protein extraction method to recover the pulse protein, the protein composition varies. Kiosseoglou and Paraskevopoulou (2011) mention that ultrafiltration tends to result in both the proteins globulins and albumins while isoelectric precipitation yields mainly globulins. This can be explained by the property differences between globulins and albumins. The pI for globulins is 4.5 whereas the pI for albumins is 6.0. Isoelectric precipitation is a method to extract the protein at pH about 4.5, and since albumins are soluble around that pH, most of them are lost in the process (Berot & Davin 1996 see Sánchez-Vioque *et al.* 1999 and Kiosseoglou & Paraskevopoulou 2011).

3.2.2. Protein hydrolysates

Aluko (2018) mentions that protein hydrolysates are used in foods for their functional properties, but most commonly for their health benefits, such as antioxidants. Protein hydrolysates can be produced by hydrolysis of raw materials, concentrates, or isolates from plant- or meat-based protein sources such as pulses, eggs, milk, oilseeds, fish, and fish by-products (Nasri 2017; Aluko 2018). Protein hydrolysates can be produced by chemical hydrolysis, enzymatic hydrolysis, or microbial fermentation. Chemical hydrolyses are more hazardous as they include strong acidic or alkaline solutions as opposed to enzymatic hydrolyses with a milder process including pH-adjustments to pH 6-8 and temperature-adjustments to 40-60 degrees Celsius (Nasri 2017). Hydrolysis is a process which cleaves the peptide bonds in proteins and creates protein hydrolysates containing components such as free amino acids, peptides, and oligopeptides. Protein hydrolysates can be divided into groups based on the charge, the size of the peptides, or on the hydrophobicity (Aluko 2018).

3.2.3. Textured protein

Textured protein, also called textured vegetable protein (TVP), is a plant-based rich-in-protein material. TVP is produced by extrusion of flour, concentrate, or isolate of legumes, mainly from soybeans but has increased to sources such as peas and fava beans as well. The extrusion creates the appearance of chunks or shreds with similar texture as meat. The protein content of TVP often lies between 60-70 percent.

3.3. Proteins in foods

Some purposes of utilizing protein concentrates, protein isolates, protein hydrolysates, or textured proteins in foods are to increase the protein content, to make use of their health benefits, or to exploit their functional properties. The need for functional properties of proteins is found in food applications such as sausages, burgers, sauces, cakes, and beverages. The focus of this study will be on plant-based proteins in the food applications meat analogues. Meat analogues can be defined as a term of a collection of food applications such as sausages, burgers, and kebab *etcetera* which are made from plants and have similar sensory attributes as if the foods were based on animals. In meat analogues, a combination of rich-in-protein materials are often used. Take for example a burger or an emulsion sausage, where protein isolates often are used to exploit the functional properties but mainly to increase the protein content, and textured proteins are used to exploit the functional properties to achieve a product with similar sensory attributes such as texture and consistency similar to meats.

The list of ingredients in meat analogues can vary depending on the type of food application. However, common ingredients in foods such as sausages, burgers, and kebab are water, ice, oil, salt, spices, fiber, starch, methylcellulose, and some kind of rich-in-protein material. The purpose to use rich-in-protein materials in foods is as explained above. Regarding the other ingredients, they have different purposes, either to preserve the quality, obtain organoleptic properties, obtain functional properties or to achieve all of them together. Hence, it is not merely the proteins that acquire functional properties. Starches and fibers are used for example for water-absorption, gelation, and thickening. In emulsion-sausages and kebab, water, ice, and oil are used for example as bases to form emulsions. Water and oil are two different substances, polar and non-polar, which do not dissolve in each other. To be able to dissolve these substances an emulsifier is required. The emulsifier in these types of food applications are both rich-in-protein materials and methylcellulose. Methylcellulose is classified as a food additive with the E-number E461. E-numbers are a collective of approved food additives with unique codes used within the European Union (EU). Methylcellulose has multiple different functions, it can be used both as an emulsifier and also as a stabilizer, or as a thickening or gelling agent.

3.4. Functional properties of proteins

Meat analogues are produced with the aim to create a food product with similar sensory attributes as the same food application based on animal protein sources. According to Kinsella (1982) the functional properties of proteins connected to food applications such as meats are WHC, gelation, emulsification, and FAC, see **Table 1**. Since the purpose of meat analogues is to imitate these food products it can be argued that these functional properties are relevant for meat analogues as well. The WHC of a protein for example, supports the water-binding and is related to the juiciness of the food product (Cornet *et al.* 2021). The FAC supports the fat-binding, the gelation properties supports the protein-protein and protein-carbohydrate interactions, and the emulsifying properties helps to form and stabilize the emulsions, see **Table 1**. These functional properties also contributes to juiciness as well as to the mouthfeel including chewiness and supports the creation of a meat-like texture. Apart from these four mentioned functional properties, the solubility of proteins will also be included in this study due to the connection to many of the other functional properties (Kiosseoglou & Paraskevopoulou 2011).

Table 1. *Functional properties desired in food applications*

Functional property	*Mechanism of action*	*Food application*
Solubility	Proteins dissolving in a solvent	Beverages
Water-holding capacity	Water-binding	Meats and Sausages
Fat-absorption capacity	Fat-binding	Meats and Sausages
Gelation	Protein-protein and protein-carbohydrates interactions	Meats
Emulsification	Form and stabilize emulsions containing hydrophobic and hydrophilic molecules	Sausages and Bologna

Source: Adapted from Kinsella 1982, p. 53 (see Söderberg 2013)

