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## Increasing plant protein attractivity to develop the offer to consumers

Guy Della Vallea\*, Catherine M.G.C. Renardb

- <sup>a</sup> INRAE UR-1268 Biopolymères Interactions et Assemblages (BIA), 44316 Nantes, France
- <sup>b</sup> INRAE, Division TRANSFORM & Qualiment, 44316 Nantes, France guy.della-valle@inrae.fr

Developing the supply to consumers is essential to increase the consumption of protein-rich legume seeds. In this perspective, this article examines five possible tracks which encompass different ways of processing legume seeds into food: applying hydrothermal treatments to improve their suitability for traditional consumption, developing new foods from protein ingredients through fermentation, adapting traditional and exotic recipes, combining cereals and legumes in traditional processes, and developing new products using more recent technologies. For each one, the available knowledge is reviewed in order to identify main research prospects. Clearly, a better understanding of the structural modifications of foods, at the different levels of organization of the material, during the process, would allow industrial actors to better meet consumer expectations. In addition, the production of ingredients based on pulses makes it possible to consider substitutes for food additives, in line with Clean Label claims, for minimally processed and healthier products. Certainly, research tracts that would contribute to a greater consumption of plant-based protein foods, require an interdisciplinary and integrative approach. However, regardless of the processing route considered, the production of pulse-based foods relies on a stable supply and selection of plant raw materials.

#### 1. Introduction

There is a need to decrease the part of animal protein in our diet, which means to increase our consumption in plant proteins, i.e. in cereals and legumes. Moreover, legumes bring at least, twice as much proteins and dietary fibres than cereals, and they are complementary to cereals, in terms of essential amino-acids, e.g. lysin and cysteine, respectively. Since cereals already take a significant share, the offer of foods based on legumes, or, at least enriched in legumes, should be improved. Actually, in spite of their agronomic interest and nutritional advantage, legumes are not sufficiently consumed, at least in Europe, especially in France where the daily individual consumption has decreased from 20 to 5 g in 80 years. Deficient sensory image, lack of convenience and so-called uncomfortable digestion are among the main inferred drawbacks (Melendez-Ruiz et al.2019), which have to be circumvented. In this purpose, five main possibilities may be envisioned according to the (food, process) target, in terms of tradition or novelty: (1) processing to improve the use properties of traditional legume foods, (2) developing new foods using plant proteins as ingredients through traditional technology such as fermentation, (3) adapting traditional and exotic recipes to local species (4) adapting processes to mix legumes and cereals in traditional foods, (5) using recent technology such as extrusion, to develop new foods, like meat-analogs. Whatever the possibility, processing will modify the complex structure of the grain, at the different levels of matter organization (Fig.1). At a macroscopic level, the grain can be schematically presented by a set of envelopes (inc. pericarp) and starchy albumen (or cotyledon). The envelopes of legume grains are less permeable than those of cereals, because they are coated with wax. The storage components (starch, proteins, lipids) forming the cotyledons are made up of

regular cells separated by cell walls constituted mostly of hemicelluloses (arabinoxylans for cereals, but also pectins and galactans for legumes).

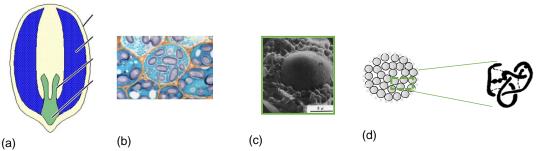


Figure 1: Legume seed structure from macroscopic to protein molecular scale: (a) dicotyledon seed and its organs, (b) pea cotyledon optical micrograph (300 μm) walls (orange), protein bodies (turquoise) and starch granules (grey); (c) MEB image (30μm) of starch granule surrounded by protein bodies; (d) schemes of protein bodies (200nm) and legumin coil. Micrographs from INRAE.

Inside legume cells, starch semi-crystalline granules are embedded in a matrix of protein bodies. At molecular level, starch is made of 30% or more of amylose, the linear component, whereas protein molecular composition may vary according to the legume species, legumin (11S) and vicilin (7S) being the major components. Given these different levels of matter organization, in this article, we will strive to follow a material science approach to define structure-process-functions relationships that describe the five main tracks to design legume-based foods, and to draw the consequent prospects for research.

