Pea and Lentil Protein Extraction and Functionality[1]

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These studies have demonstrated that peas and lentils can be used as protein sources for flours, concentrates and isolates. Less research attention has been devoted to lentil protein extraction, probably because of the greater cost of lentils as compared to peas. Pin-milling and air classification is well adapted to extracting pea flours to produce pea protein concentrates. Apparently, air-classification can be applied successfully to starch rich legumes, but will not give satisfactory results with lipid rich legumes.

Wet processes, including alkaline and salt and acid solubilization, together with isoelectric precipitation or ultrafiltration, have been developed. The pea and lentil protein extracts of these processes exhibit comparable and complementary functionality to homologous soybean products. Air-classified pea protein concentrates are different from soy protein concentrates because of residual starch which can be useful for particular functional applications. Pea isolates appear to exhibit better foaming properties and more solubility than soy isolates, but pea isolates have to be more concentrated than soy isolates to produce viscous dispersions. The economic feasibility of pea and lentil protein extracts is related directly to protein content of the flour, unique functionality of the extracts, marketability of the by-products of extraction and the cost of peas or lentils.

Dry peas *(Pisum sativum)* and lentils *(Lens culinaris)* contain 20–30% lysine-rich protein. Air-classification and alkaline solubilization with isolelectric precipitation separate legume storage globulins and albumins into concentrates and isolates. Lipoxygenase oxidation in the protein extracts results in decreased nutritive value, digestibility and solubility. Solubility, amino acid composition, conformation, surface hydrophobicity, susceptibility to denaturation and inter- and intra-molecular binding influence functionality of extracted legume proteins in food systems. Solubility, emulsification, foaming, fat-binding, water-binding, gelation, swelling and viscosity are important functional properties of protein extracts.

Acid extraction reduced pigment concentration and lipid oxidation by-products in pea protein extracts. Lipoxygenase was inactivated during low pH extraction. Cysteine and methionine concentrations were retained in acid protein extracts. Heat treatment increased surface hydrophobicity and functionality of pea proteins. Oxidation during storage of protein extracts corresponded to increases in surface hydrophobicity, foam volume and emulsion stability. Although acid extraction appears promising, degradative oxidation may outweight functionality enhancement and economic considerations may be prohibitive.

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The Pacific Northwest states of Washington and Idaho produce approximately 95% of the dry peas (*Pisum sativum* L.) and lentils (*Lens culinaris* Medik) harvested in the United States. Much of the U.S. dry pea and lentil crop is exported to the United Kingdom, South America or the Far East. Both dry peas and lentils are underutilized crops with prices ranging from $10.75 to $12.85/cwt for peas and $17.25 to 36.20/cwt for lentils, depending on market environment (1).

Consumption of plant proteins is increasing, largely due to use of plant proteins as functional ingredients in formulated foods. Advantageous functional properties that proteins may contribute to food systems include color, flavor, texture, solubility, viscosity, cohesion, gelation, foamability, water or oil absorption and emulsification (2). Extraction and utilization of proteins from legumes such as peas and lentils have received appreciable research attention, yet are not without substantial chemical and physical constraints.

COMPOSITION OF PEAS AND LENTILS

Wide genetic and environmental variability is reflected in the chemical composition of peas and lentils. In general, peas and lentils contain from 15 to 40% protein (3,4). Legume proteins are primarily storage proteins comprised of two principle globulins, legumin and vicilin. Storage globulins are synthesized in the vacuole of the developing seed and packaged into 1–10 Mm diameter "protein bodies" (5).

Legumin is composed of equimolar α (MW ca. 40 K) and β (MW ca. 20 K) subunits, has an overall MW of 340–360 K and a sedimentation coefficient (S) of 11–12 (6). Vicilin has a MW of 175–180 K, with reported subunits of 75, 56, 43, 33, 25 and 12 K and an S value of 6–7 (7). Vicilin is glycosylated, but the glycosylation of legumin is questionable (8). Legumin/vicilin ratios vary with cultivar and range from 1.0 to 4.2 (9,10). A third globulin, convicilin, with a subunit MW of 71,000 and MW of 290,000 containing a limited quantity of carbohydrate has also been identified (11). The mean isoelectric point of pea globulins is pH 4.4–4.6, contrasted with an isoelectric pH of 6.0 for the albumins (7).

