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Review

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Highlights - 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point)

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Abstract (224)

Germination is a traditional process and a re-emerging trend in healthy foods, generating an increase in scientific research on their nutritional traits and phytochemical contents. Pulses are essential in diet being rich in protein, complex carbohydrate and vitamins, and constitute an excellent complement to cereals. This review examines the physiological and biochemical changes during the germination process in pulses, taking into consideration the genotype, environmental conditions, and hormone control. Furthermore, proteins, carbohydrates, minerals, vitamins, and antinutritional compounds of pulses are described and the impact of germination process on their values. The earlier germination process needs a further process, such as cooking, drying, or roasting. The final product of the longer germination process (sprouting) is considered a ready-to-eat food. To this end, impacts of food processing, such as soaking and cooking, in nutritional values are also evaluated to complete the nutritional analyses of germinated pulses. The association of soaking, germination, and cooking increases the nutritional values of pulses by increasing protein/starch digestibility and vitamins content and by decreasing antinutritional compounds, such as phytate, protease, and α-amylase inhibitors. The final plant-based product allows versatility in formulation to produce novel food products and/or ingredients with a better nutritional content. This can encourage the scientific community, industry, and government to invest in research and development to increase plant-based food, to replace other products or to develop new ones.

Keyword: Pulses, Seeds**,** Germination, Processing, Food, Nutrition

1. Introduction (1053)

Legumes belong to the *Fabaceae* family (a.k.a. *Leguminosae*) and represent one of the largest groups of angiosperms (LPGW, 2013, 2017). According to Food and Agriculture Organization (FAO) and Codex Alimentarius Commission, dry legumes (*i.e*. pulses), used for feed and food, are distinguished from leguminous oil seeds (*e.g.* soybeans, groundnuts) by their low-fat content and can be called pulse (FAO & Alimentarius, 2019). By contrast with major cereals (*i.e.* rice, wheat, and corn), pulse breeding programs have been less dynamic over the last century, resulting in high environmental sensitivity and important fluctuation in the yield of these crops even for modern varieties. *Medicago truncatula* (Barrel Medic) has become a model legume due to the completion of its genome sequencing over the past decade (Young *et al.,* 2011; Tang *et al.,* 2014). It is demonstrated that the *M. truncatula* genome has high synteny with pulse crops. The genetic diversity of pulses can be used to optimize nutritional value and yield, by reducing its biotic and abiotic stress (Kumar *et al.,* 2011, 2019; Pratap *et al.,* 2021). Pulses represent a major source of plant proteins for human or animal consumption. These crops are particularly relevant to promote sustainable agriculture and agroecological practices due to their ability to fix atmospheric nitrogen by endosymbiosis with rhizobial bacteria and their low requirement on fertilizers (Calles *et al.,* 2019). Furthermore, introducing pulses into cropping systems as a source of diversification with cereals or oilseeds (*e.g.* intercropping, mixed-cropping, co-culture systems) can increase the economic and environmental performances of subsequent crops (Preissel *et al.,* 2015; Reckling *et al.,* 2016; Ditzler *et al.;* 2021).

The worldwide production of pulse was estimated at 92.4 million MT in 2018 with a remarkable increase of 36 million MT over the period 1998-2018 (Rawal & Navarro, 2019). chickpeas (*Cicer* genus), beans (*Phaseolus* and *Vigna genus*), lentil (*Lens genus*), pea (*Pisum genus*) and vetch (*Vicia* genus) are the main cultivated pulses. The world leaders in pulses production and exportation are India and Canada. In Europe, legume production area declined over the last 5 decades from 5.8 million ha in 1961 (4.7% of the arable area) to 2.0 million ha in 2014 (1.6% of the arable area). This decrease is due to several factors, such as the availability and low costs of synthetic nitrogen fertilizers relative to farm product prices, imported protein feed, such as soybean, intensification of livestock farming, and political support for cereal production due to their value on the market (Voisin *et al.,* 2014). However, the increasing knowledge in Europe that legumes improve the agronomic performance of farming systems and provide protein-rich food and feed, has recently heightened the political debate to increase the productivity of these crops (Watson *et al.,* 2017). For instance, between 2015 and 2019, the production of lentil and pea (*Pisum genus*) has increased by 46% and 22%, respectively (FAOstat, 2020). Pulses have large importance for livelihoods in southern countries, especially in Africa. Their production and process are responsible for the main source of income for the farmers, as they fetch a good price in local and international markets (Snapp, 2018). The most produced legumes in Western Africa are the cowpea (*Vigna unguiculata*; 7 M tons of production), Bambara-bean (*Vigna subterranea*; 149 ktons of production), and chickpea (*Cicer arietinum*; 2.2 ktons of production) (FAOstat, 2019). Cowpea is one of the most economically important pulses for farmers which provides an affordable economic source and has a major contribution to the householder's food security (Paliwal *et al.*, 2020).

From a nutritional point of view, pulses have great health benefits, being excellent suppliers of complex carbohydrates, proteins, fibers, minerals, and essential vitamins, such as those of the B complex (Mudryj *et al.,* 2014; Bojňanská *et al.,* 2016). Their nutritional benefits can contribute to the diversification in the diet in high-income country (HIC) and help to eradicate hunger and malnutrition in low and middle-income country (LMIC) (Considine *et al.,* 2017). However, pulses have some antinutritional factors, such as tannins, phytic acid, oligosaccharides, lectins, protease, and amylase inhibitors which can impact their nutritional

value (Hall *et al.*, 2017). In the history of plant domestication and plant breeding for food purposes, the biochemical composition of pulses has been strongly oriented to avoid concerns about antinutritional effects and toxicity for the end consumer. Biological processes such as germination or fermentation and physical processes, such as soaking and cooking can impact positively the taste and the nutritional value of pulse, by increasing the content of vitamins and also by decreasing the antinutritional factors (Kumar *et al.*, 2021a). Pulses are usually consumed after soaked or cooked. However, germination is also a traditional process that usually takes place at home and is a re-emerging trend in healthy foods. The earlier germination process needs a further process, such as cooking, drying, or roasting. The final product of the longer germination process (sprouting) is considered a ready-to-eat food (Bresciani & Marti, 2005). In the diet-related literature, some semantic mistakes were observed about the wording associated with germination terms leading to confusion between germination *sensu stricto* and seedling growth.

