ACS FOOD SCIENCE & TECHNOLOGY

Open Access

pubs.acs.org/acsfoodscitech

Review

Pigeon Pea, An Emerging Source of Plant-Based Proteins

Adilson Roberto Locali-Pereira, Adeline Boire, Claire Berton-Carabin,* Sebastião Roberto Taboga, Véronique Solé-Jamault, and Vânia Regina Nicoletti

Cite This: *ACS Food Sci. Technol.* 2023, 3, 1777−1799

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Making use of neglected pulse crops is a way to promote biodiversity and combat malnutrition in emerging countries, as well as a strategy to provide alternative and resilient sources of plant-based proteins. Pigeon pea (*Cajanus cajan*) is a pulse native to tropical and subtropical regions, such as Asia, India, and South America. Despite its great nutritional potential, pigeon pea is an underutilized crop, and its production is restricted to smallholder cultivators. Pigeon pea exploration for food application is a means to boost the cultivation and valorization of this crop, in addition to contributing to meeting the worldwide demand for high quality plant-based proteins obtained from sustainable crops. This review synthesizes the main research findings involving pigeon pea exploration for food applications, from the processing of its seeds and flours to the extraction and modification of its proteins, highlighting its potential as a food ingredient. Several initiatives have been carried out with the aim of investigating and improving the functional qualities of pigeon pea. Aqueous fractionation has been investigated under different conditions, showing it to be a viable process to produce protein-rich ingredients. Modifications of proteins, as in enzymatic hydrolysis processes, allow for releasing bioactive peptides of interest for applications in functional foods. Despite the overall potential, a knowledge gap has been identified regarding bulk rheological and interfacial properties of pigeon pea proteins, which need to be further investigated.

KEYWORDS: *Cajanus cajan, pulse, alternative protein, antioxidant activity, digestibility, protein extraction*

1. INTRODUCTION

Pigeon pea is a pulse native to emerging countries in Asia, Africa, and South America.[1] The FAO's *The Global Economy of Pulses*[2] includes pigeon pea among the most important pulses in terms of global production and consumption quantities, along with common bean, chickpea, dry pea, lentil, cowpea, mung bean, and urd bean. The world production of pigeon pea is about 5.5 million tons, contributing to 5.8% of total pulse production across the world. India is both the largest consumer and producer of pigeon pea, accounting for 90% of global production.[3,4] Myanmar, Malaw, and Nepal are some of the major exporters of pigeon pea, with a limited domestic demand. Pigeon pea is also cultivated in small quantities in some countries in Latin America and the Caribbean.[2]

Pigeon pea is considered one of the most drought-tolerant pulses, due to a deep root system, and a germplasm with a higher osmotic adjustment in relation to other pulses.[2,5] This allows pigeon pea plants to perform better, even with low water potential, by moderating photosynthetic functions and stomatal conductance and delaying leaf senescence.[5,6] Nevertheless, despite these convenient agronomic characteristics, its production is almost entirely restricted to small growers. In addition, its average yields vary significantly depending on agronomic practices, density of planting, and seed variety,[7,8] since it is primarily grown as an intercrop.

A key factor in boosting large-scale production of pigeon pea seeds is the development of new cultivars. While most pulses are naturally only self-pollinators, pigeon pea shows a certain degree of cross-pollination, carried out by honey bees. This opens a route to develop hybrids with improved agronomic features.[2] One of the key breeding targets is the development of short-duration cultivars that can mature in about 130−145 days, instead of 250−280 days for long-duration cultivars. This would reduce the time of field occupancy and minimize the risks of frost damage.[9] The development of new drought-tolerant cultivars of pigeon pea has been carried out by the International Center for Agricultural Research in the Dry Areas (ICARDA), in Lebanon, and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), in India.[4,9] Cultivars more suitable for human consumption, with shorter cooking times and better technological properties, have also been the focus of studies by the Brazilian Company of Farming Research (EMBRAPA).[10,11]

Beyond agronomic challenges, a better understanding and expansion of the possible uses of pigeon pea for food applications are essential to reduce the gap between small-scale farmers and large-scale production.[2] Investigations into the potential of pigeon pea-derived ingredients have gained prominence in recent literature, driven by the growing demand for alternative sources of plant-based proteins. The studies cover aspects from seed pretreatments to improving nutritional quality and evaluating the functional properties of

Received: July 17, 2023
Revised: October 12, 2023
Accepted: October 18, 2023
Published: November 7, 2023

flours[1,8,12−14] to fractionation processes to produce protein concentrates and isolates.[15−21]

However, to the best of our knowledge, no comprehensive review has covered the main findings regarding the production and properties of pigeon pea-based protein-rich ingredients in the past decade. Highlighting such advances is important to enhance the use of this pulse, in addition to understanding which aspects still need more attention in the coming years. This review summarizes the main research outcomes related to pigeon pea, focusing on seed processing and protein extraction, as well as on the functional properties of interest to the food industry. This review also intends to disclose the potential of this nutritionally valuable pulse as an approach to encourage its further exploration as a food ingredient in future years.

2. PIGEON PEA: A BRIEF HISTORICAL PERSPECTIVE

Pigeon pea (*Cajanus cajan (L.) Millsp.*), a pulse belonging to the *Fabaceae* family, grows in semiarid, tropical, and subtropical regions of the world. The English name for this pulse derived from Barbados island, in the Caribbean Sea, where the seeds were used to feed pigeons.[22] Depending on the region, pigeon pea can also be referred to as *Congo pea* and *Red gram*. The origin of this pulse is somewhat contradictory. While many believe the species is native to Africa, there is evidence supported by modern genetic data that its origin is from East India.[7] It is believed that the culture was introduced in South American countries in the 17th century, brought by the slave route from Africa.[4]

Cajanus cajan is a perennial tree (Figure 1) that can measure up to two meters in height with a growth time of 6 to 9

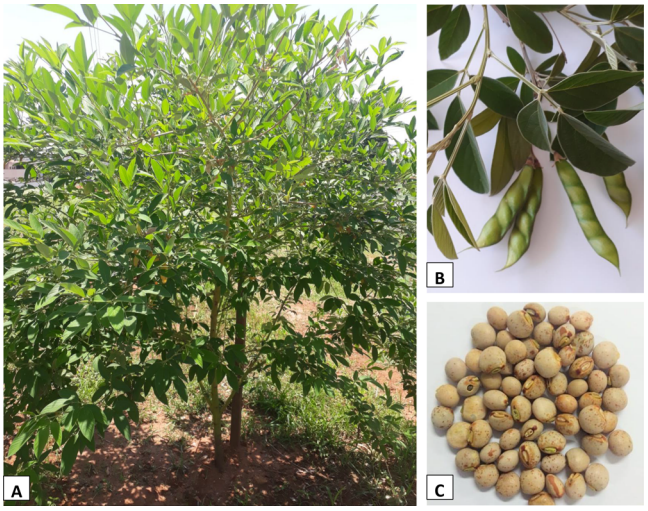

Figure 1. Pigeon pea (*Cajanus cajan* (L.) *Millsp.*) tree (a), green pods (b) and dry seeds (c).

months. The seeds are about 2-mm thick, and their color can vary from black to cream.[7,8,23] Its cotyledons are made up of several plant cells where the starch granules are dispersed in a protein matrix (Figure 2). Pigeon pea is a crop that adapts well to hot climates and low humidity, contributing to soil fertility and to sustainable agriculture in regions that are already facing the first consequences of global climate change.[4,13,24,25]

The world production of pigeon pea reached about 5.5 million tons in 2021[26] (Figure 3A). Comparatively, the world production of dried beans is on average 24 million tons, chickpea production is about 13 million tons, dry pea

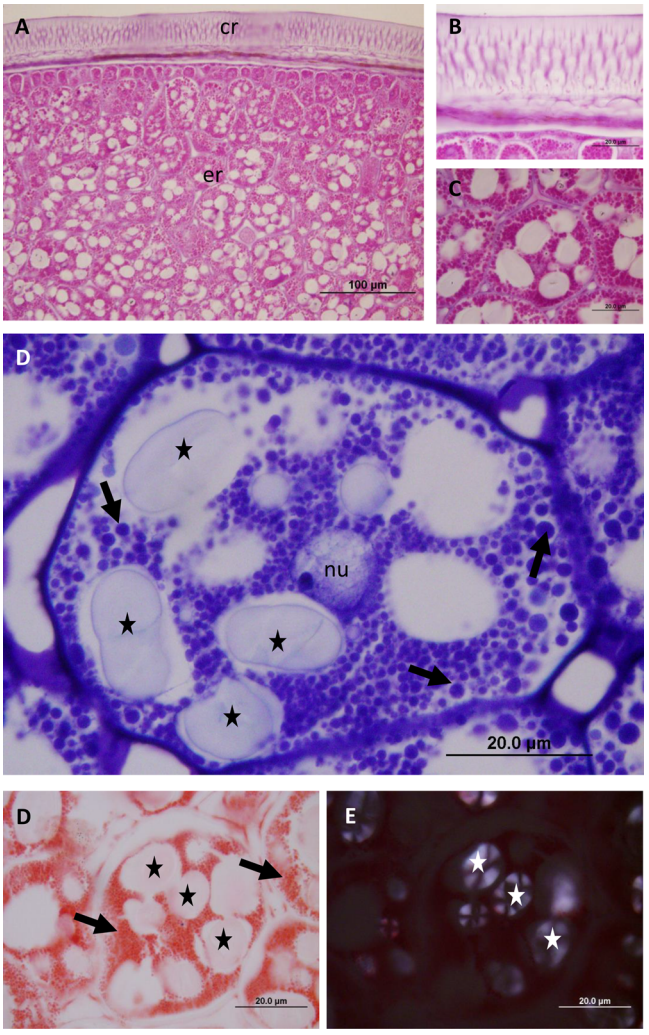

Figure 2. Histological sections of pigeon pea (Cajanus cajan) seed, obtained from formalin-fixed seeds embedded in paraffin and sectioned at 5-μm sections using rotative microtome. (A) General view of seed stained by haematoxylin-eosin. Coat region (cr) and endosperm region (er). (B) Detail in seed coat region that in the mature seed is dry and usually consists of dead cells. (C) Detail in the endosperm region occupying most of the seed volume. (D) Endosperm cell stained by toluidine blue at pH 4.0 for identification of anionic groups of the granular proteins in the cytoplasm (arrows); The nucleus is indicated by (nu) and starch granules are chromophobes and are indicated by stars. (E) Endosperm cell stained by Xylidine Ponceau at pH 2.5 for identification of cationic groups of total proteins. The protein granules are identified in red (arrows) and the starch is chromophobes too by this stain (stars). (F) The same region of the figure E visualized under polarized light for identification of starch granules with characteristic birefringence (stars).

production is about 11 million tons, cowpea production is about 7 million tons, lentil production is about 5 million tons, and faba bean production is about 4 million tons (FAO, 2019).

India is the country that mostly consumes and cultivates pigeon pea, accounting for about 90% of its world production (Figure 3B). Since 2010, however, pigeon pea production has been gradually increasing in Africa, the Caribbean, and South and Central America. In India, this pulse is used mainly in the production of typical soups made from legume seeds, called *dhal*.[3,13] In the Caribbean and Southeast Asia, grains are

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

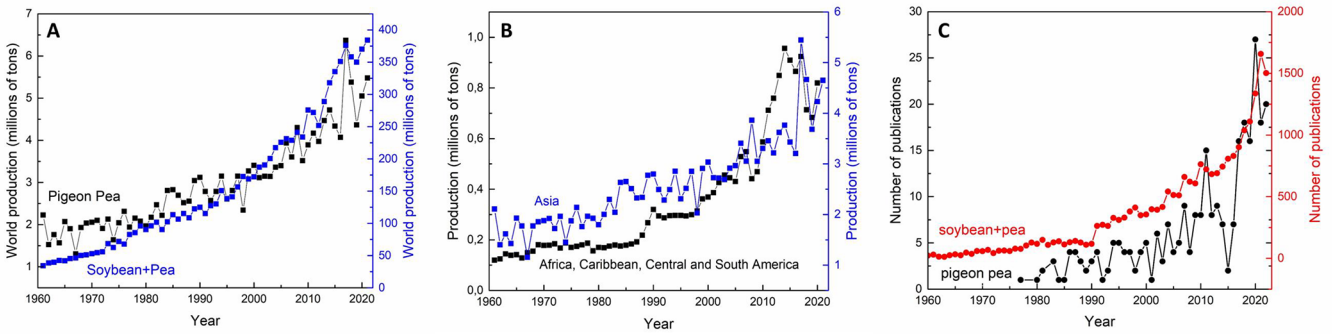

Figure 3. Dashboard with production data, scientific publications and nutritional aspects of pigeon pea (*Cajanus cajan*). (a) World production and (b) production in Asia, Africa, Caribbean, Central and South America of pigeon pea seeds compared to soybean and pea (1960−2022). Source: FAOSTAT. (c) Evolution of scientific publications on pigeon pea in the 'Food Science and Technology' field over the years compared to soybean and pea. Source: Web of Science data base.

harvested while still unripe and consumed as fresh vegetables.[7] In South American countries, such as Brazil and Argentina, pigeon pea for pulse consumption represents only small- to medium-sized productions to serve as subsistence agriculture.[24,25] An alternative reason that drives pigeon pea cultivation in Brazil is its use as an intercrop for helping nitrogen fixation in the soil, in addition to the use of leaves and branches as green manure or as fodder for animal feed.[27]

Despite the easy adaptation to different soils, pigeon pea production has remained stagnant in the last five decades.[7] Bringing knowledge about the nutritive potential of pigeon pea to the population in general is another measure that can be taken to make the best use of this pulse.[28,29] The number of scientific articles related to pigeon pea grew about 12.5 times in the period between 2015 and 2020, according to data from the Web of Science platform (Figure 3C). In the same period, all articles published in the Food Science and Technology category grew by 50% and publications referring to peas and soybeans increased by 65%. The number of publications involving pigeon pea, however, is still small, but it shows potential for further expansion in future years.

3. NUTRITIONAL AND ANTINUTRITIONAL PROFILE

Proteins represent 18 to 28 wt % (dry basis) of the composition of pigeon pea seeds.[1] A high protein content was also reported in the leaves (19.4 wt %, dry basis).[30] The amino acid profile of seed proteins is similar to that of soybean, with about 43 wt % of the total amino acids being essential amino acids. The presence of valine, leucine, isoleucine, glutamic acid, phenylalanine, and lysine stands out. The contents of these last three amino acids were found to be higher in pigeon pea than in chickpeas; however, it is low in methionine and cysteine, two sulfur-rich amino acids.[8,17,29,31]

Carbohydrates correspond to the largest fraction of pigeon pea seeds, constituting about 60 wt % (dry basis) of their total composition. Miano et al.[11] found that the soluble fiber content ranged from 4.2 to 7.9 wt %, whereas the insoluble fiber content was between 19.0 and 26.4 wt %. The largest portion of the total carbohydrates in pigeon pea is starch: about 50%, with 23.2% referring to the amylose fraction.[24]

Lipids account for a small fraction of the pigeon pea composition (approximately 1.5−5.0 wt %, dry basis), as in other similar pulses. The fatty acid profile is similar to that of soybean, with the predominant fatty acids being linoleic acid and palmitic acid, which represent about 54.8 and 21.4 wt % of the total lipid composition, respectively.[29]

The ash content in pigeon pea is about 3.5 wt % (dry basis) of the seed composition. Oshodi, Olaofe, and Hall[29] quantified the composition of minerals in pigeon pea seeds and obtained significant values of potassium (1308 mg100 g^{-1}), magnesium (110 mg 100 g^{-1}), and calcium (81.4 mg 100 g^{-1}). On the other hand, the authors observed a low sodium content (9.9 mg 100 g^{-1}). In a study by Yang et al.,[30] calcium (581 mg 100 g^{-1}), magnesium (138.8 mg 100 g^{-1}), iron (51.5 mg 100 g^{-1}), sodium (32.5 mg 100 g^{-1}), manganese (6.8 mg 100 g^{-1}), copper (1.4 mg 100 g^{-1}), and zinc (0.7 mg 100 g^{-1}) were identified. Pigeon peas are richer in calcium than common beans and chickpeas.[32,33]

In addition to these nutrients, pigeon pea seeds also present phenolic compounds (23.2 mg gallic acid equivalent g^{-1}), flavonoids (15.1 mg quercetin equivalent g^{-1}), tannins (0.4 g gallic acid 100 g^{-1}), tocopherols (1.1−9.3 mg 100 g^{-1}), and vitamins B1 (0.4 mg 100 g^{-1}) and B2 (0.3 mg 100 g^{-1}).[14,34,35] Some polyphenols and flavonoids have been identified in *Cajanus cajan* leaves,[36] but studies focusing on the identification of these compounds in the seeds are scarce in the literature. Nix et al.[37] observed the presence of phytoalexins, cajanol, cajanin and two isoprenylated flavones in pigeon pea soaked-seeds.

