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Pea protein as a suitable protein substitute and a functional ingredient

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Abstract
Pea protein is a plant based high quality protein, rich in branched chain amino acids, easily available, cost-effective protein. Its well-balanced amino acid composition makes it a suitable preference among researchers. It has low allergenicity, higher digestibility and lesser health controversy compared to soy protein. A mature pea on dry basis, is comprising of 26% protein (of which 16-24% albumin and 50-55% globulin fraction). The major globulin fraction further categorized as legumin [11S], vicilin [7S] and convicilin [8S]. These fractions are responsible for functional properties such gelling property, water holding capacity, emulsification property, foaming and fat binding ability. Pea protein fractions also possess various health benefits including antioxidative, antihypertensive, anti-inflammatory, cholesterol lowering, modulating gut microbiota activity etc. Due to these attribute pea proteins is emerging as a preferred choice among the commercial traders as compared to other proteins. Further different extraction methods like dry and wet fractionation, alkali solubilition, salt extraction is used to obtain pea protein concentrates or isolates. Pea protein can be used as a pharmaceutical applications, edible film coating material, extruded product, food emulsifier, food supplements etc. Therefore, this review will cover pea protein characterization, extraction methods, functional properties, applications and possible future aspects.

Keywords: Pea protein, isolation, application, functional property

1. Introduction
Pea, *Pisum sativum* L. belongs to the family Leguminosae [or *Papilionacea*], generally known as garden pea/field pea/green pea/English pea or common pea. These are the small round shaped seeds, present within the pod of *Pisum sativum.* The peas pod botanically, belongs to fruits as they are grown from a pea flower (Lu, 2020) [31]. Pea comes under the category of one of the oldest domestic crops consumed all over the world. The global pea production is about 13.5 million metric tons annually and cultivated in more than 90 countries (FAOSTAT, 2018) [16]. However, the chemical composition of pea may vary according to the difference in soil, climate, temperature, relative humidity, variety all over the globe. In present scenario, Soy protein is the most common source of plant-based protein for decades and soya products are excellent vegan protein replacement over animal protein. Pea has its prominent place among different vegetables due to its high nutritional value especially protein, carbohydrate, vitamin A and C, phosphorous and calcium. Pea protein as a novel protein is gaining popularity all over the globe. It provides high-quality nutritive protein with several health benefits due it to its high yield at comparatively lower cost and input (Roy *et al*., 2010; Lam *et al*., 2018; Strauch and Lila, 2021) [39, 28, 47]. Pea proteins have comparatively lower allergenicity, higher digestibility and nutritional value, lesser health controversy as compared to soya, a modified environment-friendly crops (Allred *et al*., 2004; Day, 2013; Krefting, 2017) [1, 13, 27]. Pea protein is a rich source of branched-chain amino acids which makes it an excellent substitute of whey protein for athletes (Banaszek *et al*., 2019) [5]. Pea protein is rich in lysine but it limit in methionine, due to which it is compensated by combing it with cereals rich in methionine and limited in lysine (Stone *et al*., 2015; Nosworthy and House, 2017) [46, 35]. Limiting methionine amino acid give an amino acid score of 0.79 for pea protein (FAO/WHO, 1991) [17]. The protein digestibility corrected amino acid score for pea protein falls in the range of 0.54 to 0.82 depending upon the extraction process, processing treatments and its variety (Nosworthy and House, 2017; Arntfield and Maskus, 2011) [35, 3]. Previous work suggested that the pea proteins, its hydrolysate, and specific peptide had antioxidative, antihypertensive, anti-inflammatory, cholesterol lowering, modulating gut microbiota activity.

It also possesses various functional properties like gelling property, water holding capacity, emulsification property, foaming and fat binding ability, which widen its application in food formulation. All these properties have widened the application of this protein. Previous studies had reported its application in pharmaceutical industry, encapsulating material, edible film, extruded products, food supplement etc. (Ge *et al*., 2020) [22]. This review paper will provide a comprehensive idea about pea protein fractions, isolation, functionality, modifications, applications and future aspects.

