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Enhancing Pulse Protein Quality through Processing and Genetic Tools

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ABSTRACT

Pulses are nutrient-dense food crops that are high in protein. To quantify protein quality, different methods have been developed, including the protein efficiency ratio, which is used in Canada, and the protein digestibility corrected amino acid score, which is used in the United States. When considering pulse proteins, there are inherent limitations that reduce their overall quality. The amino acid composition of pulse crops is not sufficient to meet human nutritional requirements, because they lack sufficient methionine/cysteine and/or tryptophan, and the presence of antinutritive factors reduces protein digestibility and bioavailability. Traditionally, these confounding issues have been overcome through processing, which can increase protein content, alter amino acid composition, and reduce the presence and activity of antinutritive factors. More recently, genetic techniques have been employed as potential solutions for issues of amino acid composition and antinutritive factors. In this overview different protein quality measurements, limitations of pulse proteins, effects of processing on protein quality, and genetic techniques for increasing pulse protein quality are discussed.

Pulses are a subgroup of legumes, specifically the dried seeds of beans, lentils, peas, and chickpeas; other legumes include soybeans, peanuts, and nondried peas and beans. Pulses have seen a significant rise in global production over the past few decades. Over the 22 year period from 1996 to 2008 pulse production rose by 24%, from 37.5 to 46.4 million tons (18). Between 2008 and 2017 annual pulse production almost doubled to 75.6 million tons worldwide, with beans, peas, and chickpeas accounting for 37.3 million tons (18).

With respect to consumption, developing countries tend to consume greater quantities of pulses than developed nations. In 2007 only 3.8 kg of pulses was consumed per capita in developed countries, while 7.94 kg was consumed per capita in developing nations (1). Similar differences were noted in the growth rate of pulse consumption: 0.4% between 1995 and 2007 in developed countries versus 0.8% in developing countries. These global trends are indicative of the rising prevalence of pulse crops but do not give an accurate indication of their nutritional value or the efficacy of different processing methods.

Protein Quality

An overview of the nutritional composition of pulses is presented in Table I. Pulses are high in protein, ranging from 15.9 to 34% (11,20,23), compared with cereals such as maize (8.8–11.9%) and rice (6.6–8.4%) (21). Although this is useful information, it is difficult to discuss the nutritional value of pulse protein without introducing the concept of protein quality. Protein quality can be considered the sum of the attributes of a particular protein source that satisfy the requirements for human growth and maintenance. There are multiple methods to classify the quality of a particular protein, with different jurisdictions relying on different methods from a regulatory perspective.

In Canada, the protein efficiency ratio (PER) is used to determine the protein rating (24). PER is an indicator of growth, in which weight gain per unit of protein consumed is determined over a 4 week period in rodents and normalized to a casein control group (24). This "corrected PER" is then multiplied by the protein content in the reasonable daily intake of the food or product to generate the protein rating. If the resulting protein rating is $\geq$20, it is considered a "good source" of protein; a food with a value $\geq$40 is considered an "excellent source" of protein.

In the United States, the method used to quantify protein quality is the protein digestibility corrected amino acid score (PDCAAS) (16). This method does not rely on growth but instead compares the amino acid composition of the protein source to a reference pattern delineating the requirements for growth and maintenance and a measure of protein digestibility. Similar to PER, this analysis is also performed in rats, with protein digestibility being quantified by determining the ratio of nitrogen consumed in the diet to that found in the feces over a period of 5 days. The amino acid score, identified as the lowest ratio between the amino acids present in the protein and the 1991 reference pattern, is then multiplied by the protein digestibility to generate the PDCAAS value. For regulatory purposes, the PDCAAS value is then used to generate a corrected protein value (PDCAAS × protein content in the reference amount customarily consumed), which is then compared to the percent daily value (50 g). If the final output is $\geq$10%,

Table I. Proximate composition, first limiting amino acids, and protease inhibitor content of different pulse classes

Pulse Class	Latin Designation	Protein (%)	First Limiting Amino Acid	Fat (%)	Dry Matter	Trypsin Inhibitor Activity (TIU/mg)	Chymotrypsin Inhibitor Activity (CIU/mg)
Pea	*Pisum sativum*	24.2–27.5[a]	Methionine/cysteine[b]	0.35–1.5[c]	91.3–91.43[c]	3.16–4.92[d]	2.84–3.34[d]
Lentil	*Lens culinaris*	15.9–31.4[e]	Methionine/cysteine[b]	1.13–1.78[f]	91.38–92.12[f]	4.98–6.29[d]	3.51–4.89[d]
Chickpea	*Cicer arietinum*	22–24[g]	Methionine/cysteine[h]	4.5–6.0[h]	91.17–92.08[i]	14.22–16.24[d]	11.78–13.59[d]
Common bean	*Phaseolus vulgaris*	21.4–23.6[j]	Methionine/cysteine[k]	1.05–1.39[k]	92.49–92.87[k]	15.18–20.83[d]	17.77–24.48[d]
Faba bean	*Vicia faba*	27–34[l]	Methionine/cysteine, tryptophan[k]	1.03[k]	92.15[k]	5.96–6.10[d]	1.12–1.67[d]

