OCL 2016, 23(4) D406
© D. Chéreau *et al.*, Published by EDP Sciences 2016
DOI: 10.1051/ocl/2016020

OCL Oilseeds & fats Crops and Lipids
Available online at:
www.ocl-journal.org

Research Article – Dossier Open Access

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

Dossier

Combination of existing and alternative technologies to promote oilseeds and pulses proteins in food applications

Denis Chéreau, Pauline Videcoq, Cécile Ruffieux, Lisa Pichon, Jean-Charles Motte, Saliha Belaid, Jorge Ventureira and Michel Lopez*

IMPROVE SAS, rue du fond Lagache, 80480 Dury, France

Received 3 March 2016 – Accepted 27 April 2016

Abstract – The continuous world population growth induces a total protein demand increase based mainly on plant sources. To meet these global nutritional challenges, existing and innovative dry and wet fractionation processes will have to be combined to better valorise plant protein fraction from pulses and oilseeds. The worldwide success of soy protein isolates originate from the intrinsic qualities of soybean proteins but also from a continuous R&D effort since mid-twenty century. Therefore, the soy protein development model can be applied to protein isolates from diverse pulses and oilseeds meals as rapeseed which has already been recognised as novel food protein in Europe. To boost the delivery of plant proteins, agrofood-industries and academics must pool their respective expertise. Innovative and issue solving R&D projects have to be launched to better valorise pulses and oilseed proteins by (i) creating oil extraction processes which preserve native proteins structure; (ii) developing novel protein extraction processes from lab up to industrial pilot scale; (iii) producing plant protein isolates having comparable foaming, emulsifying or gelling functionality than animal; and (iv) generating hydrolysed proteins with high digestibility adapted to human nutrition. It is also essential to initiate research programs to innovate in wet and dry fractionations of plants or to design *in vitro* models to evaluate proteins digestibility and allergenicity. The increased awareness regarding plant protein valorisation resulted in the creation by agro-industries and academics of the open platform IMPROVE which propose a combination of competencies and equipment to boost market uptake of Plant Based Proteins.

Keywords: Plant / proteins / fractionation / nutrition / functionality

Résumé – Combinaison de technologies existantes et alternatives pour promouvoir les protéines d'oléagineux et de légumineuses dans les applications alimentaires. L'augmentation continue de la population mondiale provoque un accroissement de la demande en protéines végétales. Pour répondre au défi, il faudra mieux valoriser la fraction protéique des graines de légumineuses et d'oléo-protéagineux. Le succès des isolats protéiques de soja est dû à leur valeur intrinsèque et à un effort soutenu de recherche depuis les années cinquante. Ce modèle de développement peut s'appliquer aux graines de légumineuses et d'oléo-protéagineux. Déjà, les isolats protéiques de colza ont obtenu le statut *Novel Food*. Afin d'accélérer la commercialisation de nouvelles fractions protéiques issues de légumineuses et d'oléo-protéagineux, les agroindustriels et les académiques doivent s'associer pour mène des projets R&D innovants visant à (i) créer des procédés d'extraction des lipidiques qui préservent la conformation native des protéines ; (ii) développer de nouveaux procédés d'extraction des protéines de l'échelle laboratoire à l'échelle pilote industrielle ; (iii) produire des isolats protéiques végétaux ayant d'excellentes propriétés fonctionnelles : moussantes, émulsifiantes ou gélifiantes ; (iv) créer des hydrolysats protéiques présentant une digestibilité améliorée. Il est également essentiel d'imaginer des outils originaux de fractionnement des végétaux par voie sèche ou humide, ainsi que de créer des modèle *in vitro* propres à évaluer la digestibilité et l'allergénicité des protéines. Conscients des verrous faisant obstacle à la valorisation des protéines végétales, des agroindustriels et des académiques ont créé la plateforme ouverte IMPROVE. IMPROVE propose une combinaison de compétences et d'équipements propres à accélérer la mise sur les marchés de nouveaux produits à base de protéines végétales.

Mots clés : Végétaux / protéines / fractionnement / nutrition / fonctionnalité

* Correspondence: michel.lopez@improve-innov.com

1 Introduction

The world's population is still expending very fast. It took more than 200 000 years to get to the 1st billion humans on earth and it will take no more than 11 years to get to the next one and reach 8 billion! In order to feed all these people, plant and animal production will be under strong pressure. An analysis of the market prices of the 3 macro nutrients (carbohydrates, fats and proteins) in the world over the last 15 years shows a global stability for carbs, a slight increase for fats and steady growth for proteins with a market price multiplied by 4 over the period. This demonstrates the key role played by proteins which are obviously the limiting factor.

1.1 Word proteins balance

The global world agricultural production represented 10 029 million tons of harvestable materials in 2014 (FAO Stat, 2014). The yearly production of proteins equals 555 million tons, 440 of which are fed to animals in order to convert plant proteins into animal proteins. Figure 1 shows some examples of the diversity of the conversion ratio.

Globally based on the world animal proteins production, the weighted average ratio is 4,9.

115 million tons of plant proteins are eaten directly by humans. Only 1.7 million tons of these proteins are extracted, purified and incorporated in food matrix as ingredients. This protein ingredients market is dominated by Soy and wheat proteins. All the other plant sources only represent 1% of this market. This demonstrates an important potential for growth, which would necessitate major R&D developments in order to meet market expectations.

1.2 Market evolution for proteins ingredient

In 2013 Frost & Sullivan estimated the Provisional Market growth at 5% per year until 2018. IMPROVE is the 1st R&D centre dedicated to plant proteins valorisation and well positioned to observe market needs and trends. We started our commercial activities in 2014 and we are already in contact with more than 300 different actors of the plant proteins sector in Europe and North America such as major international players, SME's or academic centres. We can summarize market demand as follows, divided into 4 families:

- Nutritional alternatives to animal proteins with good digestibility.
- New proteins sources aiming to move passed classical soy or wheat proteins with specific attention to non-allergenicity (European Directives 2003/89/EC and 2006/142/EC lists).
- New functional proteins with foaming, emulsifying or gelling properties.
- Texturized proteins which can be used as meat replacements.

All these needs must comply with acceptable pricing, reliable sourcing, food safety and organoleptic properties. It is essential to achieve a better understanding of plant proteins properties.