2. Pea protein characterization

Albumins and globulins are the two major pea proteins, out of which globulin is the major protein. The mature pea contains about 18-32% with an average of 26% protein on a dry matter basis, out of which 16-24% is albumin protein and 50-55% is globulin protein (Gatehouse *et al*., 1984) [21]. Seed proteins can be traditionally classified according to the extraction of defatted crushed seed by different solvents. Albumins are water-soluble fraction, globulin is salts soluble fraction, prolamine is fractionated by ethyl alcohol and glutelins by alkali or acid. Albumins and globulins are the two major pea proteins, out of which globulin is the major protein. The storage protein globulin can be further categorized as legumin (11S), vicilin (7S) and convicilin (8S), which are composed of several subunits. The 7S and 11S field pea proteins have similar compositions and structures to corresponding soya proteins (Tzitzikas *et al.*, 2006) [50]. The composition of different amino acids in pea protein showed that pea protein being rich in different essential, non-essential and conditionally essential amino acids is a good quality protein.

2.1 Legumin

Legumin is a heat stable protein, has a condensed quaternary structure stabilized through disulphide, hydrophobic and electrostatic interactions, which makes it a heat stable protein. The pea legumin (molecular weight ~320-410 kDa) occurs in the form of hexamer consisting of six disulphide bonded subunit pairs with beta-sheet rich structures (Lu *et al*., 2020) [31]. Each pair (molecular weight 60-65 kDa) consists of one large acidic and one small basic polypeptide units of molecular mass of ~40kDa and ~20kDa, respectively linked through a single disulphide bond (Barac *et al*., 2010) [6] (Figure 1). The legumin acidic and basic units are referred to as α and β subunits and main legumin subunit, big and small legumin subunits are referred to as Lg-1, Lg-2 and Lg-3, respectively (Figure 2). So Lg-1α refer to the acidic subunit of main legumin (Gatehouse *et al*., 1984) [21]. The hydrophilic alpha unit is positioned at the surface and the basic hydrophobic beta units are at the interior of the molecule which minimizes the contact with water (Reinkensmeier *et al*., 2015) [38].

2.2 Vicilin and Convicilin protein

The vicilin has a molecular mass of ~150kDa and exists in the form of a trimer which is made up of heterogeneous protein subunits having a mass 47-50kDa each. This protein lacks cysteine residues, due to which it lacks disulphide bridging. The composition of vicilin subunits varies due to differences in post-translation processing i.e proteolysis and glycolysis. Due to proteolysis, it breaks down into smaller polypeptides and glycolysis leads to an increase in the vicilin solubility (Stone *et al.*, 2015) [46]. Viciln is a highly surface-active protein as it contains balanced hydrophilic and hydrophobic amino acids at its surface with comparative smaller flexible structure (Liang and Tang, 2013) [30]. Heterogeneity of pea vicilin is highly complex than legumin. The vicilin polypeptide precursors have two cleavage sites A and B for post-translational proteolysis, proteolysis at these sites cleave the polypeptide into three subunits referred to as α, β and γ with the molecular mass of 19-20kDa, 13-13.5kDa and 12-16kDa (if glycosylated), respectively. Proteolysis at site A alone yields α (19-20kDa) and β+ γ (25-30kDa) and proteolysis at site B yield α+ β (30-36kDa) and γ (12-16kDa) (Tzitzikas *et al*., 2006; Gatehouse *et al*., 1984) [50, 21] (Figure 2). When the vicilin protein was isolated from pea flour at alkaline conditions and fractionated by salt at acidic medium, two different fractions vicilin 1 and vicilin 2 were found and vicilin 2 contains convicilin (molecular mass ~70kDa) when analyzed over SDS-PAGE (O'Kane *et al*., 2004a) [36]. Convicilin is ~70kDa protein and occurs in the trimeric form of molecular mass ~210kDa (Tzitzikas *et al*., 2006) [50]. Earlier before 1980, 70kDa polypeptide of conviclin was considered as a part of vicilin (O'Kane *et al*., 2004) [36], however, Croy *et al*., (1980) [11] found convicilin as a separate protein that can be purified. It has about 80% homologous amino acid residue to undissociated vicilin unit, extensive homologous in the amino acid residues 122 to 166 from C-terminal (vary according to isoform) and varies at extended N-terminal which contain some hydrophobic residue and highly charged with acidic residue (Tzitzikas *et al*., 2006) [50].

2.3 Albumin

Albumin, a water-soluble is a minor fraction of pea seed protein, accounts for only 16-24% of total protein. This fraction is rich in sulphur amino acids which increases its nutritional value and have higher normal amino acid distribution as compared to globulins. Pea seeds contain different albumins units out of which PA1a and PA1b are most common. PA1a (53 amino acids) and PA1b (37 amino acids) has a molecular mass of about 6-8kDa and 22-26kDa, respectively. In PA1b unit 6 cysteine amino acids are involved in 3 intramolecular disulfide bonds. The pI of pea albumin is between 5.5 to 6 and will not precipitate along with globulin during pH extraction (Makri *et al.*, 2005) [32]. The storage globulin proteins are very digestible but contain a lesser amount of sulphur-containing amino acids, however, albumin protein is a good source of sulphur amino acids but contains antinutritional factors (Gueguen and Barbot, 1988; Rubio *et al*., 1994) [25, 40]. Thus, albumin compensates for the amino acids of globulins but decreases the bioavailability (Le Guen *et al*., 1995) [29].

