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Agronomy Program – Food Science
Independent Project in Food Science• Master Thesis • 30 hec • Advanced A2E
Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap, no 378
Uppsala, 2013

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Credits: 30 hec
Level: Advanced A2E
Course title: Independent Project in Food Science
Course code: EX0425
Programme/education: Agronomy – Food Science

Place of publication: Uppsala
Year of publication: 2013
Title of series: Publikation/Sveriges lantbruksuniversitet, Institutionen för livsmedelsvetenskap
Serie no: 378
Online publication: http://stud.epsilon.slu.se

Keywords: Functional properties, legume proteins, egg protein, soy protein, pea protein, vegan, protein quality, consumer acceptance

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Abstract

Egg protein is used in a variety of food products due to its excellent functional properties (solubility, emulsification, foaming and gelling) and protein quality. Concerns about high cholesterol, allergies, animal welfare, high food costs, as well as the food productions negative impact on the environment has led to an increased interest in alternative protein sources that can act as egg replacers in food.

This literature study has the aim to compare the functional properties and protein quality of soy and pea with egg, this in order to evaluate their potential as egg replacers in traditional egg foods without dairy or animal ingredients. The findings showed that soy and pea protein possess similar solubility pattern as egg protein. Soy and pea protein have emulsifying properties that are similar to egg protein and changing the pH can regulate the thickness of the emulsion. Both soy and pea protein are able to form foams but pea protein is shown to be a better foaming agent than soy protein. Egg protein form stronger foams in room temperature than the two legume proteins. The gelling properties were shown to be best for egg and soy protein, but soy proteins do not form proper gels at higher temperatures and are not suitable for heat- induced food gels. Pea proteins form weak gels and are not applicable for food gels.

The drawbacks with using legume proteins as egg replacers are their content of anti-nutritional substances as well as their limitation in some sulfur containing amino acids, which affect the protein quality in a negative way. The protein quality for egg, soy and pea protein was evaluated by comparing the results from different scoring methods (e.g. PDCAAS) aimed for this. Through this evaluation, soy protein was found to be a complete protein. Pea protein was shown to be an incomplete protein, but can be complete if mutual supplementation is applied.

The study also showed that foods based on soy and pea protein do not have the same texture, color or odor as food products based on egg protein, and that the consumer acceptance for this kind of products is low. Further studies have to be carried out on optimization of formulas for legume-based products in order for them to gain consumer acceptance and succeed on the market.

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Abbreviations

SPI	Soy protein isolate
SPC	Soy protein concentrate
PPI	Pea protein isolate
PPC	Pea protein concentrate
EA	Emulsion activity
ES	Emulsion stability
FC	Foam capacity
FE	Foam expansion
FS	Foam stability
PDCAAS	Protein digestibility-corrected amino acid score
CS	Chemical score
PER	Protein efficiency ratio
NPU	Net protein utilization
BV	Biological value

1. Introduction

Legumes are an important protein source as a substitute for animal proteins (Liener, 1962). There are a variety of dairy- and animal free food products (i.e. vegan products) on the market that are based on legumes (e.g. tofu, tempeh, meat analogues, and dairy alternatives) (Liu, 2000; Singh *et al.*, 2000, as cited in Friedman and Brandon, 2001, p. 1070) but few that mimic products based on eggs (i.e. mayonnaise and baked goods).

Concerns about high-cholesterol, allergens, animal welfare, the food industries impact on the environment, and high food costs have, however, resulted in an increased interest in using legume protein as egg replacers in foods (Khouryieh *et al*., 2006; Hughes *et al*., 2011; Levan *et al*., n.d). Legumes have a high protein content as well as high protein quality, and recent studies shows that they also have the potential to fight malnutrition in the developing countries by making them a part of the daily diet (Butt and Batoola, 2010).

Eggs are used in foods because of their excellent functional properties (e.g. solubility, foaming, emulsifying and gelling/coagulation), high protein quality and sensory quality (Kato *et al*., 1993). In order for legume proteins to be a successful ingredient in foods, they must possess suitable functional properties, as well as having a good protein quality and sensory characteristics (Heng, 2005). One drawback with using legume proteins in foods is the facts that they have limiting amino acids (Leterme *et al*.,1990) and that they contain anti-nutritional factors that affect the digestibility and thus, the bioavailability of proteins in a negative way (FAO, 2011). Another drawback with using legume proteins is their distinct "beany" and "hay-like" flavor that is hard to mask (Rackis *et al*., 1979, as cited in Aspelund and Wilson, 1983, p. 539). These off-flavors may contribute to a reduced consumer acceptance for food products and thus also the success for these kinds of food products on the market (Owusu-Ansah and McCurdy, 1991).

Soy protein has been used for a long time in non-vegan foods (e.g. as meat extenders) to improve the functional properties and nutritional value of these foods (Soya, n.d). Interest in legumes that can act as a substitute for soy protein in foods has increased and one highly potential legume for this purpose is pea (O'Kane *et al*., 2004). Accepting the fact that pea allergy is rare (San Ireneo *et al*., 2000), studies also show that the functionality and protein quality of pea may be as good as those of soy protein (O'Kane *et al*., 2004).

Despite the fact that the interest from the food industry in legume proteins as potential egg replacers has increased, published studies on this area are limited. Thus, the aim of this study was to compare the functional properties of soy and pea protein, as well as their protein quality, organoleptic and kinesthetic properties, and consumer acceptance with those of egg, in order to evaluate if they have a potential as total egg replacers in traditional egg-based foods free of dairy and other animal products.

2. Methods, materials and delimitations

2.1 Methods

This study was performed as a literature survey where the following questions were in focus to reach the aim:

- What determines the functional properties of a protein, and which functional properties are associated with egg proteins? Do legume proteins also have these specific properties, and are there any similarities and/or differences concerning their functional properties? Are there any legumes of special interest?

- Are there any drawbacks and/or advantages with using legume protein as egg replacers?

- What is the protein quality of legumes compared to eggs?

- Is it possible for legume-based foods to be successful on the market?

2.2 Materials

The references behind this study have been collected through the databases: Web of knowledge, Scopus, Primo, ProQuest, PubMed, Science direct, Google scholar and Google books. Literature, in the form of books, from the San Francisco public library has also been used.

2.3 Delimitations

The delimitations in this study have been:

- Only the four most known functional properties associated with egg proteins (i.e. solubility, emulsification, gelation and foaming) were studied.

- Only the influence of structure and environmental factors (e.g. pH, temperature and ionic strength) has been studied. The fact how genes and plant varieties affect the functional properties has not been studied.

- The improvement of the functional properties of proteins through different modification methods has not been studied.

3. Functional properties of food proteins

The functional properties of a protein are:

“Those physical and chemical properties, which affect the behavior of proteins in food systems during storage, processing, preparation and consumption. It is these characteristics, which influence the ‘quality’ and organoleptic attributes in food.”

(Kinsella, 1982, p. 51)

The functional properties of a protein are affected by both intrinsic and extrinsic factors. The intrinsic factors are: shape, size, amino acid composition and sequence, the distribution of net charges, the ration between hydrophobicity/hydrophilicity, secondary, tertiary and quaternary structures of the protein as well as the protein’s capacity to interact with other components in the food system (Damodaran, 1997). The extrinsic factors that affect the functionality of proteins are: pH, temperature, moisture, chemical additives, mechanical processing, enzymes and ionic strength (Kinsella, 1982). There are proteins that are associated with specific functional properties, such as egg proteins with coagulation, or soy proteins for their use in forming food gels (Vaclavik and Christian, 2003). Some example of functional properties can be seen in **Table 1** (Kinsella, 1982).

Table 1. Functional properties of proteins in food applications

General property	**Functional criteria**
Organoleptic	Color, flavor and odor.
Kinesthetic	Texture, mouthfeel, smoothness, grittiness, turbidity.
Hydration	Solubility, wettability, water absorption, swelling, thickening. Gelling, syneresis, viscosity.
Surface	Emulsification, foaming (aeration, whipping), film formation.
Binding	Lipid-binding, flavor-binding.
Structural	Elasticity, cohesiveness, chewiness, adhesion, network crossbinding, aggregation, dough formation, textruisability, fiber formation, extrudability.
Rheological	Viscosity, gelation.
Enzymatic	Coagulation (rennet), tenderization (papain), mellowing (proteinases).
Blendability	Complementarity (wheat-soy, gluten-casein).
Antioxidant	Off-flavor prevention (fluid emulsions).

(Kinsella, 1982, p. 52)

In order to evaluate if a protein is applicable and suitable in certain food systems and food products, it is important to characterize the functionalities of the protein (Kinsella 1982; Vaclavik & Christian 2003). For the proteins to be used in foods they must possess or contribute characteristics that are appropriate in interaction with other food components (e.g. water and lipids) or be suitable for processing. The functional properties that are required from a protein vary with different food applications and food systems. The three most important functional properties of food proteins in general are solubility, emulsification and foaming (Kinsella, 1982). However, the functional properties that are associated with hens egg in food applications are:

solubility, emulsification, foaming and gelling (Pomeranz, 1991; Yang and Baldwin, 1995) and are therefore the four functional properties that are going to be discussed more in detail in this study.