The remaining metabolic proteins, 13–14% of total N of peas, are predominantly water soluble albumins (12,13). The amino acid composition of peas and lentils varies with cultivar, providing major quantities of arginine, glutamic acid, aspartic acid, lysine and leucine (14–16), and restricted limiting quantities of methionine, cystine and tryptophan. The concentration of methionine in mg per g of total N for peas and lentils is 70 and 50, respectively, and for wheat and milled rice is 106 and 145, respectively (17). To satisfy human protein requirements, legume proteins, such as peas and lentils must be complemented with a cereal grain rich in sulfur amino acids such as corn, wheat or rice (18).

Compared to the globulins, pea albumins contain more of the essential amino acids tryptophan, lysine, threonine, cysteine and methionine. The globulin protein fraction is rich in arginine, phenylalanine, leucine and isoleucine.

Since typical procedures for the preparation of protein extracts from legumes select for globulins, the protein quality of isolates is reduced by the selection of proteins with reduced concentrations of sulfur amino acids (13).

Pea lipids can be broadly classified as neutral triglycerides or polar phospholipids (19). The ratio of neutral to polar lipids ranges from 0.9 to 2.3 among cultivars (19-22). The polyunsaturated fatty acid content of phospho- and galactolipids is greater than triglycerides, thus increasing susceptibility to oxidation.

The proximate composition of pea flour is 21.5-23.0% protein (N × 6.25), 1.1% crude fat, 54.2-56.6% starch, 1.5% fiber and 2.6% ash (23). The proximate composition of lentil flour is 19.5-26.3% protein, 0.9% crude fat, 53.6% starch, 1.2% fiber and 2.3% ash (23).

PROTEIN EXTRACTION PROCESSES

Many industrial processes exist for the extraction and concentration of proteins from legumes (24). Dry processes, such as pin-milling and air-classification, are designed to differentiate starch-rich legume seeds into two populations of particles differentiated by both size and density. Air-classification separates milled peas and lentils into a light or fine fraction (the protein concentrate) and a heavy or course fraction (the starch concentrate) (10,25). Protein concentrates produced by air-classification of peas or lentils generally contain 38-65% protein (10,23,26,27). Air-classification processes are well adapted to peas and lentils because of the large diameter and fairly uniform distribution of starch granules in these legumes (10).

A commonly used wet process (28) solubilizes a legume flour in an alkaline solution (pH 9-10, ca. 1.0 N NaOH), separates insolubles by centrifugation and precipitates protein isolates by acidification of the supernatant near the isoelectric point, pH of 4.5, of the globulins (10,28). Flocculated and precipitated proteins are collected by centrifugation. Pea isolates prepared with an alkaline process contain 90-95% protein by definition, with an overall protein yield of 80% (10,29,30). Protein isolates of lentils often contain less nitrogen than protein isolates of other legumes because nitrogen loss in the whey is great (29). Extraction of proteins from pea flour with cheese whey was 18% less efficient than water at pH 8.0 and 48% less efficient at pH 6.8 (31). Pea globulins demonstrated an affinity for whey proteins and albumins in low ionic strength solutions. Ultrafiltration can be used instead of acidification to recover extracted proteins—the overall recovery yield (62%) and protein content of the spray-dried isolate (90%) are similar to acidification and centrifugation (32). The main problem preventing use of ultrafiltration has been low flow rate and plugging of membrane when the protein concentration increases (10).

Alkaline extraction of proteins causes several adverse chemical reactions such as: (i) racemization of amino acids (33); (ii) formation of the nephrotoxic compound lysinoalinine (34-36); (iii) reduced protein digestibility (37,38); and (iv) losses of essential amino acids such as cysteine and lysine (37,39,40). High temperatures, alkaline pH values and long treatment times increase damage to protein isolate quality (39).