2.4 Prolamin

The prolamin is ethanol (60-70%) soluble protein fraction and it is rich in glutamine and proline that's why named prolamin. However, propanol (50%) also solubilizes some prolamin fractions, the addition of reducing agents enhance prolamin extraction from disulfide-linked polymers. It comprises two fractions (a) gliadin (monomer) solubility in ethyl alcohol (70%) or dilute acetic acid; and (b) glutenin (polymer) solubility in sodium dodecyl sulfate (SDS) (2%, w/v) or propanol (50%, v/v) containing mercaptoethanol (2%, v/v) (Mills *et al*., 2012) [33]. The prolamin is generally found in cereals and has a specific name for different sources such as Zein (maize or corn), hordein (barley), gliadin (wheat), secalin (rye), avenin (oats), kafarin (sorghum) (Shewry and Halford, 2002; Colin, 2017) [44, 10].

2.5 Glutelin
Both prolamin and glutelin are hydrophobic proteins, characterized by poor water solubility, rich in glutamine and proline amino acids (González-Pérez and Arellano, 2009) [23], and soluble in acid and bases. Pea seeds consist of a minor amount of glutelin and it is one of the protein fractions constituting gluten. Glutenin is the most common type of glutelin, primarily responsible for baking characteristics in bread. It is soluble only in dilute bases or acids, detergents, reducing or chaotropic agents, abundant in hydrophobic amino acids like valine, proline, phenylalanine and tyrosine (Lu *et al*., 2020) [31].

4. Functional properties
Functional properties are the physicochemical characteristics that affect the protein's behaviour during production, storage and consumption. Functional properties include fat binding, flavour binding, water binding, solubility, foaming, emulsification, gelation, foaming and thickening properties.

4.1 Solubility
The protein solubility depends on the configuration of the protein, the number of hydrophilic and hydrophobic groups and its arrangement. Legume protein has good solubility at basic pH, moderate at acidic pH and lowest at pI. Solubility reached a maximum in the range of 8 to 9 pH (Fernandez-Quintela *et al*., 1997) [19]. The pH and solubility of pea protein and its product follow U shape relationship (Tömösközi *et al*., 2001) [48]. The type of protein extraction technique and drying conditions applied greatly affect the solubility. Barac *et al*. (2010) [6] compared the functional properties of six pea genotypes at different pH and found that there is a variation in solubility of all six genotypes and obtain high solubility at alkaline pH i.e. pH 7 and 8. At pH 3, the obtained solubility was 227.5 g kg-1 and 614.4 g kg-1 for L1 and Maga genotypes, respectively whereas at pH 8, 664.7 and 845.5 g kg-1 solubility for these genotypes.

4.2 Emulsification property
The emulsification property (EP) is described as the ability of the protein to emulsify and stabilize the emulsion. The emulsifier should be easily adsorbed on the fat surface and must tend to lower the oil-water surface tension. Alkaline extracted pea protein mainly contains 7S (trimeric) and 11S (hexameric) globular proteins which stabilize oil-water emulsions (Can Karaca *et al*., 2011) [8]. The EP is different for different pH values, acidic pH produces smaller sized oil droplets than neutral pH (Sharif *et al*., 2018) [42]. In an acidic medium, lentil and soy proteins stabilize the small fat droplets due to dissociation of multimer into monomer which promotes the surface adsorption, however in the case of pea proteins the mechanism is different they self-assemble during acidic pH and stabilize the fat (Sridharan *et al*., 2020) [45]. It was also reported that the unordered and unfolded proteins are easy to adsorb and arrange at the surface. The EP of PPI was found to be equivalent to commercial soya isolate (Vose, 1980) [51]. The pea globulins vicilin and legumin were studied for surface properties and found that the native vicilin is more active at the air-water interface as compared to legumin (Dagorn-Scaviner *et al*., 1987) [12]. The EP of primary pea proteins are pH-dependent, the emulsion is very unstable at pI and EP is minimum at pI (pH 4-5). The EP increases due to severe dissociation at pH value higher or lower than the pI and it is more noticeable for legumin (Tsoukala *et al*., 2006) [49]. It was found that the emulsification capacity of commercial isolates of whey protein, egg protein, soy protein and pea proteins (pH 7, 1% concentration) was 210.4 g/g, 197.9 g/g, 172.9 g/g and 177.1 g/g, respectively, however, the PPI prepared to have the same emulsion.

