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How might oilseeds help meet the protein challenge?
Comment les oléoprotéagineux peuvent-ils répondre au défi protéines ?

Dossier

Plant protein for food: opportunities and bottlenecks

Jean-Michel Chardigny[1,⋆] and Stéphane Walrand[2]

[1] Département Alimentation Humaine, INRA, 21000 Dijon, France
[2] INRA, UMR 1019, Unité de Nutrition Humaine, Clermont Université, CRNH Auvergne, 63000 Clermont-Ferrand, France

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Abstract – Dietary proteins represent a key issue for the future regarding worldwide food security. Besides animal sources, plant proteins represent an opportunity to mainly contribute to protein demand. Whether some plant protein sources could appear as deficient in some essential amino acids, mixtures from different sources could represent opportunities to further propose adapted supply regarding specific demand. Such opportunities includes legumes as well as by-products of oil processing, *i.e.* canola and sunflower cakes. The nutritional benefits of such new sources are still under investigation considering benefits and limits like allergenicity. Finally, consumer behavior and acceptability remains the final bottleneck for developing new protein sources.

Keywords: Dietary proteins / animal and plant protein sources / essential amino acids / oil processing-derived proteins

Résumé – Les protéines végétales pour l'alimentation : des opportunités et des verrous. La production des protéines alimentaires représente un enjeu majeur au regard de la croissance actuelle de la consommation mondiale. En dehors des sources animales, les protéines issues des végétaux pourraient contribuer de façon plus importante à la demande protéique future. Néanmoins, certaines sources de protéines végétales sont déficitaires en certains acides aminés essentiels, les rendant nutritionnellement impropres à une consommation exclusive. Aussi, l'étude et la définition de la complémentarité des différentes sources protéiques végétales pourraient permettre de proposer une offre adaptée en fonction de demandes spécifiques. Ces nouvelles sources comprennent les légumineuses ainsi que des sous-produits de la production huilière, à savoir les tourteaux de colza et de tournesol. Les spécificités de ces nouvelles sources sont toujours à l'étude et devront considérer à la fois leurs bénéfices nutritionnels mais également leurs limites comme l'allergénicité. Enfin, le comportement et l'acceptabilité de ces nouveaux aliments par les consommateurs représentent encore des verrous qu'il faudra lever pour le développement de nouvelles sources de protéines issues des végétaux.

Mots clés : Protéines alimentaires / sources animales et végétales de protéines / acides aminés essentiels / oléoprotéagineux

1 Introduction

In France, ANSES (previously AFSSA) recommends a daily protein intake of 0.83 g/kg body weight/day for the adult population (AFSSA, 2007). Moreover, protein sources must be of a "good quality". Although this concept remains unclear, the objective is to cover the needs for essentials amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine). Table 1 shows the levels of essential amino acids from animal and some plant sources. Although the amino acid composition of animal proteins is closed to the human needs, the association of different sources allows to cover amino acid requirements for plant proteins. For example, cereals are generally deficient in lysine and rich in sulfur amino acids (methionine). On the other hand, pulses are poor in methionine but rich in lysine, which thus allows a good complementarity. Metabolically speaking, body protein synthesis requires a good balance between all amino acids (Fig. 1).

The notion that protein intake has to be balanced between animal and plant sources on a 1:1 ratio is also recommended by the UN. However, in our countries, the current spontaneous consumption is rather rich in animal proteins (2/3 of contributions) and leaves an important place to plant proteins through new and innovative supply. This situation differs around the world according a lot of parameters, including incomes, GDP, etc...

⋆ Correspondence:
jean-michel.chardigny@clermont.inra.fr

Table 1. Aminoacid content in different protein sources (g/100 g protein). Comparison with the reference protein from FAO. Adapted.

	Ile	Leu	Lys	Met (+Cys)	Phe	Thr	Trp	Val
Reference protein	4.2	4.8	4.2	4.2	2.8	2.8	1.4	4.2
Egg	6.9	9.0	7.2	5.8	5.9	5.0	2.4	7.4
Meat	7.7	6.3	8.1	3.3	4.9	4.6	1.3	5.8
AmnoacSoybean	5.6	7.6	6.3	3.6	5.4	3.9	1.2	5.4
Chickpea	4.7	7.8	7.4	3.3	6.0	3.9	0.8	5.2
Soft wheat	3.9	6.5	2.7	3.8	4.4	3.0	1.1	4.5
Alfalfa	4.7	8.7	6.3	3.3	4.9	4.7	1.9	6.0
Spirulin	5.6	8.7	4.7	3.2	4.5	5.1	1.5	6.5

Ile : Isoleucine, Leu : Leucine, Met : Methionine, Cys : Cysteine, Phé : Phenylalanine, Thr : Thréonine, Trp : Tryptophane, Val : Valine.