The classical term "globulin" implies solubility in solutions containing moderate quantities of salt (40). Pea and lentil globulins are soluble in 0.5-1.0 M NaCl (41) and can be collected as a protein isolate by centrifugation. A Canadian patent (42) describes a method for precipitation of isolates by extracting protein with a salt solution of at least 0.2 M ionic strength.

The risk potential of lysinoalanine and decreased protein quality in alkaline solubilized protein isolates prompted exploration of acid extraction of legume proteins. Yields of 56-74% of total protein in lentils was extracted after solubilizing the protein in pH 2.0 extraction buffers (29,43). Nickel (44) developed a novel process in which pea protein was separated from starch and fiber by a multi-step solubilization at pH of 2.5 to 3.0, followed by centrifugation. Eighty-five percent of pea flour proteins were extracted with pH 1.5 solutions of hydrochloric, phosphoric or lactic acids (45). Similar solutions of sulfuric acid only solubilized 40% of the proteins present in pea flour.

Enzyme modification, cellulases, of the protein solubilization medium sometimes enhances extractability of nitrogenous constituents (46). The protein yield following modification is dependent on enzyme activity and buffer concentration.

During the solubilization and extraction of vegetable protein isolates, several non-proteinaceous materials such as polyphenolics (47), chlorophyll and carotenoids (29), phytate (48) and lipids and lipid oxidation products (10,19,49) may co-extract. Davis (44) observed that lipids present in green and yellow pea flour co-extracted to yield protein concentrates with 2.0 and 1.9% lipid, respectively. Alkaline extraction of pea flour (1.7% lipid) resulted in a protein isolate that contained 8.5% lipid (10). The beany, bitter, cardboard-like flavor of many legume protein isolates is attributed to conversion of polyunsaturated fatty acids to aldehydes, ketones and alcohols (50). Lipid oxidation may occur during storage of legume seeds (51,52), alkaline extraction processes (53) or storage of the protein isolate (10) to produce undesireable oxidized flavors that prohibit incorporation of the protein isolates in food products. Lipid oxidation influenced in peas by lipoxygenase (E.C. 1.13.11.12) may decolorize pigments, result in protein-lipid cross-linking and lead to losses in extraction efficiency and nutritive quality through decreased solubility, digestibility and availability of essential amino acids.

PROTEIN FUNCTIONALITY

Among the most important aspects of vegetable proteins are their functional properties. The functional properties of proteins denote any physicochemical property which affects the processing and behavior of protein in food systems, as judged by the quality attribute of the final product (54-56). Functional properties reflect complex interactions between the composition, structure, conformation, physicochemical properties of the protein per se, other food compounds and the nature of the environment in which the proteins are associated or measured (54). Functional properties such as solubility, emulsification, water-binding, viscosity, gelation, foaming, cohesion, color and flavor are important aspects of plant proteins proposed for use as food ingredients (54). The use of plant proteins by the food industry depends primarily on the balance between functionality and price (56).

Properties of plant proteins which influence functionality include solubility, amino acid composition, conformation, surface hydrophobicity, susceptibility to denaturation and the number and strength of intra- and inter-molecular bonds (56–59). The complexity of protein functionality can be overwhelming; improvement in one functional property will not assure or even imply improvement or maintenance of another functional property (60). Proteins play a role in very complex food structures, and although it is important to understand the specific function of a protein ingredient and the desired quality of a food product, protein functionality and food product quality may not be directly associated.

Air-classified pea and lentil concentrates demonstrated characteristic functional properties of protein solutions (Table 1). Pea concentrates exhibited high values for oil absorption, oil emulsification and foam formation (26). Gelation was relatively good for both pea and lentil concentrates. Air classified lentil protein concentrate exhibited low oil absorption, oil emulsification and viscosity. Water absorption was acceptable for both the pea and lentil protein concentrates (26).