3.4.1. Solubility – Soluble and insoluble proteins

Aryee *et al.* (2018, p. 30) describe solubility as *"the ability of a given solute to dissolve in a solvent"*. The authors mention that proteins can be divided into three different categories: fibrous, membrane, or globular and it is merely the globular proteins that are soluble *(ibid.)*. Protein solubility depends on the ratio of the protein molecule's hydrophilic and hydrophobic surface patches as well as electrostatic interactions. Albumins for example, have a higher ratio of hydrophilic surface patches and are water-soluble, whereas globulins have a more hydrophobic surface and become more soluble with slightly higher ionic strength which is often achieved by addition of salt (Oomah *et al.* 2011). Solubility is also pH dependent and increases at pH values above and below the pI, at which the net charge is zero (Aryee *et al.* 2018; Kiosseoglou & Paraskevopoulou 2011). Taking lentil as an example, Joshi *et al.* (2018, p. 2904) illustrate the solubility curve of globular proteins of lentil in a figure showing that the protein is less soluble at the pI and more soluble above and below the pI (Fan & Sosulski 1974; Hsu *et al.* 1982; Bamdad *et al.* 2009 see Joshi *et al.* 2018). Another factor affecting the solubility is denaturation. In addition to solubility, a number of functional properties of a protein depends on whether the protein has been denatured or not. Abrahamsson *et al.* (2013) describes denaturation to be a result of for example increased temperature, enzymatic impact, or a change in pH. When protein denaturation occurs the protein's secondary and tertiary structure changes (Aryee *et al.* 2018). Changes in the proteins' structures lead to hydrophobic amino acids being exposed to the surface, which has a negative impact on some of the functional properties of proteins but may improve others. Protein denaturation results in more insoluble proteins. Solubility can therefore indicate if the protein has been denatured or not (Kinsella 1982 see Joshi *et al.* 2017). However, some functional properties are dependent on the denaturation of proteins, Aryee *et al*. (2018) show that the WHC increases when the protein has been denatured.

Kiosseoglou and Paraskevopoulou (2011) conclude that rich-in-protein materials such as isolates, and concentrates are in multiple cases more soluble around the pH value of 4.5 if the recovery was made by ultrafiltration compared to isoelectric precipitation. This can be explained by the fact that ultrafiltration results in the extraction of both albumins and globulins, whereas isoelectric precipitation yields mainly globulins which are soluble with salt compared to the water-soluble albumins (Kiosseoglou & Paraskevopoulou 2011; Oomah *et al.* 2011).

3.4.2. Water-holding capacity

The WHC – also called water-absorption capacity (WAC) – is measured by how much water one gram of protein powder can hold and depends on the hydrophilic and hydrophobic amino acids and the ratio of these in a protein molecule (Aryee *et al.* 2018; Kiosseoglou & Paraskevopoulou 2011). In contrast to the denaturation-solubility relation the WHC is often improved after protein denaturation by heating

(Aryee *et al.* 2018). As with solubility, the type of recovery method have an impact on the WHC. Boye *et al*. (2010) conclude that protein materials recovered with isoelectric precipitation have higher WHC compared to proteins recovered with ultrafiltration. Kiosseoglou and Paraskevopoulou (2011) also mention that the type of pulse can be a factor to different results in the WHC.

3.4.3. Fat-absorption capacity

The FAC – also called oil-absorption capacity (OAC) – is measured by how much fat one gram of protein powder can hold and depends on the ratio of hydrophilic and hydrophobic amino acids in a protein molecule. Higher amounts of hydrophobic amino acids results in higher FAC due to their ability to bond to fat molecules. As with WHC, FAC is affected by the type of pulse and the recovery and processing method (Aryee *et al.* 2018; Kiosseoglou & Paraskevopoulou 2011).

3.4.4. Emulsification

An emulsion is a homogenization of polar and nonpolar molecules such as oil and water. Oil and water – which are normally not solvable in each other – can dissolve with the help of proteins due to their emulsifying properties. To be able to form emulsions amphiphilic protein molecules are required. The ratio of hydrophilic and hydrophobic amino acids in a protein molecule is also relevant for the emulsifying properties. In order to form emulsions, proteins need to unfold and expose the hydrophobic amino acids at the interface. Emulsions require both the hydrophilic amino acids being exposed to the water molecules and the hydrophobic amino acids being exposed to the oil molecules. Hence protein molecules adsorb oil droplets at its interface creating stable and homogenized emulsions (Aryee *et al.* 2018). There are two different indexes that are used to measure the emulsifying properties of proteins; the emulsifying activity index (EAI), which measures the amount of oil proteins can adsorb; and, emulsifying stability index (ESI), which measures the stability of the emulsification over time *(ibid.)*. These indexes can vary depending on the type of pulse and protein material. According to Kiosseoglou and Paraskevopoulou (2011) protein isolates are more suitable for food applications that requires properties such as gelation or emulsification.

3.4.5. Gelation

To achieve protein gelation the protein molecule needs to be unfolded, and the hydrophobic amino acids need to be exposed to the surface. This can be acquired by controlling external factors such as temperature and heat, enzymes, or chemical reagents. In gel formation the proteins are bonding with other protein molecules, i.e., protein-protein interactions, and polysaccharides to form networks stabilized by electrostatic interactions (Aryee *et al.* 2018). Gelation can be measured with the index ‘least gelling concentration’ (LGC). Boye *et al.* (2010, p. 423) define the LGC as “*the lowest concentration required to form a self-supporting gel”*. The authors also mention that there is a correlation between LGC and gelation capacity, lower LGC tend to lead to higher gelation capacity.

3.5. Factors affecting the functional properties

Kiosseoglou and Paraskevopoulou (2011) mention that the functional properties of protein isolates and protein concentrates can differ as a result of the preparation method used to extract the proteins from the pulses. For example, the conditions of temperature, pH, and salt content can vary and are therefore of specific importance in protein extraction.

Nishinari *et al.* (2014) and Tang (2017) mention that pH, temperature, ionic strength, and different processing conditions are factors that affect functional properties such as emulsification, solubility, and gelation. Tang (2017) also concludes that conformational flexibility of the proteins’ structural levels plays a role in the emulsifying properties of the protein. Apart from external factors such as environmental and processing conditions, internal factors such as protein characteristics also play a role in some of the functional properties of proteins. Karaca *et al.* (2011) conclude that the solubility, hydrophobicity, and the surface charge of the proteins affect the emulsifying properties of legume protein isolates such as pea.