The germination of pulses has been reviewed in recent papers (Gan *et al.*, 2017; López-martínez *et al.*, 2017; Ohanenye *et al.*, 2020). The previously mentioned reviews describe the nutritional and/or antinutritional aspects of pulses and/or the biological germination process including seedling growth and plantlets properties. The present review aims to clarifies the changes of the nutritional and antinutritional features driven by the metabolic transitions during germination focusing on seed biology (including dry, germinating and germinated stages) from plant physiology and food process point of view. Therefore, seed structure and the biological germination process will be described, with particular analyses about drying resistance, seed vigor, dormancy, and imbibition/germination conditions. Nutritional and antinutritional aspects will be analysed by their metabolic pathways, and by the food process impacting their contents in pulse. Currently, there is no review in the literature that simultaneously includes a large description of germination process and the impact of food process on the nutritional and antinutritional compounds in pulse. Others reviews were interested in the germination process and its nutritional impacts, but mainly for cereals (Hübner & Arendt, 2013; Omary *et al.*, 2012; Singh *et al.*, 2015; Benincasa *et al.*, 2019). Therefore, this review is a pioneer in the subject and, it thus promotes the sharing of information for the scientific community, industry, and government.

2. Germination biological process (238)

The germination of pulses is a traditional food process, which has been commonly practiced in the Orient for centuries. However, in western countries this practice has been accepted only in recent years, perhaps due to increasing recognition of its flavor and nutritional benefits (Prodanov *et al.*, 1997). The germination process causes important changes in the biochemical, nutritional, and sensory characteristics of pulses. For example, starch and protein *in vitro* digestibility increases, fats are broken down and the content of water-soluble vitamins, and amino acids increases. In addition, it reduces the amount of antinutritional factors, such as phytic acid, α-galactosides, trypsin inhibitor, and lectins (Prodanov *et al.*, 1997; Vidal-Valverde *et al.*, 2002). Germinated pulses can be used to produce new food with a high nutritive value, such as legume flour and isolate protein that can be incorporated in food industry. However, germination process has its limits. The pulse need to be placed in a growth-conductive environmental conditions, with an adequate temperature, relativity humidity, oxygen pressure and light, which for an industrial scale, and further consumption of germinated pulses requires specific equipment with a fine control of environmental conditions (Mayer & Poljakoff-Mayber, 1982; Prodanov *et al.*, 1997). For this purpose, it is important that the scientific community works on the optimization of germination parameters, so the socio-economic actors,

the industrialists, and the society can benefit from the advantages of the germination process and consequently increase the consumption of germinated pulses.

a. Seed Structure (322)

For a better understanding of germination process, it is important to describe the seed structure. In angiosperm, seed contains three compartments namely the zygotic diploid embryo, the triploid endosperm, both originate from the double fertilization event, and the testa (seed coat), derived from maternal tissue (Linkies *et al.*, 2010; Baroux and Grossniklaus, 2019). Pulses are mainly non-endospermous dicotyledon seeds as the endospermic tissue is completely consumed by the growing embryo during the developmental fates and is almost missing in dry mature seed (Yan *et al.*, 2014). The embryo is the result of the fertilization of the egg cell in the embryo sac by one of the male pollen tube nuclei. Thus, seed storage compounds are mainly deposited in the cotyledons of the embryo that account for almost all of the seed mass. Most of the pulses comprise the embryo with massive and reserve rich-cotyledons (1), the embryonic axis (2), the testa (3) or seed coat, the hilum (4), and micropyle (5) (Figure I). The seed coat is made up of the integument around the ovule and protect the seed from the external environment. The protective physical and chemical nature of the seed coat can discourage insect predation, provide water-retaining barrier, restrict oxygen uptake, and exchange of gases between the embryo and environment (Bewley & Black, 1994). Seed filling, acquisition of desiccation tolerance, dormancy/germination, and nutritional quality are mainly under the control of zygotic genes and influenced by the mother plant environment (Scheelbeek *et al.*, 2018). During seed development of *M. truncatula* many proteome and transcriptome changes were observed. Genes involved in carbohydrate metabolism, glycolysis, and amino acid metabolism are among the most regulated. In young developing seeds, starch transiently genes accumulate and later disappears, probably due to a donation of carbon skeletons for the synthesis of other reserve compounds during seed development. This can explain the low content of endosperm in total seed mass (Gallardo *et al.*, 2007; Yan *et al.*, 2014).

b. Seed germination process (1598)

In the scientific literature, the term “germination” may vary from a “physiological/biologist” or “processing/technical” point of view. For a plant physiologist, the germination process begins with water uptake by the seed and ends with the elongation of the radicle. After this phase, germination process is over and the seedling growth is marked by post-germination events such as mobilization and dilution of major storage reserves (Rajjou *et al.*, 2012). In this case, germinated/sprouted pulse maintains the properties of the seed, which needs further processing, such as cooking, drying, or roasting before consumption (Benincasa *et al.*, 2019). For pulses, the germination process marks the end of seed quiescence, a rest state established by the seed at maturity, with low moisture content (5-15%) and metabolic activity almost stagnant (Bewley & Black, 1994). At the end of maturation program, seeds have a major loss of water leading to a dry period called desiccation (Angelovici *et al.*, 2010). Seed maturation concerns the end of seed development including the period of reserve accumulation with a reorganization of metabolism and synthesis of storage compounds (*i.e.* starch, proteins, and lipids), the acquisition of desiccation tolerance and both germination and longevity potential. Germination potential (seed vigor) is a complex physiological trait that ensures a rapid and uniform radicle protrusion and seedling establishment under a wide range of environmental conditions *(*Rajjou *et al., 2012;* Wu *et al.*, 2017). The life span of seeds (*i.e.* longevity) is an important aspect for the quality of the seed and depends on genotypes and storage conditions (Rajjou *et al.*, 2012). For instance, seeds that are stored under high-temperature and high-moisture conditions will lose more quickly their germination capacity and their viability over time. Seed lots with a high-