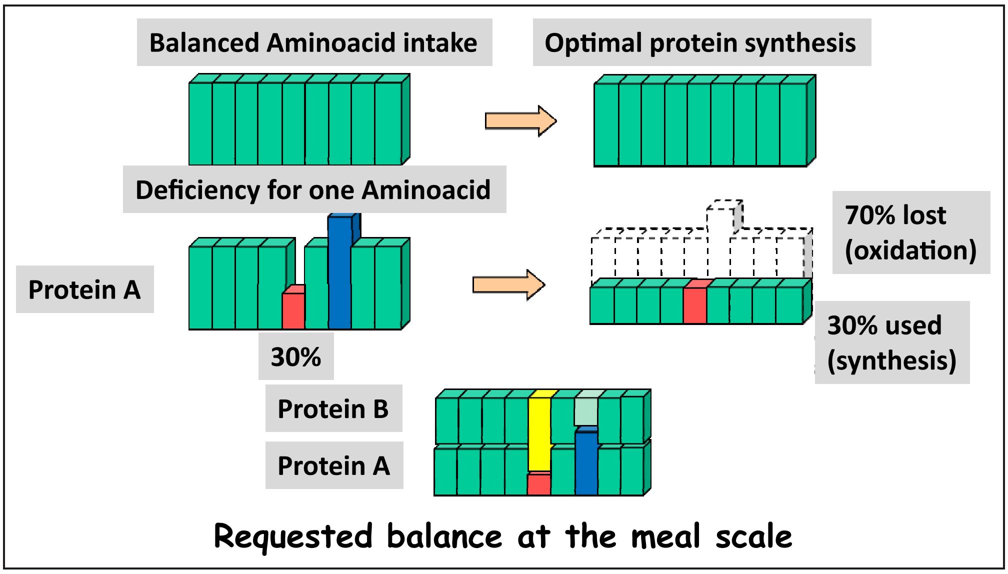

Fig. 1. Aminoacid request at the meal scale. Adapted from D Rémond.

These recommendations for the general population are even of a greater importance for older subjects. With age, due to anabolic resistance, protein synthesis requires higher levels of circulating amino acids, in particular essential amino acids. Consequently, the balance between protein synthesis and protein degradation (to maintain body protein mass) becomes more difficult to maintain (Warand and Boirie, 2005). Therefore, the recommended daily intake increased to about 1 g/kg body weight/day, promoting proteins called as "fast digestible proteins", *i.e.* rapidly absorbed and able to rapidly increase the amino acidemia. Protein needs are also increased and quality is of importance in athletes and people with intense physical activity.

2 Protein and world food security

According to FAO, 1/7 of the world population suffers from hunger and 1 billion people have inadequate protein intake. Several prospective studies (Esnouf *et al.*, 2011; BIPE, 2015) predict a major strain on the protein sources in the coming decades, while palm oil will cover the quantitative requirements in fat.

At the same time, the animal protein intake is high in industrialized countries, about 65 to 70% of the total protein intake, and this phenomenon is settling in emerging countries in connection with the increase in GDP. Yet the production of animal protein is more expensive regarding water and energy resources than plant-based protein. It is therefore important to rebalance the contributions between animal and plant proteins -or other alternatives- in sustainable food systems allowing access to quality protein intake to the entire world population.

The vegetable protein sources are mainly pulses (beans, chickpeas, lentils...) and grain products (bread, biscuits, rice...). They also brought in food by-products made from soy, in "catering" dishes, or in some specialty food and dietary preparations (sports, seniors...). Plant proteins are then incorporated into the formulation of foods as vegetable protein products (VPP). These VPP are fractions rich in proteins, obtained by fractionation of raw materials such as cereal grains or seeds of legumes and oilseeds, but also tubers (like potatoes) or leaves (foliar proteins from alfalfa). The main VPP are derived from soy and wheat (gluten). Pea and faba beans occupies a much smaller market. Lupine and potato are emerging sources (Gueguen *et al.*, 2016). Research is also developed to better use other sources like rapeseed and sunflower cakes with a preservation of the protein fraction during the oil extraction process.