#### 2. Hydrothermal modifications of seeds

One of the main limits of the traditional home consumption of pulses (pea, lentils, beans, etc) is the time required for their preparing, necessary to extract anti-nutritional factors by soaking, and to favor water absorption. Some food companies are tackling this issue by implementing a hydrothermal pre-treatment to the grains so that home cooking takes less time. The grain is pre-cooked at a temperature  $T_c$  high enough to favour grain hydration and initiate the swelling of starch granules. The value of  $T_c$  depends of the water content of the grain, and it may be predicted from the knowledge of state diagrams (Fig.2), which represent the variations, with the moisture content, of the state change temperatures of the main components (protein, starch), as determined by DSC<sup>1</sup>.

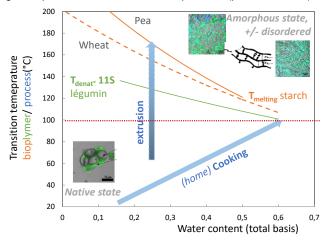


Figure 2: State diagram of legume biopolymers, with two processing paths, and micrographs (CSLM², 100μm, INRAE) showing the composite starch (grey) / protein (green) morphology, and scheme of denatured protein, above transition temperature. Arrows indicate two possible hydrothermal processing pathways.

Lefevre et al. (2021) showed on legume flour fractions, enriched in each of the constituents (starch/proteins) by grinding, hulling and turbo-separating, that on the one hand the melting temperature of starch varies between

<sup>&</sup>lt;sup>1</sup> Differential Scanning Calorimetry

<sup>&</sup>lt;sup>2</sup> Confocal Scannning Laser Microscopy

88°C and 130°C, for water contents (in total wet basis) between 80% and 30%, with no significant difference between lentils (L), beans (B) and chickpeas (CP). On the other hand, the protein denaturation temperature depends on the botanical origin of the seed, it can vary from 98°C (L), 100°C (B), 112°C (PC) at 80% water, to 131 °C (L), 128°C (H) and 138°C (PC) for a water content of 30% (Lefevre et al. 2022). These temperature values differ from those represented in Fig. 2, obtained from the pure constituents, here starch and legumin (11S), extracted from the flours by the wet process.

Indeed, as heat diffusivity is much larger than water diffusivity, about 10<sup>-7</sup> to 10<sup>-10</sup> m²/s respectively, the hydration of the grain is the limiting mechanism and, at least, as crucial for grain cooking as for its germination (Miano & Duarte Augusto, 2018). Although hydration kinetics can be controlled and modelled simply, the role of the different structural entities (cell wall, endosperm) on water diffusion is still unknown and needs further investigation to ensure the control of the (pre-)cooking process. Both, for cereals and legumes, the accessibility of water by capillarity is promoted by different organs (germ, hilum). And the storage components (starch, proteins, lipids) forming the cotyledon are assembled in regular cells with spaces conducive to the circulation of water, whereas walls may act as barriers. This last hypothesis may explain why lentil batches less suitable for canning, have faster hydration and lower polysaccharides content than batches more adapted to canning (Ghanem et al., 2023). However, the contribution of each component and the precise mechanisms of water absorption and diffusion still need to be ascertained for a better control of hydrothermal treatments. An accurate characterisation of plant raw materials would also contribute to tackle their variability and guide their selection from the field.

#### 3. Use of plant proteins as ingredients for fermented foods

Producing plant protein as ingredients in foods appears as a relevant alternative to the use of additives, not only for Clean Label necessity, but also to operate protein functionality in structuring, gels, emulsions and foams. However, besides the functionality, the yield and purity of the extracted protein fraction, may vary greatly according to the process (dry/wet), the sustainability of which also needs to be taken into account (Rivera et al., 2022). Whilst dehulling is a prerequisite for all processes, concentrates, obtained from dry process, contain about 50% proteins, whereas isolates, obtained from wet process, have a [% P] close to 70%. So there are still trade-offs to be sought to implement mild and sober processes for extracting ingredients with optimal functionality.