vigor are of paramount importance for proper germination, seedling emergence, increase crop yield, and reduce the cost of agriculture production, by prolonging their storage time (Wu *et al.*, 2017). Seed storage compounds, dynamic regulation of gene expression, and hormone pathways (especially ABA) influence seed vigor (Wu *et al.*, 2017). Reactive oxygen species (ROS), lipid peroxidation, loss of cellular membrane integrity, enzyme inactivation, weak energy metabolism, and DNA degradation contribute for seed deterioration during storage (Wu *et al.*, 2017; Kumar *et al.*, 2021b). Before the germination process begins, seeds can be in a physiological state called dormancy, in which the seed will not germinate even under optimal conditions (Rajjou *et al.*, 2012). Seed dormancy provide ecological advantages by adjusting progeny dispersal to the favorable growth period but represent a major drawback in agriculture since it limits the rapid and uniform germination. Therefore, this trait was targeted by plant domestication and modern breeding programs in order to remove seed dormancy in crops including pulses (Ladizinsky, 1987). The duration of the dormancy state is extremely variable and is determined genetically and influenced by environment of the mother plant (Prodanov *et al.*, 1997; Penfield & MacGregor, 2017). The germination can be prevented either because the embryo is limited by its surrounding structures (coat-enhanced dormancy) or by the embryo dormancy (Bewley, 1997). In pulses, it was largely documented that dormancy is mainly due to seed coat water-impermeability and hardness and is define as a physical dormancy (Baskin *et al.*, 2000; Smýkal *et al.*, 2014). The seed coat is an important source of phytohormones for the developing seed (Verdier *et al.*, 2013). The dormancy of the seed and the inhibitory effect on the germination process are triggered by the phytohormone abscisic acid (ABA), which is responsible to allow the maturation process to be sustained (Rajjou *et al.*, 2012; Ohanenye *et al.*, 2020). The seed is considered as nondormant when ABA metabolism/signaling is deficient (Bewley, 1997). To begin the germination process, the seed must be placed in growth-conductive environmental conditions, which means an adequate composition of the soaking and rinsing media, a suitable temperature and composition of the gases in the atmosphere, and light (Mayer & Poljakoff-Mayber, 1982; Prodanov *et al.*, 1997). Each plant species and variety have its requirement for these conditions which is determined by their genome and their physiological state (*i.e.* dormant, nondormant, aged), and the interactions between genotype and the environmental (Bewley, 1997). The germination process begins with the imbibition of water by the quiescent and non-dormant dry seed and ends with testa rupture, cell elongation of the embryo, which is marked by the radicle protrusion (Bewley & Black, 1994; Weitbrecht *et al.*, 2011). Post-germination events include the mobilization of the major storage reserves, cell division, nutrient dilution (*e.g.* carbon, nitrogen, sulfur, phosphorus), and growth of the seedling to the surrounding area. The germination process is a three phases water uptake by the mature dry seed (fig. II). Phase I is a rapid (i.e. few hours) initial water uptake where the solutes of the seeds will reach out to the medium and cell repair systems will be activated. This process is determined by the composition and the permeability of the seed coat. In pulse, the testa is largely impermeable to water and can restrict the metabolism and growth of inner tissues, which can impact the germination process. However, when placed in appropriate environmental conditions, water enters through the micropyle and the area around the hilum (fig. I and III) (Bewley, 1997; Turner, 2010). Imbibition causes a swelling effect in the seed, which produces an imbibition pressure and changes in osmotic. Phase II is marked by a limited water uptake (lag phase) and strong metabolic reactivation (Benincasa *et al.*, 2019). It is influenced by protein turnover and by the synthesis/degradation of regulatory hormones (Bewley, 1997; Weitbrecht *et al.*, 2011). ABA is a hormone that is rapidly degraded upon imbibition during the early phase of germination. Nine-cis-epoxycarotenoid dioxygenase (NCEDs) and ABA 8'-hydroxylases (CYP707As) are the major enzymes for ABA biosynthesis and degradation, respectively (Weitbrecht *et al.*, 2011). To trigger the germination process, seed must establish a specific catabolism and biosynthesis inhibition to reduce ABA level and synthesize another hormone,

such as the gibberellins (GAs), which is essential for cell elongation and inhibit ABA actions. ROS also controls germination process and are formed in the dry state of the seed *(Rajjou et al.*, 2012; Weitbrecht *et al.*, 2011). ROS is involved in the regulation of the ratio ABA/GA by promoting ABA catabolism and GA biosynthesis (Rajjou *et al.*, 2012). Seed vigor depends on the ABA/GA ratio, and germination can only occur when biosynthesis of GA is preferred (Ohanenye *et al.*, 2020). To this end, hormone regulation and interactions play an important role in determining the physiological state of the seed and in regulating the germination process (Weitbrecht *et al.*, 2011). Other hormones, such as ethylene, brassinosteroids, salicylic acid, cytokinin, auxin, jasmonic acid, and oxylipins, also influence germination (Rajjou *et al.*, 2012; Shu *et al.*, 2016; Ohanenye *et al.*, 2020). Ethylene acts in the late phase of germination, meanwhile, GA is important during the early and the late phase of germination (Weitbrecht *et al.*, 2011). GAs stimulate the synthesis and activities of amylases, proteases, and β-glucanases, resulting in the germination of seeds. Additionally, some important environmental conditions can stimulate germination metabolism, among them nitric oxide gas (NO°) and nitrate (NO_3^-) (Rajjou *et al.*, 2012; Weitbrecht *et al.*, 2011). Throughout germination *sensu stricto* (Phase I and II), from water uptake to radicle protrusion, seeds present a heterotrophic metabolism depending on their storage materials to survive without incoming and residual outcoming of biomass constituents. A further increase in water uptake (phase III) occurs only after germination is completed, where nutrients are released from the reserves and lead them to the embryo (Turner, 2010). All the hormonal modification and nutriments reserves accomplished in phases I and II will be mobilized by the seedling establishment of photosynthetic activity (Botha *et al.*, 1992). This will trigger cell multiplication, DNA synthesis, and elongation of embryonic axes. Radicle extension through the structures surrounding the embryo is the event that terminates germination and marks the beginning of seedling growth (Phase III, post-germination) (Bewley, 1997; Turner, 2010). After seed germination recognizable by radicle protrusion, the embryo will grow up to establish a seedling displaying photosynthetic competence. This late step is characterized by the heterotrophic-to-autotrophic metabolic transition accompanied by dilution and conversion of seed storage compounds into simpler metabolites (*e.g.* sugars, amino acids, and free fatty acids) which will migrate quickly and feed the new cells for the developing tissues (Ha *et al.*, 2017). Thus, from food science and processing point of view, the term "germination" may sometimes include seedling growth (the post-germination process). In this condition, the seedling/sprouts from pulse has no longer seed properties and it can be consumed immediately as ready-to-eat food product. This double meaning for the germination word may confuse the scientific community and induce an obstacle to compare scientific results. For example, is found in literature the impacts of the germination process on the nutritional and antinutritional factors during 24h until 6 days. In these cases, the authors use the word germination to describe two different processes, germination *in situ* and post-germination. As discussed before, both seed germination and seedling growth induce different metabolic pathways and will result in different food properties (seeds and seedling). It is important to highlight that under most conditions shorts period of seed imbibition represent a process suitable for the germination *sensu stricto* whereas a process requiring a long period of seed imbibition is suitable for seedlings production and metabolic specificities of post-germination growth. This misunderstanding of the terminology leads to disrupt research and the knowledge of the real impact of the germination process on nutritional and antinutritional factors. For this purpose, it is important to raise awareness in the scientific community to use the proper terminology to describe a complex process such as germination.