Although rich in several macro- and micronutrients, the species also has antinutritional factors, as in most pulses. Antinutritional factors are compounds that interfere with nutrient absorption by the human body, and they may, for instance, inhibit the action of digestive enzymes and impair the bioavailability of proteins. Some polyphenols, for example, can form insoluble complexes with proteins, making their absorption difficult.[8] Other antinutritional factors found in pigeon pea include saponins, protease inhibitors, phytic acid, phytolectins and oligosaccharides.[1] Ene-Obong[38] determined levels of trypsin inhibitors of up to 14.4 mg g^{-1} of flour, whereas tannins had values of 0.97 mg of catechin equivalent g^{-1} of flour, and phytates and phytic acid had values equal to 8.48 mg g^{-1} of flour. Some pretreatments in seeds can help reduce their levels of antinutritional compounds. Soaking for 12 h followed by cooking for 60 min was shown to eliminate tannins and reduce the trypsin inhibitor compounds in pigeon pea by 77%.[39] Germination, for example, was shown to be efficient in reducing some antinutritional factors in pigeon pea by action of activated proteolytic enzymes.[14] The protein

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

extraction process may also help reduce the concentration of antinutritional factors in the produced fractions. Adenekan et al.[8] reported that the protein extraction process from pigeon pea, using different solvents (water, methanol, acetone, and ammonium sulfate), resulted in a drastic reduction in tannin and phytate levels. Trypsin inhibitor and cyanogenic glycoside were not detected in the protein isolate. All isolation methods used were shown to be effective in significantly reducing the concentrations of the antinutritional factors evaluated.

Despite its high nutritional value, there is still a need to better characterize the composition of pigeon pea, especially regarding the composition of carbohydrates, lipids, and proteins. Currently, studies only report proximal compositions, but information about their composition profile is still scarce and needs to be better characterized with the aid of analytical chromatography techniques, for example. The same occurs for its minor compounds, which, in addition to identification, also need clarification regarding their impacts on the functionality and digestibility of the seeds.

4. SEED PROCESSING AND FUNCTIONAL PROPERTIES OF PIGEON PEA FLOUR

Pulse seeds are often subjected to pretreatment before processing, in either the food industry or in-home cooking. The main objective is to enhance their nutritional properties by reducing the antinutritional compounds and improving sensory aspects. Seed treatment can involve nonthermal (soaking, germination, ultrasound, fermentation, etc.) or thermal (cooking, roasting, microwave, etc.) processes.[24,28,40]

4.1. Nonthermal Processes. A simple nonthermal process that is employed is the soaking of the seeds. Hydration makes the seeds softer, in addition to promoting the activation of cell wall enzymes responsible for increasing the solubility of some compounds in the husk and reducing cooking time.[41]

Kate et al.[42] studied the dynamics of mass transfer in pigeon pea seeds during soaking. The authors noted that the beginning of the process is characterized by filling the empty cotyledon cavities with water and possible leaching of solids. In a second stage of the process, there is a loss of solids (about 5% of the total weight). Such solids are mainly made up of seed husks and loosely delimited solids on the surface of the cotyledons.

Miano et al.[11] evaluated different pigeon pea cultivars during hydration kinetics in a water bath (25 °C/660 min). The seed hydration percentage ranged from 57.2 to 99.7 wt % (amount of hydrated seeds/total amount of seeds). The authors observed that some seeds did not hydrate after the process, which was attributed to heterogeneity between grains at harvest (presence of mature and unripe seeds) and variations of cultivars in relation to the permeability of the husks.

In a study conducted by Vásquez et al.,[33] the influence of ultrasound application and the use of $NaHCO_3$ solutions on the hydration kinetics of pigeon pea was evaluated. Whereas the application of ultrasound accelerated hydration kinetics, the addition of $NaHCO_3$ inhibited grain hydration by approximately 23%. The decrease in the hydration rate due to salt addition (2 wt %) was attributed to the high pH (8.4), since hydration in alkaline medium can cause changes in the composition of the shell and the cotyledon of seeds, modifying cell diffusion mechanisms.

Dehulling is a nonthermal process commonly used after soaking. Pigeon pea is usually dehulled to improve digestibility and reduce antinutritional compounds that may be present in the husks. Some compounds are responsible for binding the bark to the cotyledon, such as the disaccharide galactomannan and the glycol protein bonds, which makes the dehulling process more difficult at the industrial level. It is known that pigeon pea is difficult to peel due to the presence of gums and mucilage between the husk and the cotyledon. Such compounds form a network of cellulosic microfibrils embedded in a non-starch carbohydrate matrix, and a mixture of enzymes (xylanase, cellulase and pectinase) proved to be efficient in dissolving the gums and mucilage present between the husk and cotyledon of pigeon pea, facilitating dehulling. The enzyme mix increased the dehulling efficiency, reduced the amount of energy needed to cook the seeds, and increased the protein content.[43] Enzymatic pretreatment has also been used for the same purpose in other species, such as basmati rice and horse grain.[44,45]

In addition to this, the germination of seeds such as soybeans, chickpeas, lentils, and beans has been investigated and revealed to cause phytochemical changes in these seeds that impact their technological characteristics. Increases in ascorbic acid, phenolic compounds, antioxidant activity and tocols are some of the changes observed in germinated seeds. Some enzymes, such as α- and β-amylase and proteases, can be activated during the germination process, causing the breakdown of carbohydrates and proteins into simpler forms. This leads to better digestibility of these nutrients, in addition to affecting their functionality, due to changes in their structures.[13,14]

Oloyo[46] studied the effects of germination of pigeon pea. Seeds were left to germinate for 5 days, and the nutritional and functional properties of the generated flours were evaluated. Regarding the proximate composition, the authors noticed a decrease in protein (21.9 to 15.3 wt %) and carbohydrate (62.6 to 59.5 wt %) contents during germination. On the other hand, the contents of lipids, fibers, and ash increased throughout germination. The authors attributed the decrease in protein and carbohydrate contents to the consumption of these macronutrients during the seed germination process. Antinutritional compounds such as oxalate and phytic acid decreased, whereas the levels of tannins, phenolic compounds, and trypsin inhibitor compounds increased.

Sharma et al.[14] observed that longer seed germination times and higher temperatures increased the levels of phenolic compounds in pigeon pea. The flavonoid contents in pigeon pea increased by more than 70% in seeds germinated at 35 °C for 48 h, compared to nongerminated seeds. The increase in the levels of phenolic compounds was also reflected in the antioxidant activity of the flours, which was higher than that of the nongerminated seeds.

In the study of Chinma et al.,[12] germination of pigeon pea seeds for 72 h also reduced the levels of phytic acid, tannins, and trypsin inhibitors by 62, 64, and 61%, respectively, compared to untreated seeds. On the other hand, there was a 76% increase in the level of total phenolic compounds, which may increase the antioxidant activity. In addition to increasing protein content by 18%, seed germination resulted in a decrease in lipid content by 29%, which was attributed to the consumption of fatty acids during germination for energy production. However, these results oppose those of Oloyo et al.[46] The duration of the germination process seems to be a critical factor modulating the levels of such macronutrients.

Acevedo et al.[13] investigated the effect of germination for 5 days on the seeds' microstructure and on the functional

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 1. Pigeon Pea Proteins Retrieved from the UniprotKB (October 2021) Database and the Corresponding Number of Amino Acids, Molecular Weight, and Isoelectric Point (pI) Computed Using the ProtParam Feature of the Expasy Web Server

Protein type	Uniprot code	Protein name	Number of amino acids	Molecular weight (g mol^{-1})	pI
Albumin	A0A151QN35	Albumin-1	101	11003	5.6
	A0A151QQ22	2S albumin	124	14459	5.0
	A0A151QQZ7	Albumin-1	102	11097	7.6
	A0A151QR00	Albumin-1	102	11085	6.8
	A0A151R635	Albumin-1	92	9720	4.5
	A0A151R6F6	Albumin-1	101	10980	6.7
Globulin	A0A151SLE6	Basic 7S globulin	259	27260	4.7
	A0A151STZ0	Basic 7S globulin 2	397	42564	9.0
	A0A151TW66	Basic 7S globulin	192	21288	6.7
	A0A151U0J2	Basic 7S globulin	379	40412	5.9
	A0A151U0M6	Basic 7S globulin 2	414	44845	8.4
	A0A151U0Q4	Basic 7S globulin	409	43794	8.7
	A0A151U0R9	Basic 7S globulin	364	38814	8.7
	A0A151U5Z8	Basic 7S globulin	413	43424	8.1
	A0A151RNW3	Glycinin	473	53413	5.1
	A0A151TUL0	Glycinin G3	446	50177	5.8
	A0A151TUN4	Glycinin G3	465	52503	5.8
Glutelin	A0A151RZS9	Glutelin type-A 1	356	38308	5.7
	A0A151SUU8	Glutelin type-A 1	356	38191	5.4
	A0A151T731	Glutelin type-A 1	356	38161	5.2
	A0A151TM61	Glutelin type-A 1	356	38509	5.6
	A0A151UBW6	Glutelin type-A 2	361	39259	5.2
	A0A151UBZ3	Glutelin type-A 2	358	38974	5.5
	A0A151UC62	Glutelin type-A 2	358	38764	5.8
Oleosin	A0A151SJ77	Oleosin 5	156	16676	9.6
	A0A151SLJ5	Oleosin 16 kDa	152	16349	9.6
	A0A151T0 × 9	Oleosin 18.5 kDa	135	14631	9.8
	A0A151TT63	Oleosin 5	104	10712	11.7

properties of pigeon pea flour. The authors observed that seed germination modified the cotyledon protein matrix but preserved the starch granule shape, whereas treatments such as soaking and boiling affected both seed microstructures. Regarding the functional properties, germination allowed a subtle increase in the water holding capacity of the flour.

Germination is one of the nonthermal processes that alters the composition of pigeon pea macronutrients the most, especially when carried out over long periods. Among the main advantages observed, the reduction of antinutritional compounds helps to improve seed digestibility. However, protein levels can decrease and the impact of this process on the functionality of ingredients produced with germinated seeds still needs to be further clarified.

Finally, pigeon pea fermentation was investigated by Lee et al.[40] The authors used *Bacillus subtilis* strains in seed fermentation, producing the fibrinolytic enzyme nattokinase. The results suggest that pigeon pea fermented with *Bacillus subtilis* can bring benefits to cardiovascular health, helping to prevent hypertension, due to increased antioxidant activity and action of the nattokinase enzyme compared to nonfermented seeds. Fermentation also increased the contents in flavonoids and total phenolic compounds.

4.2. Thermal Processes. Processes that involve heat can cause changes in the conformation of proteins and gelatinization of starch granules in the pulses, affecting their functional properties. Starch and proteins are the macronutrients that contribute to changing the functional and textural properties of pulse flours after heating. In aqueous suspensions and at high temperatures, starch granules swell and open due to breakage of the amylopectin double helix, while amylose leaches through the swollen granules, leading to gelatinization.[24] The gelation properties of legume proteins can be attributed to the globulin fraction present in these seeds.[28] The thermal properties of pigeon pea have been investigated using differential scanning calorimetry (DSC) to determine the starch gelatinization temperature and protein denaturation temperature, in addition to rheological analyses, to determine the pasting temperature of flours.

The gelatinization temperature of pigeon pea starch is reported to be between 82.0 and 83.6 °C, whereas the protein denaturation temperature is around 96 °C.[24,47] Fernández Sosa et al.[17] determined a denaturation temperature of 90.4 °C for the globulin fraction but failed to detect the denaturation temperature for the albumin fraction using DSC analysis, due to the high flexibility of the tertiary conformation of the corresponding polypeptides. The denaturation temperature of pigeon pea protein isolates was also shown to be dependent on pH, ranging from around 92 °C for pH 2.1 to 103 °C for pH 8.3.[19]

Another important thermal property for processing pulses is the pasting temperature, defined as the minimum temperature necessary to cook the flours.[13] When this temperature is reached, changes in the structure of starch granules begin, leading to their gelatinization and increased viscosity.[14] The pasting temperature for pigeon pea is reported to be between 81.6 and 87.5 °C (10 to 20 wt % solids), and the use of different pretreatments may affect these values.[14,23,24,47]

Cooking is a recurrent process applied to pulses in the industry, as well as, of course, in home cooking. Miano et al.[11]

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

observed that cooking pigeon pea (previously hydrated) for 20 min at 98 °C was sufficient to make the grains soft (minimum penetration force in a firmness test). A similar value was determined by Tiwari et al.[23] The heat treatment resulting from cooking was shown to affect the functional properties of pigeon pea flour. Soaking (6 h) followed by prolonged cooking of the seeds (60 min) reduced the pasting temperature from 81.6 to 74.1 °C, in addition to increasing the water holding capacity and delaying the creaming process of emulsions stabilized by flour.[13] Steam cooking of pigeon pea was evaluated by Tiwari et al.[23] and proved to be an efficient pretreatment for seed dehulling. Onimawo and Akpojovwo[28] submitted pigeon pea seeds to a roasting process (80−100 °C/1 h) and evaluated the functional properties and antinutritional compounds of the resulting flours. The flours produced after roasting showed moisture contents of 5.2−4.5%. The authors determined higher values of water holding capacity and oil holding capacity for seeds that underwent roasting, and they attribute this to the heat dissociation of proteins, swelling of crude fiber, and gelatinization of starch. However, a decrease in the emulsifying and foaming properties was observed. This result may be related to the denaturation of pigeon pea proteins at high temperatures. Regarding the antinutritional compounds, the roasting process at 80 °C reduced the cyanide contents in the flours by approximately 28%, whereas a further reduction (approximately 66%) was attained by roasting at 100 °C. The phytic acid content was also reduced by roasting by approximately 20%.

Seed treatment by microwaves led to gelatinization of starch granules, due to high temperatures and the presence of water.[1] Acevedo et al.[13] applied microwaves to pigeon pea seeds submerged in water (seed-to-distilled water ratio of 1:10 g mL^{-1}) at different power levels (50, 70, and 100%). The authors observed that microwave treatment decreased protein solubility over a broad range of pH values, compared to untreated flour. This decrease in solubility was attributed by the authors to increased surface hydrophobicity due to changes in the secondary structure of proteins caused by heating, in order to expose the hydrophobic amino acids and result in the formation of disulfide bridges. It was also observed that the treatment helped to delay the creaming process of emulsions stabilized by pigeon pea flour.

5. PIGEON PEA PROTEINS

The protein composition of pigeon pea has been evaluated to some extent by different research groups, addressing aspects such as protein quantification, identification, amino acid profile, and protein secondary structures. The outcomes are summarized in this section, to identify similarities with other pulses, specificities, and aspects that still need to be clarified regarding pigeon pea proteins.

5.1. Protein Composition Derived from Genome Sequencing. Pigeon pea genome sequencing[48] provides a first insight into seed protein composition. The protein sequences resulting from the sequencing are available in the public deposit UniProt Knowledgebase (UniProtKB). A total of 28 sequences of full-length proteins are retrieved using the search query "*C. cajan*" and protein type (albumin, globulin, glutelin, prolamin) or "*C. cajan* storage" (October 2021). This includes 6 albumins, 8 7S-globulins, 3 11S-globulins, 7 glutelins, and 4 oleosin sequences, as reported in Table 1. No prolamin sequence was found. The UniProtKB Align software was used to compare the similarity of each protein primary sequence within each protein group (unpublished data). The albumin, 7S-globulin, and oleosin display very low sequence homology, below 6%. The 11S-globulin and glutelin display higher homology (34−40%). This suggests that a single genotype presents a large diversity of proteins. Each protein type is encoded by a family of genes implying a polymorphism, similarly to most seed storage proteins.[49]

From the protein sequences, several physicochemical properties can be estimated using the protParam feature of the Expasy Web server. The molecular weight of the albumin fraction ranges from 9.7 to 15 kg mol^{-1}, with the isoelectric point (pI) comprised between 4.6 and 7.6 (Table 1). The molecular weight of the 7S-globulin fraction ranges from 21 to 45 kg mol^{-1}. Through SDS-PAGE and peptide mass fingerprinting analyses, Sousa et al.[50] identified the following groups of proteins in this range of molecular weight: vicilin, phaseolin α-type, and phaseolin β-type. This suggests a homology between pigeon pea 7S-globulins and pea/kidney bean 7S globulins. However, the average theoretical isoelectric point (pI) of pigeon pea 7S-globulins is around 7.5, which is much larger than that of pea/kidney bean 7S globulins (pI 4−6).[51] This may lead to specific physical chemical and functional properties of pigeon pea 7S-globulins. The 11S-globulin fraction identified in the pigeon pea genome is expected to have molecular weight around 50−53 kg mol^{-1} and pI around 6. This is similar to pea legumin A, identified by peptidomic analysis.[50,51] Concerning the glutelin sequences, the molecular weight is about 38−39 kg mol^{-1} and the pI around 5.2−5.8. In UniProtKB, they are annotated to belong to the 11S globulin family, similarly to rice glutelin.[52] To our knowledge, no detailed characterization of pigeon pea glutelins has been undertaken to confirm their presence in pigeon pea seeds/flour and their structural similarity to 11S-globulins and rice glutelin. A last group of proteins has been identified through the genome sequencing: oleosins. They are expected to be around 10−16 kg mol^{-1} and to have basic pI (9.6−11.7). Oleosins are localized on the surface of oil bodies (oleosomes) and are abundant in oil seeds.[53] Since the lipid content of pigeon pea is low, the amount of oleosin is expected to be small.