The type of functional requirements that are needed of a protein in different food systems is shown in **Table 2**. It is important to remember that no single protein exhibits all the functional properties (Vaclavik and Christian, 2003).

Table 2. Functional properties performed by functional proteins in food systems.

Functional property	Mode of action	Food system
Solubility	Protein solvation	Beverages
Water absorption and binding	Hydrogen bonding of water; Entrapment of water (no drip)	Meat, sausages Breads, cakes
Viscosity	Thickening; water binding	Soups, gravies
Gelation	Protein matrix formation and setting	Meats, curds, cheese
Cohesion-adhesion	Protein act as adhesive material	Meats, sausages, baked goods, pasta
Elasticity	Hydrophobic binding in gluten; Disulfide links in gels	Meats, bakery
Emulsification	Formation and stabilization of fat emulsions	Sausages, bologna, soups, cakes
Fat absorption	Binding of free fat	Meats, sausages, doughnuts
Flavor-binding	Adsorption, entrapment, release	Simulated meats, bakery etc.
Foaming	Form stable film to entrap gas	Whipped toppings, chiffon desserts, angel cakes

(Kinsella, 1982, p. 53)

Proteins must show good and multiple functionalities in order to perform well in food systems. This requires a deeper understanding of the structure-function relationship, which sometimes can be hard to determine. One reason why proteins possess such different functional properties is the fact that all proteins are built up by different amino acids (Nakai, 1983). The amino acid composition affects the functional properties of a protein according to how they are disposed in the polypeptide chain, as well as what type and how many of those amino acids that are present (Kinsella, 1981).

Something worth mentioning, but that will not be discussed further in this study, is that to improve the functionality and nutritional quality of the protein, modification of the proteins can be applied (Barac *et al*., 2010). Enzymatic hydrolysis is the most common and simplest method. During this process the protein is treated with an enzyme, acid or alkali that degrades the protein to its amino acid constituents (Lasztity, n.d.).

3.1 Solubility

The solubility of a protein is the most important functional property since the protein needs to be soluble in order to be applicable in food systems. Other functional properties like emulsification, foaming, and gelation are dependent on the solubility of proteins (Vaclavik and Christian, 2003). Solubility can be described as when equilibrium exists between hydrophilic and hydrophobic interactions. The solubility of a protein is related to the pH, where it is minimal at the isoelectric point, making the environmental pH the most important factor when it comes to the degree of protein solubility. The solubility is also influenced by temperature and ionic strength, (Bolontrade *et al*., 2013). Freezing, heating, drying and shearing are also factors that have an influence of protein solubility in food systems (Vaclavik and Christian, 2003). Insoluble proteins are not good for food applications and thus it is important that denaturation caused by e.g. heat is controlled so that the protein solubility not will be affected in a negative way (Raikos *et al*., 2007).

3.2 Emulsions

Emulsions consist of two liquids that are immiscible, where one of the liquids is dispersed in the other in form of small droplets. Emulsions can be classified according to the distribution of the oil and the aqueous phase. A system where the oil droplets are dispersed in the aqueous phase is called oil-in-water emulsion (O/W). Food systems like this are mayonnaise, milk, cream, soups and sauces. The opposite of an O/W emulsion is water-in-oil (W/O) but there are also water free emulsions and multiple emulsions (O/W/O or W/O/W). The droplets in an emulsion are called the dispersed (or internal) phase, whereas the surrounding liquid is referred to the continuous (or external) phase (Dickison and McClements, 1995, as cited in McClements, 2005, p.3).

When water and oil are homogenized they rapidly separate into two layers, one layer of oil, which has high density, and one layer with water that has low density. This is called phase separation and has to do with the fact that the droplets fuses together with adjacent droplets that are similar to themselves. To get a stable emulsion (both in a short and long term perspective) it is of great importance to add an emulsifier. An emulsifier is a surface-active molecule that allows the two phases to homogenize. Surface-active molecules are mostly amphiphilic i.e. they have both hydrophobic and hydrophilic parts, which allow the two liquids to blend together.

3.3 Foaming

Foams consist of a gas phase, a liquid phase and a surfactant (e.g. proteins) and whipping or shaking form foams. Foods made up by foams are e.g. whipped toppings, meringues, ice creams, chiffon desserts and angel cakes (Kinsella, 1981; Yang and Baldwin, 1995). Angel cakes and other baked goods are solid foams. Foams are formed through unfolding and absorption of the protein, at the air-water interface, as well as film formation around the air bubbles. Different proteins have different abilities to form and stabilize foams, and just as in the case of proteins and their different emulsifying properties, this is related to different physical properties of the proteins. For a protein to have superior foaming properties, it must possess high solubility in the liquid phase as well as the ability of quickly forming a film around the air bubbles in the food system (Kinsella, 1981).

The extrinsic factors that affect the foaming properties are e.g. pH, temperature and ionic strength. Foam stability and the proteins ability to form foams are also of big importance. In order for a protein to form stable foams the interfacial film should be rigid and not let the entrapped air escape (i.e. it should be almost impermeable). The protein should also have the ability to form strong bonds like hydrogen bonding and hydrophobic interactions. The protein should also possess limited denaturation at the surface to keep viscosity and rigidity (Kinsella, 1981).

3.4 Gelling / coagulation

The globular proteins' gelling properties are of big importance in foods (Van Kleef, 1986; Beveridge *et al*., 1984). According to Ikeda and Nishinari (2001) is protein gelation one of the most important functional properties when it comes to modify the structure and texture of foods. One example is the importance of the gelation properties of egg in foods like cakes, omelets and confectionary. The texture of foods and thus, the gelation properties of a protein, affect consumer acceptability (Kiosseoglou and Paraskevopoulou, 2005).

Globular proteins, such as egg white and soybean protein, are able to form gels upon heating (Doi, 1993). For a gel to form it is important that the functional groups (e.g. hydrophobic groups) within the protein are exposed. This makes it easier for the groups to interact and form a three dimensional network. Gel formation is complicated, and affected by the concentration of protein, amount of water, ionic strength, time and temperature as well as pH and interaction with other components in the food system (Raikos *et al*., 2007). The process for gelation in short, is:

Native protein $\xrightarrow[\text{Heat}]{}$ Protein denaturation $\xrightarrow[\text{Heat}]{}$ Gel / coagulum formation

The heat will make the native protein to denaturate, and during the denaturation disulfide bonds will be formed and hydrophobic amino acid residues are exposed (Shimada and Matsushita, 1980). After denaturation and further heating, the proteins will aggregate and interact with other proteins and form either a gel or a coagulum. Which type that is formed depends on conditions like molecular weight, heating time and protein concentration (Raikos *et al*., 2007; Shimada and Matsushita, 1980). The gel structure is a more structured network compared to the coagulum that is a disorganized aggregation (Raikos *et al*., 2007).

4. Legume proteins

Legumes are cheap and a high quality alternative to food based on animal products. They contain high amounts of proteins, dietary fibers, minerals and vitamins that are essential for good human health (Abd El-Hady and Habiba, 2003).The protein content in legumes varies between 17-30% depending on origin and the proteins are present as globulins (60-90%) and albumins (10-20%) (Sathe *et al*., 1984).

Today, soybean proteins are the most used and researched pulse proteins on the market, but the interest in functional properties and nutritional quality of unconventional legume proteins as an ingredient in new food products has increased (Chavan *et al*., 2001). The alternative legume proteins that are being researched are the ones that are believed to possess the same, or similar, functional properties and nutritional qualities as soy protein. The proteins should also have a price competitive to that of soybean (Marcone *et al*., 1998; Vose, 1980). One alternative pulse protein that is said to have big potential for food applications are pea protein (*Pisum sativum* L.) (O'Kane *et al*., 2004). Except the potential good functional properties of pea proteins, they are also said to be lesser in anti-nutritional substances than soy protein (Gwiazda *et al*., 1979), and are not classified as an allergen (like soy and egg proteins). This has to do with the fact that the allergic reaction to peas has been infrequent in humans (San Ireneo *et al*., 2000).

4.1 Soybean protein (*Glycine max* L.)

Soy proteins are used in foods because of their excellent emulsifying and gelling properties, which mimic the functional properties of egg proteins (Ratnayake *et al*., 2012). Soybeans as well as soybean products are classified as a health food due to their content of e.g. omega-3 fatty acids, isoflavones, dietary fiber, essential amino acids and high protein content (Variyar *et al*., 2004) One drawback concerning soybeans is their very distinct flavor that is hard to mask, and thus, their application are limited to just some food products (Endres, 2001).