3. Nutritional quality of pulses (180)

Pulses are considered to be essential in diet to cope with nutritional deficiencies and constitute an excellent complement to cereals, which are the largest source of calories in diets around the world (Boye *et al.*, 2010; Snapp, 2018; Bessada *et al.*, 2019). To reduce diet-related disease, it is recommendatory to increase fiber intake without increasing caloric intake (Neacsu *et al.*, 2017; Ohanenye *et al.*, 2020). Plant-based protein diets can help to reach this goal, since they are rich in fiber, and has a lower glycemic index (GI) than cereal (Abeysekare *et al.*, 2012; Mudryj *et al.*, 2012). For instance, *Cajanus genus* represents the pulse with the lowest percentage of GI, being a great food source for diabetes patients. Meanwhile, *Cicer genus* has the highest GI percentage, which can help malnourish patients (Sandhu & Lim, 2008). The Table I provide an overview of macromolecule content for different pulse genus showing that *Lens*, *Vigna*, and *Phaseolus genus* have the highest protein concentration, with an average of 25%, 24%, and 23%, respectively. In addition, they present a low-fat concentration of less than 2%.

a. Protein (1268)

The recommended human protein intake should represent 10 – 30% of the total daily calorie intake (or 0.8 g of protein per kg of body weight) (Ohanenye *et al.*, 2020). The quality of pulse protein is determined by its digestibility, bioavailability, and the composition of essential amino acids (Boye *et al.*, 2010; Gulzar, 2017). The protein content and composition depend on plant genotype and on the seed metabolism activity in each stage of development in interaction with the mother plant environment. Before the quiescent stage, the seed has a high metabolic activity responsible to synthesize storage proteins (Oracz & Stawsja, 2016). Methionine (sulfur-containing amino acid) is the main amino acid synthesized and is responsible for synthesizing storage proteins during seed filling. Met is synthesized in seed coat and in the embryo by enzymes, *e.g.* Met synthase and AdoMet synthetase. The enzymes limitation levels and their compartmentalization in tissues during seed development indicates the metabolic shift from a highly active to a quiescent state as the embryo store all nutriments (Oracz & Stawsja, 2016; Gallardo *et al.*, 2017). Genetic diversity, environmental factors, such as agronomic practices, geographic location, growing season, and different techniques used for protein analysis may cause variation in the protein composition in pulse (Shevkani *et al.*, 2019).

Proteins are complex polymers build-up from different sequences of amino acids (AA) connected through amide-peptide-bonds. There are 20 proteinogenic amino acids classified in human nutrition as essential amino acids (EAA - lysine, isoleucine, leucine, methionine, phenylalanine, valine, threonine, histidine, and tryptophan) and nonessential (NEAA – alanine, aspartic acid, asparagine, glutamic acid and serine) and conditionally essential amino acids (CEAA – arginine, cysteine, glutamine, glycine, proline, tyrosine). In human cells, the EAA are not synthesized, being necessarily an uptake from diet, while NEAA is synthesized (Bessada *et al.*, 2019). The endogenous synthesis of CEAA cannot meet the metabolic needs and additional dietary source is required. It is worth noting that plant proteins contain all 20 AA, however, the AA distribution pattern is less optimum in the plant than in animal food. In pulse, methionine, cysteine, and tryptophan are proportionally lower than would be optimal for human needs. Meanwhile, cereals present a rich amount of some of these EAA (Mariotti and Gardner, 2019). So, mixing pulses and cereals provides a well-balanced EAA intake (Bessada *et al.*, 2019). Table II shows the amino acid composition of different species of pulse. For example, methionine content in cowpea *(Vigna genus*), chickpea (*Cicer genus*), lentil (*Lens genus*), and pea (*Pisum genus*) represent 2.2, 1.1, 0.8, and 1.1% of total protein.

Besides the composition of EAA, protein digestibility is another important factor in pulse protein quality. Different methods have been developed over the years to evaluate the nutritional quality of pulses. The most used methods are *in vitro* digestibility (IVPD) and Protein Digestibility Corrected Amino Acid Score (PDCAAS) (Boye *et al.*, 2012; Bessada *et al.*, 2019). IVPD of uncooked food legumes ranged from 34% to 80% (table III). This value is lower than the IVPD of raw animal products (*e.g.* 92% for beef and 95% for chicken) (Boye *et al.*, 2012). The PDCAAS values ranged from 28% to 80% and reflect the lack of EAA such as methionine and tryptophan, previously mentioned. The low protein digestibility could be explained by the structural conformation of pulses proteins. Pulse has 4 types of proteins with different solubility properties: globulins (salt-soluble), albumins (water-soluble), glutelins (alkali-soluble), and prolamins (alcohol-soluble). Globulins, albumins, and glutelins are storage proteins. Their function is to provide nitrogen, sulfur, and storing carbon units in the seed, which further can be used during germination (Guéguen *et al.*, 2016; Khazaei *et al.*, 2019). Globulins are the major protein type in certain genotypes of pulses and can represent up to 80% of the total proteins (table IV). Globulins have a globular structure and no catalytic activity (Bessada *et al.*, 2019). Legumin 11S and vicilin 7S are the predominant types of globulin. Legumin is a hexameric oligomer of 330-410 kDa and each of the six polypeptides is composed of one basic and one acidic subunit, linked with a disulfide bond (Mession *et al.*, 2012). Vicilin is a trimeric oligomer of 150-190 kDa, containing three monomers of 50 kDa linked by non-covalent bonds (Bessada *et al.*, 2019). The major secondary structures of pulses proteins are β-sheets and α-helix, with small numbers of β-turns and random coils (Yu, 2005). The hydrophobic character of β structures is responsible to enhance the protein-protein interactions and aggregates formation, which leads to lower accessibility to proteolysis sites, and thus reducing their digestibility (Carbonaro *et al.*, 1997). Albumins are the most nutritive proteins in terms of amino acid composition (higher in cysteine and methionine than globulins) (Wang *et al.*, 2003; Bessada *et al.*, 2019; Shevkani *et al.*, 2019). Albumins are considered as proteins with a biological activity which includes metabolic and enzymatic proteins, protease and amylase inhibitors, and lectins (Bessada *et al.*, 2019; Shevkani *et al.*, 2019). Protease inhibitors (trypsin and chymotrypsin inhibitor) are responsible to limit the protein digestibility in pulse. Given their resistance to pepsin and the stomach's acid pH, they reduce the absorption of proteins in the human digestive tract by inhibiting protease activity (Jain *et al.*, 2009; Bessada *et al.*, 2019). Their amount goes up from 1 to 10% of their total protein content. Their main function in seeds is defense-related, which acts against insect attack (Parca *et al.*, 2018). Protease inhibitors also contribute to preserve the metabolic quiescent state and longevity of seeds (Viñegra de la Torre et al., 2019). In human health, protease inhibitors have anticarcinogenic benefits and can control blood clotting (Tiwari *et al.*, 2011; Parca *et al.*, 2018). The content of this anti-nutritional factor is an important parameter to determine the nutritional quality of pulses protein (Bessada *et al.*, 2019). Glutelins account for 10-20% of the total seed proteins (Guéguen *et al.*, 2016; Roy *et al.*, 2010). Other proteins such as prolamins can be found, but in a smaller amount (less than 5%) (Boye *et al.*, 2010; Shevkani *et al.*, 2019). Another possible explanation to increase protein digestibility could be related to the partial decrease of phytic acid and polyphenols (Chitra *et al.*, 1996; Alajaji & El-Adawy, 2006; Bessada *et al.*, 2019). These compounds interact with protein to form complexes, which decreases the solubility of protein and makes them less susceptible to proteolytic attack (Alonso *et al.*, 2000). It should be noted that the IVPD does not measure true digestibility since the digestibility is calculated and does not account concentration and availability of EAA. Some disadvantages of the PDCAAS method have been highlighted: the impact of anti-nutritional factors associated with proteins is not included and the protein digestibility does not represent precisely the bioavailability of each AA (Boye *et al.*, 2012; Nosworthy *et al.*, 2018).