4. Analysis methods

Functional properties of plant proteins desired in meat analogues are for example emulsification, WHC, FAC, solubility, and gelation. The methods used to analyze each of these functional properties of rich-in-protein materials can vary. Some examples used in other studies are described in this chapter.

4.1. Solubility

Balmaceda *et al.* (1984 see Fernandez-Quintela *et al*. 1997) measure the solubility as follows: make a suspension of protein material and water with a 1:100 ratio (w/v) and stir it for 15 minutes at room temperature. Centrifuge the suspension at 4000g for 15 minutes. Determine the nitrogen content of the supernatant – the liquid containing the soluble proteins – using the Kjeldahl method. To create a solubility curve, analyze the solubility at different pH values (2-9) – adjust with NaOH or HCl – and NaCl (salt) content (0.1, 0.3, and 0.5 M) *(ibid.)*.

4.2. Water-holding capacity

Balmaceda *et al.* (1984 see Fernandez-Quintela *et al*. 1997) measure the WHC as follows: make a suspension of 1 gram protein material and 15 milliliters deionized water. Adjust the pH with either NaOH or HCl to pH 7 and stir for 15 minutes. Centrifuge the suspension at 4000g for 15 minutes. Remove the supernatant and weigh the remaining mixture *(ibid.)*. Subtract the added protein isolate of the remaining mixture and divide the result by the same amount as the added protein material. The final result concludes the WHC expressed in grams water per gram protein isolate (g water/g protein material).

Sosulski (1962 see Vioque *et al.* 2012) explains another way to measure the WHC: make a suspension of 3 grams protein material and 25 milliliter water. Stir the suspension for 1 minute 6 times with a 10-minute interval. Centrifuge the suspension for 25 minutes at 1000g. Remove the supernatant and dry the precipitate for 25 minutes at 50ºC. Finally, weigh the remaining mixture *(ibid.)*. Subtract the added protein material of the remaining mixture and divide the result by the same amount as the added protein material. The result concludes the WHC expressed in g water/g protein material.

4.3. Fat-absorption capacity

Balmaceda *et al.* (1984 see Fernandez-Quintela *et al*. 1997) measure the FAC as follows: make a suspension with the ratio of 1 gram protein material per 1 milliliter oil. Adjust the pH with either NaOH or HCl to pH 7 and stir for 15 minutes. Centrifuge the suspension at 4000g for 15 minutes. Remove the supernatant and weigh the remaining mixture *(ibid.)*. Subtract the added protein material of the remaining mixture and divide the result by the same amount as the added protein material. The final result concludes the FAC expressed in grams oil per gram protein material (g oil/g protein material).

Lin *et al.* (1974 see Vioque *et al.* 2012) explain another method to measure the FAC: make a suspension of 0.5 grams protein material and 6 milliliter oil. Mix the suspension for 1 minute and wait 30 minutes before continuing. Centrifuge the suspension for 25 minutes at 1600g and weigh the supernatant *(ibid.)*. Subtract the added protein material of the remaining mixture and divide the result by the same amount as the added protein material. The result concludes the WHC expressed in g oil/g protein material.

4.4. Emulsification

Owusu-Apenten (2004) measures the emulsifying capacity (EC) as follows: make a suspension with protein material and oil with a protein concentration of 11 milligram per milliliter oil. Use 25 milliliter oil initially and then add gradually until the emulsion breaks. Mix the suspension at speed 13 (140 rpm) and at a temperature below 28ºC. To measure the ES, heat the emulsion to 68.8ºC and measure the fluid that is released in the process *(ibid.)*.

4.5. Gelation

Sathe and Salunkhe (1981) measure the gelation as follows: fill test tubes with suspensions of different concentrations of protein material ranging from 2-20 percent and 5 milliliter ionized water. Firstly, heat the test tubes in boiling water for 1 hour. Secondly, hold them under the tap in cold water to cool them. Thirdly, keep cooling them in a fridge at a temperature of 4 ºC for 2 hours. Measure the gelation capacity by checking if the gel holds in the test tubes when turning them upside down. Determine the LGC of the protein material as that concentration of the sample that stays in the test tube when turned upside down, hence creates a firm gel *(ibid.)*.

5. Protein sources

Proteins are used in food applications for the means to increase the nutritional content as well as to utilize their functional properties. This study includes the plant-based protein sources soybean, pea, and fava bean. In this chapter the nutritional facts of these three legumes are briefly explained and the functional properties of the proteins such as solubility, WHC, FAC, EC, ESI, EAI, and gelation are explained more in depth.

5.1. Soybean protein

An example of a crop that has been grown, processed, and utilized for years is the soybean *(Glycine Max)*. The legume was domesticated for more than 3,000 years ago in northern China. Today, China is the fourth largest producer of soybean, after the United States (U.S), Brazil, and Argentina. The soybean is a legume with great potential regarding both nutritional content and functional properties. It is a nutritious protein crop mostly grown for animal feed. Merely a small part of the global production is intended for human consumption (McGee 2004). Apart from proteins, soybeans are a good source of unsaturated fatty acids, fibers, minerals such as iron and various B vitamins. Dried soybeans contain 34 percent protein and about 18 percent of both carbohydrates and fat (SLV 2020). On the other hand, the production of soybeans results in a number of unsustainable matters. On a global level, the production in some areas causes deforestation and GHG emissions. On an individual level, the consumption can have negative effects on the body due to the content of oligosaccharides and antinutritional factors (ANF) such as saponins, tannins, and lectins. When consuming soybeans these components can cause both gases and flatulence and also inhibit the uptake of nutrients such as proteins (McGee 2004; Nylander 2014). To reduce the content of these components, the soybeans need to be prepared or processed. Examples of preparation methods are soaking, germination, boiling, or roasting. Processed soybeans can be found in foods and beverages such as soy drink, tempeh, natto, soy sauce, tofu, and miso *(ibid.)*.