Heat treatment of pea protein concentrates alters functionality. Heat treatments of increasing severity reduce the nitrogen solubility and oil emulsification of pea protein concentrate. Water-holding capacity increased continuously and oil absorption increased to a maximum, then decreased with increasing severity of heat treatment. Foaming properties were adversely affected by all heat treatments except 70°C. Electrophoresis protein bands were observed to change, and the vicilin:legumin ratio decreased as heat treatment severity increased (62).

Air-classified concentrates generally exhibit less functionality than isolates, especially foaming and emulsifying properties, but the presnce of residual starch in protein concentrate can be useful in relation to viscosity and binding properties (10).

Alkali solubilized, acid precipitated, spray-dried pea and lentil protein isolates are generally cream-colored and quite bland in flavor (10). Variation in functionality of the isolates can be due to the flour extracted and to the extraction process. Sodium proteinate isolates exhibited more functionality than isolectric isolates (10,30). Pea protein isolates are generally more soluble than corresponding soybean isolates. Chemical analysis, functional properties, color and flavor of pea protein isolate compared favorably with a soy counterpart (30). Drum drying decreased nitrogen solubility index and increased water absorption. Freeze and spray-drying resulted in isolates with high emulsification and water absorption values. Spray drying produced the best foam, color and flavor properties (30).

The water retention capacity of pea protein isolate is estimated to be 2.7–2.8 times the initial weight of water at pH 7, somewhat less than soy protein isolates that absorb 4–5 times their weight in water (25). The protein concentration from pea protein isolates must be greater than the protein concentration from soy protein isolates to obtain dispersions with the same viscosity (10). Emulsifying properties of pea protein isolates are comparable to soy protein isolates. Foaming properties and production of fibers from isoelectric or proteinate pea protein isolates are comparable or superior to soy protein isolates (10).

Protein isolates prepared by alkaline extraction of dry green peas were highly pigmented and contained lipid-derived off-flavors. Storage of alkaline solubilized pea protein isolates resulted in chlorophyll bleaching and development of "hay-like" or "grassy" aromas (24).

Extraction of legume protein isolates in approximately 0.05 M salt solutions is primarily an analytical research technique (10,30,63,64) because of the limited quantities of isolate obtained. Extraction of globulins in salt solutions provides proteinates with excellent functionality. Dagorn-Scaviner *et al.* (65) prepared pea globulin extracts by a two step chromatographic procedure, separating vicilin and legumin by ion exchange chromatography and then purifying them by gel filtration. The interfacial behaviors of vicilin and legumin at water/air interfaces were studied and vicilin, because of its lower molecular weight and more flexible tertiary structure, was reported to be a more effective surface active globulin than legumin (65).

Acid extraction of peas produced protein isolates which were less pigmented and contained fewer secondary products of lipid oxidation than isolates prepared with alkaline extraction (24). Inactivation of lipoxygenase occurred during preparation of the acid isolates. Acid prepared pea protein isolates contained an increased proportion of albumins and had 18% greater cysteine and methionine concentrations as compared to alkaline isolates. Although protein denaturation at low pH led to decreased solubility of acid protein isolates, other functional properties compared favorably with alkaline isolates (24).

An 83% pea protein concentrate extracted in water at pH 5, dialyzed and then centrifuged exhibited excellent water solubility, fat absorption, foaming and emulsification properties. Less than 50% of the protein concentrate was precipitated with a moderate heat treatment (12). Pea protein preparations separated from starch and fiber by a multi-step solubilization at pH of 2.5–3.0 (44) and centrifuged had better solubility in water than wheat gluten or soy isolates. Water absorption of the pea protein isolate was less than soybean protein concentrates or isolates, but much greater than the gluten protein preparations. The fat absorption of the pea protein isolate was poor, yet comparable to gluten. The pea protein concentrate exhibited good emulsifying activity comparable to soy protein isolates. The phytate content (66) and lipid (24) extracted into the protein concentrate may result in quality deterioration.

Although acid extraction of pea and lentil protein isolates appears promising, several problems remain.