It is noteworthy that during seed germination proteases are both activated and synthesized (Müntz, 1996; Tsuji et al., 2013; Poncet et al., 2015), inhibitory factors (*e.g.* phytic acid and protease inhibitor) are degraded, and thus strengthen the enzyme's activity and increasing protein digestibility (Ghavidel & Davoodi, 2011). Additionally, due to post-translational modifications (PTMs), seed storage proteins (SSP) display a large isoforms diversity involved in the modulation of relevant biological processes (Arc *et al.,* 2011; Mouzo *et al.,* 2018). Notably, a modification in the phosphorylation status of 7S globulin was observed in germinating common bean seeds (López-Pedrouso *et al.,* 2014). Indeed, an increase of phosphorylated isoforms promotes 7S globulin degradation in germinating seeds supporting the idea that phosphorylation-dependent proteolysis could play an important role in the mobilization of seed storage proteins (SSP). Phosphorylation may induce conformational modifications and promote protease accessibility and digestibility of SSP in germinating seeds.

b. Carbohydrates (960)

The amount of carbohydrates in pulse goes up to 60-65% of the seed weight, including mono/di/oligo/polysaccharides (Tiwari *et al.,* 2011). Starch is one of the most abundant plant polysaccharides and represents the major fraction of the total carbohydrate of almost all the pulses (98 to 99 % of the granule dry weight) and energy in the human diet (Tester *et al.,* 2004; Ratnayake & Jackson, 2008; Tiwari *et al.,* 2011). Pulse starch granules vary in size with a length in the 8 to 70 µm range, with a shape mostly oval to round, and molecular structure and composition depending on the species (Hoover *et al.,* 2010) and the Genetic Environment interactions (GxE). Starch is mainly composed of two α-glucan, essentially unbranched amylose and highly branched amylopectin (Tester *et al.,* 2004). The amylose content represents 12 to 48% of starch dry weight in pulse species (Hoover *et al.,* 2010). As a mixture of amylose and amylopectin in varying proportions, starch can be classified as soluble (rapidly hydrolyzed by digestive enzymes or RDS), insoluble (digested at a relatively slow rate or SDS) or resistant starch (RS) (not hydrolyzed or digested as it passes through the gastrointestinal tract). The starch digestibility is measured by the percentage of RDS, SDS, and RS (Hoover *et al.,* 2010). The starch digestibility is determined by the control by the enzymic hydrolysis and measurement of the released glucose using glucose oxidase. RDS and SDS are measured after incubation with pancreatic amylase and amyloglucosidase at 37°C for 20 min and 120 min, respectively. RS is the starch fraction not hydrolyzed in the small intestine within 120 min incubation and then fermented in the colon (AACC, 2000; Englyst *et al.,* 1992). Pulse starches are less digestible than cereal starches in the granular form (Fuentes-Zaragoza *et al.,* 2010). The RDS content of pulse starches ranged between 4.2% and 30%, the lowest being found in pigeon pea starch and the highest in chickpea starch. SDS and RS contents of pulse starches ranged between 17 - 58% and 8.1 – 79%, respectively (table V). The main factors contributing to the greater enzymatic resistance of native granular starches from pulses is their high amylose content, in the 31 to 49% range that helps to preserve the granular integrity and, the internal restricted porosity that contributes to a more ordered crystalline structure of starch (Wongprayoon *et al.,* 2018; Ren *et al.,* 2021). Likewise, pulses content α-amylase inhibitors which are responsible for reducing starch digestion. This compound has a therapeutic role for obesity and diabetes control since they reduce blood glucose and raised insulin levels (Champ, 2002). Bean is the principal pulse reported with a natural source of α-amylase inhibitors (Campos-Vega *et al.,* 2010). The α-amylase inhibitors are known for their heat-labile nature. In chickpea, when extracts are boiled for 10 minutes, amylase inhibitors become inactive (Singh, 1988). Additionally, in lentil, pea winged bean, and lima bean, the α-amylase inhibitors are undetected and field bean, black-eyed pea, and chickpea contain low levels of α-amylase

inhibitors (Campos-Vega *et al.*, 2010). To this end, since pulses are generally consumed after boiling and the inactivation of α-amylase inhibitors is given at a low cooking time, it is not of practical importance to study further on this antinutritional component, except only if pulses are consumed unheated.