3 Plant protein composition

Animal based foods compared with other food types contain on average greater amounts of protein per portion consumed. Other important factors apart from quantity of protein also need some consideration, *i.e.* protein quality in terms of digestibility and amino acid composition. The nutritional quality of food proteins can be defined by their ability to cover the needs in essential amino acids for growth and tissue maintenance. Moreover, due to the lack of *de novo* synthesis and storage, essential amino acids intake and requirements have to be covered at the meal scale, unlike fats and carbohydrates which have the capacity to be stored the body.

The reference method for evaluating the quality of dietary protein was PDCAAS (Protein Digestibility Corrected Amino Acid Score) (FAO, 1990) corresponding to the digestibility of the protein multiplied by the chemical index. But in 2013, FAO has proposed a new index, the DIAAS (Digestible Essential Amino Acid Score) which reflects not only the amino acid composition of proteins, but also their bioavailability (digestibility in the small intestine) (FAO, 2014).

However, these indices are still insufficient to meet the needs of specific populations such as the elderly. While the percentage of this category of people is increasing, protein requirements for these populations are particularly important, both at quantitative and qualitative levels. Especially a 30% prevalence of inadequate protein intake has been proposed - without talking about qualitative issues- (see above).

The amino acids, methionine, lysine, tryptophan and threonine, most commonly limit the nutritive values of proteins in the human diet (Pieniazek *et al.*, 1975). These amino acid concentrations are generally lower (in different proportions) in plant-based sources of proteins. The correct combination of plant-based protein foods could increase the protein quality of the complete meal by compensating for the individual plant foods' amino-acid deficits. As mentioned above, the vegetable protein sources are multiple. Besides legumes and cereals, other potential sources for human consumption are available as protein fractions of oilseeds such as sunflower and rapeseed. These "cakes" are however valued in animal feed, but a higher added-value could probably emerge for some of the volumes produced (cascading approach). Overall, the vegetable protein sources are vectors of water-soluble vitamins and fiber. This last point is a very positive aspect as current dietary intakes are well below recommendations (25–30 g/day). Another quality criterion is the ability to release bioactive peptides with interesting biological activities for health. Although, the potential biological actions of plant peptides have not been studied, di- or milk tri-peptides have been described as having anti- hypertensive properties, immunomodulating, satietogenic, etc. If the bioactive peptides from milk or meat products are clearly identified, the presence of bioactive peptides derived from vegetable proteins remains a field to investigate. Of note, peptides with opioid activity are found in pepsin hydrolysates of wheat gluten and α-casein.

Apart from different plant products being limited in certain amino acids, anti-nutrient factors in legumes, starches, cereals, vegetables and fruits also bind many nutrients, including proteins, further inhibiting the complete absorption of the nutrient from food into the human body after ingestion. In developing communities, vegetable protein sources are often mixed with cereals for complementary feeding to increase protein quantity in meals. Both these foods contain high levels of phytic acid, which can inhibit trace element and mineral absorption, including the absorption of iron, zinc, calcium and manganese (Schönfeldt and Gibson Hall, 2012). The limitations due to potential intake of phytoestrogens is also often emphasized. Phytoestrogens are a group of non-steroidal compounds naturally produced by plants, which due to their similar molecular structure with estradiol (17β-estradiol) have the ability to cause estrogenic or anti-estrogenic effects. They are therefore considered as "endocrine disruptors". In its March 2005 report, ANSES recommended (AFSSA and AFSSAPS, 2005), for example for soy foods (tonyu, miso, tofu, yogurt and soy desserts) the following labelling: "Contains Xmg of isoflavones (phytoestrogens family). Consume in moderation (limit daily consumption to 1 mg/kg body weight). Not recommended for children under 3 years."

A recommendation is added in a recent decree (24 June 2014) establishing the list of plants, other than fungi, authorized in food supplements and the conditions of their use, for example:

– For soybeans, "The recommended daily portion should not lead to a daily ingestion of isoflavones greater than 1 mg/kg body weight (expressed as aglycone of the main component). The label must indicate a warning against use in women with a personal or family history of breast cancer."
– For alfalfa, the labelling must include a message advising "against use in women with a personal or family history of breast cancer," because of the contents coumestrol, coumarin, alkaloids.

4 Dietary plant protein

Animal proteins (meat, milk, fish, and egg products) are often regarded as a reference in terms of nutritional quality but the resources available to produce these proteins are not unlimited. In addition, some dietary animal proteins, with particular characteristics, are of interest in pathophysiological situations characterized by change of body protein metabolism, *e.g.* whey proteins for older people or athletes.