Indeed, legume protein-based ingredients are good candidates for the design of new healthy and sustainable food products similar to dairy foods. The use of legume protein-based ingredients can however be hampered by sensory defects: unpleasant odor and taste, often linked to a "green" or "beany" aroma, particularly attributed to aldehydes, alcohols and ketones derived from the action of lipoxygenase. Among negative factors, flavors such as bitterness, or astringence, can be brought by sapid compounds such as saponins and phenolic compounds (isoflavones, flavonols, tannins, hydroxycinnamic acids, etc.) (Sharan et al., 2021). So clearly the interactions of proteins with these secondary metabolites is a scientific issue.

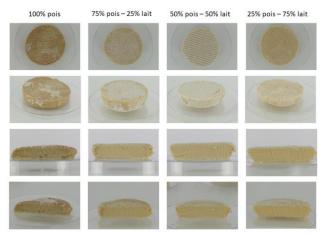


Figure 3: examples of "camenvert" elaborated from pea isolates (PPI), lower PPI content leading to highest consumer sensory acceptance (photographs from Saint Eve et al., 2021).

Actually, few studies have been devoted to the fermentation processes of new legume based-foods in comparison to microbial species involved in dairy fermentation processes, which are quite well known. Indeed, pea proteins have been shown able to create gels. Lactic fermentation has been found to improve pea protein aroma by reducing or masking "green-note" (or "beany") off-flavours. So, legume proteins may provide a wide range of nutrient sources for a variety of proteolytic microorganisms, they can be mixed or not with milk and subjected to fermentation with various microbial consortia to affect the content of aroma compounds, including those responsible for "beany" off-flavours, with consequences on consumer perception (Ben- Harb et al., 2021). Recently, these works have led to the development of a legume-protein based cheese analog, namely « camenvert » (Fig.3), and to the incorporation in yoghurts and replacing egg-white in meringues. Sensory studies have demonstrated consumer acceptance for these new foods, but the supporting structure-function relationships are still unknown, and, especially, the interactions of microbial consortia together with adaptive microbial metabolism should be further investigated by combining sensory, biochemical, physiological and meta-omic analyses, to better control their growth mechanisms in plant protein gels and foams.

#### 4. Operating the diversity of traditional and "exotic" recipes

The traditional processes developed in Southeast Asia such as fermentation, for example tempeh from soy, can be an interesting lever to overcome the sensory defects and develop food products with diversified properties. There is also a wide variety of soy foods, simple gels (tofu), fermented whole seeds (natto) or fermented gels (furu, misozuke, chao, etc.), with typical flavors and aromas. In the case of soy derivatives, the mode of preparation influences in particular the isoflavone content (Fernandez-Lopez et al. 2016). Ultrafiltration, for example, reduces the isoflavone/protein ratio from 6.5 to 1.2. The fermentation of tonyu by microorganisms equipped with α-galactosidases reduces the presence of oligosaccharides (Hati et al. 2014), which opens the way to their selection for the development of soy foods such as "yoghurt", "cheese" or drinks. These "exotic" foods, often based on soy, fermented or not, can also be adapted for other legumes species, a purpose for which a gastronomy creative approach can be developed. Indeed, starting from recipes, model foods (i.e. seeds, flours, dough, mash, soup, etc) can be proposed to determine texture-structure relationships. Then, once the process studied, not to say modelized, the recipe can be extended to a wider market, from home-cooking to canteens and restaurants. Besides usual food science and engineering issues, this approach requires first to integrate the "chefs" 'know-how. It can be started, for instance, by accurately delineating the concept of culinary quality - or culinarity - used in gastronomy (This, 2005), as illustrated by conceptual map in Fig.4, showing that measuring this property is still a scientific challenge.

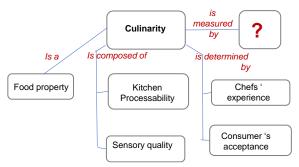


Figure 4: Example of conceptual map used to define a professional concept, here culinarity. Note that several maps can be implemented in an electronic knowledge book, containing more detailed knowledge in specific sheets (see Suciu et al, 2021 for an illustration in food process modelling.)