Dietary fiber (DF) is a combination of chemically heterogeneous substances or a complex mixture of indigestible polysaccharides (such as cellulose, hemicellulose, oligosaccharides, pectins, gums), waxes, and lignin as plant cell wall material (Tiwari *et al.*, 2011). DF is the edible parts of plants that are resistant to digestion and absorption by human endogenous enzymes with complete or partial fermentation in the large intestine. It is classified into two types (insoluble and soluble) based on their solubility in a pH-controlled enzyme solution (Tosh & Yada, 2010). The insoluble fractions (*e.g.*, cellulose, lignin, and some hemicelluloses) promote the movement of material through the digestive system. The soluble fraction (*e.g.*, pectins, galactomannan gums, mucilages, and some hemicelluloses fractions) are natural gel-forming fibers. Pulses are a very high source of dietary fiber and the concentration of insoluble fraction is dominant, with a percentage between 63-90% of the TDF (table V) (Tiwari *et al.*, 2011; Tosh & Yada, 2010). Unfortunately, the data of soluble, insoluble, and total dietary fiber for raw pulses and the comparison between pulses classes or cultivars are limited in literature (Tosh & Yada, 2010).

Through seed germination process, amylases catalyze amylose and amylopectin to simple sugars (glucose, maltose, and sucrose) thereby increasing digestibility (Benincasea *et al.*, 2019). In fact, when the surface of starch isolated from germinated mung bean was compared with raw seeds, microscopy analyses showed some dents or potential holes on the surface in germinated seeds overs the smoother surface in raw. This porous surface may be caused by enzymatic action and hydrolysis during germination (Ma *et al.*, 2018; Liu *et al.*, 2020). This fact agrees with another study that showed a maximum of α-amylase activity after 3 days of germination (Ghavidel & Davoodi, 2011). Microscopy analyses in starch of raw and germinated mung bean showed similar round and elliptical shaped granules, thus, the germination process does not affect the shape of mung beans starch granules (Liu *et al.*, 2020). However, resistant starch from germinated lentil expressed block and irregular structure, thus contradicting the last study. This last study indicates that germination and enzymatic isolation processes are responsible for damaging starch granules and cutting glucan chains into shorter length ones (Ma *et al.*, 2018). These latest studies showed the complexity of the germination process in the influence of starch structure in pulse. To this end, is of interest to invest in research on starch structure of germinated pulses, since most studies are done for cereals (Li *et al.*, 2012; Li *et al.*, 2017).

c. Minerals (413)

Minerals are considered essential micronutrients for human health since they play an important role in metabolism. The mineral content in pulses depends on the variety, growing, and environmental conditions (Hall *et al.*, 2017). Pulses are rich in iron (Fe), magnesium (Mg), zinc (Zn), and potassium (K). The recommended daily intake (RDA) of Fe, Mg, Zn, and K in adults is 10-15 mg/day, 350 mg/day, 12-15 mg/day, and 2000 mg/day, respectively (National Research Council, 1989). Table VI shows the mineral content in pulses. Among the pulses, *Lens* and *Cicer genus* present the highest amount of iron, with an average of 70 µg/g and 68 µg/g, respectively. Although pulses are considered to be rich in some minerals, unfortunately, the intake of 100 g of the raw pulse does not reach the RDA. For example, 100 g of lentils has 50%, 47%, and 49% of RDA for iron, zinc, and phosphorus, respectively.

The low bioavailability of pulse minerals is related to the high phytic acid (a.k.a. phytate) amount. Phytic acid (myo-inositol 1,2,3,4,5,6 – hexakisdihydrogenphosphate) represents a group of phosphorus compounds widely found in legumes, grains, and vegetables. It accounts for about 78% of the total phosphorus in pulses (Campos-Vega et al., 2012). In pulse, phytate is stored in protein bodies, in the aleurone layer, and in scutellum cells (Ali & Elozeiri, 2017). The major structure of phytate is the inositol-6-phosphate (IP6) which is a cyclic compound that contains six phosphate groups ($C_6H_{18}0_{24}P_6$). Phytase is the enzyme responsible to reduce IP6 in their analogs IP5, IP4, IP3, IP2, IP1, or unphosphorylated myo-inositol (Egli *et al.*, 2002). The IP6 concentration tends to be higher in raw dry bean, blackeye pea, and pigeon pea than in lentil, green and yellow split pea, and chickpea (Campos-Vega *et al.*, 2010). Phytate is responsible for a strong chelation affinity with cations, principally Zn^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mn^{2+}, and Cu^{2+}, reducing their solubility and availability to human absorption (Benincasa *et al.*, 2020). On the other hand, phytates have beneficial effects on human health, such as their anticarcinogens action and their ability to decrease heart diseases or diabetes risk (Campos-Vega *et al.*, 2010). Phytate in germinating seeds is hydrolyzed by the enzyme phytase, releasing phosphate, cations, and inositol that are used for physiological and metabolic requirements (*e.g.* enzymes of starch metabolism and ATP synthesis) (Ali & Elozeiri, 2017). To this end, as germination process advances phytate content decreases, thus bioavailability of phosphorus and minerals increases, enhancing the nutritional value of germinated pulses (Benincasa *et al.*, 2020).

d. Vitamins in pulse (862)

The B complex is the main source of vitamins found in pulse. They are water-soluble, not stored by the body and a continuous daily supply is required in our diet. Foods containing B vitamins need proper storage and preparation to minimize vitamin loss since they are easily destroyed or washed out during food storage or preparation. The B complex group includes eight B vitamins constituting: thiamine (B_1), riboflavin (B_2), niacin (B_3), pyridoxine (B_6), folate (B_9), vitamin B_{12}, biotin, and pantothenic acid (Moore, 2012). These nutrients have an important physiologic role as they work as co-enzyme in many functions in metabolism and are responsible for numerous oxidoreduction reactions (Hall *et al.*, 2017). Additionally, their deficiency can cause various diseases, such as beriberi, pellagra, dermatitis, oral and genital lesions, and chronic disease, which is a major public health problem in many countries (FAO *et al.*, 2013). Pulses have been recognized as a great source of B vitamins, especially vitamin B_1, B_2, and B_9 (table VII). Thiamin and folate are the main vitamins in *Vigna genus*, with an amount of 771 µg/100g and 420 µg/100g, respectively. This means, with just 100 grams of raw *Vigna genus*, more than 70% and 100% of the daily intake of thiamin and folate, respectively, are obtained. In general, 100 grams of pulses do not have a considerable daily intake of riboflavin, with the largest intake being only 26% coming from *Phaseolus* and *Lens genus*.