Soybean proteins are used as a food ingredient in infant formulas, flours, protein isolates and concentrates as well as in textured form. Examples of soy foods are: imitation cheese, miso, tempeh, tofu and meat substitutes, and new soy foods are frequently developed (Liu, 2000; Singh *et al*., 2000, as cited in Friedman and Brandon, 2001, p. 1070).

The functional properties that can be ascribed to the soybean proteins are solubility, water absorption and binding, viscosity, gelation, cohesion-adhesion, elasticity, emulsification, fat absorption, flavor binding and color control. Among the plant proteins, soybean proteins are the most studied (and thus the best understood plant protein) and are often used in comparison with other plant proteins in order to evaluate their functional properties (Mcwatters and Cherry, 1977).

Mainly soy protein isolates (SPI) and soy protein concentrates (SPC) are used in the food industry (Varzakas *et al*., n.d.). SPI has the highest protein content (90%) and are thus the most expensive (Riaz, n.d.). SPI are made from defatted soybean flakes, where the sugars and dietary fiber have been removed. It is used in a variety of foods. Some examples are dairy type products, fruit drinks, power bars, soups and sauces, meat analogs, bread and baked goods, breakfast cereals and protein powders (Soyfoods, 2013). SPI do not affect color and flavor of the end product to any great extent (Riaz, n.d.). SPC are made by removal of the carbohydrates from soy flakes or soy flours. It has a protein content of 65-70% and is used in foods like baked goods, breakfast cereals and meat products to increase nutritional value and functional properties (Soya, n.d.).

Native soybeans have a protein content of 40% and they comprise the storage proteins albumin and globulin, where globulins are the dominant ones. The globulins are salt-extractable while the albumins are water soluble (Derbyshire *et al.*, 1976). The globulins can be grouped into 7S globulins and 11S globulins according to their sedimentation coefficients (Shigeru Utsumi *et al.*, 1997). The 7S globulins can be further divided into β-conglycinin, γ-conglycinin and basic 7S globulin (Bg) and all of them differ in their functional properties. As an example does Bg have a higher isoelectric point (pH 9.05-9.26) than the other globulins. The function of Bg is yet unknown (Shigeru Utsumi *et al.*, 1997). β-conglycinin is a trimer and consists of four subunits: major α', α and β and minor: γ (Shigeru Utsumi *et al.*, 1997). The 11S globulins are also known by the name glycinin, which consists of disulfide-linked acidic and basic amino acids. There are two groups consisting of five subunits in the soybean glycinin that have been identified: $A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a} (group I) and A_3B_4, $A_5A_4B_3$ (group II) (Adachi *et al.*, 2003; Mujoo *et al.*, 2003). In soybeans it is the glycine and β-conglycinin that gives soy proteins their functional properties (Lee, 2011).

4.2 Pea protein (*Pisum sativum* L.)

Peas have a high content of proteins, minerals vitamins, starches and fibers. They are used in human foods like: soups, puddings, snacks, and stews or as sprouted. Peas are also used in animal feed, where they are mixed with cereals or canola oil in order to improve the protein quality (Betker, 1990; Hoang, 2012). Studies show that pea proteins may be a good substitute for soybean proteins as a functional additive in food products intended for human consumption (Barac *et al.*, 2010; Maninder *et al.*, 2007; Aluko *et al.*, 2009), but in order to increase the utilization of pea proteins, their functional properties must be further evaluated (Aluko *et al.*, 2009).

Pea protein concentrate (PPC) and pea protein isolate (PPI) have the biggest potential as food ingredients (Choi and Han, 2001). PPC is made from pea flour, where the protein has been removed from the starch granules by air-classification (Owusu-Ansah and McCurdy, 1991), resulting in a protein content of 47% (Sosulski and McCurdy, 1987). PPI is also made from pea flour but by aqueous extraction and isoelectric precipitation of the protein (Owusu-Ansah and McCurdy, 1991). The protein content in pea isolate is approximately 80% (Sosulski and McCurdy, 1987).

Peas have a protein content around 25 %, but the content varies depending on pea variety (Aluko *et al.*, 2009; Gueguen and Barbot, 1988). Pea protein consists of legumin (11S), vicillin (7S) and albumins (2S), where 11S and 7S are the most abundant ones (O'Kane *et al.*, 2005). The legumin and vicilin have similar amino acid composition and subunit structure as the glycinin and β-conglycinin of soy proteins (Derbyshire *et al.*, 1976).

5. Functional properties of soy and pea protein

In order for the consumer to accept legume proteins in foods, and to optimize its utilization, the functional properties of the protein must be studied. It is the functional properties and nutritional value as well as the sensory characteristics of the legume proteins that are crucial for the quality and acceptance of the end product (Adebowale and Lawal, 2004). As mentioned in the beginning of this study, the functional properties of proteins are affected by environmental factors as pH, temperature and ionic strength. Due to limited published studies concerning some of these factors in relation to the functionality of soy and pea protein, they will only be discussed if applicable studies regarding this have been found.

5.1 Solubility

Protein solubility is affected by extrinsic factors like pH, temperature and ionic strength (Bolontrande *et al*., 2013). The effect of pH on soy protein solubility (i.e. solubility profile) gives a u-shaped curve, where the highest solubility is shown to be on both sides of the isoelectric point, (pI) 4.5, with a high solubility above the pI and a low solubility below the pI (Lee, 2011). Lee *et al*. (2003) showed that commercial SPI and SPC had similar solubility profiles, but that the amount of soluble proteins in the two samples differed at same pH values. Since legume proteins have to go through thermal heating in order to remove the anti-nutritional factors, the effect of heating on protein solubility is extremely important (Lee, 2011). There are, however, few studies on how heat treatment affects soy protein solubility. Ionic strength also affects protein solubility. Renkema *et al*. (2001) studied the effect of NaCl on soy protein solubility as a function of pH. The results showed that high NaCl concentrations increased the solubility of the protein near its isoelectric point.

Pea proteins also shows a u-shaped curve as a function of pH, with a high solubility above the pI, and a moderate solubility below the pI (Adebiyi and Aluko, 2011; Tömösközi *et al*., 2001). Tömösközi *et al*. (2001) showed that PPI had the same solubility profile as other legume proteins. Tian (1998) found that PPI had higher solubility than SPI, and the same was stated in a study performed by Naczk *et al*. (1986). Heat treatment studies regarding pea proteins are few. One study found showed, though, that heat treatment reduced the solubility of pea proteins (Habiba, 2002).

5.2 Emulsifying properties

It has been reported that SPI shows great emulsifying properties. This is related to its high solubility and high protein content (Gwiazda *et al*., 1979).
Jideani (2011) write that SPI, as well as SPC, are good emulsifiers but that SPC shows lower emulsifying capacity than SPI. Environmental factors, such as pH, affect the emulsifying properties of soy protein and this was studied by McWatters and Cherry (1977). They saw that soybean flour was able to create a mayonnaise-like emulsion that was extremely thick (at pH 6.5 and pH 8.2). At lower pH values, a salad-like dressing emulsion was created.

Emulsifying properties can be evaluated by the protein's emulsion stability (ES) and emulsion activity (EA). The ES is a measure of the stability of the emulsion over a certain time span and EA is a measurement of how much oil a protein can emulsify per unit protein (Boye *et al*., 2010). Gwiazda *et al*. (1979) presented the result that SPI and SPC had different emulsifying properties. SPI showed an EA of 96%, and an ES of 92%. SPC had an EA of 55.6% and an ES of 56.8%. In the same study, pea protein concentrate showed an EA of 60.6 and an ES of 65.3%.

It has been reported that PPI have similar or better emulsifying properties than SPI (Vose, 1980). Aluko *et al*. (2009) showed that PPI actually had better emulsifying capacity than SPI. There is another study that shows a different result; Tömössközi *et al*. (2001) found that PPI had quite good emulsifying capacity but low emulsion stability in comparison to SPI. In a study done by McWatters and Cherry (1977) it is shown that the emulsifying properties of pea protein are minor compared to soy protein but it is still able to produce both semi-thick and thick mayonnaise-like emulsions at different pH values.
The effect of temperature on the emulsifying properties of pea protein is that, when the temperature increase the emulsifying properties decrease. It has also been reported that addition of NaCl increase the emulsion capacity of both pea and soy proteins but that the emulsion stability decreases with increased NaCl concentrations (Tian, 1998).

5.3 Foaming properties

To evaluate the foaming properties of a protein, foam stability (FS), foam capacity (FC) and foam expansion (FE) can be measured. FE and FC are measured in volume (%) when whipped, while the volume of the foam over time (normally 0-30 min) gives the protein's FS (Boye *et al*., 2010). In a study done by Boye *et al*. (2010) SPI showed a FE of 41.8% and a FS of 93 %.