The soybean is a well-studied legume with great potential for foods due to its nutritional content. Soybeans contain all of the essential amino acids and are therefore unique in terms of protein and amino acid content compared to other plant-based protein sources. Soybean proteins have the highest score possible in terms of protein digestibility corrected amino acid score (PDCAAS), namely 1.0. PDCAAS is a method used to assess the protein quality, it indicates the potential of the amino acids to be digested. Due to this high score in protein quality, soybean proteins are considered equivalent to proteins in meat-based protein sources (Fukushima 2011). In food applications such as meat analogues, it is merely the soybean proteins that are used. To extract proteins out of soybeans different methods can be applied. Extractions of proteins in soybeans result in either soy protein isolate (SPI) or soy protein concentrate (SPC). As previously mentioned, isolates and concentrates include different types and compositions of proteins. Hughes *et al.* (2011) use a specific correction factor when measuring and calculating the PDCAAS score of SPI and SPC and concludes that both SPI and SPC have score of 1.0. According to FAO (2018), the content of ANFs is of particular importance to consider when evaluating the protein quality with the PDCAAS method due to the fact that ANFs inhibit the uptake of nutrients such as proteins. Fernandez-Quintela *et al.* (1997) reports that the ANFs of protein isolates of soybean, pea, and fava bean are lower compared to the legume seeds.

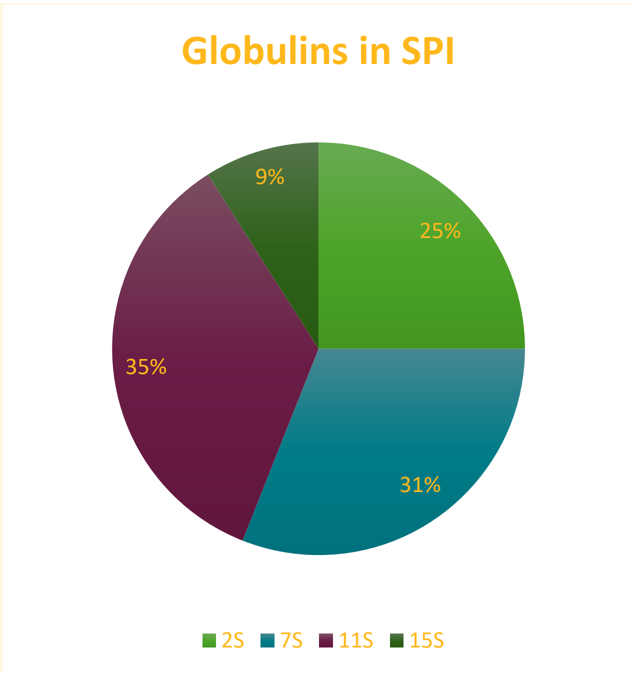

Figure 2. *Globulin composition (2S, 7S, 11S, and 15S) in SPI (Chakraborty et al. 1979).*

Apart from the protein quality of soybeans, they are utilized in food applications for the acquired functional properties (McGee 2004; Nylander 2014). In food applications such as meat analogues however, it is merely the proteins of soybeans that are used. SPI and SPC acquire the functional properties desired in these food applications. SPI contains the globulins 2S, 7S, 11S, and 15S. These globulins are divided into groups depending on the sedimentation coefficient (s), i.e., the molecular mass and the extraction capacity. The majority of globulins in legumes consist of 7S and 11S, 7S globulins are called β-conglycinin and 11S globulins are called glycinin (Nishinari *et al.* 2014). Kimura *et al.* (2008) compare the functional properties of 7S and 11S globulins in proteins such as soybeans, fava beans, and peas. The authors clearly state that 7S and 11S globulins are the major storage proteins in plant-based protein sources. Both pea-, fava bean-, and soybean proteins contain these globulins. In the study by Chakraborty *et al*. (1979) the globulin composition of SPI was analyzed to 25, 31, 35, and 9 percent of 2S, 7S, 11S, and 15S, respectively, see **Figure 2**. Tang (2017) mention that the ratio of 11S:7S in soy protein can impact both the emulsifying ability as well as the emulsifying stability. Regarding the emulsifying properties, Karaca *et al.* (2011) measured the EAI of SPI produced by isoelectric precipitation to 44.2 square meter (m^2) per gram protein, the ESI to 86.0 minutes (min) and the EC to 520 grams oil per gram protein (g oil/g protein). As previously mentioned, other factors affecting the emulsifying properties are solubility, hydrophobicity, and surface charge of proteins. The surface charge of SPI was measured to about –22.6 millivolt (mV), the surface hydrophobicity was measured to about 55.2 H_0-ANS and the solubility to 96.5 percent, see **Table 2** *(ibid.)*. Solubility is however dependent on both pH and salt concentration. Fernandez-Quintela *et al*. (1997) reported that the lowest solubility of SPI is achieved between pH 4.00-6.00 and maximum solubility is found above and below these pH values. The authors also analyzed other functional properties of SPI, the FAC was analyzed to 1.1 g oil/g protein material and the WHC was analyzed to 1.3 g water/g protein material, see **Table 2**. Lopez de Ogara *et al.* (1992 see Fernandez-Quintela *et al*. 1997) analyzed a correlation between WHC and the gelation capacity, lower WHC was shown to result in a lower gel formation capacity.