Thiamine, known as the B_1 vitamin, is present in a free form in living organisms, and as its phosphorylated derivatives, such as thiamine monophosphate, diphosphate, triphosphate, and adenosine thiamine triphosphate. Thiamine diphosphate is the main type in cells, their concentration goes up to 90% of total thiamine and its derivatives. Phosphorylated derivatives of thiamine are responsible for the control of cell metabolism by allosteric regulation of enzymes, the transmission of nerve signals in synapses, and regulation of protein synthesis by riboswitches in microorganisms and plants (Tylicki *et al.*, 2018). Hence, the insufficient intake of thiamine impacts the proper functioning of nervous, cardiovascular, and locomotive systems, due to an overall decrease in bioenergetics processes, carbohydrate metabolism, and its inter-connection with amino acid metabolism. Thiamine deficiency is responsible for the disease beriberi, which occurs in human-milk-fed infants whose nursing mothers are deficient

(Chunming *et al.*, 2001). Thiamine deficiency may occur in the elderly, patients after major surgery, pregnant and breastfeeding women, smokers, drug and alcohol addicts, diabetic, and in youth persons who prefer a high carbohydrate diet (Tylicki *et al.*, 2018). The recommended daily intake of thiamine is 1.1 mg/day for women and men (Elmadfa *et al.*, 2009).

Riboflavin, or vitamin B_2, is a co-enzyme responsible for numerous oxidation and reduction reactions central to human metabolism (Powers, 2003). The two active forms of riboflavin are 5'-phosphate or flavin mononucleotide (FMN) and 5'-adenosine diphosphate or flavin adenine dinucleotide (FAD) (Mack & Grill, 2006; Schwechheimer *et al.*, 2016). Riboflavin is synthesized by plants and by most microorganisms but not by bigger animals. To this end, humans have to get their daily intake of B_2 vitamin through food. The main food source of riboflavin is mostly milk and dairy products, but cereals, meat, fatty fish, and certain fruit and vegetable, especially dark green, are also good sources. The deficiency of B_2 vitamin results in a sore throat, hyperemia, oedema, oral mucous membranes, corneal vascularization, and skin lesion (Chunming *et al.*, 2001; Schwechheimer *et al.*, 2016). The recommended daily intake of riboflavin lies between 1.1 and 1.3 mg/day (Schwechheimer *et al.*, 2016).

Another major B vitamin is folate (B_9 vitamin). Folates are present in different forms in the seeds, such as folic acid (PteGlu), 10-formylfolic acid (10-CHO-PteGlu), 5-formyltetrahydrofolate (5-CHO-H_4folate), 5-methyltetrahydrofolate (5-CH_3-H_4folate), and tetrahydrofolate (H_4folate). 5-CH_3-H_4folate is the main form found in cowpea, chickpea and fava bean (Hefni & Witthöft, 2013; Coffigniez et al., 2019b). Folate co-enzymes mediate two major interrelated metabolic cycles, which are responsible for the synthesis of thymidylate, purines, and methionine (Moll & Davis, 2017). Since humans cannot synthesize folates, they depend on plants or animal-based food, or dietary supplements. Pulse crops, including lentils, cowpea, chickpea, and bean contain significant amounts of folates (Zhang *et al.*, 2018). Folate deficiency is strongly linked to the development of neural tube defects in fetuses and to increase risk of chronic diseases, such as cardiovascular disease, childhood leukemia, and certain types of cancer (Moll & Davis, 2017; Zhang *et al.*, 2018). One of the major causes of folate deficiency is the inadequate dietary intake, which comes along with old age, alcohol abuse, and poverty as risk factors (Moll & Davis, 2017). The daily recommended folate intake varies between 200 and 400 µg for adults (Krawinkel *et al.*, 2014; Moll & Davis, 2017). During pulse germination, there is an increase in folate synthesis due to a demand for methyl groups, which participate in the pathways of cell division. The PteGlu, 10-CHO-PteGlu and 5-CHO-H_4folate convert into 5-CH_3-H_4folate form (the main form of folate in pulse). This conversion usually occurs when the seed physiologically prepare to germinate (Coffigniez *et al.*, 2021). Thus, it would be logical to think that during germination there is an increase in folate content due to its synthesis (Scott *et al.*, 2000).

e. Antinutritional factor in pulse (820)

Pulses are a great source of protein, carbohydrates, and vitamins, but unfortunately, pulses also have antinutritional factors that impact their nutritional value. Besides the antinutritional factors discussed before (protease and α-amylase inhibitor, and phytate) there are other compounds such as polyphenolic compounds, α-galactosides, and lectins that negatively impacts the nutrition value of pulse. They are divided into two major categories: those that are sensitive to high temperatures, such as lectins, protease and α-amylase inhibitors, and others that are stable or resistant to temperatures, which include polyphenolic compounds (Soetan & Oyewole, 2009). Even they are considered as antinutritional factors, these compounds can also

have health benefits and will be described later on (Campos-Vega *et al.*, 2010; López-martínez *et al.*, 2017).

Tannins, phenolic acids, and flavonoids are the major polyphenolic compound in pulses. It is in the seed coat/hull that high amounts of phenolics are found in the soluble and insoluble-bound forms (Shahidi & Yeo, 2016). After analyzing the whole-seed and decorticated samples of chickpea for polyphenolic compounds, it was noticed that seed-coat contributes about 75% of the total phenolic compounds. Although they are considered as an antinutritional factor, they also have health benefits to human consumption and are considered as phytochemicals by some studies (López-martínez *et al.*, 2017). For example, they can reduce the risk factors for coronary heart disease (CHD) and the side effects of menopause. They also have an antioxidant characteristic that can prevent oxidation events in food (Tiwari *et al.*, 2011; Shahidi & Yeo, 2016; Xu *et al.*, 2020). After a complex germination and post-germination process, the total content and antioxidant activity of phenolic compounds might either decrease or increase (Xu *et al.*, 2020). The early stage of germination initiates the synthesis of natural phenolic compounds from glucose or aromatic amino acids, through the oxidative pentose phosphate, glycolytic, and shikimate pathways. These phenolic compounds will be further polymerized or bounded with macromolecular nutrients, such as polysaccharides, proteins, and lipids, stored in cell walls or vacuoles, thus decreasing their content. Continuing the germination process, the macromolecular nutrients are metabolized by enzymes that result in the release of phenolic compounds from their bound form (Xu *et al.*, 2020). With the beginning of the post-germination process, new plant cells proliferate and the synthesized soluble phenolics can be secreted to the cell wall to form new bound phenolics, thus increasing their content (Gan *et al.*, 2017). To this end, controlling the germination process is important to regulate the polyphenolic compounds in the pulse.