5.2. Protein Composition Derived from Differential Solubility and Chromatography. The protein compositions of the seed coat, embryo, cotyledons, whole seed, and flour of pigeon pea were determined by successive solubilization following an Osborne-like procedure.[54,55] Four groups of proteins were identified: albumins, soluble in water; globulins, soluble in 0.5 M sodium chloride solution in 0.01 M phosphate buffer (pH 7.0); glutelins, soluble in 0.1 M sodium hydroxide; and prolamin, soluble in a 70% ethanol/water mixture. Globulins were the main proteins found in pigeon pea flour (60−66%), followed by glutelins (19−25%), albumins (7−9%), and prolamins (2−5%).[55] The protein composition was found to depend on the pigeon pea variety and on the investigated seed compartment. The cotyledon and embryo were found richer in globulins than the seed coat.[55] Differential solubility gives an overall picture of the flour/seed protein composition. However, it is unreliable for a quantitative estimation of each protein group, since a substantial level of albumins may be found in the globulin fraction. SDS-PAGE has been used to describe it qualitatively. Most prominent bands of pigeon pea proteins were located at 47 and 64 kDa, corresponding to two 7S-globulin subunits.[56] Smaller bands were observed at 21, 35, and 36 kDa, in the region of the 11S-globulins.

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 2. Amino Acid Profile of Pigeon Pea Proteins

Amino acids	Composition (g 100 g^{-1} of pigeon pea protein)								
Essential amino acids									
Histidine	3.09	4.41−4.78	3.61	2.66	3.98−6.24	1.81−1.91	3.29	3.41	3.60
Isoleucine	3.74	3.48−3.82	3.92	5.16	3.46−3.68	6.30−6.50	3.54	4.13	3.90
Leucine	6.97	7.61−8.88	6.79	13.79	6.88−8.37	9.27−11.05	7.22	8.57	7.20
Lysine	5.96	7.05−7.66	7.40	6.05	7.05−7.56	5.00−6.54	6.38	5.86	6.80
Methionine	0.89	0.84−1.77	0.70	1.32	0.75−0.97	1.98−2.17	1.09	0.32	1.00
Phenylanine	6.78	7.42−8.87	3.54	17.64	6.80−8.58	9.46−10.50	10.10	9.71	9.70
Threonine	2.87	3.27−4.01	1.36	3.02	3.27−3.93	7.16−8.40	3.45	2.64	3.80
Tryptophan	0.61	0.20−0.78	0.09	0.53	0.20−0.32	2.18−3.91	1.15	-	-
Valine	4.01	3.46−4.18	6.71	14.32	3.46−3.87	11.00−12.01	4.02	5.73	4.40
Nonessential amino acids									
Alanine	3.61	4.00−4.24	15.47	5.46	3.82−4.04	2.56−3.70	4.31	6.28	4.60
Arginine	5.59	6.91−7.24	2.79	10.06	6.91−7.57	3.18−4.40	6.05	3.23	6.30
Asparagine	-	-	-	2.65	-	3.77−4.23	-	-	-
Aspartic acid	8.44	10.77−11.55	1.26	0.43	9.53−11.59	3.11−3.61	8.78	10.56	10.40
Cystine	0.66	0.43−1.01	-	-	0.43−0.60	4.06−5.12	1.01	-	1.20
Glutamic acid	15.03	18.04−20.45	6.48	8.10	19.90−22.50	1.67−1.95	20.34	24.71	19.00
Glycine	2.96	3.12−3.78	1.60	1.22	3.12−3.63	10.32−11.14	3.56	3.95	3.80
Proline	3.87	4.83−5.64	0.72	-	4.83−5.06	2.44−3.00	4.79	5.41	4.30
Serine	3.77	4.59−6.33	2.20	2.33	5.60−6.27	4.13−5.29	4.84	4.77	5.00
Tyrosine	2.49	2.47−2.79	1.86	4.41	2.60−2.77	6.95−7.12	2.70	0.44	3.00
Reference	[20]	[21]	[30]	[3]	[16]	[8]	[38]	[29]	[31]

Liquid chromatography techniques such as size exclusion and ion-exchange chromatography have been used to purify and better characterize pigeon pea proteins at lab scale. Krishna, Mitra, and Bhatia[57] purified globulins by solubilizing the proteins in phosphate buffer solution (pH 7.2) followed by protein precipitation (pH 4.7) in a $(NH_4)_2SO_4$ saturated solution. Fractionation of purified globulins by size exclusion chromatography resulted in fractions α, β, and γ, with the first two corresponding to 11S-legumin, present in small quantities in pigeon pea, and 7S-vicilin, the main pigeon pea protein, respectively.

Vicilin from pigeon pea seeds was also purified from the precipitated globulins fraction by a zonal isoelectric precipitation procedure in a Sephadex G-50 column followed by further purification in a DEAE-Sephacel column.[58] While globulin proteins had subunits ranging from 72 to 20 kDa, purified vicilin had two subunits at 72 and 57 kDa. According to the authors, pigeon pea vicilin differs from those of *Vicia* and *Pisum* species by the absence of low molecular weight subunits. However, a similar pattern is found in vicilins of *Phaseolus vulgaris* and *Glycine max*, which suggests that *Cajanus cajan* is closer to common bean and soybean on this matter.

In another work, γ-proteins, rich in sulfur amino acids, were purified from pigeon pea globulins.[59] The γ-proteins presented two subunits (32 and 20 kDa) linked by disulfide bonds. Amino acid analysis showed that this protein has 3 to 4 times more sulfur amino acids in its composition, when compared to legumin and vicilin.

More recently, Fernández Sosa et al.[17] extracted an albumin and a globulin fraction from pigeon pea with a sequential extraction procedure in water and in Tris-HCl buffer, respectively, and subsequent purification of the fractions by size exclusion chromatography. The authors characterized the fractions by fluorescence spectroscopy, differential scanning calorimetry, and surface hydrophobicity measurements and found that the structure of the globulins was less flexible and more compact compared to that of the albumin fraction.

In a study by Bravo et al.,[18] the main subunit of 7S-vicilin from pigeon pea (≈50 kDa) was purified and proteolyzed to investigate the antibacterial, antihypertensive, and antioxidant properties of the resulting bioactive peptides. Despite not presenting antibacterial properties against the microorganisms evaluated (*Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus*), pigeon pea peptides showed high antioxidant activity and antihypertensive activity comparable to those of Captopril, a well-known and potent angiotensin-converting enzyme (ACE) inhibitor. The results suggest that bioactive peptides from pigeon pea have potential for application in dietary supplements and even in alternative medicine.

It has been established that pigeon pea seeds contain several protein groups based on Osborne's definition. Globulins, in particular 7S-globulin, are the major type of storage proteins. The protein composition was found to vary depending on the variety and on the seed compartment. However, to the best of our knowledge, no attempt using analytical chromatography has been undertaken to quantify each protein group, as done for pea proteins, for example.[60] This would provide a better description of the variability of pigeon pea protein composition that is probably a major driver of the nutritional and the functional quality of pigeon pea. Still, the purification and characterization of 7S-vicilin has brought fundamental knowledge on the structural properties of the major pigeon pea protein. Furthermore, large-scale purification of pigeon pea proteins, based on mild processes such as preparative chromatography and achieving reasonable yield, would allow for investigating the functional properties of pigeon pea proteins.

5.3. Amino Acid Profile and Protein Secondary Structure. The amino acid profile of pigeon pea reveals that the species has a substantial amount of essential amino acids in its composition (Table 2), and it has been reported to be very close to that of chickpeas.[31] The levels of amino acids such as valine, isoleucine, phenylalanine, leucine, and lysine in pigeon pea are above those recommended for infant feeding according

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

to Oshodi et al.[29] The authors point out that the same is true for adult individuals. Mwasaru et al.[61] found that the amino acids present in pigeon pea are mostly hydrophilic (approximately 60 wt %), whereas the hydrophobic residues represent about 35% of the total amino acids, and the cyclic and sulfur residues represent approximately 5 and 1.5%, respectively.

No significant difference was observed in the amino acid profile of protein isolates obtained by the isoelectric precipitation method when compared to pigeon pea flour (native protein), which indicates that the extraction process had little influence on the protein composition.[8] A similar result was observed by Olagunju et al.[16] in the production of pigeon pea protein hydrolysates using different proteases. Although some specific amino acids, such as glutamic acid, valine, and tryptophan had higher levels in the hydrolysates compared to the nonhydrolyzed protein isolate, the amino acid profile of the proteins was similar for both materials. Olangunju et al.[16] observed that hydrolysis with Thermoase increased the content of branched-chain amino acids (BCAAs) by 7%, as well as that of most hydrophobic amino acids (alanine, glycine, leucine, valine, proline, isoleucine, and phenylalanine); this result was attributed to the specificity of Thermoase to cleave proteins from peptide bonds constituted by hydrophobic amino acids, thus increasing the content of associated amino acids and, consequently, increasing the hydrophobicity of the peptides, which might improve the antioxidant activity of protein extracts in lipid-containing foods.

Regarding the protein secondary structure, Sun et al.[1] observed the predominance of β-sheet structures (47%) in pigeon pea proteins; random coil, α-helix, curved, and β-antiparallel structures constitute 16, 14, 13, and 10% of the overall structure, respectively. The authors also determined a total of sulfhydryl groups and disulfide bonds of 22.6 and 7.9 μmol g^{-1} for pigeon pea proteins. The proportion of β-sheet and β-strand can be related to protein digestibility, and a decrease in the proportion of β-sheet and/or an increase in the random coil contribute positively to an increase in digestibility. There are indications that the pigeon pea globulin fraction might be more digestible than the albumin fraction, due to the lower proportion of β-strands found in its structure.[18]

6. METHODS FOR PIGEON PEA PROTEIN ISOLATION

According to Tapal et al.,[3] there is no commercial production of pigeon pea protein fractions, not even in countries where it is grown in large quantities, such as India. In parallel, pigeon pea has received increasing attention from researchers in recent years,[2] which may indicate the beginning of the reversal of this scenario.

Several studies have focused on the production of pigeon pea protein isolates and concentrates, in addition to protein hydrolysates.[3,15,16,62−65] Protein hydrolysates are widespread in the food industry, as they enable the reduction of antinutritional factors, as well as increase protein digestibility and release of bioactive peptides.[3,20,66,67] In the next sections, the main points regarding the extraction of pigeon pea proteins are presented.

Pulse proteins can be extracted using chemical, physical, or biotechnological methods. The most commonly used chemical method is the aqueous fractionation process that can be divided into three stages: (i) flour defatting using solvents such as petroleum ether, *n*-hexane, or *n*-pentane to remove lipids and other lipophilic compounds that can interfere in the protein extraction; (ii) extraction of proteins using salts, water, alcohols, or buffer solutions; and (iii) precipitation of proteins using ammonium sulfate, ethanol, methanol, acetone, or acids (hydrochloric acid, citric acid, etc.).[68]

Among the physical methods, the main processes are assisted by technologies that improve protein recovery. Some examples are pulsed electric field-assisted extraction, microwave-assisted extraction, high-pressure-assisted extraction, and ultrasound-assisted extraction.

Among the biotechnological methods, the enzyme-assisted extraction and the bacteria-assisted extraction are the most prevalent. Enzyme-assisted extraction is an approach that allows the recovery of high-quality plant proteins, due to the action of proteases that promote greater release of proteins from the polysaccharide matrix present in the seeds.

6.1. Extraction Process and Protein Recovery. Pigeon pea proteins have mostly been extracted using the conventional chemical process of aqueous fractionation by alkaline solubilization followed by isoelectric precipitation (Figure 4 and Table 3).[61,66] This method

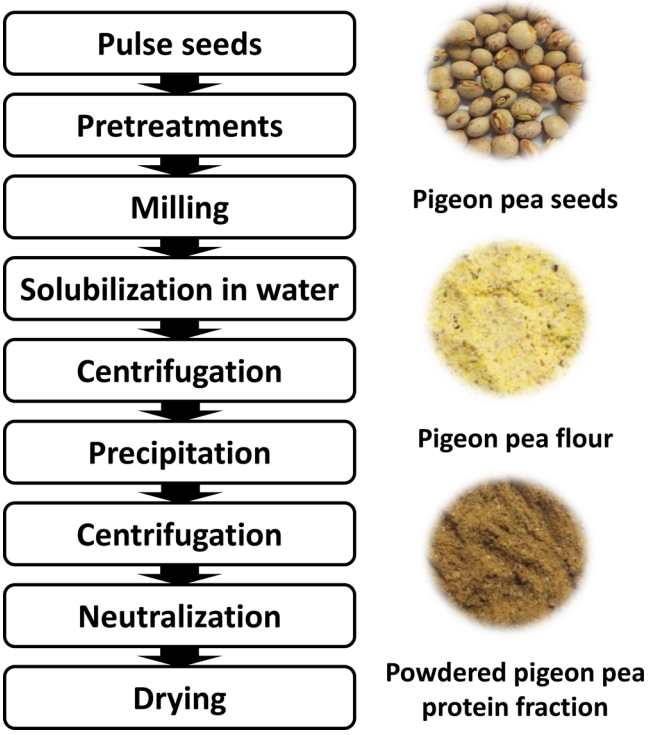

Figure 4. Conventional aqueous fractionation process to produce protein fractions from pigeon pea seeds.

consists of solubilizing proteins at alkaline pH (pH 8.0−12.0) and then precipitating the protein fractions at a pH close to their isoelectric point (pH 4.0−5.0). Precipitated proteins can be separated by centrifugation, ultracentrifugation, and filtration. Parameters such as solubilization pH, solvent used, temperature, ionic strength, extraction time, and solids:solvent ratio can affect protein extractability and process yield.[61] The optimization of pigeon pea protein extraction, carried out by Mwasaru et al.,[61] showed that the optimal process conditions were at pH 8.5, with a solids:solvent ratio ranging from 1:5 to 1:25, which resulted in extraction yields around 75%. In the work by Fernández Sosa et al.,[17] the use of a higher alkalinization pH (11.0) allowed greater protein recovery (67.5%) compared to that at lower pH values (yield between 49.9 and 51.2%). The authors also evaluated the extraction of albumin and globulin fractions from pigeon pea and obtained extraction yields of 30.6% for albumin and 6.6% for globulin. No extraction method could be considered the best to meet all the criteria evaluated in the work, but the authors emphasized that the isoelectric precipitation method with pH variation for protein extraction is a simple, cheap, and fast method to obtain protein ingredients. Mwasaru et al.[64] also evaluated the production of pigeon pea protein isolates at different solubilization pH values. Protein recovery ranged from 35.1 to 58.1%, whereas protein content in the isolates ranged from 78.1 to 83.4 wt %. The

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 3. Pigeon Pea Protein Extraction Process[a]