Fuhrmeister and Meuser (2003) showed that the foam forming properties of pea protein isolate were best at pH 5 and 7. The stability of the foam showed to be much lower than that of egg white. In a study done by Fernández-Quintela *et al*. (1997) the FE of pea protein showed to be around 15 % and the FS around 94 %. The FC was greater in acid and alkaline regions. The FS increased with pea protein concentration and ionic strength (Akintayo, *et al*., 1999). Another study showed, however, that the FS of pea protein was around 76-79%. The foam volume also decreased relatively fast compared to other legume proteins. It was also shown that PPI had a significantly higher FC than SPI. The foam stability of PPI was better than SPI at pH 5.0 but minor in other pH values (Toews and Wang, 2013). Tian (1998) showed that the addition of NaCl improved the foaming properties of pea protein, but only up to an addition of 0.5% (w/v). Increased temperature also improved the foaming properties.

5.4 Gelling properties

Studies have shown that the concentration of soy proteins affects the hardness of the gel and that the gelation properties of soybean proteins depend on temperature, pH, and ionic strength. In SPI the ratio between β-conglycinin and glycinin can influence gelation (Renkema *et al*., 2001) Varzakas *et al*. (n.d.) studied the gelling properties of SPI and SPC. The results showed that both SPI and SPC showed different gelling strength at different protein concentrations and temperatures. The conclusion drawn was that strong gels were formed at low temperature and high protein concentrations.

O'Kane *et al*. (2004) write that pea protein forms more unstructured gels than soy protein and thus their gelling properties are not that good as those of soy. Akintayo *et al*. (1999) reported that pea protein concentrate (72 % protein) had low gelling properties Another study showed that pea protein isolate forms a paste instead of a rigid gel (Adebiyi and Aluko 2011). Nunes *et al*. (2006) studied pea protein as a replacer of dairy and egg proteins in a gelled vegetable dessert. The results showed that pea proteins produced good gels that were highly applicable as a food product.

6. Egg protein

Hen eggs are used as a key ingredient in a variety of food products. Eggs can be used as a whole, as yolk or as white fractions. Egg possesses good functional properties, good nutritional value, as well as good sensory characteristics that all are of big importance for food applications (Kato *et al*., 1993).

One drawback with consuming eggs is their high content of cholesterol (Hoang, 2012). One egg contains 200 mg cholesterol, and according to American Heart Association (2013), should the consumption of egg not exceed the guidelines of 2 eggs a day. It is also stated that one of the most common food allergies is egg protein allergy, which also is a drawback, and a reason for not consuming eggs (Hoang, 2012).

Eggs are said to be "polyfunctional", i.e. they contribute with more than one functional property at the same time (Pomeranz, 1991; Yang and Baldwin, 1995).
It is important to fully understand the functional properties of egg proteins in order for the food industry to be able to develop new food products. Not until recently, the functional properties of eggs have been researched. This probably has to do with the complicated structure and composition of the egg. While the protein composition and structure of egg white has been widely researched, egg yolk has not got that much attention (Kisseohlou, 2003).

Hen eggs consist of 12-13% protein (by weight) that are present in the egg membrane (4% protein), egg white (12% protein) and egg yolk (31% protein) (Ibrahim, 1997). Eggs are used in foods like mayonnaise, salad dressing, baked goods, noodles and ice cream, and it is the four major functional properties of the egg that have made this possible (Pomeranz, 1991).

6.1 Egg white

The egg white consists of 10.5% protein and 85% water and there are 40 different proteins found in the white. The egg white is built up by four layers with similar protein composition, except of higher content of ovomucin in the more viscous layers (Coultate, 2009). There are six major proteins of hens egg white, which all varies in content (see **Table 3**).

Table 3. The proteins found in egg albumen and the approximate percent of total protein content

Protein[1]	**Approximate total protein**[1]
Ovalbumin *A_1, A_2 and A_3* [A]	54
Conalbumin*	12
Ovomucoid	11
Ovomucin	1.5-1.3
Lysozyme	3.4
Ovoglobulins *G1,G2 and G3*[B]	8

[1] Coultate, 2009 [A] Three types of ovalbumin exist: A_1, A_2 and A_3 and the difference between them are the degree of phosphorylation (Doi and Kitabatake, 1997), [B] Ovoglobulin is present in three forms: G1, G2 and G3 (Mine, 1995), * Conalbumin is also known by the name ovotransferrin (Coultate, 2009)

6.2 Egg yolk

Egg yolk has lower protein content and higher fat content compared to egg white, with a protein content of 31.2% and a fat content of 15.3%. Egg yolk is an emulsion where the continuous phase consists of livetin and riboflavin-binding protein (RBP) while the dispersed phase contains both low-density lipoprotein (LDL) as lipovitellinins, and high-density lipoproteins (HDL) as lipovitellins and phosvitin (Pomeranz, 1991) (see **Table 4**).

Table 4. The protein composition and content in egg yolk

Proteins	**Content (%)**[B]
Livetins [A, B]	
α - livetin	0.2
β - livetin	0.3
γ – livetin	0.2
RBP[A]	-
Lipovitellenins (LDL) [B]	
Apovitellenin I	0.7
Apovitellenin II	0.1
Apovitellenin III	0.1
Apovitellenin IV	0.6
Apovitellenin V	0.6
Apovitellenin VI	0.7
Lipovitellins (HDL) [A, B]	
α - lipovitellin	2.0
β – lipovitellin	1.0
Phosvitin [A, B]	1.0

[A] Pomeranz, 1991, [B] Jujena and Kim, 1991, LDL= Low density lipoprotein, HDL= High density lipoprotein

6.3 Functional properties of egg white and egg yolk protein

6.3.1 Solubility

Gomes and Pelegrine (2012) reported that egg white protein solubility was affected by both pH and temperature changes. A minimum solubility was shown at 60°C, due to protein coagulation. In a study on egg white solubility at different pH and NaCl concentrations done by Ferreira Machado *et al*. (2007) it was reported that maximum solubility was shown at pH 9.0 and minimum at pH 4.6 in every NaCl concentration tested.

Just as with egg white proteins, studies show that egg yolk proteins possess different solubility in different pH and temperature regions (Le Denmat *et al*.; Assis *et al*., 2010).

The proteins in egg white and egg yolk has an average isoelectric point (pI) of 5.4 and 5.3 respectively, and in this regions the proteins are less soluble (Gaonkar *et al*., 2010).

6.3.2 Emulsifying properties

Mayonnaise is a well-known food emulsion (oil-in-water) that is stabilized by egg yolk (Mine, 1998). Some examples of other foods based on egg yolk emulsions are dressings, sauces, custards, puddings and ice creams (American Egg Board, 2013). Egg yolk stabilizes emulsions by preventing two immiscible liquids from separating by creating an interfacial film between the two. The physico-chemical properties of emulsions are influenced by the composition, as well as the concentration of the interfacial film (American Egg Board, 2013; Anton and Gandemer 1999). It is the protein components in egg yolks that make them good emulsifiers (American egg board, 2013). These proteins are lipoproteins (LDL and HDL), phospholipids and the proteins phosvitins and livetins, where low-density lipoproteins (LDL) are the most important ones for food emulsions (Anton and Gandemer, 1997).

Food emulsions are often made at different pH values and thus, the effect of pH on the emulsifying properties of egg yolk is important. Kurt and Zorba (2009) reported that pH has an effect on the emulsifying properties of egg yolk proteins. The emulsion capacity (EC) increased with increased pH and the optimum pH values were between 4.61 and 7.43. Examples of food emulsions with different pH values are béarnaise and mayonnaise that are made at pH 7 and pH 4 respectively (Anton and Gandemer, 1999).

Egg yolk is said to be a flexible emulsifier since it can stabilize emulsions that are both cold and warm (e.g. mayonnaise and hollandaise sauce) (American Egg Board, 2013).

6.3.3 Foaming properties

Many proteins are able to form foams, but egg proteins are the most effective. Good foaming properties of the egg white are important in baked goods and certain confectionaries (Yang and Baldwin, 1995). The reason why egg white shows such good foaming properties is the fact that it contains a mixture of different proteins. These proteins all possess different physical properties and thus, all contribute with different functionality in the formation and stabilization of foams. Ovalbumin, which is the most abundant protein in the egg albumen, is heat sensitive and denatures easily if heated. When heat-treated and denaturated, ovalbumin form foams easily in the presence of other proteins in the albumen. If alone, the whipping time for obtaining foams is longer (MacDonell 1955, as cited in Stadelman and Cotterill 1995, p. 420).Globulins (G2 and G3) enhance the initial formation of foams by lowering the surface tension (MacDonell 1955, as cited in Stadelman and Cotterill 1995, p. 420). Sauter and Montoure (1972) investigated the role of lysozyme (G1 globulin) in egg white foaming. The results showed that lysozyme improves foam volume. Additionally the results showed that egg white with a higher amount of lysozyme produced more voluminous foams that those with a minor content of the globulin. Concerning the FC, a study done by Wong and Kitts (2003) showed that dried egg white had a greater foaming capacity than SPI, while other studies shows that there are no difference in foaming capacity between the two. McWatters and Cherry (1997) reported that soy protein was able to form very thick foams (at all pH tested, accept pH 4) that resembled of egg white foams.