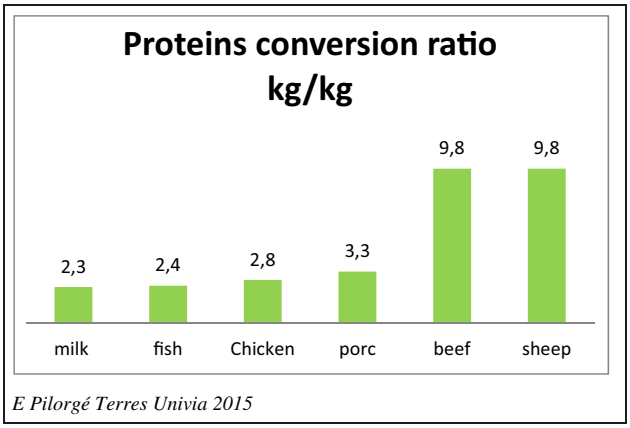

Fig. 1. Diversity of proteins conversion ratio (kg of plant proteins/kg of animal proteins).

1.3 Potential of oilseeds proteins

More than 7 million tons of rapeseed and sun flower were produced in France in 2013. This represents more than 1 million tons of proteins. They are many possibilities to extract, fractionate and purify these proteins for food or feed applications. With well-designed processes preventing proteins from denaturation, we could supply the market with alternative proteins having high digestibility index, well balanced amino acids profile and interesting functional properties. From a regulatory stand point rapeseed proteins have already passed a Novel Food agreement[1]. Sun flower seed are commonly used as food in countries like Spain, this should facilitate future agreements. The challenge for the industry is now to move from the traditional hexane process to new processes combining the market needs with the potential of the oil seeds proteins. As major investments will be needed to convert actual industrial facilities, this evolution will need time. It can be initiated on niche markets providing high added value.

2 Proteins diversity in oilseed and protein rich beans

A major part of the human diet consists of cereals, pulses and proteins isolates extracted from oilseed beans.

2.1 Classification

Classification divides seed proteins into storage, structural and biologically active proteins. The major biologically active proteins include lectins, enzymes and enzyme inhibitors. These minor proteins have nutritionally more balanced amino acid composition than storage proteins. The seed storage proteins have the sole purpose of providing proteins required

[1] COMMISSION IMPLEMENTING DECISION of 1 July 2014 authorising the placing on the market of rapeseed protein as a novel food ingredient under Regulation (EC) No. 258/97 of the European Parliament and of the Council.

Table 1. Seed proteins solubility (Osborne's classification).

	Albumin	Globulin	Prolamin	Glutelin
Solubility	water	salty solution	ethanol 70%	pH > 11
Function in plant	physiologic	storage	storage	storage
Soybean	10%	90%		
Pea	20%	65%		15%
Fababeans	25%	55%		20%
Sunflower	20%	60%	5%	15%
Rapeseed	50%	25%	5%	10%
Mil	8%	4%	46%	42%
Wheat	5%	10%	45%	40%

Table 2. The nomenclature of storage seed proteins classified according to their sedimentation coefficient.

Classification	Soybean	Pea	Lupin	Rapeseed	Sunflower
2S	α-conglycin	PA1	conglutin-δ	napin	SFA
7S	β-conglycin	vicilin	conglutin-β		
	γ-conglycin	conviciline			
11/12S	glycinin	legumin	conglutin-α	cruciferin	helianthinin

during seed germination. Seed proteins were empirically classified by Osborne (1924) based on their solubility as follows: water extractable (albumins); extractable in dilute salt solutions (globulins); extractable in aqueous alcohol (prolamins); extractable by weakly acidic or alkaline or dilute SDS solutions (glutelins). Later on seed proteins has been also defined on the basis of their sedimentation coefficients (Mandal and Mandal, 2000).

As noticed in Table 1, prolamins and glutelins are major proteins in monocots *e.g.* cereals whereas albumins (2S) and globulins (7S-12S) comprise the storage proteins of dicots *e.g.* pulses and oilseeds beans. The seed storage proteins are deposited mostly in special storage organelles called protein bodies.

Later this document will focus on major pulses and oilseeds proteins. The nomenclature of storage seed proteins classified according to their sedimentation coefficient is presented in Table 2.

2.2 2S albumin storage proteins

The 2S albumins are widely distributed in dicot seeds and have been most widely studied in the Cruciferae, notably oilseed rape (in which they are called napins).

The napins consist of two polypeptide chains with M, values of 9000 and 4000, which are linked by interchain disulphide bonds. They are synthesized as single precursor proteins that are proteolytically cleaved with the loss of a linker peptide and short peptides from both the N and C termini. This appears to be the most typical 2S albumin structure (Shewry *et al.*, 1995).

Variant types of 2S albumin also occur. Those of pea appear to lack interchain disulfide bonds, whereas the 2S albumins of sunflower remain uncleaved. Despite differences in their subunit structure and synthesis, all the 2S albumins are compact globular proteins with conserved cysteine residues (Shewry *et al.*, 1995).

2.3 Globulin storage proteins

The globulins are the most widely distributed group of storage proteins. They can be divided into two groups: the 7S vicilin-type globulins and the 11S legumin-type globulins. Both groups show considerable variation in their structures, which results partly from post-translational processing. In addition, both have nutritional significance in that they are deficient in cysteine and methionine. The globulin storage proteins have been studied in most detail in peas and soybean (Shewry *et al.*, 1995; Guéguen and Lemarié, 1996).

2.3.1 7S Globulins

The 7S globulins are typically trimeric proteins of M, ~150 000 to 190 000 that lack cysteine residues and hence cannot form disulfide bonds. Their detailed subunit compositions vary considerably, mainly because of differences in post-translational processing.

2.3.2 11S Globulins

The 11S legumins are the major storage proteins in most legumes, other dicots (*e.g.* brassicas, and cucurbits) and some cereals (oats and rice). The mature proteins consist of six subunit pairs that interact non-covalently. Each of these subunit pairs consists in turn of an acidic subunit of $MW \sim 40\,000$ and a basic subunit of $MW \sim 20\,000$, linked by a single disulfide bond (Fig. 2).