Reference	Extraction method	Flour treatment	Flour:solvent ratio	Solubilization pH	Precipitation pH	Centrifugation conditions	Fraction	Protein content (%)	Properties evaluated
[20]	Isoelectric precipitation method followed by enzymatic hydrolysis with alcalase and bromelain (1%, w w^{-1}, enzyme/substrate ratio).	Defatting and milling	1:10	10.0	5.0	8,000*g* (4 °C/30 min)	Protein isolated, alcalase hydrolysated protein (DH = 10.65%), and bromelain hydrolysated protein (DH = 4.82%)	87	Molecular weight profile (SDS-PAGE and SEC); Functional properties (solubility, surface hydrophobicity, WHC, and OHC); Amino acid profile; Antioxidant activity (DPPH, NO scavenging, and ORAC); Anti-inflammatory activity.
[56]	Isoelectric precipitation method followed by enzymatic hydrolysis with pepsin, papain, and thermolysin (1:100, w w^{-1}, enzyme/substrate ratio).	Milling	1:10		4.0	7,000*g* (4 °C/30 min)	Pepsin hydrolysated protein, papain hydrolysated protein, and thermolysin hydrolysated protein		Molecular weight profile (SDS-PAGE); Surface hydrophobicity; Amino acid profile; Particle characterization (particle size, zeta potential, and polydispersity index); DPP-4 inhibition assay.
[21]	Isoelectric precipitation method followed by enzymatic hydrolysis with Thermoase. An enzyme/substrate ratio of 1:100 is used.	Soaking in boiling water, dehulling and milling	1:15	9.0	4.5	11,000*g* (20 °C/30 min)	Protein concentrated, Thermoase hydrolysated protein (DH = 14%) and peptide fractions (<1 kDa, 1−3 kDa, 3−5 kDa, 5−10 kDa, *e* > 10 kDa)	75.96−80.40	Amino acid profile; Antioxidant activity (DPPH and FRAP); Inhibition of linoleic acid oxidation; Inhibition of ACE activity; Inhibition of renin activity; Inhibition of carbohydrate-hydrolyzing enzymes (α-amylase and α-glucosidase inhibition).
[67]	Peptide extraction by the ultrasonic-assisted method (40 kHz, 100% power, 10 min) followed by enzymatic hydrolysis by pepsin (2,000 U mL^{-1}) and pancreatin (100 U mL^{-1}) at 37 °C. Peptide fractionation was performed by subjecting the hydrolysate to dialysis.	Soaking, boiling, dehulling, fermentation, and milling	1:10			7,000*g* (15 min)	Peptide fraction (<1 kDa, 1−3.5 kDa, 3.5−14 kDa, >14 kDa).		*In vitro* digestion simulation; Peptide fractionation; Peptide absorption; Angiotensin-I-converting enzyme inhibitory activity assay.
[17]	Isoelectric precipitation method. Albumin and globulin fractions were extracted according to their solubility in different solvents.	Milling and defatting in hexane		8.0−11.0			Protein isolated and albumin and globulin fractions	90.84	Molecular weight profile (SDS-PAGE); DSC; SEC; Fluorescence spectroscopy; Surface hydrophobicity; Protein denaturation with urea; FTIR; Protein solubility.
[1]	Isoeletric precipitation method.	Milling, soaking, ultrasound, and microwave treatments	1:10		4.0	18,000*g* (4 °C/45 min)	Protein isolated		*In vitro* simulated gastrointestinal digestion; Molecular weight profile (SDS-PAGE); Protein solubility; Free sulfhydryl group and disulfide bond determination; Surface hydrophobicity; Particle characterization (particle size, zeta potential, and polydispersity index); Fourier transform infrared (FTIR).
[3]	Isoelectric precipitation method followed by hydrolysis using the enzyme pepsin (concentration ranging from 2 to 10%, w w^{-1}) for 2 h at 37 °C.	Defatting and milling	1:10		4.5	8,000 rpm (30 min)	Pepsin hydrolysated protein (DH = 24−38%)	74	*In vitro* digestibility; Molecular weight profile; Antioxidant activity (DPPH and reduction power); Encapsulation of curcumin with pigeon pea protein isolate (SEM, particle size, and curcumin concentration in microcapsules).
[15]	Isoelectric precipitation method followed by enzymatic hydrolysis. For enzymatic hydrolysis, an enzyme/substrate ratio of 1:100 is used. Three treatments were carried out: hydrolysis with alcalase (pH 8.0, 50 °C), pancreatin (pH 7.5, 37 °C), and pepsin +					9,000*g* (4 °C/30 min)	Protein isolated, alcalase hydrolysated protein (DH = 24.22%), pancreatin hydrolysated protein (DH = 27.17%), pepsin-pancreatin hydrolysated protein (DH =	23.85−56.82%	Molecular weight profile (SDS-PAGE); Antioxidant activity (DPPH, SRSA, HRSA, ABTS, ORAC, and FRAP); Inhibition of linoleic acid oxidation).

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 3. continued

Reference	Extraction method	Flour treatment	Flour:solvent ratio	Solubilization pH	Precipitation pH	Centrifugation conditions	Fraction	Protein content (%)	Properties evaluated
	pancreatin (pH 2.0 for pepsin followed by 7.5 for pancreatin, 37 °C). The peptides were fractionated by ultrafiltration.						18.53%), and peptide fractions (<1 kDa, 1−3 kDa, 3−5 kDa, 5−10 kDa, *e* > 10 kDa)		
[16]	Isoelectric precipitation method followed by enzymatic hydrolysis with pancreatin (pH 8.0, 37 °C) and pepsin-pancreatin (pH 2.0, 37 °C) for 4 h. Also, a sequential hydrolysis with pepsin followed by pancreatin for 2 h each was done. The enzyme/substrate ratio was 1:20 (w w^{-1}).		1:20	9.0	4.5	9,000*g* (4 °C/30 min)	Protein isolated, pepsin hydrolyzed protein, pancreatin hydrolyzed protein, and pepsin-pancreatin hydrolyzed protein		Amino acid profile; Antioxidant activity (DPPH, HRSA, ABTS, and FRAP); Inhibition of linoleic acid oxidation); ACE inhibitory activity; Renin inhibitory activity; Antihypertensive activity.
[8]	Methanol precipitation method, water extraction method, ammonium sulfate extraction method and acetone precipitation method.	Dehulling and milling		11		6,000 rpm (20 °C/ (4 °C/30 min)	Protein isolated	91.35−91.83	Antinutritional factors (phytic acid content, trypsin inhibitor, and cyanogenic glucoside content); Functional properties (WHC, OHC, emulsion capacity and stability, foaming capacity and stability, protein solubility, and bulk density); Amino acid profile; Nutritional properties (NB, BV, NPU, TPD, and PER).
[62]	Enzymatic hydrolysis method using papain enzyme (2%) at 55 °C.	Soaking in water for 5 h, dehulling, milling, and defatting in hexane	1:1	8.0		3,000 rpm 10 min)	Papain hydrolysated protein		Production and characterization of maize-pigeon pea hydrolysate flour blends (bulk density, WHC, OHC, swelling capacity, dispersibility, emulsification capacity, foaming capacity and stability, pasting properties); Sensory evaluation of snacks produced with maize-pigeon pea hydrolysate flour blends.
[61]	Isoelectric precipitation method.			8.5			Protein isolated	83.4	Protein solubility; Emulsifying properties; Whipping properties; Gelation properties.
[63]	Isoelectric precipitation method.	Dehulling and milling	1:10	8.5	4.5	8,000 rpm (30 min)	Protein concentrated	72	Foaming properties; Gelation properties.
[64]	Isoelectric precipitation method and micellization technique. The isoelectric precipitation method was evaluated to vary the extraction pH, and the method of micellization was performed with the use of 0.25 M NaCl solution (pH 6.5).	Milling	1:10	8.5, 9.5, 10.5, 11.5 and 12.5	4.5	5,000*g* (15 min)	Protein isolated	78.1−83.4	Hydrophobicity; Molecular weight profile (SDS-PAGE); Isoelectric focusing; Color; DSC.
[65]	Isoelectric precipitation method and micellization technique. The isoelectric precipitation method was evaluated to vary the extraction pH, and the method of micellization was performed with the use of 0.25 M NaCl solution (pH 6.5).	Milling	1:10	8.5, 9.5, 10.5, 11.5 and 12.5	4.5	5,000*g*	Protein isolated	78.1−83.4	Functional properties (protein solubility, WHC, OHC, emulsifying properties, whipping properties, and gelation properties).
[31]	Isoeletric precipitation method.	Milling and defatting in hexane				12,000*g* (15 min)	Albumin and globulin fractions	88.9−99.4	Amino acid composition of different seed components.

[a]Abbreviations: ABTS, 2,20-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid; BV, biological value; ACE, angiotensin converting enzyme; DG, degree of hydrolysis; DPPH, 2,2-diphenyl-1-picrylhydrazyl; DPP-4, dipeptidyl peptidase-4; DSC, differential scanning calorimetry; FRAP, ferric reducing ability of plasma; FTIR, Fourier transform infrared spectra; HRSA: hydroxyl radical scavenging activity; NB, nitrogen balance; NPU, net protein utilization; OHC, oil holding capacity; ORAC, oxygen radical absorbance capacity; PER, protein efficiency ratio; SDS-PAGE, sodium dodecyl sulfate−polyacrylamide gel electrophoresis; SEC, size exclusion chromatography; SEM, scanning electron microscopy; SRSA, superoxide radical scavenging activity; TPD, true protein digestibility; WHC, water holding capacity.

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

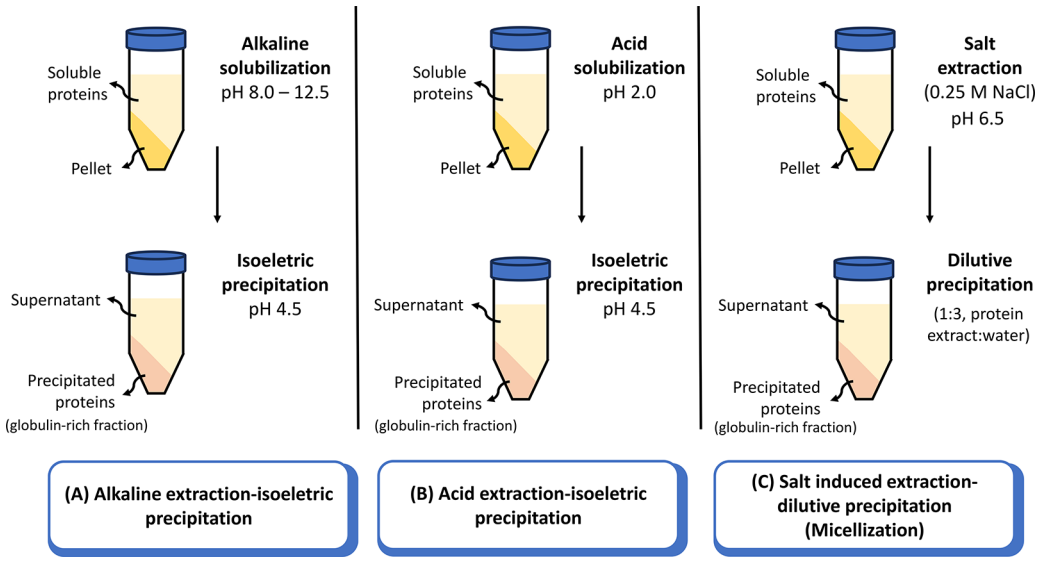

Figure 5. Extraction methods for isolation of pigeon pea proteins. (a) Alkaline extraction followed by isoelectric precipitation, (b) Acid extraction followed by isoelectric precipitation, and (c) salt-induced extraction followed by dilutive precipitation (micellization). Source: methods described by Mwasaru et al.[61] and Tapal et al.[3]

authors also evaluated the micellization method in protein extraction, using 0.25 M NaCl solution at pH 6.5. They achieved a higher protein recovery (40.2%), with the extract showing 82.8 wt % protein as well as a higher solubility as compared to that of the isoelectric precipitation method.[64] Besides, ultrasound-assisted extraction was investigated by Sun et al.[1] and resulted in a 26% increase in the extractability of pigeon pea proteins compared to untreated seeds.

In addition to the process of alkaline extraction followed by isoelectric precipitation, some works had also used acid extraction followed by isoelectric precipitation[3] and salt-induced extraction followed by isoelectric precipitation[64] (Figure 5). These methods allow the isolation of globulin-rich fractions from pigeon pea, mainly. Albumins can also be extracted in these processes and be present in low quantities in protein concentrates or isolates.[17] Tapal et al.[3] used pH 2.0 to solubilize the proteins, followed by isoelectric precipitation at pH 4.5. The protein content determined in the isolate was 85%, and the extraction yield was 37%.

Other solvents such as methanol, acetone, and ammonium sulfate were shown to be good precipitants of proteins from pigeon pea, allowing extraction of pigeon pea protein isolates containing about 91% of proteins.[8] On the other hand, the work by Xu et al.[20] showed that protein hydrolysis impaired the recovery of proteins. While the protein isolate produced by isoelectric precipitation had 87 wt % of proteins, the hydrolysates produced with the enzymes alcalase and bromelain had 60 and 20 wt %, respectively. The authors attributed the low protein content identified in hydrolysates to the conversion of proteins into small peptides and free amino acids, which makes them more difficult to quantify by the Bradford assay.

Beyond chemical extraction processes, the use of enzymes in protein extraction processes has been investigated for peas, pinto beans, mung beans, and soybeans to improve the protein recovery.[68] Furthermore, bacteria-assisted processes were evaluated by Emkani et al.[69] for extracting an albumin-rich fraction and a globulin-rich fraction from pea through a process assisted by lactic fermentation. The process increased the protein content of the albumin fraction as compared to the traditional process of protein precipitation using HCl or lactic acid. Nevertheless, the protein content in the globulin fraction was reduced using the process assisted by lactic acid fermentation. Such biotechnological routes, even though not commonly explored yet, may deserve attempts on pigeon pea protein fractionation.

The aqueous fractionation processes have some disadvantages, as they are time-consuming, require high-water consumption, and involve possible losses in protein functionality due to exposure to very alkaline or acidic pH values.[68] A more sustainable approach that can be employed is the dry fractionation of pulse flours. Dry fractionation is a promising route to preserve protein functionality, as it only involves milling and air-classification or electrostatic sorting processes. This avoids the need for water solubilization and subsequent drying steps to obtain protein fractions, which represents a much more sustainable process.[70] It could also be used as a preliminary step of aqueous fractionation to enrich the raw material in proteins.[71] For pigeon pea proteins, however, only processes that used the wet route are reported in the literature. Dry fractionation could be an important process to be explored for pigeon pea in future years, mainly to understand the functionality of highly concentrated protein ingredients (70−90% proteins, using aqueous fractionation methods) in comparison with ingredients mildly concentrated in proteins (50−60% proteins, by dry fractionation). With a milder fractionation in perspective, the need to better understand the profile of non-proteinaceous compounds in pigeon pea is reinforced, as suggested in section 3.

6.2. Possible Applications of Coproducts from the Protein Extraction Process. The pellet produced from the aqueous fractionation of plant proteins is rich in carbohydrates that are eliminated by centrifugation during the process. Although less studied, pigeon pea carbohydrates also have properties of interest to the food industry, which implies that the coproducts of the protein extraction process are likely to produce food ingredients.

Olagunju et al.[21] isolated native starch granules from pigeon pea seeds and modified them through acetylation. Acetylation enabled greater water and oil holding capacities, a lower retrogradation tendency, and a lower glycemic index compared to those of native pigeon pea starch, highlighting the potential of pigeon pea modified starch for applications as a thickening and stabilizing agent. Guleria and Yadav[72] evaluated the rheological properties of native, cross-linked, oxidized, and hydroxypropylated starches from pigeon pea. The chemical modifications resulted in changes to several properties of starches, reducing paste clarity, gel hardness, swelling power, and solubility. The cross-linked starch presented better thermal stability and, consequently, a more stable structure. Finally, Singh et al.[73] evaluated cellulose nanocrystals from pigeon pea stem waste. Cellulose was extracted from the stems using the chlorite bleaching and NaOH treatment method, followed by production of cellulose nanocrystals by acid hydrolysis with HCl. The cellulose nanocrystals showed good thermal stability (>324.3 °C), being highlighted as an interesting material for application in the food packaging sector.