If multiple alternative and diverse sources -Insects, fungi, algae . . . –could be developed in the future, plant proteins are, in the short term, the opportunity for a broader supply in addition to animal proteins. Although, they are cheaper to produce, plant proteins will however face many bottlenecks both in terms of availability of technology and consumer acceptability. For instance, pulse represent a valuable source of proteins (peas 24%, faba beans 30%, lupine 36%), but they are rarely used and are facing a degraded image and unfavorable sensory perceptions. Hence, using the complementarity concept in terms of amino acid composition between plant protein sources (grains/legumes), it will be possible to develop new food products and meat analogues of nutritional and organoleptic qualities optimized. Among plant sources

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and particularly legumes, soybean is the one which is essentially used. It is well balanced in essential amino acids and widely available in various forms (drinks, desserts, textured product…). For other crops (wheat, rapeseed, sunflower, etc.), the protein fraction is a co-product of starch or oil products and is mainly valued in animal nutrition. The tonnages available are important. Their use as human food is a challenge because of limited nutritional value by the often unbalanced amino acid composition, a lower digestibility and as for other sources, their potential for allergenicity. Thus, some locks are remaining despite a real potentiality.

Plant proteins are incorporated into the formulation of foods as fractions rich in proteins, *i.e.* VPP, obtained by fractionation of raw materials. We generally consider 3 types of VPP, according to their protein content, *i.e.* flours, concentrates and isolates. Their protein contents are between 50 and 65%, 65 and 90% and greater than 90% respectively. The enrichment technologies are adapted to raw materials, depending on their structure (seeds, leaves, tubers) and physicochemical properties of proteins. Overall, the technologies are distinguished by "dry" (*e.g.* milling) and "wet". The latter are particularly based on the differential solubility properties of proteins.

The VPP (flours, concentrates, isolates) are incorporated into formulas as nutritional ingredients to increase the protein content of the finished product and/or as functional ingredients (by exploiting the emulsifying, foaming, gelling properties). They are thus technological aids for food formulation or to improve their texture and physical stability. Vegetable proteins like animal proteins often have a functional role for giving the food texture and organoleptic properties. This is the case for example in bread and pasta whose quality depends closely on the viscoelastic properties of wheat protein (gluten). The use of VPP is a new lever for developing a food supply "ready to cook" in response to consumer demand.

To develop more applications, research efforts can still be made both at the genetic level and technologies to enhance the functionality and the nutritional characteristics of VPP, especially isolates and concentrates. With the exception of wheat, genetics has paid little interest in the relationship between the composition and polymorphism of proteins and their technological functionality for use in human food. The selection represents an important lever as far as the needs of the 1st and 2nd processing industry are well defined, both in terms of functional properties that control for undesirable compounds or generators "off flavors". Similarly, innovations are possible in the field of manufacturing processes to enrich the VPP into functional protein fractionation technology, or to better control the impact of treatments on protein conformation and structure. Finally, recent studies have shown that interactions between plant proteins and other biopolymers, proteins or polysaccharides, can cause functional properties improvements. All these areas of innovations have been privileged in the study of CVT Allenvi (http://www.cvt-allenvi.fr/).

5 Nutritional quality

Because of a higher branched chain amino acid (BCAA) content (Tang and Phillips, 2009), and rapid increases in blood amino acid concentration, whey protein is often considered nutritionally superior to other isolated protein sources (Pennings *et al.*, 2012). In a recent review, Van Vliet *et al.* (2015) compared the anabolic response in skeletal muscle after plant versus animal protein intake. They reported that from a general point of view, plant-protein are less efficient to enhance postprandial anabolic rates, *i.e.* postprandial muscle protein synthesis, when compared to animal counterparts. For example, it has been well established that the ingestion of soy protein results in lower postprandial anabolic rates than does the ingestion of beef (Phillips, 2012), whey (Tang *et al.*, 2009; Yang *et al.*, 2012), or milk (Wilkinson *et al.*, 2007), both at rest and during recovery from exercise. This begets the question as to whether chronic intake of plant- *vs.* animal-based proteins would result in divergent phenotypic outcomes, particularly differences in muscle mass. Overall, previous studies have reported that the consumption of animal proteins during exercise training intervention resulted in greater gains in muscle mass than an isonitrogenous amount of plant proteins (Hartman *et al.*, 2007; Volek *et al.*, 2013; Campbell *et al.*, 1999). However, the ingestion of higher amounts of protein may reduce the proposed differences in the capacity of different protein sources (plant *vs.* animal) to modulate the gains in skeletal muscle mass during prolonged exercise interventions (Campbell and Leidy, 2007).