Table 2. *Functional properties of SPI, PPI, and FbPI*

	SPI	***PPI***	***FbPI***
Surface charge[a] (mV)	−22.7 ± 0.06	−21.0 ± 0.26	−23.0 ± 0.70
Hydrophobicity[a] (H_0-ANS)	55.32 ± 0.58	84.76 ± 1.16	55.23 ± 2.23
Solubility[a] (%)	96.53 ± 0.04	61.42 ± 0.77	89.65 ± 0.24
Emulsifying capacity[a] (g oil/g protein)	520.00 ± 13.33	477.78 ± 3.85	513.33 ± 0.00
Emulsifying stability index[a] (min)	85.97 ± 5.33	12.40 ± 0.04	69.39 ± 3.71
Emulsifying activity index[a] (m^2/g)	44.20 ± 0.92	42.87 ± 0.80	44.29± 0.55
Water-holding capacity[b] (g water/g protein material)	1.3 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Fat-absorption capacity[b] (g oil/g protein material)	1.1 ± 0.1	1.2 ± 0.1	1.6 ± 0.2

Source: a) Karaca et al. 2011; b) Fernandez-Quintela et al. 1997

5.2. Pea protein

The history of peas started about 9,000 years ago in the Middle East and the different varieties originates from different places in the world. The family of peas *(Pisum Sativum)* include varieties such as yellow pea, green pea, grey pea, black-eyed pea, and pigeon pea. However, the black-eyed pea and the pigeon pea are not domesticated from the pea but are rather relatives to the mung bean and the common bean, respectively. In the Middle Ages the legume played an important role as a protein crop in Europe (McGee 2004). Lately, the role of peas in several countries of Europe has been as a vegetable and also as a main ingredient in some dishes such as *Crème Ninon* and '*ärtsoppa'* – soups containing green peas and yellow peas, respectively. The utilization of peas has expanded in recent years and the legume is currently in the uprise of being a meat substitute due to its nutritional content. Peas are a good source to proteins, carbohydrates, and fibers. Dried peas contain almost 50 percent carbohydrates and about 20 percent protein (SLV 2020). However, peas do not contain all the essential amino acids in a sufficient amount as soybeans do, they are deficient in the amino acids methionine and cysteine. In fact, most of the pulse proteins acquire these deficiencies and it is therefore recommended to mix pulse proteins with cereal grains that contain these amino acids in a sufficient amount in a diet. Compared to soybeans peas contain ANFs, but in considerably lesser amount. Hence, before consumption peas do not require preparation methods to the same extent as other legumes in regard to digestibility inefficiencies. However, ANFs can affect other qualities such as texture, appearance, and palatability which argues for the importance to remove these before use (Arntfield & Maskus 2011). The authors mention that the ANFs are removed in the process when producing protein isolates. Furthermore, as previously mentioned, it has been reported that the amount of ANFs in protein isolates of soy, peas, and fava beans are lower compared to the original legume seeds (Fernandez-Quintela *et al.* 1997).

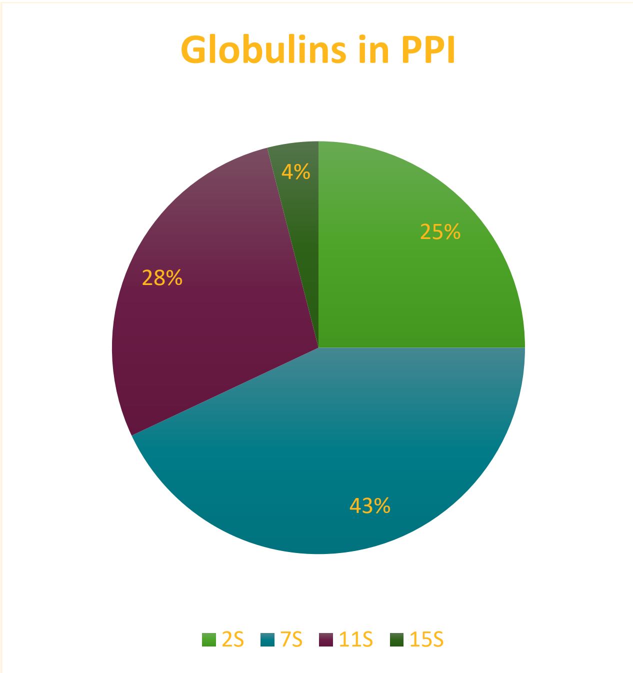

Figure 3. *Globulin composition (2S, 7S, 11S, and 15S) in PPI (Chakraborty et al. 1979).*

Pea protein isolate (PPI) and pea protein concentrate (PPC) are examples of rich-in-protein materials of peas which are used in food applications such as meat analogues. PPI and PPC are used in meat analogues both due to the protein content as well as their functional properties. The protein content and the protein composition of PPI and PPC can vary due to the preparation and extraction method used. But in general, isolates contain a higher amount of protein compared to concentrates and the majority of proteins tend to consist of globulins. Arntfield and Maskus (2011) analyzed the protein content of PPI to between 80-90 percent and Chakraborty *et al.* (1979) analyzed the globulin composition in PPI to 25, 43, 28, and 4 percent of

2S, 7S, 11S, and 15S, respectively, see **Figure 3**. Similar to soybeans, the majority of globulins in PPI consist of 7S and 11S, and the ratio between these globulins can affect some of the emulsifying properties (Tang 2017). The emulsifying properties of plant protein isolates can also be affected by solubility, hydrophobicity, and surface charge. Karaca *et al.* (2011) analyzed the surface charge of PPI produced by isoelectric precipitation to –21 mV, the surface hydrophobicity to 84.8 H_0-ANS and the solubility to about 61.5 percent, see **Table 2**. According to Fernandez-Quintela *et al*. (1997) the lowest solubility of PPI is found around pH 4.00-6.00, whereas higher solubility is found above and below those pH values. The authors found that PPI reached about 80 percent solubility around pH 8.00-9.00. Karaca *et al.* (2011) concluded that the results of solubility, hydrophobicity, and surface charge displays in the results of the emulsifying properties of PPI. The authors analyzed the EC of PPI to about 478 g oil/g protein, the ESI to 12.4 min, and the EAI to 42.9 m^2 per gram isolate. Other functional properties of legume proteins desired in meat analogues are WHC, FAC, and gelation. Fernandez-Quintela *et al*. (1997) analyzed the WHC and FAC of PPI to 1.7 g water/g protein material and 1.2 g oil/g protein material, respectively, see **Table 2**.