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 4. Functional Properties of Pigeon Pea Flour and Protein Extracts

Reference	Treatment	Pasting temperature (°C)	Water holding capacity (g g^{-1})	Oil holding capacity (g g^{-1})	Least gelation concentration (%)	Thermal properties (°C)	Emulsifying properties	Foam properties	Surface hydrophobicity	Solubility (%)
		Pigeon pea flour								
14	Ground pigeon pea seeds were used as a control (A). Two processes were evaluated: soaking (10 h/25 °C) (B) and germination (48 h at 25 (C), 30 (D), and 35 (E) °C).	(A) 87.50; (B) 87.70; (C) 89.40; (D) 90.70; (E) 93.85								
8	Milled pigeon pea seeds.		1.25	1.31			Emulsifying capacity (%): 0.40 Emulsion stability (%): 0.40	Foaming capacity (%): 18.20 Foaming stability (%): 25.30		12.1
13	The flours were produced with native seeds (without treatment) (A); germinated for 5 days (B); soaked (6 h) and boiled in water for 20 (C), 40 (D), and 60 (E) min; and treated in microwaves at powers of 50 (F), 70 (G), and 100 (H)% (output power 800 W).	81.6 (A); 82.8 (B); 83.1 (C); 75.2 (D); 74.1 (E); 81.4 (F); 82.2 (G); 82.5 (H)	1.00 (A); 1.50 (B); 1.72 (C); 1.73 (D); 1.74 (E); 0.99 (F); 0.98 (G); 1.00 (H)	1.11 (A); 1.14 (B); 1.13 (C); 1.13 (D); 1.11 (E); 1.13 (F); 1.12 (G); 1.11 (H)						
24	Milled pigeon pea seeds.	81.63			6–8	83.6 (starch gelatinization); 96.0 (protein denaturation)				
23	Dehulled and ground seeds were used as control (A). Different dryings were carried out as pretreatments on the seeds in order to facilitate dehulling, which are wet method (B), hydrothermal method (C), and dry method (D).	84.7 (A); 84.7 (B); 87.5 (C); 85.5 (D)	1.05 (A); 1.00 (B); 1.35 (C); 1.10 (D)	1.25 (A); 1.20 (B); 1.30 (C); 1.10 (D)	6 (A); 8 (B); 8 (C); 8 (D)		Emulsifying capacity (%): 40–80 Emulsion Stability (%): 30–60	Foaming capacity (%): 10–22 Foaming stability (%): <20		
47	Flours produced from seeds defatted from different Indian cultivars (AL-15 (A) and AL-201 (B)) were evaluated.	83.4 (A);	1.37 (A);	0.98 (A);	12 (A);	81.8 (A);		Foaming Capacity (%): 34.5 (A); 37.3 (B)		

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 4. continued

Reference	Treatment	Pasting temperature (°C)	Water holding capacity (g g^{-1})	Oil holding capacity (g g^{-1})	Least gelation concentration (%)	Thermal properties (°C)	Emulsifying properties	Foam properties	Surface hydrophobicity	Solubility (%)
		Pigeon pea flour								
		83.5 (B)	1.39 (B)	0.96 (B)	14 (B)	82.2 (B)		Foaming Stability (%):		
								80.0 (A);		
								80.0 (B)		
							Emulsifying capacity (%):	Foaming capacity (%):		
							80.0 (A);	600 (A);		
			4.47 (A);	2.50 (A);	4 (A);		75.0 (B);	325 (B);		
28	Ground seeds (without treatment) were used as control (A). Flours produced with roasted seeds in two different processes were evaluated: roasting at 80 °C/1 h (B) and roasting at 100 °C/1 h (C).		4.82 (B);	2.81 (B);	4 (B);		50.0 (C)	300 (C)		
			4.94 (C)	2.98 (C)	4 (C)		Emulsion Stability (%):	Foaming Stability (%):		
							62.5 (A);	410 (A);		
							55.6 (B);	295 (B);		
							55.0 (C)	235 (C)		
		Pigeon pea protein extracts								
	Protein isolate (A), protein hydrolysate produced by the enzyme alkalase (B), and protein hydrolysate produced by the enzyme bromelain (C) were evaluated.		3.90 (A);	3.20 (A);					100−120 (A);	
20			4.00 (B);	3.00 (B);					<30 (B);	68.7 at pH 12.0 (A)
			7.60 (C)	6.00 (C)					<30 (C)	
						90.4 (B);			1843 (A);	71 (A);
									2600 (B);	21 (B);
17	Albumin (A) and globulin (B) fractions of pigeon pea were evaluated. Protein isolates produced with different alkalinization pH (8.0 (C), 9.0 (D), 10.0 (E), and 11.0 (F)) were also evaluated.					94.7 (C);			2377 (C);	70 (C);
						95.9 (D);			2138 (D);	69 (D);
						96.7 (E);			2122 (E);	74 (E);
						96.5 (F)			2622 (F)	60 (F)
							Emulsifying capacity (%):	Foaming capacity (%):		
							1.40 (A);	33.3 (A);		
							1.40 (B);	35.37 (B);		
8	Protein isolates produced by different methods were evaluated: aqueous extraction (A), methanol precipitation extraction (B), ammonium sulfate extraction (C), and acetone precipitation extraction (D).		1.55 (A);	1.40 (A);			2.17 (C);	35.10 (C);		97.03 (A);
			1.60 (B);	1.45 (B);			2.23 (D)	33.23 (D)		97.13 (B);
			1.51 (C);	1.45 (C);			Emulsion Stability (%):	Foaming stability (%):		90.70 (C);

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 4. continued

Reference	Treatment	Pasting temperature (°C)	Water holding capacity (g g^{-1})	Oil holding capacity (g g^{-1})	Least gelation concentration (%)	Thermal properties (°C)	Emulsifying properties	Foam properties	Surface hydrophobicity	Solubility (%)
			Pigeon pea protein extracts							
			1.50 (D)	1.40 (D)			91.20 (A);	54.57 (A);		92.63 (D)
							91.47 (B);	53.83 (B);		
							89.10 (C);	55.73 (C);		
							90.43 (D)	52.33 (D)		
							Emulsifying capacity (%):	Foaming capacity (%):		
							39.50 (A);	34.0 (A);		
							43.09 (B);	21.0 (B);		
							42.26 (C);	40.2 (C);		
					14 (B);		39.34 (D);	54.3 (D);		
					14 (C);		43.75 (E);	59.0 (E)		
61	Pigeon pea protein isolate solutions containing 0 (A), 0.1 (B), 0.2 (C), 0.3 (D), 0.4 (E), and 0.5 (F) M of NaCl were evaluated.				10 (D);		42.42 (F)	64.2 (F)		96.9 (pH 12.0) (E)
					6 (E);		Emulsion Stability (%):	Foaming stability (%):		
					6 (F)		44.98 (A);	77.8 (A);		
							47.86 (B);	29.3 (B);		
							50.67 (C);	21.7 (C);		
							57.36 (D);	24.7 (D);		
							51.47 (E);	32.1 (E);		
							56.35 (F)	35.6 (F)		
								Foaming capacity (%):		
								80 (A);		
								110 (B);		
								60 (C);		
								40 (D);		
								80 (E);		
								100 (F);		
	The functional properties of pigeon pea protein concentrate were evaluated for the impact of different factors. Protein solutions containing 0.00 (A), 0.25 (B), 0.50 (C), 1.00 (D)% (w w^{-1}) of NaCl were evaluated. The influence of ionic strength on protein solutions was evaluated under the following conditions: 0.0 (E), 0.5 (F), and 1.0 (G). The influence of the protein extract concentration was evaluated in 2 (H), 4 (I), 6 (J), 8 (K), and 10 (L)% (w/w). The influence of pH on the protein extract was evaluated at 2 (M), 4 (N), 6 (O), 8 (P), and 10 (Q).							50 (G);		
								80 (H);		
								110 (I);		
								126 (J);		
								140 (K);		
								152 (L);		
63					6 (A);			140 (M);		
					2 (B);			90 (N);		
					12 (C);			80 (O);		
					14 (D);			110 (P);		
					4 (F);			128 (Q)		

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Table 4. continued

Reference	Treatment	Pasting temperature (°C)	Water holding capacity (g g^{-1})	Oil holding capacity (g g^{-1})	Least gelation concentration (%)	Thermal properties (°C)	Emulsifying properties	Foam properties	Surface hydrophobicity	Solubility (%)
				Pigeon pea protein extracts						
64	Protein isolates were obtained by micellization (A) and isoelectric precipitation using alkalinization pH's of 8.5 (B), 9.5 (C), 10.5 (D), 11.5 (E) and 12.5 (F).		1.24 (A); 0.84 (B); 1.25 (C); 1.26 (D); 2.10 (E); 2.13 (F)	2.10 (A); 2.45 (B); 1.79 (C); 1.77 (D); 1.77 (E); 2.03 (F)	8 (G); 4 (M); 10 (N); 6 (O); 12 (P); 6 (A); 6 (B); 6 (C); 8 (D); 10 (E); 10 (F)		Emulsifying capacity (%): 30–50; Emulsion stability (%): 10–50	Foaming stability (%): 90.9 (A); 55.5 (B); 66.6 (C); 71.4 (D); 90.9 (E); 55.5 (F); 67.5 (G); 90.9 (H); 66.6 (I); 50.0 (J); 41.6 (K); 40.0 (L); 42.0 (M); 54.0 (N); 58.8 (O); 50.0 (P); 45.4 (Q); Foaming capacity (%): 15–40; Foaming stability (%): 65–80		79.22 (pH 12.0) (A)
65	Protein isolates were obtained by micellization (A) and isoelectric precipitation using alkalinization pH's of 8.5 (B), 9.5 (C), 10.5 (D), 11.5 (E), and 12.5 (F).					93.78 (A); 92.00 (B); 92.70 (C); 91.22 (E)			3089 (A); 3053 (B); 3465 (C); 3479 (D); 1826 (E); 1673 (F)	

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

7. FUNCTIONAL PROPERTIES

The functional properties of plant-based proteins can be affected by intrinsic and extrinsic factors. Among the intrinsic factors are the size and shape of the proteins, net charge distribution, amino acid sequence, structure (secondary, tertiary, and quaternary), interaction with other components of the food matrix, hydrophobicity/hydrophilicity ratio, and others. The extrinsic factors include moisture, temperature, pH, ionic strength, chemical additives, enzymes, and mechanical processes.[74] In addition, pretreatments applied to seeds and the selected protein extraction processes can affect the functional properties of the resulting flours and protein extracts (Table 4). Functional properties such as solubility, water holding capacity, oil holding capacity, surface hydrophobicity, emulsifying capacity, and foaming capacity, among others, are defined as physicochemical properties that provide information on how proteins behave in food systems, when applied either as a processing aid or as a macro-constituent of the product.[8]

This section presents several functional properties investigated for pigeon pea proteins. The functionality of pigeon pea proteins showed similarities to other plant-based proteins such as cowpea, dolichos bean, jack bean, and field pea.[13,24,47,61,64] There is still a need for studies that compare the functionality of pigeon pea with other plant sources and that evaluate the ability of pigeon pea proteins to be analogous to proteins obtained from animal sources (e.g., egg protein, whey protein, etc.).

7.1. Protein Solubility, Surface Hydrophobicity, and Zeta Potential. A protein's solubility can be considered a good index to determine its potential for applications in food systems.[8] As the pH of a medium is increased to more alkaline regions, there is an increase in the solubility of proteins, a phenomenon that is associated with the amphoteric character of these molecules. At alkaline pH, proteins are strongly negatively charged, showing a greater interaction with water and, consequently, leading to greater solubility. In parallel, modifications in the conformation of proteins by alkalinization can cause their hydrophobic groups to become buried within their structure.[20,61]

In most works on pigeon pea, as well as in studies involving other plant-based proteins, the protein solubility is usually determined by the preparation of a protein suspension, followed by centrifugation, and determination of the protein concentration in the supernatant. It is important to highlight that these methods can also quantify proteins that are not necessarily soluble but are in the supernatant phase in the form of small aggregates which do not sediment under the applied centrifugation conditions.

For a better understanding of the mechanisms that involve protein solubility, surface hydrophobicity is a commonly investigated property. This structural property affects the bioavailability and nutritional quality of proteins, having a direct relationship not only with their solubility but also with other functional properties, as observed by Fernández Sosa et al.[17] These authors investigated the extraction of protein isolates and of albumin and globulin fractions from pigeon pea and reported that the extraction pH and the nature of the protein fraction impact the solubility of proteins in aqueous media. While the albumin fraction and protein isolates extracted at different alkaline pH values (8, 9, 10, and 11) presented solubility in water ranging from 60 to 74%, the globulin fraction presented a lower solubility in such conditions, around 21%. However, in buffer solution (pH 8, μ = 0.3 M) the solubility of globulin was higher than that of albumin and other protein isolates (82%). The authors attributed the decreased solubility of protein isolates in the buffer solution to the presence of NaCl and Na_2HPO_4 salts (the "*salting out*" effect). Regarding the surface hydrophobicity, higher values were determined by the authors for protein isolates and the globulin fraction, whereas the albumin fraction had lower hydrophobicity.

The ionic strength of the medium directly affects the solubility of proteins, as investigated by Mwasaru et al.:[61] the presence of NaCl decreased the solubility of pigeon pea protein isolate in acidic medium, whereas at the alkaline end, the solubility of proteins increased; protein suspensions in 0.5 and 0.2 M NaCl showed the lowest solubility for the acid and alkaline extremes, respectively, whereas the highest solubility was in 0.4 M NaCl solution at pH 12.0 (96.9%). According to the authors, at low pH, carboxyl groups protonate and the protein acquires a globally positive charge, resulting in decreased Cl^- ion repulsion and hydrophobic interactions, leading to the formation of insoluble aggregates. On the other hand, at high pH values there is an increase in the negative charge on proteins, which combined with the *salting in* effect of NaCl, serves to dissociate protein aggregates and thus increase solubility. It was also observed that the presence of NaCl affected more the solubility of pigeon pea proteins than that of cowpea proteins.

The action of hydrolysis on protein solubility was assessed by Xu et al.[20] The study showed a lower solubility of the hydrolysates compared to the protein isolate in the entire pH range evaluated, which the authors attributed to alterations in the protein profile. In fact, the action of proteases can result in hydrolysates with peptides of more heterogeneous protein profile (lower solubility). Regarding hydrophobicity, higher values were observed for isolates compared to hydrolysates; the low surface hydrophobicity of hydrolysates may be associated with the production of peptides, decreasing the hydrophobic structures on the surface of the hydrolysates.

The protein extraction method and the fractions obtained affect the surface hydrophobicity values. Mwasaru et al.[64] reported that pigeon pea proteins extracted by isoelectric precipitation showed greater hydrophobicity than those extracted by micellization. The solvents chosen for the extraction processes, in turn, also play an important role in the protein solubility. Adenekan et al.[8] demonstrated that, while aqueous extraction and methanol extraction led to about 97% solubility, ammonium sulfate and acetone extractions resulted in slightly lower solubilities (90.7−92.6%).

Finally, protein denaturation at high temperatures (>90 °C), as in microwave processing of flour, has been reported to result in decreased water solubility of pigeon pea proteins. The microwave treatment also resulted in a reduction in the particle size of protein isolates (166 nm), compared to protein isolates produced from flour without pretreatment (457 nm). However, the polydispersity index showed no differences, indicating a heterogeneous distribution of particle sizes (>0.3). The zeta potential ranged from −32.7 to −35.2 mV. The authors used protein samples dispersed in Milli-Q water (1 mg mL^{-1}) in the assays.[1]

The thermolysin enzyme produced smaller particles in the pigeon pea protein hydrolysate (264.6 nm), as compared to papain (400 nm) and pepsin (578.2 nm) enzymes. The

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

polydispersity of the particles ranged from 0.4 to 0.6, and the zeta potential of the hydrolysates was around −15 mV, indicating a low electrostatic stability and potential tendency of the hydrolysates to aggregate.[56]

7.2. Water and Oil Holding Capacity. The water and oil holding capacities (WHC and OHC, respectively) of pulse-based ingredients are of great importance for the incorporation of these ingredients in meat, dairy, confectionery, and bakery products. Whereas WHC is essential for hydration processes and ingredient incorporation in water-continuous matrices, OHC helps in texture and better retention of aromas and flavors.[13]

The presence of -COOH, -NH_2, and -OH groups in the structure of proteins is largely responsible for the WHC of proteins. The unfolding of the exposed quaternary structure increases the protein's affinity for water molecules which could contribute to the greater WHC. OHC has been associated with lipid−protein interactions resulting from noncovalent bonds, electrostatic bonds, and hydrogen bonds.[13,20]

Both the flour and the protein extracts of pigeon pea have good WHC and OHC properties. Flour is rich in carbohydrates and fiber, hydrophilic compounds that can easily interact with water and contribute to the WHC value.[24] However, the WHC and OHC of pigeon pea protein isolates have been shown to be greater than those of their flours.[8,13,20,23,24]

In flours, one way to increase these capacities is the addition of some pretreatment steps. It has been shown that soaking, cooking, germination, and roasting of pigeon pea contribute to the increase in WHC and/or OHC of their flours. On the other hand, treatments based on the use of microwaves were not able to increase these indices.[23,24,28]

In the case of protein extracts, Mwasaru et al.[65] highlighted that extraction at a more alkaline pH (pH 12.5) led to an increase in WHC compared to extracts obtained under lower alkalinization conditions (pH 8.5). The inverse was observed by the authors for OHC, with higher values being obtained in less alkaline regions.