[a] Source: Hood-Niefer et al. (25).
[b] Source: Sarwar and Peace (40).
[c] Source: Nosworthy et al. (31).
[d] Source: Shi et al. (42).
[e] Source: Grusak (20).
[f] Source: Nosworthy et al. (33).
[g] Source: El-Adawy (12).
[h] Source: Boye et al. (6).
[i] Source: Rachwa-Rosiak et al. (38).
[j] Source: Akibode and Maredia (1).
[k] Source: Nosworthy et al. (32).
[l] Sources: Duc (11) and Hacıseferoğulları et al. (23).

the food is considered a "good source" of protein; if the final output is ≥20%, it is considered an "excellent source" of protein. Although the current regulatory guidelines require the use of a rat model for the determination of protein digestibility in the PDCAAS calculation, there has been growing interest in the development of an in vitro assay for protein digestibility that accurately reflects in vivo values—in vitro PDCAAS (IVPDCAAS). A good relationship, $R^2 = 0.75$ (beans), 0.92 (peas), or 0.94 (lentils), has been identified between PDCAAS and IVPDCAAS in processed pulse crops (31–33)

Although not currently accepted for regulatory purposes, it is worth mentioning the most recently developed method for assessing protein quality—the digestible indispensable amino acid score (DIAAS) (17). This assessment method is quite similar to PDCAAS and arose as a response to concerns regarding the accuracy of PDCAAS. There are two primary differences between PDCAAS and DIAAS. First, PDCAAS uses a measurement of protein digestibility captured in fecal analysis, whereas DIAAS measures the digestibility of individual amino acids at the end of the small intestine. This has the benefit of providing specific information regarding amino acid digestibility, which is important when identifying absorption rates and limiting amino acids in products, as well as reducing any confounding participation of the colonic microflora. The other significant difference between PDCAAS and DIAAS is the revision of the reference pattern, which has a direct impact on the amino acid score and, therefore, the final assessment of protein quality. Although this reference pattern may better reflect human requirements, it is important to note that the U.S. Food and Drug Administration (FDA) requires the use of the 1991 reference pattern for any protein content claims (US FDA CFR 21CFR101.9).

When considering the PER, PDCAAS, and DIAAS values for protein sources, animal-based proteins have higher values than plant-based sources. For PER, the values for processed pulses range between 0.64 and 2.08, while casein regularly exceeds 2.5 (31–33). PDCAAS scores for casein and egg white are truncated to 100, while processed pulses range between 47.1 and 75.2 and cereals such as whole wheat reach 40.0 and rolled oats reach 57.0 (16,31–33). DIAAS has not been as thoroughly investigated as PER and PDCAAS, but the initial Food and Agriculture Organization of the United Nations (FAO) report describing DIAAS methodology presents values of 122 for whole milk powder, 64 for peas, and 40 for wheat, further indicating that animal-based protein sources are generally higher in protein quality than those obtained from plant-based sources (17).

Limitations of Pulse Proteins

Although the protein content of pulses is high compared with other plant-based protein sources, there are limitations to their protein quality. When comparing the amino acid composition of pulse classes to human nutritional requirements, they are limiting in the sulfur amino acids methionine and cysteine and/or tryptophan. The first limiting amino acid does vary among pulses. Pea and lentil are first limiting in the sulfur amino acids (40). In common beans, tryptophan is the limiting amino acid in navy, black, and pinto beans (26,40), whereas the protein quality of red kidney bean protein is limited by its methionine content (49). In faba beans, the limiting amino acid differs depending on the cultivar investigated, with some sources citing tryptophan (40), whereas others identify the sulfur amino acids (2). Chickpeas have been identified as being limited in the branched-chain amino acid valine (12). In addition to the lack of particular amino acids, the presence of antinutritive factors in pulses can lower protein digestibility or sequester other nutrients, thereby preventing optimal nutrient absorption.