The foaming properties of egg white are influenced by environmental factors as pH, temperature and ionic strength. It is known that egg white shows better foaming properties in room temperature. Under these conditions the foam formation is quicker and foam volume is greater (Lomakina and Míková, 2006). It is also known that egg yolk decrease the foaming capability of egg white, and that is almost impossible to get an egg white completely free from egg yolk (Kim and Setser, 1982). There are also foam products that contain both egg white and egg yolk, namely soufflés and omelets. The whole egg, or just the yolk alone, can also be used in sponge cakes (Yang and Baldwin, 1995).

6.3.4 Gelation / coagulation properties

Gelation/coagulation is a functional property that is highly important in the food industry (Campbell *et al*., 2003). The making and texturization of foods like cakes, creams, omelets, confectionary and sauces are dependent on the eggs ability to form gel networks upon heating (Kiosseoglou, 2003).

When eggs are used in baked goods, the protein molecules will aggregate and form insoluble networks (a gel or coagulum). It is these networks that give e.g. cakes and muffins their height, volume and stability (American Egg Board, 2013). Studies show that the egg white proteins coagulates at different temperatures when heated, starting at 61.5°C and reach complete coagulation at 73.0 °C (Johnson and Zabik, 1981). The proteins of egg white are sometimes referred to as "coagulation type proteins" (Gossett *et al*., 1984) and according to Coultate (2009) it is the rapid denaturation of ovalbumin that contributes to the setting of gel when egg white is heated.

Egg yolk proteins also form gels. According to Kiosseoglou (2003) it is likely that it is the apolipoproteins in the LDL micelles that play a major role in the gel formation.

7. Protein quality

Food proteins are divided into high quality (complete) protein and low quality (incomplete) protein. A complete protein contains all the essential amino acids, while incomplete proteins have limiting amino acids. Limiting amino acids are the ones that are present in such low amounts that they are not able to take part in the synthesis of other proteins. Animal proteins are complete proteins, while plant proteins are incomplete proteins. If the intake of protein mainly consists of incomplete protein sources the body is not able to make certain amino acids. In order to get a more complete protein, protein from different sources, like legumes and cereals can be combined. This is called mutual supplementation (Gropper *et al*., 2012).

Legume proteins are generally high in lysine, but the content of sulfur containing amino acids, like methionine and cysteine, is limited. Both soy protein and pea protein has a high content of lysine and low content of methionine, cysteine and tryptophan (Leterme *et al*., 1990). **Table 5** and **Table 6** show the amino acid composition of egg, soybean and pea.

Table 5. The amino acid content (AA g / 16 g N) of soybean (*Glycine max* L) and pea (*Pisum sativum* L)

Amino acid	Soybean	Pea	Amino acid	Soybean	Pea
	Ref [1]	Ref [2]		Ref[1]	Ref[2]
Essential amino acid			***Non essential amino acid***		
Histidine	2.53	2.52	**Alanine**	4.26	4.27
Isoleucine	4.54	3.33	**Arginine**	7.23	6.84
Leucine	7.78	6.58	**Aspartic acid**	11.70	10.68
Lysine	6.38	6.84	**Cysteine**	1.33	1.55
Methionine	1.26	1.03	**Glutamic acid**	18.70	16.92
Phenylalanine	4.94	4.19	**Glycine**	4.18	4.32
Threonine	3.86	3.59	**Proline**	5.49	3.76
Tryptophan	1.28	0.94	**Serine**	5.12	4.79
Valine	4.80	3.89	**Tyrosine**	3.14	3.16

[1] FAO, 1992, [2] Leterme *et al*., 1990

Table 6. The amino acid content (AA g/100 g total protein) of whole egg

Amino acid	Whole egg		Whole egg
Essential		***Non essential***	

amino acid		*amino acid*	
	Ref[1]		Ref[1]
Histidine	2.4	**Alanine**	5.4
Isoleucine	5.6	**Arginine**	6.1
Leucine	8.3	**Aspartic acid**	10.7
Lysine	6.3	**Cysteine**	1.8
Methionine	3.2	**Glutamic acid**	12.0
Phenylalanine	5.1	**Glycine**	3.0
Threonine	5.1	**Proline**	3.8
Tryptophan	1.8	**Serine**	7.9
Valine	7.6	**Tyrosine**	4.0

[1] Coultate, 2009

There are several ways to determine the quality of proteins. One of the most admitted and approved method is the protein digestibility-corrected amino acid score (PDCAAS) (Hughes *et al*., 2011). According to McMann (2000, p.7) is PDCAAS *"based on several factors; a food proteins profile of essential amino acids; the digestibility of the protein and the protein's ability to supply essential amino acids in the amounts needed to meet the requirements of growing human beings."* The PDCAAS is calculated by using the formulas prescribed by FAO/WHO (1991, as cited in Hughes *et al*. 2011, p.12708):

1) **Amino acid score** = Amino acid content of test protein / Reference amino acid pattern

2) **PDCAAS** = Amino acid score (of the most limiting amino acid) x true digestibility (%)

At first, calculation of the amino acid score is performed. This is done by dividing the content of the most limiting amino acid in the test protein by the content in one of the reference proteins. Thereafter, the result is multiplied with the true digestibility of the test protein. As an example: If a protein has a chemical score of 0.70 and a true digestibility of 80 %, the PDCAAS is calculated to 0.56 (Insel *et al*., 2004).

The highest PDCAAS a food protein can get is 1.0 or 100% (Hughes *et al*., 2011; McMann, 2000) but it is also possible that the protein get a score over 1.00. This is usually truncated to 1.00 because the amino acid in excess are often not required and thus catabolized (Tome, 2012). A score of 1.00 means that the protein provides proper amounts of all the essential amino acids, assumed that the intake is in appropriate amounts (Hughes *et al*., 2011).

The PDCAAS of soy protein show varying numbers in various studies, where SPI showed to have a PDCAAS ranging from 0.92 to 1.00 and SPC 0.99-1.00 (FAO/WHO, 1991; Sarwar, 1997, as cited in Hughes *et al*., 2011, p. 12707). There are also studies done that just show the PDCAAS value from soy protein, without defining the protein type further i.e. if its SPI or SPC. These studies showed the PDCAAS values of 0.94 and 0.99 (Gropper *et al*., 2012; Tome 2012). The reason why there is a variation in the PDCAAS values, was investigated by Hughes *et al*. (2011). In the study the SPI and SPC had to be truncated to 1.00 in the first testing, but the second testing showed values ranging from 0.95-1.00. The authors write that the variations may depend on errors in the analytical methods. Egg white protein has a PDCAAS of 1.00 and pea protein concentrate 0.73 (Hughes *et al*., 2011). No studies concerning PPI were found. For a summary of the PDCAAS for the various protein sources see **Table 7**.

In the United States, using PDCAAS is required before labeling foods (Hughes *et al*., 2011). Gropper *et al*. (2012) write that before labeling foods with information about the amount of protein (g) as well as the Daily Value (%) for proteins, PDCAAS is used to determine the protein quality. If the food protein has a PDCAAS equal or higher in quality than milk protein, 50 g of protein is sufficient. However, if the PDCAAS is lower in quality than that of milk protein, an intake of 65 g protein is required to meet the Daily Value.

Table 7. The protein digestibility-corrected amino acid score (PDCAAS) given for egg, soy and pea

Protein source	PDCAAS
Egg	1.00[1]
SPI[A]	0.92-1.00[2]
SPC[B]	0.99-1.00[3]
Soy protein	0.94, 0.99[4]
PPC[C]	0.73[1]

[A] Soy protein isolate, [B] Soy protein concentrate, [C] Pea protein concentrate, [1] Hughes *et al*., 2011, [2] FAO/WHO, 1991, [3] Sarwar, 1997, as cited in Hughes *et al*., 2011, p. 12707, [4] Gropper *et al*., 2012; Tome, 2012

Some studies found have criticized PDCAAS (Schaafsma, 2005; Hughes *et al*., 2011) and FAO (2011) write that this method may not be appropriate for novel protein with known anti-nutritional substances (factors that can disrupt the protein digestion and metabolism, see section 7.1), and that PDCAAS may overestimate the protein quality in these foods. Therefore, some other methods for measuring the protein quality will now be presented.

There are several other ways to determine the quality of a food protein. One simple way is to compare the amino acid pattern of the test protein with the amino acid pattern of a reference protein (usually egg or milk protein). This is called amino acid score (AAS) or chemical score (CS) (Gropper *et al*., 2009).

- **Chemical score (CS)** = mg of essential amino acid / mg essential amino acid in 1 g reference protein x 100

The essential amino acid that has the lowest chemical score is the limiting amino acid. The CS is not a good measure alone since it does not account for protein digestibility or amino acid bioavailability (FAO, 1992).