5.3. Fava bean protein

The fava bean *(Vicia Faba)*, also called faba bean or broad bean, is one of the first ever domesticated plant and originates from central or western Asia. Today, China is one of the largest producers of fava beans (McGee 2004). The legume is a good source of proteins, fibers, carbohydrates, and minerals such as iron. In the matter of nutritional content, fava beans are more similar to peas rather than soybeans. Compared to soybeans, fava beans contain a lesser amount of protein, a greater amount of carbohydrates and a considerably lesser amount of fat. Dried fava beans contain about 40 percent carbohydrates, 25 percent protein, and only about 2 percent fat (SLV 2020). Fava bean protein do not contain all the amino acids in a sufficient amount. As for pea protein, fava bean protein is deficient in the amino acids methionine and cysteine. Nowadays, fava beans are produced both for feed and food. Fava beans can be found in dishes such as *ful medames* and on the shelves of food stores as canned, dried, or frozen. Similar to soybeans, fava beans contain ANFs and require preparation or processing before consumption to reduce the risk of flatulence, or the protein uptake to be inhibited. A unique property of fava beans is the content of the glucosides vicine and convicine. These glucosides can cause favism for people that are unable to produce the enzyme G6PD which is connected to the metabolism in the body. Favism is a disease which in serious cases can cause fatal anemia (McGee 2004). In other words, vicine and convicine need to be removed from fava beans to reduce the risk of favism when consuming the legume. Vioque *et al.* (2012) show that extracting protein from fava beans and producing fava bean protein isolate (FbPI) can nearly eliminate the content of these glucosides.

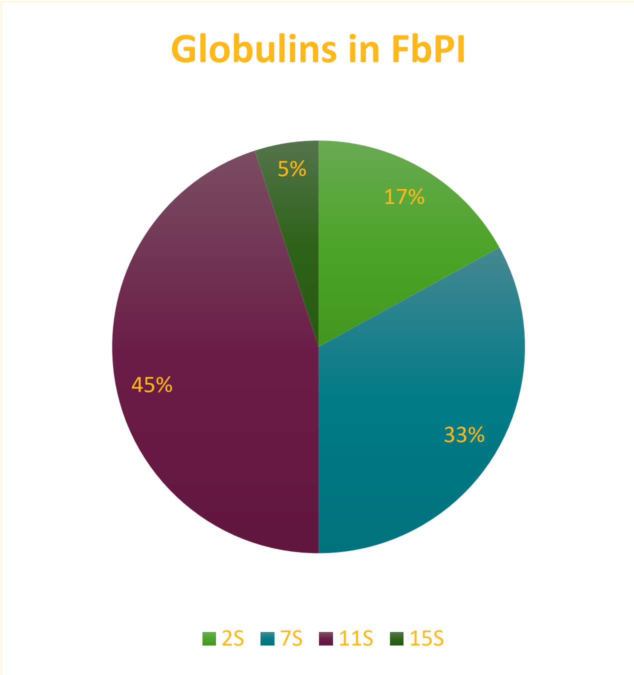

Figure 4. *Globulin composition (2S, 7S, 11S, and 15S) in FbPI (Chakraborty et al. 1979).*

As with isolates and concentrates of peas and soybeans, FbPI and fava bean protein concentrates (FbPC) are used in food applications such as meat analogues due to their protein content and functional properties. FbPC contain proteins about 70 percent while FbPI contain about 80-90 percent. As with PPI and SPI, the majority of globulins in FbPI consist of 7S and 11S. Chakraborty *et al.* (1979) analyzed the globulin composition in FbPI to 17, 33, 45, and 5 percent of 2S, 7S, 11S, and 15S, respectively, see **Figure 4**.

Regarding the functional properties of FbPI, Karaca *et al.* (2011) analyzed the surface charge of FbPI produced by isoelectric precipitation to –23.0 mV, the surface hydrophobicity to about 55.2 H_0-ANS and the solubility to above 90 percent, see **Table 2**. Fernandez-Quintela *et al.* (1997) reported that the lowest solubility of FbPI is found around pH 4.00-6.00 and higher solubility is found above and below those pH values. For FbPI, maximum solubility is found between pH 8.00-9.00. As previously stated, solubility, hydrophobicity, and surface charge can affect the emulsifying properties. Karaca *et al.* (2011) found that the EC of FbPI produced by isoelectric precipitation is about 513 g oil/g protein, the ESI is 69.4 min and the EAI is 44.3 m^2 per gram isolate. Other functional properties such as WHC, FAC, and gelation was reported by Fernandez-Quintela *et al*. (1997). The authors found that the WHC and the FAC of FbPI is 1.8 g water/g protein material and 1.6 g oil/g protein material, see **Table 2**. As mentioned previously, Lopez de Ogara *et al.* (1992 see Fernandez-Quintela *et al*. 1997) analyzed a correlation between WHC and the gelation capacity, lower WHC was shown to result in a lower gel formation capacity. As with some of the other functional properties presented in this study, the gelation capacity and the LGC of protein materials of legumes such as pea and fava bean can vary due to external factors such as pH, salt, and extraction method (Langton *et al.* 2020; Raikos *et al.* 2014).

6. Discussion

In this chapter the focus is to discuss the findings of the study. The chapter starts with a discussion of the sustainability aspects of rich-in-protein materials in plant-based foods and continues with a discussion of the functional properties of soybean, pea, and fava bean proteins. Lastly, the methods to analyze these functional properties are summarized.