Protein hydrolysis can also contribute to produce ingredients with greater WHC and/or OHC. Xu et al.[20] showed that the WHC of a pigeon pea protein hydrolysate produced with bromelain almost doubled, compared to the non-hydrolyzed protein isolate. The same behavior was observed for OHC. The authors attributed these results to changes in protein structures, causing greater or lesser exposure of polar groups (e.g., -COOH, NH_2-, -OH).

7.3. Foam Properties. A foam is formed when air is injected into a liquid and trapped in the form of bubbles. What defines a good foaming ability of a given material is the presence of surface-active molecules, such as proteins that can reduce surface tension at the air−water interface[8] and form viscoelastic interfacial films.

Surface hydrophobicity is positively correlated with foaming properties, as the initial anchoring of proteins at the air−water interface is facilitated by surface hydrophobic patches. The ability of proteins to unfold at the interface is another important aspect of foam formation. Molecular flexibility can expose previously buried hydrophobic portions, which can contribute to a more rapid reduction in surface tension, increasing foamability.[75,76]

While whey and egg are proteins widely used in the production of foams in foods, soybean, peas, chickpea, and wheat are examples of plant-based proteins used as substitutes for animal proteins as foam-forming agents. Globulins are the most explored vegetable protein fraction regarding foaming capacity, because the conventional method of aqueous fractionation of vegetable proteins results in a globulin rich-fraction (more soluble in high pH conditions). However, studies have shown that globulins have poor interfacial and foam-stabilizing properties when compared to animal-derived proteins, due to their highly ordered structure and limited ability to unfold and adsorb at the interface.[8] Conversely, the albumin fraction (generally present in the pellet from the aqueous fractionation process) has a foaming activity superior to that of globular proteins.[77,78]

Several studies evaluated the foaming properties of pigeon pea flours and derived protein extracts. However, to date, no studies have further investigated the impact of the interfacial properties of these proteins on the foaming capacity.

Mwasaru et al.,[65] in a study with pigeon pea protein isolates, showed that foam volume expansion decreases with increasing pH. The authors also highlighted that the use of saline solutions made it possible to increase the formed foam volume, as it improves protein solubility. On the other hand, the presence of salts impaired the rheological properties of the formed films, decreasing the foam stability over time. Akintayo et al.[63] observed that the foaming capacity of pigeon pea protein concentrates increased when low concentrations (0.25%, w v^{-1}) of NaCl were added. The foaming capacity had also a direct relationship with the increase in the concentration of protein concentrate, with better foam volumes being observed at concentrations equal to 10% (w v^{-1}). Both the foaming capacity of pigeon pea protein isolates and the resulting foam stability were superior compared to those obtained with pigeon pea flour.[8] Although it forms a rather small volume of foam, which is characteristic of pulses' flour, pigeon pea flour generated foams with good stability (>80%).[38]

7.4. Emulsifying Properties. Pigeon pea flours and protein extracts were shown to have the potential to stabilize oil-in-water (O/W) emulsions.[8,23,28,61,65] Adenekan et al.[8] reported that pigeon pea protein extracts showed better results in the stabilization of emulsions than pigeon pea flours (obtained by milling the seeds). The authors obtained emulsion stability values that reached 90%, with this percentage being the measure of the emulsion layer that remained stable after heating (85 °C/15 min).

Mwasaru et al.[65] observed that the pH of protein extraction significantly affected their emulsifying properties. While the emulsifying capacity increased slightly as the extraction pH increased from 8.5 to 11.5, extraction conditions with higher alkalinity (pH 12.0) decreased the emulsifying capacity of pigeon pea protein isolates. The authors explained that the emulsification functionality depends on the botanical source, in addition to the protein solubility and conformational stability.

The addition of NaCl was also shown to influence the emulsifying properties of protein isolates. In a work conducted by Mwasaru et al.,[61] the addition of 0.1 and 0.4 M of NaCl contributed positively to increasing the emulsifying capacity, due to the better solubility conditions of the proteins.

Roasting pigeon pea seeds at temperatures of 80 and 100 °C was shown to have a negative impact on emulsifying properties, according to Onimawo and Akpojovwo.[28] The authors observed reductions in emulsifying capacity and emulsion stability compared to flour that was not produced from roasted

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

seeds. The decrease in emulsifying properties may be related to the denaturation of proteins at high temperature.

7.5. Gel Forming Properties. Gelation is a functional property that is linked to the formation of a percolating network that provides a solid-like matrix with the retention of water, lipids, sugars, and flavor in foods, in addition to affecting their final consistency. Pigeon pea flour was able to form a self-supporting gel,[24] highlighting its potential for application in foods that require a firm gel consistency. Part of this is due to the presence of starch in the flour, which contributes to gel formation.

Acidic pH conditions were shown to be more beneficial for the formation of gels, compared to alkaline pH values, as observed by Akintayo et al.[63] and Mwasaru et al.[65] These authors also observed that gelation was improved under conditions of moderate ionic strength, even causing a reduction from 14 to 6% in the least concentration for gel formation.

According to Tapal et al.,[3] the production of protein hydrolysates from pigeon pea can also affect the least concentration for gel formation. The authors determined that too high levels of enzyme (8−10 wt %) did not result in gel formation. Conversely, the addition of enzyme up to a concentration of 6 wt % was ideal to obtain firm gels.

Fernández Sosa et al.[19] evaluated the gelling properties of pigeon pea protein isolates extracted by alkaline solubilization (pH 8.0) followed by isoelectric precipitation. The gelling properties of pigeon pea protein isolate were dependent on pH and ionic strength, with gels obtained at a pH close to the isoelectric point of proteins (pH = 3.9) showing a denser network and higher hardness, in texture profile analysis (TPA), and greater water retention capacity, compared to the cases of more alkaline pH values. The authors also observed pH-dependent changes in the color of the gels, varying from light brown at pH = 2.1 to dark brown at pH = 8.3, which is an important aspect for consumer acceptance.

8. BIOACTIVE PROPERTIES AND DIGESTIBILITY

8.1. Bioactive Peptides. Bioactive peptides are nutraceutical agents that have health benefits, acting in disease prevention. Reactive or hydrophobic groups of proteins are found within their structure; that is, the sequence of reactive peptides remains inactive within their primary structure. The release of these peptides through enzymatic protein hydrolysis is possible and has been shown to be promising for pigeon pea.[15]

By means of bioinformatics analysis, Boachie et al.[56] evaluated 40 pigeon pea proteins and showed that almost 50% of the amino acids were associated with the inhibition of DPP-4, an enzyme linked with the treatment of diabetes mellitus (type 2). The evaluation using bioinformatics projected the pepsin enzyme as the best protease to trigger the release of bioactive peptides with DPP-4 inhibitory functions. However, *in vitro* assays conducted by the authors showed that the thermolysin enzyme released the most active DPP-4 inhibitors. Differences between the bioinformatics simulation and the *in vitro* assays were attributed by the authors to the nature and behavior of the protein under laboratory conditions (acid pH for pepsin catalysis and the structural conformation of proteins) which are unincorporated parameters to existing bioinformatics tools for enzymatic hydrolysis.

Olagunju et al.[21] also evaluated the bioactive activity of pigeon pea peptides in controlling postprandial blood glucose levels. The results showed that the hydrolysate and peptide fractions (obtained through membrane ultrafiltration) may have potential as natural inhibitors of α-glucosidase, the enzyme responsible for hydrolyzing starch and increasing the level of glucose in the bloodstream.

Olagunju et al.[16] evaluated the anti-hypertensive effect of pigeon pea protein isolates and protein hydrolysates through an assay with hypertensive rats. The hydrolysates were instantaneous in reducing blood pressure, confirming the effectiveness of the enzymatic hydrolysis process. Pepsin hydrolysate had the fastest action in lowering blood pressure, with a maximum lowering effect of −30.91 mmHg after 2 h of oral administration. The authors found a similar blood pressure lowering effect reported for mung bean hydrolysate prepared with alcalase and for hemp seed and yellow pea pentapeptides.[79−81]

8.2. Digestibility. Food digestibility can be studied using *in vitro* and *in vivo* methods. The *in vitro* strategy consists of subjecting foods to conditions that simulate the digestion process (e.g., treatment with mixed digestive enzymes, pH changes, etc.). The *in vivo* approach allows for understanding the real effects that nutrient digestion has on a living body, usually using rats or pigs. Protein digestibility can provide insights into the stability of proteins during digestion and how they withstand the digestive process. *In vitro* assays have some advantages over *in vivo* assays, such as greater reproducibility, greater speed, and lower cost. However, such methods may provide an overestimated result for the actual nutritional value, as it disregards biologically unavailable amino acids. In recent years, an international group of researchers developed the INFOGEST protocol (http://www.cost-INFOGEST.eu/) with the aim of standardizing *in vitro* digestion methods and enabling a better comparison between results.[82,83]

The Protein Digestibility Corrected Amino Acid Score (PDCAAS) is an index used to assess the nutritional quality of proteins and more reliably estimate the protein value of foods for human consumption. The maximum value of the PDCAAS is 1.0, which means that after the digestion process, one unit of protein provides 100% of the essential amino acids required for human consumption. However, studies have shown that the PDCAAS generally underestimates the value of high-quality proteins and overestimates the value of other proteins. In addition, the presence of antinutritional factors may make the PDCAAS measurement inappropriate for predicting plant-based protein quality.[82]

Another method used to assess food digestibility is the Digestible Indispensable Amino Acid Score (DIAAS). Measured in the small intestine (ileum) of pigs, it is shown to be a more adequate and accurate estimate for humans, avoiding the limitations of the PDCAAS procedure (evaluated in rats). However, the applicability of the DIAAS for proteins of plant sources is limited by some aspects, such as low representation of plant-based foods within the scoring structure; failure to translate differences in nitrogen-to-protein conversion factors between plant-based and animal-based foods; focus on isolated nutrients rather than the food matrix; and inadequate recognition of increased digestibility of heat-treated and processed plant-based foods.[84]

Some studies were carried out to evaluate pigeon pea digestibility, mainly using *in vitro* methods or assays conducted in rats. Although the findings of these works may have inherent

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

limitations of the used methods, they provide an idea of how pretreatments can affect pigeon pea digestibility. In a study conducted by Sousa et al.[50] on the *in vitro* digestion of some pulse flours and isolated proteins, quantification of the amount of free amino acid groups after digestion showed that pigeon pea fractions were the evaluated ingredients that released the greatest amounts of equivalent glutamic acid, compared to sorghum, peanuts, black beans, and wheat fractions. Sun et al.[1] evaluated the digestibility of pigeon pea flours submitted to different treatments. Gastrointestinal digestion was conducted by submitting the flour samples to simulated digestive fluids containing electrolytes, enzymes (α-amylase, pepsin, and pancreatin), $CaCl_2$, and water. The flour processed by microwaves showed greater digestibility (71.6%) when compared to the control flour (54.4%). According to the authors, the loss of secondary protein structures (due to microwave treatment) may explain the improvement in digestibility.

Tapal et al.[3] determined the digestibility of pigeon pea protein isolates using pepsin and pancreatin enzymes. The degree of hydrolysis achieved in the *in vitro* digestibility assay was about 50%. It was observed that the method used had an impact mainly on the degradation of proteins with molecular mass between 45 and 66 kDa. The authors highlighted that the peptides produced by hydrolysis have bioactive potential against oxidative stress and hypertension, according to bioinformatics analysis using the BIOPEP database.

Sharma et al.[14] determined pigeon pea digestibility using α-amylase (for starch digestion) and pepsin and pancreatin (for protein digestion). Both starch and protein digestion were improved by seed germination. Germination at 35 °C for 48 h resulted in a starch digestibility of 39.1% and a protein digestibility of about 98.3%, while nongerminated seeds had results close to 20 and 70%, respectively.

Membrane filtration improved the digestibility of peptide fractions extracted from pigeon pea, which ranged from 90.6 to 93.6%.[21] The size uniformity of the peptides, made possible by filtration, can contribute to better action of the proteases in relation to the hydrolysate that did not undergo ultrafiltration and had peptides of various sizes.

Adenekan et al.[8] evaluated the *in vivo* digestibility of pigeon pea protein isolates using rats. The authors observed values of nitrogen balance, biological value, net protein utilization, true protein digestibility, and protein efficiency ratio equal to 0.66 g, 90.0%, 93.0%, 93.1, and 1.4, respectively.

8.3. Antioxidant Activity. In a study conducted by Tekale et al.,[85] 37 compounds including some short peptides were identified in *Cajanus cajan* seeds. According to the authors, pigeon pea is a source of important bioactives that contribute to its antioxidant and iron chelating activity.

Yang et al.[30] determined IC_{50} (antioxidant concentration to achieve 50% inhibition) values of 2536 and 1250 $\mu g\ mL^{-1}$ for aqueous extracts of pigeon pea seeds, by the DPPH (2,2-diphenyl-1-picrylhydrazyl) and NO radical elimination methods, respectively. The authors determined a positive correlation between the levels of phenolic and flavonoid compounds and the values found in the antioxidant activity assays.

Sharma et al.[14] reported that germination increased the antioxidant capacity of pigeon pea. Antioxidant activity was determined by the DPPH, metal chelating activity, and reducing power methods, with values of 47.9, 87.7, and 197.3% for seeds germinated at 35 °C for 48 h, while nongerminated seeds presented values of 21.6, 42.0, and 98.3%, respectively.

Xu et al.[20] evaluated the antioxidant activity of pigeon pea protein isolate and hydrolysates. The protein isolate showed greater antioxidant activity (evaluated by the DPPH and NO radical scavenging methods) than the hydrolysates. Nevertheless, a different result was observed in a study conducted by Tapal et al.,[2] in which the protein hydrolysate showed better antioxidant capacity by the DPPH method than the protein isolate. Similar behavior was observed for the reducing power assay. According to the authors, the antioxidant activity of hydrolysates depends on the composition of peptides generated after hydrolysis. The antioxidant activity of protein hydrolysates was also superior to that of protein isolates in a study conducted by Olagunju et al.,[66] using the DPPH, ABTS, FRAP, and hydroxyl radical scavenging methods.

Olagunju et al.[16] evaluated the antioxidant activity of pigeon pea hydrolysates and peptides separated by ultrafiltration through different methods, such as DPPH, ABTS, FRAP, superoxide radical scavenging activity, ORAC, and inhibition of linoleic acid oxidation. In general, low-molecular-mass peptides, especially the <1 kDa fraction, exhibited superior antioxidant properties when compared to higher molecular weight fractions. The hydrolysates produced from the enzyme alcalase and pancreatin hydrolysates were better than the hydrolysates produced by the combination of pepsin and pancreatin.

9. APPLICATION OF PIGEON PEA IN NEW FOOD PRODUCTS

There is a wide range of applications for plant-based proteins in the development of food products, including use in food supplementation, emulsifying, foaming, and gelling agents, hydrogels for pharmaceutical applications, and use in edible coatings.[86] Some functional properties have already been investigated for pigeon pea flours and protein extracts (as presented in the previous section). Yet, the use of pigeon pea seeds and their protein-rich ingredients in new formulations with sensory acceptance assessment is still little or nonexistent in the literature.

Torres et al.[35] produced flours from germinated pigeon pea seeds (4 days/20 °C) and investigated their application in pasta formulations. A formulation of 100% semolina was used as control, and formulations added with 5, 8, and 10% (w/w) of germinated pigeon pea flour were evaluated for their nutritional composition, cooking time, and sensory acceptance. The protein content of the germinated flour was 29%. Pigeon pea flour supplementation resulted in shorter cooking times (20−30% shorter than those of the formulation with 100% semolina) and better protein, fat, dietary fiber, and mineral content. No difference was observed by consumers in the overall acceptance of pastas produced only with semolina and pastas produced with pigeon pea flour supplementation.

A protein binder (protein content = 32%, w/w) from pigeon pea was used in the formulation of beef sausages by Mongi and Gomezulu,[87] with concentrations ranging from 0 to 6% (weight of protein binder/weight of beef meat). The descriptive analysis showed that the 6% concentration led to greater acceptance compared to the other concentrations tested, showing that the increase in the amount of pigeon pea proteins in the formulations increased the sensory profile and consumer acceptance. However, control sausages made with chemical phosphate binder were the most accepted by

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

consumers. Studies that also use protein binders from other plant sources as controls are encouraged to better understand the performance of pigeon pea proteins in this type of application.