The protein efficiency ratio (PER) is another way of determining protein quality. This method accounts for to what extent the body can use the protein in terms of digestibility and availability, and also reflects the amino acids composition (Insel *et al*., 2004).

- **Protein efficiency ratio (PER)** = weight gain in g / protein intake in g

The PER method is based on how well the protein contributes to the growth in young rats and in recent years some questions have been raised towards this method. It is now known that PER overestimates values of certain animal protein, and underestimates values of certain plant proteins needed for human growth. Rats grow much faster, and thus, needs more essential amino acids than humans (Boutrif, 1991).

Net protein utilization (NPU) is a measure of protein utilization within the body. The more nitrogen the body keeps, the higher NPU value and protein quality the protein has (Insel *et al*., 2004). This method is also performed by doing tests on young rats and it has the same drawbacks as the PER method (FAO, 1985).

- **Net protein utilization (NPU)** = nitrogen retained / nitrogen intake x 100

The biological value (BV) of a protein is a measure of how much protein the body absorbs and keeps for other processes in the body This method is also performed on laboratory animals (Insel *et al*., 2004).

- **Biological value (BV)** = nitrogen retained / nitrogen absorbed x 100

In **Table 8**, the values for the chemical score (CS), protein efficiency ratio (PER), nitrogen protein utilization (NPU) and biological value (BV) of whole egg, soy and pea are given.

Table 8. The chemical score (CS), protein efficiency ratio (PER), nitrogen protein utilization (NPU) and biological value (BV) of whole egg, soy and pea

Protein	CS (%)	PER[A]	NPU (%)	BV (%)
Whole egg	100[1]	3.8[2]	94[2]	100[2]
Soy	47[3]	3.32[3]	61[3]	73[3]
Pea	37[3]	1.57[3]	47[3]	64[3]

[1] Coultate, 2009, [2] U.S. Dairy Export Council, 2011, [3] Hegested, 1971, [A] Unit: the gain in body weight per g protein consumed

7.1 Anti-nutritional factors

Anti-nutritional factors (ANF) are naturally present or can be formed during processing of legume proteins (FAO, 2011; Sarwar Gilani *et al*, 2005). The seeds of legumes contain ANF like protease inhibitors, lecitins, tannins, saponins and phytates (Liener, 1994). These factors can affect the protein digestibility, and thus, amino acid bioavailability in a negative way (FAO, 2011). Different ways to remove or inactivate some of the ANF have been established through physical (e.g. dehulling) and chemical methods (e.g. soaking, heating, irradiation) (Melcion and Van der Poel 1993, as cited in Fernández-Quintela *et al*., 1997 p. 332). Factors as genetic selection, fermentation and germination are also used for the same purpose (Frias *et al*., 1995; Kozlowska *et al*., 1996; Kothekar *et al*., 1996). The content of ANF in soybean and pea varies depending on variety (Adsule and Kadam, 1989; Hedley, 2001, as cited in Vidal-Valverde *et al*., 2003, p. 298; Becker-Ritt *et al*., 2004).

In a study by Khattab *et al*. (2009) different pulse proteins were investigated for ANF reduction; different heating methods showed to be the best. The authors strongly suggested that those methods were carried out before human consumption. Fernández-Quintela *et al*. (1997) showed that the tannin and phytase activity decreased after protein isolate preparation of soy and pea protein. The trypsin inhibitor activity was also reduced in SPI by 27 % and in PPI with 47 %. The tannins were reduced by 67% in SPI and the phytates by 30%. The phytase reduction in PPI was 46%.

8. Organoleptic aspects, kinesthetic aspects and consumer acceptance of soy and pea protein foods

In order for legume proteins to be used as a substitute for animal proteins, it is of big importance that the quality as well as the traditional characteristics of the food is maintained. It is also important to remember that the organoleptic and kinesthetic properties e.g. color, flavor, taste, texture and appearance of foods, are related to the proteins in the food (Endres 2001).

Egg protein contributes with texture and color in food products, and it is the eggs abilities to bind ingredients in foods that prevent the food product from losing its shape, crumbling or falling apart. The xantophylls (lutein and zeaxhantin) in the egg yolk gives e.g. sponge cakes, pasta and mayonnaise their yellow color. The color can differ depending on the hens diet, and it is possible to change the color by adding corn or alfalfa leaf to the feed. (American Egg Board, 2013).

In order for pulse proteins, such as soy and pea protein, to be successful and gain consumer acceptance, it is important that the flavor (aroma and odor) of the product appeal to the customers (Heng, 2005). One of the constraints with using soy and pea protein in food products is the distinct off-flavors that are hard to mask. It is the volatile, saponins, and non-volatile, ketone and aldehyde compounds that are responsible for this (Murray *et al*., 1979). These off-flavors are often described in terms like “beany” and “green” and are formed during autooxidation or lipoxygenase activity (Rackis *et al*., 1979, as cited in Aspelund and Wilson, 1983, p. 539). The flavor compounds interact with the proteins in soybean and peas and therefore they are also present in isolates and concentrates, which limit the uses and lower the consumer acceptance for these products (Meyer, 1970; Kalbrener *et al*., 1971; Eldridge, 1978; Smith and Circle, 1978; Wolf and Cowan, 1975 as cited in Aspelund and Wilson, 1983 p. 539). Some studies show that it is possible to remove the off-flavor compounds (Maheshwari, Ooi and Nikolov, 1995; Samoto *et al*., 1998). One way to do this is to remove the lipids. If the lipids are removed the proteins will not be able to bind to them (Wu *et al*., 2001). It is also of big importance to choose the right extraction method. Wu *et al*. (2011) reported that the extraction method may be efficient in the terms of removal of off-flavor compounds, but may have a negative effect on the functional properties of the protein, like denaturation of the protein or decreased protein solubility.

Even though many researchers have studied the functional properties of soy proteins, and to some extent those of pea proteins (see part 5 in this study), there are limited published studies on the applicability and consumer acceptance of these proteins as total egg replacers in foods free of dairy and animal products. There are however some studies found that now will be presented.

Schyver and Smith (2005) investigated what factors that affect soy food consumption. The results showed that those who consumed soy foods were the ones that wanted to exclude or minimize animal products in their diet, wanted to adapt a healthier lifestyle or had environmental concerns. The main reason why consumers continued to eat soy was the fact that they thought it tasted good. The non-consumers in this study thought the sensory attributes of soy products were unfavorable, but the main reason behind not consuming soy foods was the fact that they were unfamiliar with the products.
Both consumers and non-consumers agreed upon the fact that in order to increase the soy consumption by non-consumers it was not necessarily to improve the taste but to improve the perception in soy foods. A study that points out the issues with perceived taste, was done by Wansink (2003). In this study a snack bar with soy as a phantom ingredient was tested. The result showed that the taste and attitudes towards the snack bar were negative. The conclusion drawn from this was the fact that consumers may exclude products labeled with soy ingredients and that perceived taste plays a substantial role in this.

Garcia *et al*. (2009) studied the acceptability of mayonnaise-type emulsions based on different concentrations of SPC and rice bran oil (RBO). It was shown that a higher content of SPC lowered the acceptability of the color in the final product. This was probably related to the fact that SPC are known to darken the products to which they are added. A high content of SPC also lowered the odor acceptability. Taste acceptability did not differ significantly among the samples (60.4-60.7 on a scale of 100). The mouth-feel score also showed that an addition of SPC over 8% was not accepted. The spreadability showed greater acceptance with higher content of SPC. The study also showed that few consumers were willing to buy this type of mayonnaise, mainly due to the bland taste. When an ultimate content of SPC had been established, the authors performed a test with this mayonnaise. The mayonnaise was presented in three different flavors and one plain. The results showed that the flavored ones were highly acceptable, and that the plain was not accepted at all (49%). Other results shown in the same study was that people who were health conscious selected this type of mayonnaise, and that the three most important attributes for purchasing this type of product were taste, mouth-feel and overall acceptability. The purchase increased (with 70%) when the health benefits of this type of products was exposed to the test panel.

Khouryieh *et al*. (2006) performed a study on partial (50%) and total (100%) replacement of egg by comparing soy flour with four commercial egg replacers (Pasta Power, based on wheat protein; Eggstend300, Biozate1, and Bipro, based on whey protein) in fresh pasta. A trained sensory panel evaluated the different pastas and the results showed that neither the taste nor the roughness of the cooked noodles was affected by total replacement of egg with soy flour. The stickiness, firmness and color were significantly affected by total replacement, and soy flour showed the lowest score concerning firmness compared to egg and the commercial egg replacers. The color of the noodles showed highest acceptability for the noodles with egg and the noodles with 100% replacement of egg by soy flour.