2.4 Amino acids composition of albumins and globulins fraction of oilseeds and pulse seeds

Oilseeds and pulses are rich in lysine and arginine and are deficient in sulphur amino acids. Variability exists within and between species and the environment and genotype contribute

Table 3. Amino acids composition of some albumins and globulins fraction of oilseeds and pulses (Guéguen and Lemarié, 1996; Hughes *et al.*, 2011*).

Amino acids	Pea		Sunflower		Rapeseed		Soybean*
	Alb.	Glob.	Alb.	Glob.	Alb.	Glob.	Isolate
Asparatic/asparagine	118	133	79	119	68	85	113–129
Threonine	56	34	32	39	37	25	36–38
Serine	50	54	40	51	58	35	49–51
Glutamic/glutamine	149	191	278	184	198	198	189–196
Proline	44	45	59	47	77	61	52–53
Glycine	59	40	60	85	49	47	40–42
Alanine	58	41	43	70	49	36	41–43
Cysteine	31	8	60	13	–	17	12–13
Valine	44	48	46	68	36	49	49–51
Methionine	13	7	36	15	–	–	12–13
Isoleucine	48	47	43	52	39	51	45–49
Leucine	48	84	62	74	69	91	78–82
Tyrosine	47	34	15	23	22	31	37–39
Phenylalanine	45	55	19	49	60	47	49–53
Tryptophan	15	6	–	–	–	5	12–14
Lysine	93	65	35	27	136	73	61–65
Histidine	26	26	11	22	39	38	23–26
Arginine	56	82	82	61	63	100	73–86

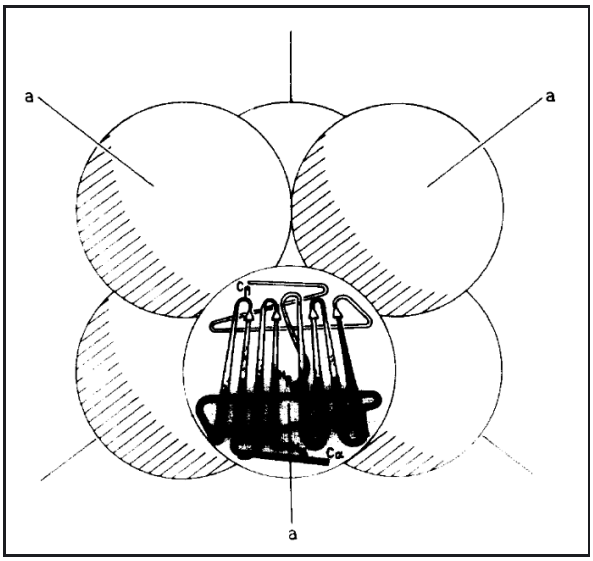

Fig. 2. Hypothetical model of the arrangement of the α-chain and α-chain of each subunit within the hexameric molecule of the 11S globulins (Plietz *et al.* 1987).

to it. The cysteine and methionine deficiency is more important in pea and fababean than in soybean proteins. Pea proteins are also deficient in tryptophan while rapeseed appears to get a well-balanced amino acid profile as shown in Table 3. Grain legumes contain lesser amounts of glutamic acid and proline than cereals (Guéguen and Lemarié, 1996).

The physiological distinction roles attribute to albumins and globulins is reflected in the different amino acids compositions of those two fractions. In general, albumins are richer in sulphur amino acids and lysine while globulins are characterised by a higher proportion of aspartic acid, glutamic acid and their corresponding amides on the one hand, and on the other, upper arginine content.

2.5 Nutritional and functional properties

The production of plant protein isolates is of growing interest to industry, because of their increasing application (cereals, legumes and oilseed proteins) in food (Fleddermann *et al.*, 2013; Joshi *et al.*, 2012; Schwenzfeier *et al.*, 2013) and non-food markets (Nesterenko *et al.*, 2013; Nordqvist *et al.*, 2013).

Plant proteins for foods, in addition to providing nutrition, should also possess specific functional properties that facilitate processing and serve as the basis of product performance. The nutritional properties of plant proteins are related to the balance of their amino acids compositions, their digestibility and the presence of anti-nutrients factors (Fleddermann *et al.*, 2013). In the other side, plant protein isolates in foods are usually used as functional ingredients (Makri *et al.*, 2005; Peng *et al.*, 2016). The major functional properties of food proteins are solubility, binding properties, surfactant properties and viscogenic texturizing characteristics (Peng *et al.*, 2016).

Protein-based systems in foods depend upon the intrinsic molecular properties of the protein being used. Thus, amino acid sequence and disposition; molecular size, shape, conformation and flexibility; surface polarity, charge, hydrophobicity, *etc.*, all influence functional behaviour in food systems. These, in turn, are affected by processing history (Stone *et al.*, 2015) (Peng *et al.*, 2016) and by the physical and chemical environment in which the protein is being used (Chang *et al.*, 2015; Kinsella, 1981).

Table 4. Existing products produced from soybeans.

Raw material	Product
Mature bean	Roasting, roasting and grinding (traditional confectionary products)
	Water-extracting of dried soy (soymilk)
	Precipitating water-extracts (soy curd as tofu)
	Fermenting cooked bean (soy taste as miso, soysauce as sho-yu…)
Defatted meal	Defatted soy flours and grift: human consumption grounded
	Soy protein concentrates: 70% of protein prepared from defatted meal by selective extraction
	Soy protein isolates: protein concentration: 96% are obtained by selective solubilisation of the protein
	Extrusion textured soy protein or Textured Soy Protein-TSP (meat analogues)
	Spun fibers of soy protein (synthetic fiber analogous meat)

3 Existing and future pulses and oilseeds sources

3.1 Soy

Cultivated soy (Glycine max (L.) Merril) is a major oilseed and protein rich annual crop. The main producers are USA with an average production from 2010 to 2014 of 78 Mt then Brazil (48 Mt), Argentina (31 Mt) and China (14 Mt) (FAO Stat). Considered as one of the five sacred food grain in ancient China, soy has been cultivated and consumed during millennia in the Orient. The Western world has limited contacts with soy and soyfood was limited until the 19th century when a massive emigration occurred from Asia towards Europe and USA. Soybean crops has been introduced in these area during this period but also in Africa. The large scale development of soy production and processing in the USA began during the 1940s and 1950s instigated by the increasing demand in oil and meal during the World War II. Soy as a new crop was successful due to the needs in oil and protein, but also to its similarity with corn culture and the benefit brought by soy crop in a rotation. Then the improvement of farming, especially with genetically modified soy introduction, lead to an expansion of soy use and consumption.