There is a wide range of antinutritional factors in pulses that limits the digestion or absorption of nutrients. Trypsin and chymotrypsin inhibitors, known to reduce protein digestibility, are present in beans, peas, chickpeas, and lentils to varying degrees (42). Trypsin inhibitor activity was greatest in chickpeas (~13 TIU/mg, dry matter) and beans (~18 TIU/mg, dry matter), with peas, lentils, and faba beans containing only 5.3 TIU/mg (dry matter) on average. A similar pattern was found for chymotrypsin inhibitor activity, with beans having the greatest activity, followed by chickpeas, lentils, peas, and faba beans. Hemagglutinin is another common antinutritive factor found in pulses that is capable of binding nutritive proteins, as well as red blood cells, thereby reducing

nutrient absorption (9). Hemagglutinins, in addition to preventing nutrient absorption, are also capable of inducing gastrointestinal effects such as bloating, nausea, diarrhea, and vomiting (30). Beyond altering protein digestibility and absorption, other antinutritive factors present in pulses, such as phytic acid, saponins, tannins, and oxalates, are capable of reducing the bioavailability of vitamins and minerals (37,41).

Pulse Processing

There are a wide variety of processing methods used to prepare pulses and their flours prior to consumption. These methods can reduce the presence, or activity, of antinutritive factors, resulting in increased nutrient availability. Processing methods can also alter the final protein content and/or amino acid composition and, thereby, affect the resulting protein quality. Processing of pulses can be divided into multiple areas: protein purification, through procedures such as air classification and chemical protein extraction; thermal treatments, including micronization, extrusion, baking, and cooking; and biological procedures such as fermentation and germination. Although this is not a comprehensive list of processing techniques and technologies, it does provide a cross-section of methods currently employed in pulse preparation and product development.

Increasing Protein Content. As expected, the purpose of protein purification is the generation of protein-rich fractions, such as protein concentrates and isolates. A general definition to differentiate between protein concentrates and isolates is that a protein concentrate has a greater protein content than the starting material, with the final protein content being <90%, while a protein isolate has a final protein content >90%; however, this is not a regulatory guideline. When comparing air classification (dry fractionation) with chemical extraction (wet fractionation), there are a few factors to consider. Air classification is a simple and effective processing technique that uses a stream of air to separate light and heavy particles in flours (46). This process can be repeated multiple times in an effort to increase the protein content of the final product. One study investigated the efficiency of air classification for protein enrichment in 11 legumes and found that although the flours initially ranged in protein content from 19.5% in lentils to 58.5% in soybean, the final protein content after air classification rose to 49.3% in lentils and 73.7% in soybean (13). Unlike air classification, which relies on particle size and density, "wet fractionation" methods for increasing protein content of the final product rely on protein solubility and, in some cases, the molecular weight of the protein of interest. There are multiple methods for chemical extraction of pulse proteins, such as alkaline and acid extraction used in conjunction with either isoelectric precipitation or ultrafiltration (6,28). This alkaline process has been able to generate protein isolates of 84.8 and 90.5% for chickpeas, 90.1% for peas, and 89.3% for lentils (8,35). It is possible to use ultrafiltration rather than isoelectric precipitation in the preparation of high protein products, such as faba bean (94.1%) and pea (89.5%) protein isolates (6,19,45). Although these techniques are commonly used to increase the total protein content in the final product, there are other processing methods that are used to more directly enhance protein quality.

Increasing Protein Quality. Micronization is a processing method that uses infrared radiation for heat transfer. A study on micronized yellow peas found no difference in protein content, but micronization was able to increase protein digestibility by 6% after processing (48). When investigating the effect of micronization on chickpea flours, one study found no alteration in protein content or amino acid composition (5). The treatment was capable, however, of significantly reducing protease inhibitor activity and increasing the desi chickpea IVPDCAAS from approximately 65 to 71 (5). These results are is similar to those of other investigations in which micronization was able to reduce phytate content by 49–55% in lentils (4) and trypsin inhibitor activity by up to 50% in beans and 30% in lentils (15).

A series of experiments was conducted on peas, beans, and lentils to determine how the protein quality of these pulses would respond to the thermal treatments of extrusion, baking, and cooking (boiling) (31–33). The PER and PDCAAS values obtained in these studies are presented in Table II. With the exception of green lentils and green split peas, boiling resulted in a greater PER value for all pulses investigated than did extrusion or baking, whereas baked products consistently had the lowest PER values, except for green split pea, green lentil, and faba bean. The PDCAAS data are not as consistent, but for the majority of pulse classes, extrusion resulted in a greater PDCAAS value than either baking or boiling. This highlights the differences between protein quality measurements, because PER indicated boiling was more beneficial for the majority of pulse classes, whereas PDCAAS suggested extrusion was more beneficial. Unfortunately, these studies did not include analysis of antinutritive factors; however, it has been

Table II. Average protein efficiency ratio (PER) and protein digestibility corrected amino acid score (PDCAAS) for processed pulses