6.1. Sustainability aspects

Soybeans, peas, and fava beans are good sources to protein. Dried soybeans contain 34 percent protein, dried peas contain about 20 percent protein, and dried fava beans contain 25 percent protein. However, it is merely soybean protein that contain all the 20 essential amino acids, fava bean and pea protein are both deficient in the amino acids methionine and cysteine. In a diet, it is important that all the essential amino acids are included due to their biological functions and to the body's inability to produce them. Apart from soybeans, legumes are often recommended to be combined with cereal grains in a diet to cover all the essential amino acids. It can therefore be argued that the amino acid composition can be important to consider when making foods with these kinds of proteins due to nutritional purposes. Purposes to utilize rich-in-protein materials are not only the nutritional content, but for their organoleptic and functional properties as well. In contrast to the content of amino acids, legumes in general contain ANFs which can inhibit the uptake of nutrients such as proteins. The content of ANFs in rich-in-protein materials of legumes can therefore be of importance when the purpose of utilizing these in food applications is to increase the protein content. However, Fernandez-Quintela *et al.* (1997) found that the content of ANFs in rich-in-protein materials such as isolates, and concentrates of soybeans, peas, and fava beans are lower compared to the original legume seeds. It can therefore be argued that the production of isolates and concentrates of these legumes are beneficial for individuals in consumption. In addition, it can also be beneficial since Vioque *et al.* (2012) found that the preparation and extraction methods can reduce the content of vicine and convicine. These glucosides can cause favism for people that are born with the inability to produce a specific enzyme needed to metabolize sugars in the red blood cells. To summarize, in a social and a health perspective protein isolates and concentrates of legumes can be preferable due to the reduction of some health risks compared to consuming edible legume seeds.

In contrast, the production of isolates and concentrates requires more steps, hence more resources, and results in more by-products compared to the production of edible legume seeds. Consuming edible legume seeds would therefore be considered as a rather better choice compared to consuming foods containing rich-in-protein materials from an environmental perspective. To conclude, on one hand we have the reduced health risks and on the other we have the environmental impact of foods based on rich-in-protein materials of legumes. However, the environmental impact can be interpreted either as lesser or greater depending on what it is compared to. Comparing it to edible legume seeds it results in a greater environmental impact. Other relevant options is to compare it with meat-based proteins since both rich-in-protein materials and meat-based proteins can be used to produce foods such as sausages, hamburgers, and kebab. The comparison might result in that foods based on rich-in-protein materials of legumes have a lesser environmental impact. This is based on the fact that meat production is a section in the food system which partly have a negative impact on the environment due to for example GHG emissions from ruminants.

Another aspect is the market demand and the consumer acceptance of edible legume seeds and foods with rich-in-protein materials of legumes. Lower consumer acceptance of the consumption of edible legume seeds can be connected to organoleptic qualities such as taste and texture. In comparison to rich-in-protein materials such as isolates and concentrates these do not have the same composition of nutrients as the edible legume seeds, hence different organoleptic profiles. Plant-based foods such as meat analogues are often produced with the aim to imitate meat in aspects such as taste, texture, mouth-feel, and bite. An example of a rich-in-protein material fitting in this category is the TVP, which has the appearance of shreds or chunks with a similar texture as meat. The consumer acceptance and the market demand for plant-based foods has increased alongside the increasing trend of vegan and vegetarian diets. In contrast to lower consumer acceptance, higher consumer acceptance of edible legume seeds and plant-based foods include factors such as health, ethics, and environment. These including factors are parts of sustainability and it can therefore be argued that the current awareness of this concept can support the consumer acceptance to continue increasing.

Comparing the levels of sustainability in this way gives an overview of the levels alone but not of sustainability as a whole. Sustainability is a concept where all the levels; the environmental, social, and the economic perspective are included and considered. At the same time, it could be argued that sustainability is a goal worth reaching for and can be described as a concept with the ability to constantly improve. It is therefore beneficial to weigh the positive and the negative aspects.

6.2. Functional properties

Protein isolates and protein concentrates both contain protein, moisture, lipids, minerals, and carbohydrates, but in different amounts. The main component of these rich-in-protein materials is protein, as previously stated. The other components can be viewed as by-products which the extraction method did not have the ability to separate or remove. The main focus of this study has been on the proteins whereas the effects other components have on the functional and organoleptic properties has not been considered in this study. However, it should be stated that these components can possibly have a greater impact or effect on the functional and organoleptic properties on concentrates than isolates due to the protein content of about 70 percent in concentrates and about 90 percent protein. Hence, the possible impact on these properties are of a lesser risk in isolates due to the amount of these components.

Kiosseoglou and Paraskevopoulou (2011) found that the preparation method used to extract the proteins from the legume seeds can affect the functional properties of protein isolates and protein concentrates. The authors also found that conditions of parameters such as temperature, pH, and salt content can vary in these preparation methods and are therefore of specific importance when extracting proteins. There are a couple of different processing methods that can be used to produce rich-in-protein materials, the results presented in this study have been generated from rich-in-protein materials produced by isoelectric precipitation. Other factors apart from the extraction and processing methods used to produce rich-in-protein materials that can affect the functional properties are hydrophobicity, solubility, and surface charge concluded by Karaca *et al.* (2011). Rich-in-protein material of soybeans are often used as a reference in many studies and can therefore be interpreted to be an optimal protein in this context, and its properties are worth aiming for. The differences of these measurements between soybean, pea, and fava bean protein are shown in the study. As seen in **Table 2**, PPI have higher hydrophobicity and surface charge, and lower solubility meanwhile lower EC and ESI compared to both FbPI and SPI. Based on these results, Karaca *et al.* (2011) conclude a correlation between higher hydrophobicity, higher surface charge, and lower solubility with lower emulsion capacity regarding EC and ESI.