Several varieties are available for wide and specific applications. The most commercially grow soy are the yellow-seeded field cultivar. Soy contain approximately 40% protein and 20% oil on an average dry basis. 85% of worldwide soy is processed into oil and protein. Major proteins of soy are globulins (90%): β-conglycinin and glycinin. Soy is the only protein-rich oil plant from which the protein extract is not considered as a by-product. Traditionally, the whole bean is used. Immature bean can be consumed as vegetable. However, mature bean needs to be processed to be edible. In fact, mature grain exhibit a bitter taste, have a poor digestibility and necessitate a long time cooking. Traditional process use fractionation and/or fermentation to improve soybean nutritional properties. Modern protein rich products are produced from meal (Tab. 4). However, most of meals are used as animal feedstuff for its high nutritional quality.

The success of soy proteins isolate mostly arise from its high nutritional quality and processing ability. Indeed, soy protein is a well-balanced protein which can be an excellent complement to lysine-limited cereal protein. Soy proteins have also an excellent processing ability such as gelling, emulsifying ability and water and oil-holding capacity (Nishinari *et al.*, 2014). Moreover, soy belongs to the fabaceae family which is desirable in rotation because they enrich the soil in nitrogen.

3.2 Pea

Grown as a vegetable for both fresh and dried seed, Pea (*Pisum sativum*, L.) is a long established fabaceae crop in Europe. The main producers are China with an average production from 2010 to 2014 of 10.6 Mt, India (4.1 Mt), Canada (3.3 Mt) and European Union (2.2 Mt) (Data: FaoStat). Pea contain 22–24% of proteins (Guéguen n.d.). Major proteins of pea are albumins (20%) and globulins (50–60%) as convicilin, vicilin and legumin. Originally, this pulse was used in cereal/legume mixture grown for arable silage due to its content in protein and starch. Nowadays it is still it main purpose: 90% of pea production in Europe is used for animal feed. However, pea protein are more and more use in food for their good emulsifying and foaming properties. Pea protein can challenge on the European market soy protein: both have a high content in lysine and a good processing ability (Karaca *et al.*, 2011). Moreover, the low content in oil of pea avoids the defatting step and simple methods like air classification (dry-fractionation) and ultrafiltration (wet-fractionation) can be used to produce concentrate or isolates (Bramsnaes and Olsen, 1979). Pea proteins isolates are currently produced at industrial scale in Europe and abroad.

3.3 Faba bean

One of the oldest domesticated legume is the faba bean (*Vicia faba*. L.) from the fabaceae familly. The main producer are China with an average production from 2010 to 2014 of 1.6 Mt, Ethiopia (0.8 Mt), Australia (0.3 Mt) and France (0.3 Mt). The faba bean is about 27–32% protein. The major proteins in faba bean are albumins (20%) and globulins (60%) as convicilin, vicilin and legumin. Faba bean is considered as one of the main sources of cheap protein and energy in Africa, Asia and Latin America. Historically, the use of faba beans in animal feed has been limited due to the presence of anti-nutritional factors as tannins or glucosides like

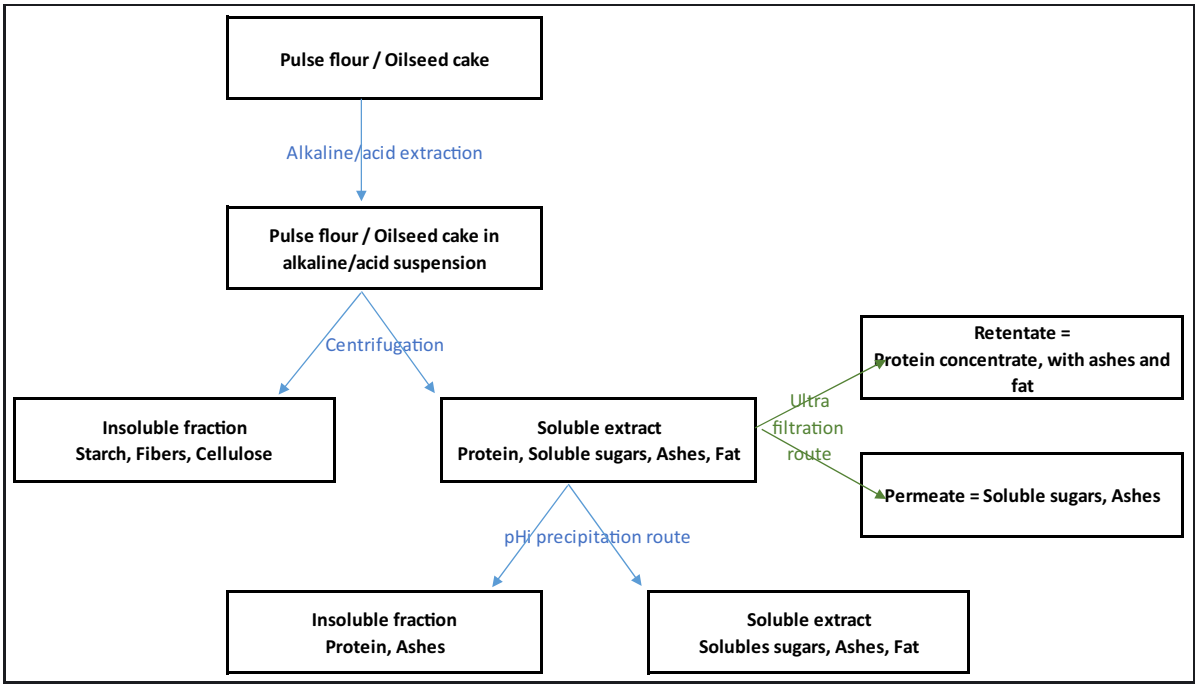

Fig. 3. The two possible ways for the classical two-steps process fractionation (Extraction/Isolation).

vicin and convicin. Recent advances in plant breeding (zero tannin) and advance in animal feed technology allow its use in feed. Faba bean is added into animal feed ration (New Zealand Feed Manufacturers Association, 2008). Traditionally in Middle East, faba bean are introduced in food in canning, sauces and falafel are common uses for both types of faba bean.