4.3 Foaming property
Foaming properties (FP) is described in terms of foam foaming capacity (FC, ability to form foam under specific condition) and foaming stability (FS, ability to retain the foam volume for a specific period). The protein which can unfold and adsorb at the air-water interface, reduces the surface tension provide better FC (Stone *et al*., 2015) [46]. The FP of PPI is affected by the extraction method, drying method, the cultivar, pH etc. The PPI obtained by the UF method provides better FP as compared to heat or acid prepared PPI (Fuhrmeister and Meuser, 2003) [20]. Chavan *et al*. (2001) [9] reported that the increase in FC may be due to an increase in solubility, unfolding ability at the interface, protein surface-active component's flexibility and limited intermolecular cohesion. The FS of PPI and salt soluble fractions enhances from pH 4 to 9 due to an increase in charge density which increases the electrostatic repulsion that decreases the air bubble coalescence rate. FC is positively related to solubility, greater is the protein migration towards air-water interface greater is the foam-forming property. The FC of the commercial product was highest for whey protein isolate (277%) followed by wheat (182.2%), soya (171.1%), egg (115.6%) and pea (81.1%) protein isolates. The FS of commercial isolates of whey protein, egg, soy, wheat and pea are about 75.5, 72.7, 67.7, 49.2 and 27.1%, respectively (Stone *et al*., 2015) [46].

4.4 Fat binding property
Protein fat binding property (FBP) influence the body, texture, flavour and mouthfeel of the product. The absorption of oil is decided by the FBP of non-polar sites of protein. Plant protein contains various hydrophobic side chains which can interact with hydrocarbons, thus contributing fat absorption. The non-polar and insoluble proteins offer higher oil binding capacity. It can also be described in terms of oil holding capacity (OHC). The OHC is greatly affected by the protein source, method of extraction, types of protein, protein hydrophobicity etc. Stone *et al*. (2015) [46] found that OHC of commercial protein isolates of wheat, egg, Soya, whey and pea protein (at pH 7 and 1% protein solution) were 2.8, 2.0, 1.8, 1.4 and 1.0 g/g, respectively. The OHC of PPI prepared by the AS-AP method was 2.8 g/g which was lower than soy protein isolates and some desi chickpea varieties (Withana-Gamage *et al*., 2011) [52], which might be due to the lower number of non-polar amino acid proteins. When the effect of the extraction method on functional properties were studied, it was found that the OHC of PPI by SE method was higher (~5.3 g/g) than AS-AP (~3.6g/g) and MP (~3.6g/g) (Stone *et al*., 2015) [46].

4.5 Water holding capacity
The water holding capacity (WHC) is affected by the amount of protein, the ratio of polar and non-polar amino acids, temperature etc. It is an important characteristic of a protein to be used as a food ingredient in products like custard, meat products, soup to increase viscosity and thickening, baked products to increase handling and freshness characteristic (Wolf, 1970) [53]. Protein with good WHC helps to decrease

the moisture loss from a packed baked product and preserve the freshness and moist attributes of the product (Ge *et al*., 2020) [22]. When the flours of green pea, lupin, hemp, buckwheat and fava beans were compared for functional properties it was found that the WHC of the flours were in the order of lupin, hemp, fava bean, buckwheat, green pea and wheat (Raikos, 2014) [37]. The WHC is affected by the extraction method used, Fuhrmeister and Meuser (2003) [20] found that the WHC of PPI of the commercial sample, acid precipitated, heat-acid precipitated samples were 4g/g, 2.7g/g, 2.2 g/g, respectively. PPC were also compared for their WHC and found that the value for AS-AP and UF samples are 4.5 ml/g and 3.9 ml/g, respectively (Boye *et al*., 2010) [7].