Tian (1998) carried out a study on the overall acceptability and beany flavor in sponge cakes and a mayonnaise-type product with pea protein as an egg replacer. The findings concerning the sponge cake showed that the panelist thought that a 25 % replacement of egg by pea protein did not give the product a beany flavor, and that a 75% replacement by pea protein was acceptable concerning the cake quality. The negative notations were that pea protein gave the sponge cake a crumbly and coarse mouth-feel at higher protein concentrations. Some of the panelist noted, though, that they liked the pea flavor in the sponge cake. This probably had to do with the different backgrounds of the participants. The acceptability of the mayonnaise-type product was high up to 25% replacement of egg, while 50% was not acceptable and the panelist described the mayonnaise as having watery texture and that the mouth-feel were coarse and oily.

Northern Pulse Growers Association (2009) performed a baking test on how well pea protein isolate and concentrate could replace whole egg in cakes and cookies. The study compared cakes made with pea protein with those made with commercial cake and cookie mixes. The result was that the pea protein cakes were more moist and that pea protein isolate created higher cakes than pea protein concentrate and that the cakes was comparable in height to the reference cake. The protein isolate also created more moisture cookies than eggs. This study did not evaluate the sensory characteristics concerning consumer acceptance.

9. Pulse proteins as egg replacers available on the market

Currently there are some products at the US market containing soy and pea protein as egg replacers. In a brochure written by the Northern Pulse Association (n.d), pea protein is presented as a functional and cheap protein that has the ability to replace egg in foods like pasta and baked goods. The company Follow your Heart, that produces a variety of egg free food products in the US, has an imitation mayonnaise called *Veganaise* that is soy free and based on pea proteins. The company also has a *Veganaise* based on soy protein as well as a mayonnaise-type Horseradish sauce (Follow Your Heart, 2013). Recently, the company Hampton Creek Foods launched a product named *Beyond Eggs* based on plant proteins that are intended to replace egg in baking (Hampton Creek Foods, 2013).

10. Discussion

10.1 Comparison of functional properties

Solubility

Egg proteins show different solubility at different pH values, temperatures and ionic strengths, and are most soluble above their isoelectric point (Gomes and Pelegrine, 2012; Ferreira Machado *et al*., 2007; Gaonkar *et al*., 2010). This is similar to the solubility patterns of soy protein and pea protein. Both soy protein and pea protein showed to have the same solubility pattern and the conclusion can be drawn that they are more or less soluble at different pH values. Soy protein concentrates and isolates also showed a u-shaped solubility pattern, but the amount of soluble protein at different pH values differed among them (Lee *et al.*, 2003). This may have to do with the fact that they contain different amounts of protein. Pea protein isolate and pea protein concentrate also showed the u-shaped solubility pattern (Adebiyi and Aluko, 2011; Tömössközi *et al*., 2001) and they may also show differences in amount of soluble protein, since they contain different amounts of protein.

Temperature and NaCl also affect the solubility of the proteins (Bolontrande *et al.*, 2011) and more studies are needed on this subject in order to fully understand how these factors affect the solubility properties of soy and pea protein. The importance of this has been stated by Lee *et al*. (2003) where the authors wrote that it is crucial to know the solubility pattern of soy protein in different environmental circumstances in order for the industry to evaluate them for potential food applications.

Emulsifying properties

Egg yolk proteins are good emulsifiers since they contain LDL and HDL proteins (American Egg Board, 2013). These proteins are not found in soy and pea protein and therefore one may expect that legume proteins do not have the same emulsifying properties as egg proteins, or that their emulsifying properties will be poor. This literature study showed, nevertheless, that both soy and pea protein possess good emulsifying properties. This denotes that both soy and pea proteins contain surface-active molecules, that are amphiphilic, and that they are able to homogenize the two immiscible liquids in the emulsion.

Just as in the case with solubility, pH affects the emulsifying properties of all the studied proteins. As seen in the study presented by McWatters and Cherry (1977), both soy and pea protein were able to create both mayonnaise and salad-like emulsions where the thickness was pH dependent. Egg proteins showed the best emulsifying properties at higher pH values, this is also the fact for soy and pea proteins. This may be related to that the amount of soluble proteins is higher above the isoelectric point.

Since both soy and pea protein created semi-thick emulsions at lower pH they may not be suitable for mayonnaise-type emulsions (that are prepared at pH 4 and lower) that are supposed to have a thick consistency. However, in a study done by Anton and Gandemer (1999) it was concluded that pH 3, which are the pH value where most of the food emulsions are prepared in, are not favorable for emulsion formation of egg yolk proteins either. Instead it was shown that egg yolk had the best emulsifying properties at pH 6. The low pH is important to eliminate microbial growth in mayonnaise, since raw egg yolk is used (Wethington and Fabian, 1950), but low pH is not as important for the legume based mayonnaise-type emulsions and they may be made in higher pH values. Changing pH values may also be a method to create thick or thin mayonnaise-type emulsions and sauces made with legume proteins.

Concerning the emulsifying properties of the legumes as concentrates and isolates, they showed different results, where the isolates seem to be the best emulsifiers. It is difficult to tell what this depends on but difference in protein content between isolates and concentrates may be a factor. PPI have better emulsifying properties than SPI (Vose, 1998). According to Aluko *et al*. (2009) this may be related to the fact that PPI contains more sugars than SPI and thus are more soluble and more capable of forming emulsions.

Foaming properties

Egg white is an excellent foaming agent due to its mixture of different proteins, which all contribute to the different functionalities required (MacDonell 1955, as cited in Stadelman and Cotterill 1995, p. 420). Soy and pea proteins do not have the same protein composition, thus their foaming properties may differ from that of egg. Soy proteins showed, however, to be good foaming agents, and that the thickness of the foam was pH dependent. The foam stability showed to be slightly better for pea protein than soy protein, but both of them were able to form foams. This means that both pea and soy protein possesses high solubility in the liquid phase and that they are able to form a film around the air bubbles in the food system.

One difference between the egg and pea proteins is that egg white shows better foaming capacity in room temperature, while the foaming capacity of pea protein increase with increased temperature (i.e. temperatures over room temperature). This may indicate that pea proteins may not be used in food foams that are prepared in room temperature (e.g. the preparation of meringues).

Gelling properties

Just like in the case of egg proteins, soy proteins are also able to form gels, and changing the protein concentration, pH and temperature can modify the hardness of the gel. Soy protein formed the strongest gel in lower temperatures (Varzakas *et al*., n.d.). Therefore soy protein may not be as good as an egg replacer in food gels that are heated and dependent on heat coagulation, like baked goods and omelets. In some studies (Akintay *et al*., 1990; Adebiyi and Aluko, 2011), pea proteins showed to have poor gelling properties, with a result that were more like a paste than a gel. As mentioned in part 5.4, a study done by Nunes *et al*. (2006) showed, though, that pea proteins were able to create a vegetable gel that was highly applicable as a food product. In this study the consumer acceptance was not taken into consideration and thus it is hard to say if this product would be accepted in the end.

To summarize this section, it can be conclude that both soy and pea proteins are soluble, and therefore suited for further food applications. They are also able to act as emulsifiers and form foam and gels (though, it is still not fully clear if pea proteins are good or bad gelling agents) just as egg proteins. The functional properties are affected by both intrinsic and extrinsic factors, and the extrinsic factors can be used to modify the functional properties as wished (e.g. thicker or thinner mayonnaise and salad dressings). The functional properties of pea protein are similar, and in some cases even better, to those of soy protein and this may have to do with the fact that pea protein has similar amino acid composition as soy proteins. It is also important to remember that the legumes in forms as isolates and concentrates have different functional properties, and that this can depend on that they are prepared in different ways. The importance of knowing how the intrinsic factors affect the functional properties of a protein is also demonstrated. More studies, concerning both intrinsic and extrinsic factors needs to be done, in order to fully understand how the legume proteins will perform in different food systems. By doing this the food applications for legume proteins will likely widen.

In order to further evaluate the potential of soy and pea protein as egg replacers, the protein quality, as well as consumer acceptance will now be discussed.

10.2 Comparison of protein quality

There are different ways to determine the quality of a protein (Gropper *et al.*, 2009). Depending on what method that is used, different results concerning the quality is obtained (see **Table 7 and 8**).

In **Table 5**, we can clearly see that both soybeans and peas are limiting in the essential amino acids methionine and tryptophan. Methionine is required for the synthesis of cysteine (Coultate, 2009) and is therefore also limiting. Both the legumes are rich in lysine and leucine. Peas have a higher content of cysteine than soybeans. We can also conclude that soybeans and peas have a similar amino acid pattern, but since peas have a lower content of methionine and tryptophan the CS will be lower for peas. If we compare this with **Table 6**, which shows the amino acid content for egg, we can see that the amount of methionine is substantially higher in egg. The tryptophan and cysteine values are not much higher than those of soybeans.