TABLE 1

Pea and Lentil Protein Concentrate Functionality (61)

	Pea	Lentil
pH	6.4	6.4
Nitrogen solubility	90.4	90.5
Water absorption	118	105
Fat absorption	97	92
Emulsification (oil emulsified)	25	19
Whippability (vol. increase)	105	110
Foam stability (decrease)	38.6%	18.9%
Viscosity (time to peak)	65 min	65 min
Viscosity	460 BU	620 BU
Gelation (proportion gel)	75	54

Lipid oxidation and related problems during storage may be decreased by removal of metal ions during aqueous extraction. Extraction of lipids from legume protein isolates will enhance flavor and functionality.

PEA AND LENTIL PROTEIN EXTRACTS IN FOOD PRODUCTS

Pea flours, concentrates and isolates have been suggested as alternative protein sources for several food products. Air-classified pea protein concentrate was blended with cheddar cheese whey, heated at 63°C for 30 min or 85°C for 20 min, concentrated and spray-dried. The spray dried product compared favorably with spray-dried non-fat dry milk (NFDM), exhibiting greater water hydration, oil absorption and oil emulsification properties than NFDM. The pea/whey concentrate exhibited greater foam stability than NFDM and produced equivalent bread volume. The flavor of the pea/whey concentrate was not markedly different than spray-dried NFDM. The low temperature, 63°C for 30 min, heat treatment for the pea/whey concentrate was preferable to the high temperature treatment (67).

Pea and lentil protein isolates were used to prepare imitation milks. Lentil protein isolate produced a milk of intermediate quality equivalent to milk prepared from soy protein isolates. Pea protein isolates resulted in milk products of poor quality. Selected properties of pea and lentil isolates used in preparing imitation milk are presented in Table 2 (68).

Pea protein preparations were precipitated to form a protein curd similar to tofu. The yields of curd from pea protein concentrate (13.6%) was not equivalent to soy protein concentrate (39.8%), but the protein yield from peas (43%) was improved compared to protein yield from soy (55%). The amino acid content and flavor of pea and soy protein curds were similar. Addition of gluten to the pea protein curd increased the concentration of limiting sulfur-containing amino acids, reduced the lysine concentration and improved the poor color and texture of pea protein curd (69).

Pasta, noodles and spaghetti were fortified with 33% pea flour or 20% air-classified pea concentrate. Fortification increased the protein concentration of noodles to 22% and spaghetti to 24%. Pea fortification reduces raw noodle strength and reduced cooking time, but noodle characteristics deteriorated and cooking losses were greater. Sensory evaluation of color, flavor and texture of pea protein fortified noodles compared favorably with control noodles. Pea flour gave pasta a desirable yellow color. Pea protein fortified spaghetti lost its tolerance to overcooking and the flavor was inferior to control spaghetti. Precooking the pea protein improved spaghetti, decreased noodle dough stickiness and improved flavor (70). Yellow pea flour was successfully substituted for cowpea flour in preparation of akla, a popular West African deep fat fried bread, according to American sensory panelists. African sensory panelists preferred cowpea akla (71).

Pea protein meal and concentrate were utilized to extend ground beef in preparation of hamburgers. Five percent pea meal added to hamburger increased cooked yields and water retention properties of beef patties (72). Extending ground beef with 10% pea protein concentrate was acceptable, making the beef patties softer, more tender and requiring less force to compress than all-beef patties (73). Texturizing the pea concentrate reduced fat retention in cooked hamburger. Flaked pea protein concentrates were superior to flours as ground beef extenders. Fat retention was lower in beef patties extended with flakes—juiceness decreased with increased pea protein. The pea protein flakes improved flavor, firmness and apparent juiceness, and decreased fat binding in cooked ground beef. Extending ground beef with pea flour and pea protein concentrates increased the quantity of available protein.

TABLE 2

Properties of Pea and Lentil Concentrates (68)

	Pea	Lentil
Solubility		
Distilled HOH	92%	86%
Buffer	85%	92%
Conductivity	87	78
Homogenization index	17.9	13.1
Viscosity	2.8	2.0
Sensory evaluation		
Odor	3.4	4.7
Taste	3.2	3.3
Color	5.8	4.0
Viscosity	8.2	8.3

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