The application of pigeon pea protein hydrolysate in a maize-based snack was investigated by Akoja et al.[62] The hydrolysate was produced with the addition of the enzyme papain (2%, w w^{-1}), but the protein content of the hydrolysate was not reported by the authors. Maize flour was added with pigeon pea protein hydrolysate at concentrations of 5, 10, 15, and 20% to produce snacks, with the 5% concentration being the one that showed the best overall acceptance by consumers, together with the control formulation (without addition of protein hydrolysate).

A study conducted by Anchang and Okafor[88] evaluated the effect of different formulations of breakfast cereals using mango, pigeon pea, and sorghum flour on the composition of antinutritional factors. Twenty-eight samples were evaluated from the combined mixture-process linear multiplication model used in the study. The authors evaluated the levels of some antinutritional compounds such as lectins, phytates, and tannins. Increasing the concentration of pigeon pea flour in breakfast cereals resulted in an increase in the lectin content. Increasing the concentrations of pigeon pea flour and sorghum flour was also accompanied by an increase in the tannin content. The protein content of the formulations was not determined by the authors.

It is noted that only flours or ingredients slightly more concentrated in proteins were used in new product formulations. The use of pigeon pea protein concentrates and isolates in product development is scarce and should be investigated, as it may allow for better performance, when a certain amount of protein is required, in addition to the fractionation process potentially removing compounds that have negative effects on consumer acceptance, such as tannins, for example. In general, pigeon pea applications appear promising for the development of cereal-based products and meat-like products. However, the possible advantages of using pigeon pea ingredients over other plant sources remain to be clarified.

10. PERSPECTIVES FOR FUTURE WORK

In recent years, there has been a growing interest in the exploration of pigeon pea proteins, mainly to produce concentrates, isolates, and hydrolysates. The works raised in this review showed promising results, using mostly isoelectric precipitation extraction methods to obtain protein extracts from pigeon pea that can be used as food ingredients. The studies carried out range from improvements in the processing of pigeon pea seeds to the investigation of their technological properties to produce functional foods. It is possible to obtain protein extracts of pigeon pea in a sustainable way, using simple methods that do not use organic solvents in the extraction. The investigation of its proteins is a way of promoting biodiversity and boosting the expansion of this pulse cultivation in future years.

Some gaps were observed in the evaluated literature that are relevant to guide future studies on pigeon pea:

(I) Minor compounds (phenolic compounds, flavonoids, antinutritional compounds, etc.) must be identified and quantified in more depth (using, e.g., chromatographic techniques), and their implications for the nutritional and functional value of pigeon pea ingredients should also be evaluated.

(II) The protein composition of pigeon pea should be characterized in detail. Protein purification has been little explored in the literature, yet it is important for a better understanding of how each protein fraction contributes to the overall functionality of pigeon pea protein ingredients. Such studies are still relevant to assist in the selection of cultivars with the desired protein profile.

(III) Alternative protein extraction methods must be evaluated, mainly aiming at better process yield and protein recovery. Studies that use processes assisted by emerging technologies and dry fractionation of proteins, in particular, are encouraged.

(IV) The rheological behavior and interfacial properties of pigeon pea protein should be further investigated to provide mechanical underpinning of certain functional properties (emulsifying, gelling) and support for the development of new products.

(V) The digestibility of pigeon pea proteins has been little evaluated and needs to be further clarified. Some studies have investigated the digestibility of protein isolates, concentrates, or hydrolysates, but there is no knowledge of the digestibility of these proteins in more complex food systems. The way in which pigeon pea proteins associate with other components, such as carbohydrates and lipids, must also be clarified.

(VI) Sensory analyses must be conducted to evaluate the acceptance of seeds and products based on pigeon pea flour and protein extracts. Comparison with foods based on animal proteins or vegetable proteins most used currently (soy, peas, etc.) is of potential interest.

Clarifying these points should be the focus of future studies on pigeon pea proteins to better understand their functional properties, improve extraction yield, assess nutritional value, and enhance consumer acceptance. These efforts aim to harness pigeon pea proteins as sustainable and nutritious alternatives to address global protein demand.

AUTHOR INFORMATION

Corresponding Author

Claire Berton-Carabin − *INRAE, UR BIA, F-44316 Nantes, France; Laboratory of Food Process Engineering, Wageningen University, 6708 WG Wageningen, The Netherlands;* orcid.org/0000-0002-5585-640X; Email: claire.carabin-berton@wur.nl

Authors

Adilson Roberto Locali-Pereira − *Unesp - São Paulo State University, Institute of Biosciences, Humanities and Exact Sciences, Department of Food Engineering and Technology, 15054-000 São José do Rio Preto, SP, Brazil;* orcid.org/0000-0003-0242-482X

Adeline Boire − *INRAE, UR BIA, F-44316 Nantes, France*

Sebastião Roberto Taboga − *Unesp - São Paulo State University, Institute of Biosciences, Humanities and Exact Sciences, Department of Biology, 15054-000 São José do Rio Preto, SP, Brazil*

Véronique Solé-Jamault − *INRAE, UR BIA, F-44316 Nantes, France;* orcid.org/0000-0002-6221-7630

Vânia Regina Nicoletti − *Unesp - São Paulo State University, Institute of Biosciences, Humanities and Exact Sciences,*

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799

Department of Food Engineering and Technology, 15054-000 São José do Rio Preto, SP, Brazil; orcid.org/0000-0002-2553-4629

Complete contact information is available at: https://pubs.acs.org/10.1021/acsfoodscitech.3c00268

Funding

The authors thank the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for the financial support provided, in the scope of the CAPES-PrInt Program (process number 88887.310463/2018-00, International Cooperation Project number 88887.310309/2018-00), and the Ministry of Europe and Foreign Affairs of France (EIFFEL Excellence Program) for the financial support of ARLP's PhD grant.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Sun, X.; Ohanenye, I. C.; Ahmed, T.; Udenigwe, C. C. Microwave Treatment Increased Protein Digestibility of Pigeon Pea (Cajanus Cajan) Flour: Elucidation of Underlying Mechanisms. *Food Chem.* **2020**, *329*, 127196.

(2) Food and Agriculture Organization of the United Nations (FAO). *The Global Economy of Pulses*; Rawal, V., Navarro, D. K., Eds.; Rome, 2019.

(3) Tapal, A.; Vegarud, G. E.; Sreedhara, A.; Kaul Tiku, P. Nutraceutical Protein Isolate from Pigeon Pea (: Cajanus Cajan) Milling Waste by-Product: Functional Aspects and Digestibility. *Food Funct* **2019**, *10* (5), 2710−2719.

(4) Sarkar, S.; Panda, S.; Yadav, K. K.; Kandasamy, P. Pigeon Pea (Cajanus Cajan) an Important Food Legume in Indian Scenario - a Review. *Legume Research* **2020**, *43* (5), 601−610.

(5) Kumar, S.; Gopinath, K. A.; Sheoran, S.; Meena, R. S.; Srinivasarao, Ch.; Bedwal, S.; Jangir, C. K.; Mrunalini, K.; Jat, R.; Praharaj, C. S. Pulse-Based Cropping Systems for Soil Health Restoration, Resources Conservation, and Nutritional and Environmental Security in Rainfed Agroecosystems. *Front. Microbiol.* **2023**, DOI: 10.3389/fmicb.2022.1041124.

(6) Basu, P.; Singh, U.; Kumar, A.; Praharaj, C. S.; Shivran, R. Climate Change and Its Mitigation Strategies in Pulses Production. *Indian Journal of Agronomy* **2016**, *61*, 71−82.

(7) Fuller, D. Q.; Murphy, C.; Kingwell-Banham, E.; Castillo, C. C.; Naik, S. Cajanus Cajan (L.) Millsp. Origins and Domestication: The South and Southeast Asian Archaeobotanical Evidence. *Genet Resour Crop Evol* **2019**, *66* (6), 1175−1188.

(8) Adenekan, M. K.; Fadimu, G. J.; Odunmbaku, L. A.; Oke, E. K. Effect of Isolation Techniques on the Characteristics of Pigeon Pea (Cajanus Cajan) Protein Isolates. *Food Sci. Nutr* **2018**, *6* (1), 146−152.

(9) Singh, Y. P.; Singh, S.; Nanda, P.; Singh, A. K. Impact of Establishment Techniques and Maturity Duration of Pigeon Pea Cultivars on Yield, Water Productivity and Properties of Soil. *Agricultural Research* **2018**, *7* (3), 271−279.

(10) Abranches, M. de O.; Silva, G. A. M. da; dos Santos, L. C.; Pereira, L. F.; de Freitas, G. B. Contribuição Da Adubação Verde Nas Características Químicas, Físicas e Biológicas Do Solo e Sua Influência Na Nutrição de Hortaliças. *Research, Society and Development* **2021**, *10* (7), No. e7410716351.

(11) Miano, A. C.; Carvalho, G. R. de; Sabadoti, V. D.; dos Anjos, C. B. P.; Godoy, R.; Augusto, P. E. D. Evaluating New Lines of Pigeon Pea (Cajanus Cajan L.) as a Human Food Source. *J. Food Process Preserv.* **2020**, *44* (7), 1−9.

(12) Chinma, C. E.; Abu, J. O.; Adedeji, O. E.; Aburime, L. C.; Joseph, D. G.; Agunloye, G. F.; Adebo, J. A.; Oyeyinka, S. A.; Njobeh, P. B.; Adebo, O. A. Nutritional Composition, Bioactivity, Starch Characteristics, Thermal and Microstructural Properties of Germinated Pigeon Pea Flour. *Food Biosci.* **2022**, *49*, 101900.

(13) Acevedo, B. A.; Thompson, C. M. B.; González Foutel, N. S.; Chaves, M. G.; Avanza, M. V. Effect of Different Treatments on the Microstructure and Functional and Pasting Properties of Pigeon Pea (Cajanus Cajan L.), Dolichos Bean (Dolichos Lablab L.) and Jack Bean (Canavalia Ensiformis) Flours from the North-East Argentina. *Int. J. Food Sci. Technol.* **2017**, *52* (1), 222−230.

(14) Sharma, S.; Singh, A.; Singh, B. Characterization of in Vitro Antioxidant Activity, Bioactive Components, and Nutrient Digestibility in Pigeon Pea (Cajanus Cajan) as Influenced by Germination Time and Temperature. *J. Food Biochem.* **2019**, *43* (2), e12706.

(15) Olagunju, A. I.; Omoba, O. S.; Enujiugha, V. N.; Alashi, A. M.; Aluko, R. E. Antioxidant Properties, ACE/Renin Inhibitory Activities of Pigeon Pea Hydrolysates and Effects on Systolic Blood Pressure of Spontaneously Hypertensive Rats. *Food Sci. Nutr* **2018**, *6* (7), 1879−1889.

(16) Olagunju, A. I.; Omoba, O. S.; Enujiugha, V. N.; Alashi, A. M.; Aluko, R. E. Pigeon Pea Enzymatic Protein Hydrolysates and Ultrafiltration Peptide Fractions as Potential Sources of Antioxidant Peptides: An in Vitro Study. *Lwt* **2018**, *97* (April), 269−278.

(17) Fernández Sosa, E. I.; Chaves, M. G.; Quiroga, A. V.; Avanza, M. V. Comparative Study of Structural and Physicochemical Properties of Pigeon Pea (Cajanus Cajan L.) Protein Isolates and Its Major Protein Fractions. *Plant Foods for Human Nutrition* **2021**, *76* (1), 37−45.

(18) Bravo, R. K. D.; Angelia, M. R. N.; Uy, L. Y. C.; Garcia, R. N.; Torio, M. A. O. Isolation, Purification and Characterization of the Antibacterial, Antihypertensive and Antioxidative Properties of the Bioactive Peptides in the Purified and Proteolyzed Major Storage Protein of Pigeon Pea (Cajanus Cajan) Seeds. *Food Chemistry: Molecular Sciences* **2022**, *4*, 100062.

(19) Fernández Sosa, E. I.; Chaves, M. G.; Peyrano, F.; Quiroga, A. V.; Avanza, M. V. Thermal Gelation of Proteins from Cajanus Cajan Influenced by PH and Ionic Strength. *Plant Foods for Human Nutrition* **2023**, *78*, 574.

(20) Xu, X.; Qiao, Y.; Shi, B.; Dia, V. P. Alcalase and Bromelain Hydrolysis Affected Physicochemical and Functional Properties and Biological Activities of Legume Proteins. *Food Structure* **2021**, *27* (January), 100178.

(21) Olagunju, A. I.; Omoba, O. S.; Enujiugha, V. N.; Alashi, A. M.; Aluko, R. E. Thermoase-Hydrolysed Pigeon Pea Protein and Its Membrane Fractions Possess in Vitro Bioactive Properties (Antioxidative, Antihypertensive, and Antidiabetic). *J. Food Biochem* **2021**, *45* (3), 1−13.

(22) Van de Maesen, L. J. G. Pigeonpea: Origin, History, Evolution and Taxonomy. *The pigeonpea*; CAB International: Wallingford, 1990; pp 15−46.

(23) Tiwari, B. K.; Tiwari, U.; Jagan Mohan, R.; Alagusundaram, K. Effect of Various Pre-Treatments on Functional, Physiochemical, and Cooking Properties of Pigeon Pea (Cajanus Cajan L). *Food Science and Technology International* **2008**, *14* (6), 487−495.

(24) Acevedo, B. A.; Avanza, M. V.; Cháves, M. G.; Ronda, F. Gelation, Thermal and Pasting Properties of Pigeon Pea (Cajanus Cajan L.), Dolichos Bean (Dolichos Lablab L.) and Jack Bean (Canavalia Ensiformis) Flours. *J. Food Eng.* **2013**, *119* (1), 65−71.

(25) Mizubuti, I. Y.; Biondo Júnior, O.; De Oliveira Souza, L. W.; Dos Santos Ferreira Da Silva, R. S.; Ida, E. I. Response Surface Methodology for Extraction Optimization of Pigeon Pea Protein. *Food Chem.* **2000**, *70* (2), 259−265.

(26) FAOSTAT. *Food and Agriculture Organization of the United Nations (FAO)*; https://www.fao.org/faostat/en/#data/QCL. Accessed in February 2023.

(27) Magrini, M.-B.; Cabanac, G.; Lascialfari, M.; Plumecocq, G.; Amiot, M.-J.; Anton, M.; Arvisenet, G.; Baranger, A.; Bedoussac, L.; Chardigny, J.-M.; Duc, G.; Jeuffroy, M.-H.; Journet, E.-P.; Juin, H.; Larré, C.; Leiser, H.; Micard, V.; Millot, D.; Pilet-Nayel, M.-L.; Nguyen-Thé, C.; Salord, T.; Voisin, A.-S.; Walrand, S.; Wery, J. Peer-Reviewed Literature on Grain Legume Species in the WoS (1980−