To explain this data, the human body requires a small set of essential amino acids in a defined proportion. These essential amino acids are provided in roughly the same proportion in most animal-based foods, but are often found in different proportions in plant-based foods. Humans have overcome imbalances in plant-based foods by consuming foods with complementary essential amino acids patterns. Historic examples of these complements include beans and corn in the Americas, or rice and soy in Asia. However, given changes in food availability and increased scientific data about food, other plant-based food pairings could serve our needs as well or better than these historical complements. Strategies to improve the anabolic properties of plant-based protein are then to be developed. Such strategies have to consider different issues. The first one is the protein quantity, as plant-based protein are generally deficient in some essential amino acids. However, this strategy suffers of limits due to increased oxidation of specific amino acids such as leucine (Yang *et al.*, 2012). Leucine or lysine/methionine fortifications have also been studied according to the deficiency of raw material. Such supplementation seems to be a good strategy, although because their rates of assimilation are different, the metabolic fate of amino acids given as dietary proteins or free amino acids mixture has to be studied (Engelen *et al.*, 2007, Zhao *et al.*, 2004). Finally, protein blends should be a good opportunity. For example, Reidy *et al.* showed that a soy-dairy protein blend (25% soy, 25% whey, and 50% casein) is capable of stimulating muscle growth to a similar extent as whey protein through a marked elevation in muscle protein synthesis. Interestingly, they compared this intervention with whey protein as the single source of protein while maintaining a similar absolute leucine concentration between the protein blend and whey protein. These data support the use of a blended protein supplement containing plant proteins compared with an isolated animal protein. A blended protein supplement containing sufficient essential amino acid content, several digestion rates, and a prolonged aminoacidemia clearly promotes muscle protein synthesis, even in the presence of plant proteins.

6 Focus on Canola and sunflower cakes

Canola is considered as an emergent source of dietary proteins (http://www.cvt-allenvi.fr/). Sunflower cakes resulting from oil processing could also be a new protein source.

Bos *et al.* (2007) have determined the nutritional value of rapeseed proteins in Humans. Using an intestinal tube to quantify ileal nitrogen flow rates and 15N-labeled protein to specifically measure the metabolic fate of the dietary nitrogen absorbed, they showed that rapeseed proteins have a poor real ileal digestibility in humans (84%). This low bioavailability is compensated for by an excellent postprandial biological value (84%). Taken together, these results indicate a postprandial retention of rapeseed proteins of 70%, comparable to that of other plant proteins. Thus, these findings show that this protein source could be of great interest for human nutrition. In particular, the high postprandial biological value of rapeseed proteins was presumably due to the high levels of essential amino acids and particularly sulfur amino acids. In addition, in a recent study, Norgaard *et al.* (2012) reported the ileal digestibility in pigs of sunflower meals and rapeseed cakes compared to the one of legumes. When compared to soybean or pea, the digestibility of these sources was lower, but it was rather higher than lupine. Another study revealed that despite very similar overall indices of postprandial dietary nitrogen digestion and retention in vivo, the ingestion of rapeseed and milk protein isolates led to marked regional differences in dietary nitrogen utilization (Boutry *et al.*, 2011). Rapeseed proteins ingestion resulted in a greater retention of nitrogen in visceral organs whereas milk proteins enhanced that in the skin. By contrast, the protein anabolic rate of corresponding tissues were not influenced by the protein source. Finally, most of the differences arising between rapeseed proteins and milk proteins postprandial metabolism were observed following the first ingestion of each protein source and persisted after adaptation.

7 Bottlenecks and perspectives

There is a set of locks to increase the consumption of plant proteins. Pulses themselves are not well received by consumers, with an outdated image and a positioning as a starchy food in the French food pyramid. Thus, they are not identified as protein vectors compared with the trilogy "meat, eggs, fish" already present in school- and textbooks. The situation in North America, particularly in Canada is different with the classification of pulses in a protein sources.

As ingredients, challenges remain in both technology (solubility, foaming and emulsifying properties, etc.) as well as in the nutritional and sensory quality of the finished product ("green" taste for example). Among the compounds of these generators "off-flavors", lipoxygenases or saponins are targeted. Improvements could be made both by genetics and formulations, possibly by combining these different levers.

In conclusion, plant proteins are certainly an opportunity to meet the future worldwide global protein needs, using complementarity or associations with other traditional (animal products) or new (algae, insects...) sources. Nevertheless, major research efforts are needed to facilitate their use in both domestic preparations and food industry. Animal proteins retain their place in food intake, but their production should be preferred from inedible biomass by humans (*e.g.* grassland for feeding ruminants).

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