Like for pea, protein isolate and concentrate can produced by simple methods (Bramsnaes and Olsen, 1979). Faba bean contain more protein and less starch than pea. Faba bean protein exhibit functional properties (emulsifying) (Karaca *et al.*, 2011). Moreover, the culture of Faba bean is easier than pea's.

3.4 Future sources of proteins

Oilseeds represent very important underexploited protein sources. Indeed, oil-extracted meal have a low value but consist 40 to 45% of protein (rapeseed). The main protein are cruciferin (albumin) and napin (globulin) for the rape seed and heliathinin and albumin 2S for sunflower. These proteins exhibit functional properties (Gonzalez-Pérez and Vereijken, 2007) and can be valorised in food industry. However two major obstacles limit oilseed protein extractions:

- Anti-nutritional factors (ANF) like protease inhibitor, glucosinolate, phenolic compounds and phytates need to be remove before incorporating in Food or Feed. The difficulty lead in extracting protein without ANF or removing ANF without losing the protein yield.
- The extensive denaturation of protein during the oil-extracting process need to be limited. Indeed, oilseed protein functional properties seems to be impacted by the high temperature requested for the oil-extraction process.

The challenge of oilseed protein is complex. Breeding effort can be done to improve of protein quality for specific higher added-value uses (Malabat *et al.*, 2003).

4 Existing and future technologies used to valorise protein fraction of pulses and oilseeds

4.1 Wet fractionation of pulse seeds and oilseed cakes

4.1.1 Classical two steps wet fractionation process

In order to prepare protein concentrates/isolates from pulse seeds or oilseed cake, the most widely used process is the two-steps process patented by Anson and Pader (1955). After an alkaline solubilization of the proteins, the insoluble material (mainly starch and fibers) is removed by centrifugation. By adding hydrochloric acid to the supernatant, the protein is precipitated iso-electrically (pH 4.0–5.0), separated by centrifugation and neutralized. The co-product contains the other soluble components, mainly sugars, soluble fibers, fat and ashes (Fig. 3).

Other versions of this historical 2-steps process (Extraction/Isolation) exist on industrial scale:

- Extraction step can be performed in acidic condition (Alli *et al.* 1993), or in water (Klamczynska *et al.*, 2001). Globally, if acid or alkaline extraction can lead to high level of purity (>90%), for water extraction, values are lower: 50–65%.
- Isolation step: instead of pHi precipitation (assisted with heat-treatment or not), the ultrafiltration way can be used to purify protein. This process was developed at the Food

Technology Laboratory (Lyngby, Denmark) by Olsen and Andersen (1978). The main difference between the pHi and the filtration process is the solubility and functionality, generally higher in case of ultrafiltration.

4.1.2 Alternative to the 2-steps processes

Alternative to the 2-steps processes (Extraction/Isolation) were also proposed at pilot scale:

- A simpler wet process, derived from those described formerly, has been developed for processing pea protein by the Prairie Regional Laboratory (Canada). The whole alkaline extract is spray-dried. In this way, protein content can reach 60%.
- "Micellization", proposed by Paredes-Lopez *et al.* (1991) is based on salting-in salting-out phenomenon of food proteins. As reviewed by Boye *et al.* (2010), after extraction of protein using an appropriate salt solution at desired ionic strength, the solution is diluted, inducing protein precipitation that can then be recovered by centrifugation or filtration, followed by drying. Purity of 88% was achieved but there's no industrial reality today, because of the high consumption of water.

4.1.3 Pre-treatments to enhance extractability

Pre-treatments to enhance extractability:

- Physical ways: adapted granulometry (100–150 µm) thanks to milling permits to decrease the proportion of extract remaining in the starchy by-product up to 25%. High-pressure homogenization or ultra-sound pre-treatment also can be used.
- Chemical ways: pre-hydrolysis with cellulase can help to liberate protein corpuscles from starch granules. Fermentation can also be a way of functionalization of the concentrates obtained further.

4.1.4 Specific case of oilseed cakes: impact of de-oiling pretreatment

Impact on the protein extractability and functionality is huge. Indeed, protein fraction is submitted to solvent exposure (mainly hexane), and mostly to high heat-treatment nearing 110 °C. In the future, alternative processes for oil extraction should be implemented to preserve protein quality of the cake to be able to produce high quality protein concentrate from rapeseed or sunflower:

- Use of alternative solvents as supercritical fluids, alcohols, aqueous mixes (Bardeau *et al.*, 2015; Phan *et al.*, 2009; Russin *et al.*, 2011; Sicaire *et al.*, 2015).
- Use of new technological approaches as enzymes cocktails (Rosenthal *et al.*, 1996) or ultrasounds (Vilkhu *et al.*, 2008).

Globally, industrial production of protein concentrates/isolates already exists since early 50ies for soy and were industrialized later for pea and lupine, mainly on a two-steps process scheme (Extraction/isolation). The next generation will clearly be the industrial implementation of the rapeseed and sunflower protein production, and an important research effort is needed on alternative way for oil extraction as mentioned above. Of course, each production plant possesses its specificity. Industrial equipment choice will have a strong impact and each step is optimized according to the raw material and the (yield-purity-functionality) equation.

4.2 Dry fractionation of pulse seeds

Dry processes is a family of processes able to generate particles functionalized for further process. It repose on the feasibility to reduce particle size and create fractions of different composition. Two types of processes are generally involved:

- Process of particle size reduction, which crushed, grind or mill particles at a targeted particle size and shape.
- Fractionations processes that separate particles according to a given physical properties: size, density, aerodynamically properties, and electrostatic properties.

Dry processes are able to produce particles optimized for molecule extraction, mixing with other ingredient, enriching the product in a compound (*e.g.* proteins, starch…), reducing the content of unwanted or molecules (minerals, anti-nutritional factors) or maximizing the bio-availability of the substrate (biological or enzymatic processes). The particle size is the main parameter to adjust and 3 categories are generally described: Coarse milling (>500 µm), fine milling (50 to 500 µm) and ultrafine milling (<50 µm). The particle size and shape are therefore important parameters to be adjusted specifically considering the application, the resources concerned and the economical balance of the process (Tab. 5).