	PER			PDCAAS		
Pulse	**Extruded**	**Boiled**	**Baked**	**Extruded**	**Boiled**	**Baked**
Yellow split pea[a]	1.80	2.08	1.89	65.44	69.19	68.89
Green split pea[a]	2.00	1.75	1.84	73.61	72.00	75.22
Red lentil[b]	1.25	1.28	1.00	65.01	57.40	53.84
Green lentil[b]	1.22	1.04	1.05	57.08	52.92	47.14
Black bean[c]	1.71	1.96	0.64	69.74	67.54	57.52
Navy bean[c]	1.67	2.01	0.78	60.82	61.23	53.62
Pinto bean[c]	1.73	2.03	0.84	66.21	75.10	47.75
Red kidney bean[c]	1.63	2.03	0.86	64.98	62.40	50.10
Faba bean[c]	0.68	1.18	0.96	58.01	54.14	66.36

[a] Source: Nosworthy et al. (31).
[b] Source: Nosworthy et al. (33).
[c] Source: Nosworthy et al. (32).

demonstrated that extrusion is capable of attenuating or abolishing the activity of protease inhibitors and reducing the overall content of phytate, tannins, and phenolics (36).

Germination and fermentation are processes used to enhance nutrient bioavailability through increased digestibility and reduced antinutritive factors. There are many variables to consider with germination, such as light exposure, water provision, and duration, that can effect the final product. Nutritionally, sprouting has been demonstrated to increase protein content in chickpeas and green peas, while also reducing tannins, phytic acid, and protease inhibitors (14,36). Fermentation can be performed using bacteria or fungi and under commercial or household environments. An important consideration with respect to fermentation is selection of the appropriate organism. An investigation into the fermentation of pea protein concentrate by *Lactobacillus plantarum* showed a reduction in protease inhibitor activity and subsequent increase in protein digestibility; however, sulfur amino acid content was reduced after fermentation—potentially due to bacterial utilization—leading to a reduction in IVPDCAAS from 67 to 55 (7). Similar results were obtained when *Aspergillus oryzae* and *A. niger* were used to ferment pea protein concentrate, in which protease inhibitor activity decreased as protein digestibility increased, but with an overall decrease in IVPDCAAS (67 to 64 for *A. oryzae* and 69 to 59 for *A. niger*) (27). Although processing is the most common method currently employed to overcome the limitations of pulse crops as nutrient sources, more attention has been devoted recently to understanding how modern genetic technologies can be used to affect pulse protein composition and availability.

Using Genetic Techniques to Increase Pulse Protein Quality

There is significant interest in the identification of locations within pulse genomes that are associated with nutritional composition and bioavailability. These can include locations where modifications can be made to increase the nutritional quality of pulses or assessment of naturally occurring genetic variation in different cultivars. A common technique used to identify these areas of interest is a genome-wide association study (GWAS) that is capable of linking genetic variability and specific traits of interest. For chickpeas, GWAS has been used to identify genes regulating protein content (44) and those linked to improved protein nutritional value in peas (29).

When considering genetic modification of pulse crops, the two primary targets for increasing protein quality are increasing the sulfur amino acid content and reducing the presence or activity of antinutritive factors. Increasing the sulfur amino acid composition of pulses can be achieved through increasing the overall protein content, thereby increasing all amino acids or, ideally, increasing the content of specific protein fractions that are high in sulfur residues. This is typically done by incorporating the genetic sequence of a protein from another plant that is rich in sulfur into the pulse genome. Expression of a sulfur-rich sunflower protein in chickpeas was moderately successful (10), as was expression of a brazil nut protein in beans (3). A combination of genes can also be used, as in the case of a bacterial aspartate-kinase and sulfur-rich brazil nut protein added to a bean line, thereby altering the metabolism of the bean to increase overall sulfur content (34). Public concerns regarding genetic modification prevent these options from being commercially viable; however, these results provide information regarding metabolic regulatory processes that can then be identified should they occur due to natural variation present in pulse crops.

Identification, and modification, of the genes associated with antinutritive factors has not received as much attention as the genes associated with increased nutrient content. In peas and beans most of the attention has focused on reducing phytate content. In peas this has been done through chemical mutagenesis to modify the genome and then identifying the mutations that resulted in lower phytate content (47). Once cultivars have been identified as differing in phytate content, it is possible to generate a linkage map to elucidate which genetic variations are responsible for phytate reduction (43). For faba beans there is an interest in reducing the antinutritive factors vicine and convicine, due to their toxic nature for some individuals. This has resulted in the use of genetic screening tools to identify naturally occurring variations related to these compounds and the development of a genetic library relating these variations to antinutritional factors (22,39).

Summary

Pulses as commercial crops continue to gain in popularity as consumers seek alternative protein sources that are of high quality. Although there are limitations for utilization of pulse proteins due to their amino acid composition and antinutritive factors, these issues can be reduced through different processing methods and determining potential genetic solutions. For this reason, it is important to continue studying the impacts of processing on overall protein quality and identifying both naturally occurring pulse cultivars and potential genetic interventions that would result in greater protein quality.

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