2018): A Comparative Analysis of Soybean and Pulses. *Sustainability* **2019**, *11* (23), 6833.
(28) Onimawo, I. A.; Akpojovwo, A. E. Toasting (Dry Heat) and Nutrient Composition, Functional Properties and Antinutritional Factors of Pigeon Pea (Cajanus Cajan) Flour. *J. Food Process Preserv* **2006**, *30* (6), 742−753.
(29) Oshodi, A. A.; Olaofe, O.; Hall, G. M. Amino Acid, Fatty Acid and Mineral Composition of Pigeon Pea (Cajanus Cajan). *Int. J. Food Sci. Nutr* **1993**, *43* (4), 187−191.
(30) Yang, S. E.; Vo, T. L. T.; Chen, C. L.; Yang, N. C.; Chen, C. I.; Song, T. Y. Nutritional Composition, Bioactive Compounds and Functional Evaluation of Various Parts of Cajanus Cajan (L.) Millsp. *Agriculture (Switzerland)* **2020**, *10* (11), 558.
(31) Devindra, S.; Sreenivasa, R. J.; Bhaskar, V.; Mulimani, V. H. Effect of Heat Treatment on α-Galactoside Content of Red Gram Seeds (Cajanus Cajan, L). *J. Food Process Preserv* **2012**, *36* (2), 97−103.
(32) Awasthi, A.; Baran, C.; Tripathi, A.; Tiwari, A.; Sharma, S.; Uttam, K. N. Assessment of the Elemental Profile of the Pigeon Pea by the Direct Current Arc Optical Emission Spectroscopy. *National Academy Science Letters* **2022**, *45*, 433.
(33) Vásquez, U.; Siche, R.; Miano, A. C. Ultrasound-Assisted Hydration with Sodium Bicarbonate Solution Enhances Hydration-Cooking of Pigeon Pea. *Lwt* **2021**, *144* (February), 111191.
(34) Ferreira, E. C.; Nogueira, A. R. A.; Souza, G. B.; Batista, L. A. R. Effect of Drying Method and Length of Storage on Tannin and Total Phenol Concentrations in Pigeon Pea Seeds. *Food Chem.* **2004**, *86* (1), 17−23.
(35) Torres, A.; Frias, J.; Granito, M.; Vidal-Valverde, C. Germinated Cajanus Cajan Seeds as Ingredients in Pasta Products: Chemical, Biological and Sensory Evaluation. *Food Chem.* **2007**, *101* (1), 202−211.
(36) Prabhakar, P.; Mukherjee, S.; Kumar, A.; Kumar, S.; Verma, D. K.; Dhara, S.; Maiti, M. K.; Banerjee, M. Optimization of Microwave-Assisted Extraction (MAE) of Key Phenolic Compounds from Pigeon Pea (Cajanus Cajan L.), Their Characterization, and Measurement of Their Anti-Diabetic and Cytotoxic Potential. *Journal of Food Measurement and Characterization* **2023**, DOI: 10.1007/s11694-023-02082-5.
(37) Nix, A.; Paull, C. A.; Colgrave, M. The Flavonoid Profile of Pigeonpea, Cajanus Cajan: A Review. *Springerplus* **2015**, *4* (1), 125.
(38) Ene-Obong, H. N. Content of Antinutrients and in Vitro Protein Digestibility of the African Yambean, Pigeon and Cowpea. *Plant Foods for Human Nutrition* **1995**, *48* (3), 225−233.
(39) Onwuka, G. I. Soaking, Boiling and Antinutritional Factors in Pigeon Peas (Cajanus Cajan) and Cowpeas (Vigna Unguiculata). *J. Food Process Preserv* **2006**, *30* (5), 616−630.
(40) Lee, B. H.; Lai, Y. S.; Wu, S. C. Antioxidation, Angiotensin Converting Enzyme Inhibition Activity, Nattokinase, and Antihypertension of Bacillus Subtilis (Natto)-Fermented Pigeon Pea. *J. Food Drug Anal* **2015**, *23* (4), 750−757.
(41) SILVA, C. A. B.; BATES, R. P.; DENG, J. C. Influence of Soaking and Cooking upon the Softening and Eating Quality of Black Beans (Phaseolus Vulgaris). *J. Food Sci.* **1981**, *46* (6), 1716−1720.
(42) Kate, A. E.; Giri, S. K.; Sivatejaswini, B. Mass Transfer Dynamics of Simultaneous Water Gain and Solid Loss during Soaking of Pigeon Pea Grains. *J. Food Sci.* **2020**, *85* (10), 3406−3414.
(43) Dabhi, M. N.; Sangani, V. P.; Rathod, P. J. Effect of Enzyme Pretreatment on Dehulling, Cooking Time and Protein Content of Pigeon Pea (Variety BDN2). *J. Food Sci. Technol.* **2019**, *56* (10), 4552−4564.
(44) Arora, G.; Sehgal, V. K.; Arora, M. Optimization of Process Parameters for Milling of Enzymatically Pretreated Basmati Rice. *J. Food Eng.* **2007**, *82* (2), 153−159.
(45) Sreerama, Y. N.; Sasikala, V. B.; Pratape, V. M. Nutritional Implications and Flour Functionality of Popped/Expanded Horse Gram. *Food Chem.* **2008**, *108* (3), 891−899.
(46) Oloyo, R. A. Chemical and Nutritional Quality Changes in Germinating Seeds of Cajanus Cajan L. *Food Chem.* **2004**, *85* (4), 497−502.
(47) Maninder, K.; Sandhu, K. S.; Singh, N. Comparative Study of the Functional, Thermal and Pasting Properties of Flours from Different Field Pea (Pisum Sativum L.) and Pigeon Pea (Cajanus Cajan L.) Cultivars. *Food Chem.* **2007**, *104* (1), 259−267.
(48) Varshney, R. K.; Chen, W.; Li, Y.; Bharti, A. K.; Saxena, R. K.; Schlueter, J. A.; Donoghue, M. T. A.; Azam, S.; Fan, G.; Whaley, A. M.; Farmer, A. D.; Sheridan, J.; Iwata, A.; Tuteja, R.; Penmetsa, R. V.; Wu, W.; Upadhyaya, H. D.; Yang, S.-P.; Shah, T.; Saxena, K. B.; Michael, T.; McCombie, W. R.; Yang, B.; Zhang, G.; Yang, H.; Wang, J.; Spillane, C.; Cook, D. R.; May, G. D.; Xu, X.; Jackson, S. A. Draft Genome Sequence of Pigeonpea (Cajanus Cajan), an Orphan Legume Crop of Resource-Poor Farmers. *Nat. Biotechnol.* **2012**, *30* (1), 83−89.
(49) Krishnan, H. B.; Coe, E. H. Seed Storage Proteins. *Encuclopedia of Genetics* **2001**, 1782−1787.
(50) Sousa, R.; Portmann, R.; Dubois, S.; Recio, I.; Egger, L. Protein Digestion of Different Protein Sources Using the INFOGEST Static Digestion Model. *Food Research International* **2020**, *130*, 108996.
(51) Helmick, H.; Hartanto, C.; Bhunia, A.; Liceaga, A.; Kokini, J. L. Validation of Bioinformatic Modeling for the Zeta Potential of Vicilin, Legumin, and Commercial Pea Protein Isolate. *Food Biophys* **2021**, *16*, 474−483.
(52) Wen, T.; Luthe, D. S. Biochemical Characterization of Rice Glutelin. *Plant Physiology* **1985**, *78*, 172−177.
(53) Tzen, J. T. C.; Lai, Y.; Chan, K.; Huang, A. H. C. Oleosin Isoforms of High and Low Molecular Weights Are Present in the Oil Bodies of Diverse Seed Species1. **1990**, *94*, 1282−1289.
(54) Singh, U.; Jambunathan, R. Distribution of Seed Protein Fractions and Amino Acids in Different Anatomical Parts of Chickpea (Cicer Arietinum L.) and Pigeonpea (Cajanus Cajan L.). *Qualitas Plantarum Plant Foods for Human Nutrition* **1982**, *31* (4), 347−354.
(55) Singh, U.; Jambunathan, R.; Saxena, K.; Subrahmanyam, N. Nutritional Quality Evaluation of Newly Developed High-Protein Genotypes of Pigeonpea (Cajanus Cajan). *J. Sci. Food Agric* **1990**, *50* (2), 201−209.
(56) Boachie, R. T.; Okoro, F. L.; Imai, K.; Sun, L.; Elom, S. O.; Nwankwo, J. O.; Ejike, C. E. C. C.; Udenigwe, C. C. Enzymatic Release of Dipeptidyl Peptidase-4 Inhibitors (Gliptins) from Pigeon Pea (Cajanus Cajan) Nutrient Reservoir Proteins: In Silico and in Vitro Assessments. *J. Food Biochem* **2019**, *43* (12), 1−11.
(57) Krishna, T. G.; Mitra, R. K.; Bhatia, C. R. Seed Globulins of Cajanus cajan. *Qualitas Plantarum* **1977**, *27*, 313325.
(58) Krishna, T. G.; Bhatia, C. R. Vicilin from Cajanus Cajan Seeds *Phytochemistry* **1985**, *24*, 2201.
(59) Krishna, T. G.; Bhatia, C. R. γ-Protein, a Sulphur Amino Acid Rich Protein from Pigeon Pea (Cajanus Cajan (L.) Millsp.). *J. Biosci* **1986**, *10* (1), 57−65.
(60) Baniel, A.; Bertrand, D.; Lelion, A.; Guéguen, J. Variability in Protein Composition of Pea Seed Studied by FPLC and Multidimensional Analysis. *Crop Sci.* **1998**, *38* (6), 1568−1575.
(61) Mwasaru, M. A.; Muhammad, K.; Bakar, J.; Che Man, Y. B. Influence of Altered Solvent Environment on the Functionality of Pigeonpea (Cajanus Cajan) and Cowpea (Vigna Unguiculata) Protein Isolates. *Food Chem.* **2000**, *71* (2), 157−165.
(62) Akoja, S. S.; Adebowale, O. J.; Makanjuola, O. M.; Salaam, H. Functional Properties, Nutritional and Sensory Qualities of Maize-Based Snack (Kokoro) Supplemented with Protein Hydrolysate Prepared from Pigeon Pea (Cajanus Cajan) Seed. *Journal of Culinary Science and Technology* **2017**, *15* (4), 306−319.
(63) Akintayo, E. T.; Oshodi, A. A.; Esuoso, K. O. Effects of NaCl, Ionic Strength and PH on the Foaming and Gelation of Pigeon Pea (Cajanus Cajan) Protein Concentrates. *Food Chem.* **1999**, *66* (1), 51−56.
(64) Mwasaru, M. A.; Muhammad, K.; Bakar, J.; Man, Y. B. C. Effects of Isolation Technique and Conditions on the Extractability, Physicochemical and Functional Properties of Pigeonpea (Cajanus

Cajan) and Cowpea (Vigna Unguiculata) Protein Isolates. I. Physicochemical Properties. *Food Chem.* **1999**, *67* (4), 435−443.
(65) Mwasaru, M. A.; Muhammad, K.; Bakar, J.; Che Man, Y. B. Effects of Isolation Technique and Conditions on the Extractability, Physicochemical and Functional Properties of Pigeonpea (Cajanus Cajan) and Cowpea (Vigna Unguiculata) Protein Isolates. II. Functional Properties. *Food Chem.* **1999**, *67* (4), 445−452.
(66) Olagunju, A. I.; Omoba, O. S.; Enujiugha, V. N.; Wiens, R. A.; Gough, K. M.; Aluko, R. E. Influence of Acetylation on Physicochemical and Morphological Characteristics of Pigeon Pea Starch. *Food Hydrocolloids* **2020**, *100*, 105424.
(67) Putra, I. D.; Marsono, Y.; Indrati, R. Effect of Simulated Gastrointestinal Digestion of Bioactive Peptide from Pigeon Pea (Cajanus Cajan) Tempe on Angiotensin-I Converting Enzyme Inhibitory Activity. *Nutr Food Sci.* **2021**, *51* (2), 244−254.
(68) Kumar, M.; Tomar, M.; Potkule, J.; Verma, R.; Punia, S.; Mahapatra, A.; Belwal, T.; Dahuja, A.; Joshi, S.; Berwal, M. K.; Satankar, V.; Bhoite, A. G.; Amarowicz, R.; Kaur, C.; Kennedy, J. F. Advances in the Plant Protein Extraction: Mechanism and Recommendations. *Food Hydrocolloids* **2021**, *115*, 106595 DOI: 10.1016/j.foodhyd.2021.106595.
(69) Emkani, M.; Oliete, B.; Saurel, R. Pea Protein Extraction Assisted by Lactic Fermentation: Impact on Protein Profile and Thermal Properties. *Foods* **2021**, *10* (3), 549.
(70) Schutyser, M. A. I.; Pelgrom, P. J. M.; van der Goot, A. J.; Boom, R. M. Dry Fractionation for Sustainable Production of Functional Legume Protein Concentrates. *Trends Food Sci. Technol.* **2015**, *45* (2), 327−335.
(71) Yang, Q.; Eikelboom, E.; van der Linden, E.; de Vries, R.; Venema, P. A Mild Hybrid Liquid Separation to Obtain Functional Mungbean Protein. *LWT* **2022**, *154*, 112784.
(72) Guleria, P.; Yadav, B. S. Effect of Chemical Treatments on the Functional, Morphological and Rheological Properties of Starch Isolated from Pigeon Pea (Cajanus Cajan). *Curr. Res. Food Sci.* **2022**, *5*, 1750−1759.
(73) Singh, S.; Bhardwaj, S.; Meda, R. S.; Verma, C.; Chhajed, M.; Ghosh, K.; Maji, P. K. Insights into Thermal Degradation Kinetics and Liquid Crystalline Behavior of Cellulose Nanocrystals from the Waste of Cajanus Cajan (Pigeon Pea). *Int. J. Biol. Macromol.* **2023**, *242*, 124507.
(74) Kumar, M.; Tomar, M.; Punia, S.; Dhakane-Lad, J.; Dhumal, S.; Changan, S.; Senapathy, M.; Berwal, M. K.; Sampathrajan, V.; Sayed, A. A. S.; Chandran, D.; Pandiselvam, R.; Rais, N.; Mahato, D. K.; Udikeri, S. S.; Satankar, V.; Anitha, T.; Reetu; Radha; Singh, S.; Amarowicz, R.; Kennedy, J. F. Plant-Based Proteins and Their Multifaceted Industrial Applications. *LWT* **2022**, *154*, 112620.
(75) Damodaran, S. Protein Stabilization of Emulsions and Foams. *J. Food Sci.* **2005**, *70* (3), R54−R66.
(76) Foegeding, E. A.; Luck, P. J.; Davis, J. P. Factors Determining the Physical Properties of Protein Foams. *Food Hydrocoll* **2006**, *20* (2−3), 284−292.
(77) Yang, J.; Sagis, L. M. C. Interfacial Behavior of Plant Proteins — Novel Sources and Extraction Methods. *Curr. Opin. Colloid Interface Sci.* **2021**, *56*, 101499.
(78) Murray, B. S. Recent Developments in Food Foams. *Curr. Opin. Colloid Interface Sci.* **2020**, *50*, 101394.
(79) Aluko, R. E.; Girgih, A. T.; He, R.; Malomo, S.; Li, H.; Offengenden, M.; Wu, J. Structural and Functional Characterization of Yellow Field Pea Seed (Pisum Sativum L.) Protein-Derived Antihypertensive Peptides. *Food Research International* **2015**, *77*, 10−16.
(80) Girgih, A. T.; He, R.; Malomo, S.; Offengenden, M.; Wu, J.; Aluko, R. E. Structural and Functional Characterization of Hemp Seed (Cannabis Sativa L.) Protein-Derived Antioxidant and Antihypertensive Peptides. *J. Funct Foods* **2014**, *6*, 384−394.
(81) Li, G.-H.; Shi, Y.-H.; Liu, H.; Le, G.-W. Antihypertensive Effect of Alcalase Generated Mung Bean Protein Hydrolysates in Spontaneously Hypertensive Rats. *European Food Research and Technology* **2006**, *222* (5−6), 733−736.
(82) Gomes, A.; Sá, A.; Maria, Y.; Moreno, F.; Augusto, B. Food Processing for the Improvement of Plant Proteins Digestibility. *Crit. Rev. Food Sci. Nutr.* **2020**, *60* (20), 3367−3386.
(83) Ogawa, Y.; Donlao, N.; Thuengtung, S.; Tian, J.; Cai, Y.; Reginio, F. C; Ketnawa, S.; Yamamoto, N.; Tamura, M. Impact of Food Structure and Cell Matrix on Digestibility of Plant-Based Food. *Curr. Opin. Food Sci.* **2018**, *19*, 36−41.
(84) Craddock, J. C.; Genoni, A.; Strutt, E. F.; Goldman, D. M. Limitations with the Digestible Indispensable Amino Acid Score (DIAAS) with Special Attention to Plant-Based Diets: A Review. *Curr. Nutr. Rep.* **2021**, *10*, 93−98.
(85) Tekale, S. S.; Jaiwal, B. V.; Padul, M. V. Identification of Metabolites from an Active Fraction of Cajanus Cajan Seeds by High Resolution Mass Spectrometry. *Food Chem.* **2016**, *211*, 763−769.
(86) Kumar, M.; Tomar, M.; Potkule, J.; Reetu; Punia, S.; Dhakane-Lad, J.; Singh, S.; Dhumal, S.; Chandra Pradhan, P.; Bhushan, B.; Anitha, T.; Alajil, O.; Alhariri, A.; Amarowicz, R.; Kennedy, J. F. Functional Characterization of Plant-Based Protein to Determine Its Quality for Food Applications. *Food Hydrocolloids* **2022**, *123*, 106986 DOI: 10.1016/j.foodhyd.2021.106986.
(87) Mongi, R. J.; Gomezulu, A. D. Descriptive Sensory Analysis, Consumer Acceptability, and Conjoint Analysis of Beef Sausages Prepared from a Pigeon Pea Protein Binder. *Heliyon* **2022**, *8* (9), e10703.
(88) Anchang, M. M.; Okafor, G. I. Some Physical, Functional and Antinutrients of Breakfast Cereals from Blends of Guinea Corn, Pigeon Pea and Mango Flour Using Mixture-Process Design. *J. Food Sci. Technol.* **2022**, *59* (8), 2972−2982.

https://doi.org/10.1021/acsfoodscitech.3c00268
ACS Food Sci. Technol. 2023, 3, 1777−1799