#### 5. Cereal-legumes mixes

Agronomical research has shown the potential advantages of cereal-legume intercropping and mixed culture, so there is an interest to promote the use of mixed flours, that would enable the design of products fortified in proteins. However, enriching cereal products with legumes modifies their processability and properties, especially rheological properties. Moreover, the addition of legume flours induces structural changes in the processed foods. This has been illustrated in the case of foods initially processed from cereal flours (Monnet et al., 2019; Bresciani et al., 2019) extruded snacks (Kristiawan et al., 2021), pasta (Berrazaga et al., 2020), biscuits, cakes (Assad-Bustillos et al., 2020; Monnet et al., 2022) and bread (Atudorei et al., 2020). These works also underline the role of structural changes, resulting in different textures. For instance, increased

cooking loss and decreased pasta resilience were found in pasta enriched with legumes, due to weaker interprotein bonds, diluting gluten network (Fig.5), which can be circumvented by higher drying temperature, in order to cross-link proteins (Laleg et al., 2017).

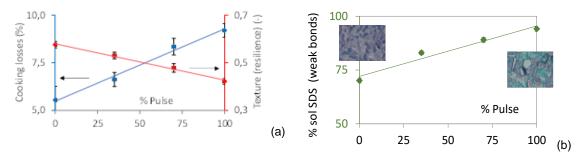


Figure 5: Properties and structure of pasta enriched with pulses: (a) culinary (cooking losses and texture) (b) hydrogen and/or hydrophobic bonds, and corresponding microscopy images (300µm) with turquoise stained proteins (from Laleg et al., 2017).

So, to design and process legume-enriched cereal products, tools and concepts such as biopolymers state diagrams may be helpful to predict the gelatinization and the denaturation temperatures of starch and protein, respectively, as a function of the water content (see Fig.2). By doing so, the necessary adjustments of the process can be inplemented, for example to compensate for the dilution of the gluten network, which is typical in the case of pasta and bread, or to improve batter aeration, in the case of cakes, or to modulate melt viscosity and expansion in the case of snack dry extrusion (Monnet et al., 2019; Jebalia et al., 2022). Clearly, adverse sensory effects hamper the consumption of these foods, but it can be balanced by relevant aromatic formulation, and therefore they should not be considered as a definitive obstacle.

#### 6. More recent technologies: protein fibration by wet extrusion

The demand for plant-based « meat analog », as an alternative food, illustrates the use of technology to develop new foods. Meat analogue, relatively cheap compared to animal meat, is a purely plant-derived product made mainly of plant protein isolates. It is developed into a texture similar to that of real meat using extrusion-cooking process at high moisture content (>50%, total basis) (Noguchi, 1989). The mechanisms of texturizing plant protein through this process encompass a first stage at temperature > 120°C, where proteins are denatured by heat and by the shearing force inside the extruder barrel. Then, the formation of a laminated, multilayer or fibrated, gel structure begins in the cooled die (< 30°C), either due to flow orientation, or to temperature gradient, inducing a phase separation between protein and hydrophilic compounds (Ubbink & Muhialdin, 2022). The texturized products obtained from wet extrusion process are typically not dried but refrigerated, and can be directly consumed. So, structure, and thus texture, can be controlled by adapting extrusion parameters, but still, much work is required to improve sensory properties and checking that denaturation does not decrease protein bioavailability and essential amino-acids content.

#### 7. Conclusions

These five tracks should be based on stabilized upstream selection and sourcing. They all require a better understanding of the changes of food structure at different levels of matter organization during processing, so that manufacturers can better satisfy consumers' expectations. The production of legume-based ingredients can also be envisioned to substitute food additives, in agreement with the Clean Label claim. In addition, nutritional impact on protein digestion should be studied, whereas systemic modelling should help to integrate data and knowledge for various sources. Clearly, the main research directions to improve consumption of plant-based protein foods require an interdisciplinary effort and an integrative approach.

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