If we calculate the CS by using the values from **Table 5-6**:

CS for soybeans: (1.26 (Met) + 1.28 (Try)) / (3.2 (Met) + 1.8 (Try)) x 100 = **50,8 %**

CS for peas: (1.03 (Met) + 0.94 (Try)) / (3.2 (Met) + 1.8 (Try)) x 100 = **39,4 %**

These calculated values are close, but slightly higher, to the other findings concerning CS of soybeans and peas, where whole egg had a CS value of 100%, soy 47% and pea 37% (see **Table 8**). Egg is superior in protein quality if this method is used, but as mentioned by FAO (1992) this may not be a good measurement of protein quality, since it does not take protein digestibility or amino acid bioavailability into consideration.

The PDCAAS showed that egg and soy protein both are complete proteins (where soy protein had values between 0.92-1.00). Pea protein concentrate had a value of 0.73, which means that it does not provide all the essential amino acids needed in the diet. This has to do with the fact that pea proteins have lower amounts of both methionine and tryptophan. Any numbers on the true digestibility of pea proteins were not found, but FAO/WHO (1989) write that soy protein has a digestibility of 90-98%, which means that the PDCAAS should be around 1.00 and even truncated for soybeans (just as seen in **Table 7**). If pea protein has a PDCAAS value of 0.73 it must also have a true digestibility in the same range, or slightly lower than soybeans (PDCAAS = 0.394 (CS) x 0.198 (true digestibility) = 0.78). This means that it is the essential amino acid composition of pea and soybeans that differ, and not the fact that peas may have a lower true digestibility. In order for pea protein to become a complete protein, mutual supplementation may be used.

The PER value showed that there was not much difference between egg and soy (3.8 and 3.32 respectively) concerning protein quality. Pea showed a lower value (1.57). The NPU value was highest for egg (94%) and lowest for pea (47%) and soy had a value of (61%). Egg had the highest BV (100%), soy a little bit lower (73%) and pea protein the lowest (64%). Soy protein still performs better than pea protein, but is not a complete protein if some of the previous methods are used. As previously mentioned, the critics towards these methods have been that they are usually performed on rats that have other amino acid requirements than humans (Boutrif, 1991). These methods may not be the best when determine the protein quality of proteins aimed for humans.

Both soy and peas contain ANF that can affect the protein digestibility and amino acid bioavailability. It has been shown (Melcion and Van der Poel 1993, as cited in Fernández-Quintela *et al*., 1997 p. 332; Frias *et al*., 1995; Kozlowska *et al*., 1996; Kothekar *et al*., 1996) that these can be removed by different methods or choosing a variety that has low amount of these. The ANF will not be big problem since different heating methods have shown to lower or completely remove the amount of ANF. It has also been shown (Fernández-Quintela *et al*., 1997) that the processing of soybeans into SPI, and peas into PPI lower the ANF. This is good, since SPI and PPI are used in most food applications. PDCAAS does not take ANF into consideration, and as mentioned earlier in this study, pea proteins are said (Gwiazda *et al*., 1979) to have a lower content of ANF than soy. If this was taken into consideration maybe the pea protein would be “better” or “similar” in comparison to soy protein, but it is unclear.

Habiba (2002) wrote that heat treatment lowered the solubility properties of pea proteins, thus it is important to remember that heating as well as other processing methods could affect the functional properties of the proteins. A suggestion is to use proteins from pea and soy that have low ANF (either variety or as isolates) as well as a good amino acid composition for food application, and to carefully choose ANF reduction methods that do not denaturate the protein and thus destroy the protein quality as well as functional properties.

One thing that is worth pointing out is that egg products like mayonnaise, cakes and confectionaries are in most cases not consumed because of their nutritional value, and thus these kinds of foods are not the prime source of nutrients in the human diet.

10.3 Consumer acceptance

In order for soy and pea proteins to be successful as egg replacers in vegan food, consumer acceptance is crucial (Heng, 2005). No one will buy a product that does not have high sensory qualities or over all high quality (i.e. protein quality). One problem shown in this study, is the off-flavors that soy and pea protein inherent. These substances can, however, be removed (Maheswari, Ooi and Nikolov, 1995; Samoto *et al*., 1998; Wu *et al*., 2001). As mentioned by Wu et al. (2011) it is also crucial to choose a method that doesn’t affect the functional or nutritive properties of the final product.

Soy and pea proteins are, just as egg proteins, able to stabilize emulsion and form foams and gels. One thing that differs is the quality of the final product (e.g. organoleptic and kinesthetic properties as well as consumer acceptance). The studies (Garcia *et al*., 2009; Khouryieh *et al*., 2006; Tian, 1998) done on organoleptic and kinesthetic properties show, that it is somehow problematic to use soy and pea protein in foods. It can be hard to get the right texture, odor and/or color, and the beany taste often shines through. For people that already consume soy food products, the beany taste may not be such a big problem, as long as it doesn't differ too much with the consumers perceived taste of soy foods. Since these kinds of products in the first place will appeal to people with dietary restrictions (e.g. vegans), they will gain consumer acceptance if the overall quality of the product is good. People that are health conscious, worries about high cholesterol, allergies and the environment may be harder to convince. Concerning the unacceptable color of these kinds of food products it is surprising that none of the studies discuss beta-carotene as a potential food colorant in order to improve the color of the product. Beta-carotene is used as a food colorant in various products and also contributes positively to the nutritional value of the product (Ranhotra *et al*., 1995).

One study (Tian, 1998) indicates that it is hard to fully replace egg in sponge cakes without getting a quality loss (up 75% of egg replacement was accepted). The same was shown, in the study by Tian (1998), for a mayonnaise-type emulsion, which had unacceptable mouth-feel and texture. In the study (Garcia *et al.*, 2009) where the mayonnaise was flavored the consumer acceptance was higher, which may indicate that the flavor compounds used was able to mask the off-flavors. By choosing the right flavoring it may be possible to reduce the beany taste.

In order for soy and pea proteins to be successful as egg replacers in foods, it is crucial to come up with an ultimate food formula that increase consumer acceptance and do not affect the functional properties of the protein. It may be difficult to come up with a product that exactly mimics the taste, color and texture of egg but that it is possible to come very close. These types of products have a big potential but that more research are needed in order to get an optimal product.

11. Conclusion

Soy and pea protein possess the similar functional properties as egg proteins (except for the poor gelling properties of pea protein) and that the estimation of protein quality of soy and peas differs with different method used. When using PDCAAS, soy was shown to be a complete, high quality protein. Mutual supplementation can make pea protein a complete protein. The consumer acceptance for foods with pea and soy protein as egg replacers varied, but showed to be highest for the flavored mayonnaise-type product. Further studies have to be carried out on optimization of formulas for these kinds of products in order for them to gain consumer acceptance.

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Appendix 1 – Popular scientific summary

There are various reasons why legume proteins have potential to be used as an alternative to egg proteins; legume proteins are a cheaper alternative to animal proteins, the production of legumes has less impact on the environment, they are a good alternative for people with egg allergies and diet restrictions, and they are also healthy. Another potential field of application is the use of legume-based foods in the developing countries; Due to raised food prices on animal products and environmental changes that have led to uncertainty of crop yields around the world, the food insecurity and malnutrition in the developing countries have increased steadily. Foods based on legume proteins have a big potential to improve the nutritional status of these populations by adding legume proteins to the daily diet, this because legume proteins are said to have a high protein quality.

Since legume proteins seems to have such potential in foods, this study focused on comparing egg proteins with soybean and pea proteins. The comparison focused on the functional properties of the proteins as well as protein quality and consumer acceptance.

The functional properties that are associated with egg proteins are solubility, emulsification, foaming and gelling, and the functional properties of a protein can be used to evaluate if they are suitable for certain food applications. As an example, eggs are used in the preparation of meringues, and it is their emulsifying properties as well as their coagulation properties that make this possible. This is because the proteins in eggs are able to create foams when whipped, and coagulate if they are subjected to heating. In order for legume proteins to be used as egg replacers, they must possess the same or similar functional properties as egg proteins. Egg proteins are high quality proteins and thus, it is also important that the legume proteins have a good protein quality in order to replace eggs in food. A protein possesses a good protein quality if it is able to provide all the essential amino acids needed for human growth. Consumer acceptance is also important if legumes are going to be successful as egg replacers.

The findings from this study showed that it is possible to use proteins from soybean and pea as egg replacers in foods free of dairy and animal products (e.g. vegan foods). They possess similar functional properties (i.e. solubility, emulsification, foaming and gelling/coagulation) as egg proteins. It was also concluded that soy proteins possess the same protein quality as egg proteins. The quality of the proteins was evaluated by looking at the amino acid composition and digestibility for egg, soybean and pea proteins. If the protein has a good amino acid composition and are easy digestible it has a high protein quality.
One drawback with using legume proteins in foods are their distinct “beany” and “hay-like” flavors that seems to lower the consumer acceptance for these kind of products. The poor texture, color and odor were also factors that influenced consumer acceptance in a negative way. The consumer acceptance was, however, highest for a flavored mayonnaise- type spread. More studies on how to create a product that has an acceptable taste, color, odor and texture needs to be carried out in order for these kinds of product to be successful.