4.2.1 Coarse particle size reduction

Coarse milling generate particle size higher than 500 µm. This process is adapted to break material structure according to their morphological differences. Since plant organs have strong compositional differences because of their role in the plants (protection, storage, …), coarse milling generate easily particles of different composition. After crushing, particles can be separate mainly by sieving and air classifying processes.

These processes can be applied for every material including soy, pulses or meal. On pulse or soy, this particle size reduction is classically used for dehulling processes. The aim of these processes is separate the hull of the resource from the endosperm in order to remove many antinutritionnal factors, fibers or abrasive fraction (silica) while enriching the product in protein content. Coarse milling can be applied to meals for particle desagglomeration after oil extraction. In that case no separation device is required.

Coarse milling are extensively used at industrial scale since it's energetically cost is low and it's appropriate to reduce resource volume and removal unwanted compounds.

Table 5. Dry processes technologies able to produce coarse, fine and ultrafine particles and their adapted applications.

Range of particle size		Coarse milling	Fine milling	Ultrafine milling
		>500 μm	50 to 500 μm	<50 μm
Possible technologies	Milling	Impact mill Cutting mill Hammer mill	Impact mill Cutting mill Hammer mill	Impact mill
	Separation	Air classifying Sieving	Assisted sieving Electrostatic fractionation	Air classifying Electrostatic fractionation
Application to ressources	Soya	Cleaning Dehulling	–	–
	Meal	Desaglomeration Husk removal	Flour production	Protein enrichment
	Pea	Cleaning Dehulling	Flour production	Protein enrichment

4.2.2 Fine particle size reduction

Fine milling generate particle which size is comprise between 50 and 500 μm. This particle size reduction break resources according to their histological structure (tissues). Because of the different role of the tissues in the plant, the particles generated by a fine milling have different compositions. After milling, sieving processes are the process generally applied. Because of aggregation effect, the sieving must be assist with aids such as chains or balls for few 100 μm sieves or ultrasound for very small sieves.

This range of particle size is classical for most of the wet extraction process since it generally represent a compromise between energy cost and surface area generated (Schutyser and van der Goot, 2011). With oily product, this level of particle size reduction generate free lipids that limit strongly the process. The soy is therefore difficult to mill finely if oil is not extracted. Meals are processable if lipids have been properly removed. Pulse are easily grind and can produce easily flours for wet extraction for examples (Boye *et al.*, 2010).

Fine milling are extensively used at industrial scale on a target of compromise between energetical cost and powder functionality.

4.2.3 UltraFine particle size reduction

Ultrafine milling generate particle size lower than 50 μm. These process breaks material structure at a cytological scale (plant cells) (Boye *et al.*, 2010). Because of the high level of material disintegration, the energetically cost is extremely high (Schutyser and van der Goot, 2011). The energetically cost must be counter by a significant gain of particles functionality. The powder generated can be treated by air classifying that provide a cut point of a few micrometers or by electrostatic separation that separate particle according to their electrostatic charges and consequently their composition.

Air classifying is particularly adapted to pulses to create fraction enriched in protein. It generate a heavy fraction rich that contain the particles which size is higher than 20 μm, which correspond to starch granule, while generating a light fraction with particle lower than 5 μm which is rich in proteins (Pelgrom *et al.*, 2013). These process can easily double the protein of pulses (Pelgrom *et al.*, 2014; Pelgrom *et al.*, 2013).

Oily product such as meal or soy cannot been treated by air classification since ultrafine particle size reduction generate free lipids. Recent development have been performed to use air-classifying on oily product such as lupine, by the use of flowability aids (Pelgrom *et al.*, 2014). Electrostatic separation have been historically developed on minerals and then transpose to cereals (Schutyser and van der Goot, 2011). Recent work transpose this technique to meal in order to enriched the protein content (Barakat *et al.*, 2015) to create two fractions of respectively 5% and 49% of protein content.

4.3 Approaches to improve functionality, nutritional value and taste of plant proteins

Plant proteins are sometimes associated to functionality, nutritional and organoleptic deficiencies which could make difficult their use as food ingredients. To solve or reduce those problems different strategies can be applied.

4.3.1 Enhance functionality

The functional properties of proteins depend of the structure, charge and hydrophobicity, therefore changes of those characteristics would produce changes of functional properties so proteins are subjected to physical chemical or enzymatic treatments.

Some physical chemical treatments can be used to modify proteins structure. Modifications of the pH of the media will produce variation of charges of proteins and depending the pH irreversible changes of the structure that can increase functionality (Ventureira *et al.*, 2012). Thermal treatments can

be used to expose hydrophobic regions of proteins and produce different textures (gels, microgels, viscosity, fibers). High hydrostatic pressure (up to 1000 MPa) was used to modify functional properties of soybean proteins enhancing gelification and emulsifying properties (Messens *et al.*, 1997).

Proteases are used to hydrolyse peptide bonds and reduce the size of proteins. This reduction of size usually yields to increase solubility and increase of the flexibility of the polypeptides structure that can lead to a better functionality. Controlled enzymatic hydrolysis increases solubility, emulsifying and foaming properties of wheat gluten (Popineau *et al.*, 2002), soybean (Zhao and Hou, 2009), pea (He *et al.*, 2012), rapeseed (Chabanon *et al.*, 2007), amaranth (Ventureira *et al.*, 2010), sunflower (Rodriguez Patino *et al.*, 2007) or chickpea (Mokni Ghribi *et al.*, 2015).

Some chemical modifications in proteins can be the acylation (*e.g.* succinylation), blocking amine group or hydroxyl group of lateral chains by acyl residues; phosphorylation, introducing acid groups into a protein; glycosylation, by attaching a sugar molecule to the protein; deamidation, produced by heat and extreme pH that transforms glutamine and asparagine in their acids increasing charge; esterification by reaction of carboxyl groups with alcohols or hydroxyl groups with carboxylic acids. Most of these chemical modifications are conducted at laboratory scale without any industrial application.