4.6 Gelling property

The gelling property of the protein contributes to the textural and sensory property of the product. Protein gelation can be divided into the cold set and heat-induced gelation. Gelling property is mainly expressed in terms of rheological properties like least gelation concentration (LGC), gelation temperature (Tgel), storage modulus (G'), loss modulus (G"), fracture stress (gel strength), young's moduli (gel stiffness), fracture strain (gel brittleness) etc. Gelling property of pea protein is mainly affected by protein fractions, method of extraction, cultivar and several other factors like protein concentration, pH, temperature, ionic strength, and cooling and heating rate (Ge *et al*., 2020) [22]. The LGC of PPC by UF (12%) was lower than PPC obtained by AS-AP (14%), however, the LGC of commercial PPI by WE (17%) were equal to the commercial PPI by AS-AP (Boye *et al*., 2010; Moreno *et al*., 2020) [7, 34]. It was found that mechanical properties of PPI by SE was dependent on salt concentration and pH, better gel was attained at low ionic strength and pH value away from PI.

6. Application Concern

Pea protein is widely used as a plant protein substitute for animal or soy protein due to its low cost, sustainability, functionality and nutrition. Some of its highlighted potentials are utilized enormously in every field as listed below;

6.1 Pharmaceutical application

Due to the amphiphilic nature of plant proteins, these can be added with encapsulated components as a wall material or as an emulsifier (Dickinson, 2012) [14]. Different plant proteins such as soya protein, zein protein, pea proteins are generally used during the encapsulation of bioactive ingredients. Pea proteins having surface-active properties are used as encapsulating material. These proteins are mostly hydrophilic in nature thus water-soluble and are used to form and stabilize oil in water nanoemulsions for the preparation of nanoencapsulation of bioactive components. PPIs were used as a matrix, film or wall material during encapsulation. Pea proteins were used for stabilization of emulsion during encapsulation of lipophilic bioactive materials like α-tocopherol, omega-3 fatty acids, conjugated linoleic acid and black-pepper oil (Ge *et al*., 2020; Lu *et al*., 2020) [22, 31]. PPI was used as a matrix material for microencapsulation of beta carotene (Graaf *et al*., 2001) [24]. PPI or PPC when used in an encapsulation system improves the encapsulation stability, provide better oxidative and thermal stability to the encapsulating material.

6.2 Edible film coating material

Recent trends for the use of biodegradable films lead to enhancing the application of pea protein as a film material. The film formation of PPI is mainly influenced by the type of plasticizer used, protein-plasticizer ratio, injection parameters, heat treatment and pH (Ge *et al*., 2020) [22]. The film-forming properties of PPI were found to be better when compared with soya bean, rice, crayfish, albumen or kidney beans proteins (Shevkani and Singh, 2015;
Felix *et al*., 2016) [43, 18]. Pea protein, when used in a film, showed excellent UV light and water vapour barriers at low relative humidity, however because of the hydrophilic nature the moisture barrier property is lowered than synthetic film. The film-forming properties like mechanical and moisture barrier property of pea protein are improved when mixed with other polymers (polysaccharide or hydrophobic protein) (Ge *et al*., 2020) [22].

6.3 Extruded products

Due to high-quality protein and nutritional value pea protein are added in different extruded products. Protein-enriched extruded products were prepared by adding PPI with rice starch, wheat starch and corn grits. It was found that PPI when added at the rate of 10% and 20% enhances the expansion property and microstructure of extrudates, however when added at higher concentration (50%) PPI exhibit poor product characteristics. Meat analogues using pea protein were also prepared by using the twin extruder technique (Ge *et al*., 2020) [22] which can provide a meat-like fibrous structure. Meat analogues using wheat gluten/PPI blend were also prepared by Schreuders *et al*. (2019) [41].

6.4 Food emulsifier

Pea proteins are also used as an emulsifier due to their high surface-active property. Pea protein has the property to decrease the water-oil interfacial tension and also provide emulsion stability (Ducel *et al*., 2004) [15]. At neutral pH, pea protein imparts better emulsifying and foaming properties as compared to soya protein (Aluko *et al*., 2009) [2]. The foaming and emulsifying properties of pea protein are previously discussed in detail in this paper.

6.5 Food supplements

Pea proteins are widely used as a food supplement or additive in different food products. The PPI can be used for the preparation of gluten-free products. Because of its high functional properties as gelation property, fat and water absorption property it could be used as a novel ingredient for the production of functional food. The functional properties of beef patties, salad dressing and spaghetti and muffins were increased by adding pea proteins. Being rich in BCAA valine, isoleucine and leucine, it helps in muscle growth, thus can be used as a nutritional supplement for sports and body building. The effect of oral supplementation of pea protein was compared with whey protein and placebo protein, it was found that the muscle growth was more by pea protein supplementation when compared with placebo protein and pea protein was comparable to whey protein (Lu et al., 2020; Babault *et al*., 2015) [31, 4].

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