In contrast to internal factors, external factors can affect some functional properties. Examples of external factors are temperature, enzymes, and pH. Increased temperature, enzymatic impact, or a change in pH values can result in protein denaturation, where the protein structure changes completely, and the protein molecule is converted from a native state to a denatured state. When protein denaturation occurs, functional properties are either enhanced or reduced. The solubility is for example reduced whereas the WHC is enhanced. Protein denaturation affects functional properties, hence functional properties partly depend on protein denaturation. It can hereby be stated that a protein always acquires a functional property to some extent, and it is therefore problematic to define, separate, and use the terms *functional proteins* and *non-functional proteins*.

In many studies, optimal conditions regarding for example temperature, ionic strength, and pH are created to analyze the functional properties of proteins. To give an example, the solubility of protein such as globulins are often dependent on the pH value and the ionic strength, which can be achieved by the addition of salt. Furthermore, most of the methods used to analyze the functional properties of rich-in-protein materials include an active change in pH and temperature. These changes are made to be able to analyze the proteins' full potential regarding their functional properties. It can therefore be concluded that these factors are crucial parameters to control to acquire the desired functional properties. Additionally, most of the functional properties are dependent on the solubility of proteins, i.e., the proteins need to be soluble. The solubility curve of globular proteins of legumes are relatively similar; the solubility tends to be the lowest around pH 4-6 and higher above and below these pH values. This can be explained by the fact that the pI of almost all globular proteins of legumes is about pH 4-5. In this case it means that the pH needs to be above or below 4-5 to acquire the desired functional properties. In food applications such as meat analogues the process method and the environmental factors might not correspond to these optimal conditions. To give an example, the pH of meat analogues is about 4-5. This raises the question of whether the functional properties of these rich-in-protein materials will not or cannot be utilized in these types of foods to their full potential. Another aspect is that when making meat analogues such as sausages, burgers, and kebab, methylcellulose is used as an emulsifier, stabilizer, thickening agent or as a gelling agent.

Methods to analyze the functional properties often include a solution preparation, a change in pH and temperature, and a centrifugation. To decide on analysis methods that are relevant to evaluate the functional properties of plant-based proteins, it is crucial to know the intended food application and the conditions of factors that can have an impact on the functional properties in this food application. Other things that might be relevant to consider is the process to produce this product, the temperature and pH values that will be reached, the amount accessible water, the salt concentration, and what mechanical process is included.

6.3. Ideas for further research

A suggestion for further research is to analyze the influence of methylcellulose on the functional properties in meat analogues to determine the impact compared to the rich-in-protein materials. Such analysis could possibly lead to the findings of the importance of the functional properties in meat analogues to determine what to focus on when developing new foods. Another suggestion is to study the influence of parameters such as pH, salt content, and temperature on the functional properties of rich-in-protein materials by empirical studies.

7. Conclusion

Rich-in-protein materials are utilized in foods both for the nutritional content as well as their functional properties. In a health perspective, it can be argued that isolates and concentrates are preferable compared to consumption of edible legume seeds in regard to the reduced content of antinutritional factors and the glucosides vicine and convicine that can be found in fava beans. Apart from the content of antinutritional factors and glucosides, optimal conditions of factors such as pH, temperature, and ionic strength are crucial to control and analyze to achieve the full capacity of proteins' functional properties. To achieve the full capacity of the proteins' functional properties it is also crucial to be familiar with the process and the conditions of pH, temperature, and salt. In contrast to the preparation methods, the conditions of these factors, the amount of accessible water, and the mechanical process are also important in the intended food application to determine which functional properties of proteins that can be utilized in meat analogues.

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Appendix 1 – Popular scientific summary

Food applications such as meat analogues with plant-based protein sources such as legumes has increased on the market and on the shelves in stores alongside the consumer awareness of for example environmental, ethical, and health issues. Soy has been the leading protein source in meat analogues for a very long time. However, due to the sustainability issues such as deforestation and the amount of greenhouse gas emissions in the food supply chain of this legume, the need for other alternatives has increased. Legumes such as pea and fava bean are potential alternatives due to the availability of them on the market and the feasibility to cultivate them in Sweden. Regarding meat analogues, legumes are not often used in these food applications in its natural form, but rather in the form of rich-in-protein materials such as protein isolates, protein concentrates, protein hydrolysates, or textured protein. These materials have a protein content reaching from 60 to 90 percent and have been produced by protein extraction of plant-based protein sources such as legumes.

The aim of this literature study was to do a screening of rich-in-protein materials from soy, pea, and fava bean and to study methods to evaluate the functional properties of these that are relevant in meat analogues.

Soy is an optimal alternative to meats due to its protein quality and its eminent functional properties. In comparison, soy contains all the essential amino acids whereas pea and fava bean are deficient in methionine and cysteine. One thing to consider before utilizing legumes in foods such as meat analogues is that they often contain antinutritional factors (ANFs) such as saponins, tannins, and lectins which can cause gases at consumption. Another downside is that fava beans contain the glucosides vicine and convicine which can cause favism for people that are not able to produce a specific enzyme. However, studies show that the production of rich-in-protein materials reduces the amount of both ANFs and the glucosides vicine and convicine.

This study found that the purpose to use rich-in-protein materials in meat analogues are to increase the protein content, to exploit their health benefits, or to exploit their functional properties. Functional properties of rich-in-protein materials from soy, pea, and fava bean relevant in meat analogues are solubility, water-holding capacity, fat-absorption capacity, gelation, and emulsification. The study also found that some of these functional properties can be affected by internal factors such as surface hydrophobicity, ionic charge, and surface charge of the protein molecules as well as external factors such as pH, temperature, and salt content. Emulsification properties of pea protein isolate was shown to be affected by solubility, surface charge, and surface hydrophobicity. Conditions of parameters such as pH, temperature, and salt content was shown to be of importance in protein extraction, in methods to analyze functional properties as well as in the process of producing meat analogues. To determine the influence of these parameters on the functional properties of rich-in-protein materials an empirical study is required.