4.3.2 Enhance nutritional value

Plant protein sources may vary from animal sources in terms of amino acid composition, digestibility and anti-nutritional factors. Even if it is often mentioned that plants can provide all human protein needs there still exists the mistaken belief that they are nutritionally inferior to animal proteins. Plants proteins can completely provide for human amino acid needs (Millward, 1999). Sometimes they have to be cross-complemented to reach sufficient essential amino acid content (*e.g.* association of cereals and pulses to enhance lysine content). Concerning digestibility of plant proteins, once plant-cell-wall constituents are removed (as in isolates or concentrates), the inherent digestibility of plant proteins may be indistinguishable from that of animal proteins. Sometimes the presence of anti-nutritional factors can limit digestibility (beans) (Millward, 1999). Different anti-nutritional factors can be found in plant proteins that can produce negative effect on protein or carbohydrates digestion as lectins, phenolic compounds, saponins, trypsin, chymotrypsin or amylase inhibitors or negative effect on the utilisation of minerals as glucosinolates, phytic acid or oxalic acid. Some anti-nutritional factors can affect vitamins or over-stimulate the immune system (allergenic) (Huisman and Tolman, 1992). Many of these factors are thermosensitive and eliminated by thermal treatments including cooking. Others can be reduced by washing or by differential solubilisations (depending the characteristics of molecules). Enzymatic treatments can be also considered to decrease the antinutritional content or improve the biodisponibility of plant proteins using for example cocktails of hemicellulases and cellulases on the ram material.

4.3.3 Enhance taste

Concerning organoleptic properties of plant proteins objectionable flavors can arise when some grains are processed into protein isolates (Rackis *et al.*, 1979). Pure proteins do not have off flavors but they appear normally during the milling because the cell breaking put in contact different compounds or molecules that have responsibility in the generation. Normally are associated to oxidative deterioration of unsaturated fatty acid in protein-bound lipids that yields small components that can bound to proteins. These molecules are responsible for the grassy/beany flavor (*e.g.* soybean). Regulating process conditions those reactions are reduced and flavor enhanced. Also flavors can be masked by the use of different components. Several patents address this issue.

5 Conclusion and perspectives

The continuous word population growth induces an increase of total protein demand based on plant sources complemented with animal sources. The world population is facing two distinguished nutritional transitions: the desire to eat more meat in countries where population gross domestic product growth and the progressive switch from animal to plant proteins diet in developed countries as EU and USA.

Diverse strategies must be combined to meet these nutritional challenges. Using as model the worldwide success of soy protein isolates, R&D investments in agriculture and agrofood technology have to be considered to expand the production of protein-rich seeds, to better valorise the already consumed plant protein fractions and to promote new generation of protein extracts from agro resources like oilseed meals in human nutrition.

To boost the market uptake of plant based proteins, agrofood-industries and academics must pool their respective expertise to deliver plants proteins with better, or at least comparable characteristics (performance, price, availability, and sustainability) than animal proteins. The specific valorisation of pulses and oilseed proteins fraction requires to create oil extraction processes which preserve native proteins structure to get new foaming, emulsifying or gelling functionalities, or result in hydrolysed proteins with high nutritional value adapted for infant, adult or elderly people nutrition. To address the diversification and enrichment of current offer in vegetal proteins, private companies and public institutes have to initiate common pragmatic R&D programs to innovate in wet and dry fractionations of plants or create *in vitro* models to evaluate proteins digestibility and allergenicity. The aim is to be able to anticipate the impacts of proteins production process changes on the interaction of those proteins with human (or animal) metabolism. Moreover, all efforts towards better valorisation as human food of proteins extracted from pulses and oilseeds will have to be conducted in parallel with promoting animal feed integrative models: dedicate high nutritional value plant proteins to human and base livestock productions on remaining by-products.

The increased awareness regarding plant protein valorisation resulted in the creation by major agro-industries and

academic institutes of the open platform IMPROVE. IMPROVE proposes a combination of competencies and equipment to:

- Boost plants natives proteins extractions combining dry and wet fractionations from lab up to industrial pilot scale.
- Create chemo-enzymatic hydrolysates to increase nutritional, functional and biological properties of plant proteins.
- Understand plant protein behaviour in food matrix to substitute partially or totally animal proteins.
- Identify and anticipate hurdles regarding plant based proteins incorporation in food.

References

Alli I, Gribbs BF, Okoniewska MK, Konishi Y, Dumas F. 1993. Identification and characterization of phaseolin polypeptides in a crystalline protein isolated from white kidney beans (Phaseolus vulgaris). *J. Agric. Food Chem.* 41: 1830–1834.

Anson ML, Pader M. 1955. US2785155 A – Extraction of soy protein.

Barakat A, Jérôme F, Rouau X. 2015. A Dry Platform for Separation of Proteins from Biomass-Containing Polysaccharides, Lignin, and Polyphenols. *ChemSusChem* 8: 1161–1166.

Bardeau T, Savoire R, Cansell M, Subra-Paternault P. 2015. Recovery of oils from press cakes by CO_2-based technology. *OCL* 22: D403.

Boye J, Zare F, Pletch A. 2010. Pulse proteins: Processing, characterization, functional properties and applications in food and feed. *Food Res. Int.* 43: 414–431.

Bramsnaes F, Olsen HS. 1979. Development of Field Pea and Faba Bean Proteins. *J. Am. Oil Chem. Soc.* 56: 450–454.

Chabanon G, Chevalot I, Framboisier X, Chenu S, Marc I. 2007. Hydrolysis of rapeseed protein isolates: Kinetics, characterization and functional properties of hydrolysates. *Process Biochem.* 42: 1419–1428.

Gonzalez-Pérez S, Vereijken JM. 2007. Sunflower proteins: overview of their physicochemical, structural and functional properties. *J. Sci. Food Agric.* 87: 2173–2192.

Guéguen J. Pea proteins: new and promising protein ingredients. *Industrial proteins* 8(1): 6–8.

Guéguen J, Lemarié J., Composition, structure et propriétés physiologiques des protéines légumineuses et d'oléagineux. In: Gordon B, ed. Protéines végétales. Paris (France): Lavoisier Tec et Doc, 1996, pp. 80–119.

He XQ, Xiao HQ, Li YZ. 2012. Study on enzymatic hydrolysis and products properties of pea. *Science and Technology of Cereals, Oils and Foods*, 05.

Hughes GJ, Ryan DJ, Mukherjea R, *et al*. 2011. Protein digestibility-corrected amino acid scores (PDCAAS) for soy protein isolates and concentrate: Criteria for evaluation. *J. Agric. Food Chem.* 59: 12707–12712.

Huisman J, Tolman GH. 1992. Antinutritional factors in the plant proteins of diets for non-ruminants. *Recent Advances in Animal Nutrition* 68: 101–110.

Karaca AC, Low N, Nickerson M. 2011. Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Res. Int.* 44: 2742–2750.

Klamczynska B, Czuchajowska Z, Baik B-K. 2001. Composition, soaking, cooking properties and thermal characteristics of starch of chickpeas, wrinkled peas and smooth peas. *Int. J. Food Sci. Technol.* 36: 563–572.

Malabat C, Atterby H, Chaudhary Q, Renard M, Gueguen J. 2003. Genetic variability of rapeseed protein composition. In: Proceedings of the 11th international rapeseed congress, pp. 205–208.

Mandal S, Mandal RK. 2000. Seed storage proteins and approaches for improvement of their nutritional quality by genetic engineering. *Curr. Sci.* 79: 576–589.

Messens W, Van Camp J, Huyghebaert A. 1997. The use of high pressure to modify the functionality of food proteins. *Trends Food Sci. Technol.* 8: 107–112.

Millward DJ. 1999. The nutritional value of plant-based diets in relation to human amino acid and protein requirements. In: The Proceedings of the Nutrition Society, Vol. 58, pp. 249–260.

Mokni Ghribi A, Maklouf Gafsi I, Sila A, *et al*. 2015. Effects of enzymatic hydrolysis on conformational and functional properties of chickpea protein isolate. *Food Chem.* 187: 322–330.

New Zealand Feed Manufacturers Association. 2008. Faba Beans: An Alternative Protein Source for Animal Feeds. *Pork R&D-Brief* 05: 48–50.

Nishinari K, Fang Y, Guo S, Phillips GO. 2014. Soy proteins: A review on composition, aggregation and emulsification. *Food Hydrocolloids* 39: 301–318.

Osborne TB. 1924. The vegetable proteins. London: Longmans, Green.

Paredes-Lopez O, Ordorica-Falomir C, Olivares-Vazque MR. 1991. Chickpea Protein Isolates: Physicochemical, Functional and Nutritional Characterization. *J. Food Sci.* 56: 726–729.

Pelgrom PJM, Vissers AM, Boom RM, Schutyser MAI. 2013. Dry fractionation for production of functional pea protein concentrates. *Food Res. Int.* 53: 232–239.

Pelgrom PJM, Berghout JAM, van der Goot AJ, Boom RM, Schutyser MAI. 2014. Preparation of functional lupine protein fractions by dry separation. *LWT. Food Sci. Technol.* 59: 680–688.

Phan L, Brown H, White J, Hodgson A, Jessop PG. 2009. Soybean oil extraction and separation using switchable or expanded solvents. *Green Chem.* 11: 53–59.

Plietz P, Drescher B, Damaschun G. 1987. Relationship between the amino acid sequence and the domain structure of the subunits of the 11S seed globulins. *Int. J. Biol. Macromol.* 9: 161–165.

Popineau Y, Huchet B, Larré C, Bérot S. 2002. Foaming and emulsifying properties of fractions of gluten peptides obtained by limited enzymatic hydrolysis and ultrafiltration. *J. Cereal Sci.* 35: 327–335.

Rackis JJ, Sessa DJ, Honig DH. 1979. Flavor problems of vegetable food proteins. *J. Am. Oil Chem. Soc.* 56: 262–271.

Rodriguez Patino JM, Miñones Conde J, Millán Linares H, *et al*. 2007. Interfacial and foaming properties of enzyme-induced hydrolysis of sunflower protein isolate. *Food Hydrocolloids* 21: 782–793.

Rosenthal A, Pyle, DL, Niranjan K. 1996. Aqueous and enzymatic processes for edible oil extraction. *Enzyme Microb. Technol.* 19: 402–420.

Russin TA, Boye JI, Arcand Y, Rajamohamed SH. 2011. Alternative Techniques for Defatting Soy: A Practical Review. *Food Bioprocess Technol.* 4: 200–223.

Schutyser MAI, van der Goot AJ, 2011. The potential of dry fractionation processes for sustainable plant protein production. *Trends Food Sci. Technol.* 22: 154–164.

Sejr Olsen H, Hinge Andersen J. 1978. The estimation of vicine and convicine in fababeans (*Vicia faba* L.) and isolated fababean proteins. *J. Sci. Food Agric.* 29: 323–331.

Shewry PR, Napier JA, Tatham AS. 1995. Seed storage proteins: structures and biosynthesis. *The Plant Cell* 7: 945–956.

Sicaire A-G, Abert Vian M, Fine F, Carré P, Tostain S, Chemat F. 2015. Experimental approach versus COSMO-RS assisted solvent screening for predicting the solubility of rapeseed oil. *OCL* 22: D404.

Ventureira J, Martinez EN, Anon MC. 2010. Stability of oil: Water emulsions of amaranth proteins. Effect of hydrolysis and pH. *Food Hydrocolloids* 24: 551–559.

Ventureira JL, Martínez EN, Añón MC. 2012. Effect of acid treatment on structural and foaming properties of soy amaranth protein mixtures. *Food Hydrocolloids* 29: 272–279.

Vilkhu, K, Mawson R, Simons L, Bates D. 2008. Applications and opportunities for ultrasound assisted extraction in the food industry – A review. *Innov. Food Sci. Emerg. Technol.* 9: 161–169.

Zhao X, Hou Y. 2009. Limited hydrolysis of soybean protein concentrate and isolate with two proteases and the impact on emulsifying activity index of hydrolysates. *African J. Biotechnol.* 8: 3314–3319.

Cite this article as: Denis Chéreau, Pauline Videcoq, Cécile Ruffieux, Lisa Pichon, Jean-Charles Motte, Saliha Belaid, Jorge Ventureira, Michel Lopez. Combination of existing and alternative technologies to promote oilseeds and pulses proteins in food applications. OCL 2016, 23(4) D406.