The α-galactosides are low molecular weight non-reducing sugars; they are sucrosylgalactosides and are characterized by the presence of an α (1 → 6) linkage between the galactosyl residue and the C-6 of the glucose moiety of sucrose (Chibbar *et al.*, 2016). In pulses they are present in the form of raffinose family oligosaccharides (RFOs); the first member of this group is raffinose, followed by stachyose, and verbascose. They represent 6-18% of the dry weight of pulse and are soluble in water and alcohol-based solutions (Bessada *et al.*, 2019). Stachyose is present in higher amounts in cotyledon and embryonic axe fractions in the seeds. Meanwhile, verbascose is the major α-galactoside in the seed coat (Njoumi *et al.*, 2019). The α-galactosidase is an enzyme responsible for the degradation of α-galactosides generating galactose and sucrose as end products (Veldman *et al.*, 1993). Due to the absence of α-galactosidase in the human and animal intestinal mucosa, these oligosaccharides escape digestion and are metabolized by colon bacteria, producing hydrogen, carbon dioxide, and methane, causing flatulence and gut gas production (Naczk *et al.*, 1997; Bessada *et al.*, 2019). During the germination process, the disappearance of oligosaccharides (verbascose, stachyose, and raffinose) is accompanied by an increase in the levels of sucrose, glucose, and galactose (Akinlosotu & Akinyele, 1991; Vidal-Valverde & Frias, 1992; Oboh *et al.*, 2000). This shows the high metabolism in the germinated pulse, promoting the synthesis and activity of the α-galactosidase enzyme (Coffigniez *et al.*, 2018).

Lectins (hemagglutinins or phytohemagglutinins) are proteins found in most plant foods, however, in human food, pulses are the main sources. They are originally defined as carbohydrate-binding proteins of non-immune origin that agglutinate cells and/or precipitate glycoconjugates (Hirsch, 1999). Depending on the species and variety, pulses have between 2-10% of lectin (Abbas & Ahmad, 2018). The *Phaseolus* and *Lens genus* are an important source

of lectins since it is the major protein found in these pulses (Campos-Vega *et al.*, 2010). Lectins form complexes with specific sugars (mono- and oligosaccharides) and other proteins, therefore they are responsible to reduce the bioavailability and absorption of these nutrients (Jain *et al.*, 2009; Derbyshire & Delange, 2011). On the other hand, lectins also present beneficial effects since they may help in obesity treatment and tumor growth (Campos-Vega *et al.*, 2010).

Table XII presents the average of the antinutritional contents in pulse. In general, pulses present a high amount of oligosaccharides and phytic acid and a low number of lectins and α-amylase inhibitors.

4. Food processing and its impacts on pulse composition (158)

As described before, pulses are a great nutritional source being rich in protein, high dietary fiber, minerals, and vitamins. There is a growing interest in processing pulse to be used as an ingredient in the food industry, either using pulses alone or in combination with cereals. Figure III shows the different food processing that can occur for pulses. Traditional processing methods include dehulling, soaking, cooking, germination, sprouting, fermentation, and roasting. More recent processing techniques, used exclusively by the food industry, include micronization, extrusion, canning, puffing, and flour milling (Malcolmson & Han, 2019). In this recent work, we were interested to investigate the impacts of the germination process on the nutritional quality of pulse. As shown in figure III, to have an edible pulse as an end-product throughout the germination process, the combination of soaking and cooking process is necessary. To this end, we will analyse the impact of these processes in the nutritional and antinutritional compounds in pulse.

a. Food process impacts on protein digestibility (549)

As previously mentioned (2.a), the average IVPD of raw pulses is 70% and can be related to the secondary structure of proteins, particularly rich in *β*-sheet. Food processes, such as soaking, germination, and cooking can impact this due to the hydrolysis of seed proteins, the reduction or elimination of different antinutrients (Jood *et al.*, 1989; Chitra *et al.*, 1996; Bessada *et al.*, 2019) but also to thermal phase change of proteins (Rui *et al.*, 2011). Heat treatment in the presence of water results in denaturation of inter and intra-molecular bonds in proteins (Carbonaro *et al.*, 1997). Consequently, the tridimensional structure of the protein unfolds and the intra-molecular cleavage sites become more accessible for enzymatic hydrolysis. Different thermal stabilities have been observed between pulses, due to the different ratios of specific proteins (Barbana & Boye, 2013). Due to their differences in structures, the thermal stability of legumin is higher than that of vicilin, due to the presence of disulfide bonds. The covalent bonds are more stable, so they need higher energy to be cleaved compared to the non-covalent bonds (Sirtori *et al.*, 2012). Therefore, the protein composition of pulses is an important parameter for thermal denaturation and protein digestibility. In addition, the protein denaturation is followed by the aggregation of the unfolded polypeptides (Carbonaro *et al.*, 1997). This thermal coagulation is due to the formation of intermolecular *β*-sheet structures *(*Rui *et al.*, 2011). Hydrophobic AA (such as phenylalanine, tryptophan, and tyrosine) and charged basic AA (lysine and arginine) are involved in such aggregates, so the accessibility of those AA is thus restricted. Because of the specific cleavage sites of trypsin and chymotrypsin, the emerging of protein aggregates can reduce the digestibility in the small intestine (Carbonaro *et al.*, 2012). Processing pulses can effectively increase the *in vitro* protein digestibility, by consequently increasing their nutritional value (Carbonaro *et al.*, 2000). To demonstrate these shreds of evidences, previous studies showed that cooking under pressure (autoclaving) at 121 °C for 10

minutes improve by 96% - 105% the protein digestibility when compared to raw legumes, for black grams, chickpea, lentil, red and white kidney bean (Rehman & Shah, 2005). Nevertheless, an increase in cooking time at this temperature decreased protein digestibility, perhaps due to a decrease in the availability of some amino acids, such as lysine. This result seems to reflect the increase in protein aggregates observed by Carbonaro *et al.,* (2012). For chickpea, IVPD was improved after boiling, autoclaving, and microwave cooking. The two last cooking techniques induced the greater increase in IVPD (Alajaji & El-Adawy, 2006). Protein digestibility was also higher when soaked instead of unsoaked grains were cooked. According to Setia *et al.,* (2019), soaking overnight at room temperature did not change the IVPD of yellow pea and fava bean. Germination also increases the *in vitro* protein digestibility but depends on the variety and germination conditions. Germinated pigeon pea, kidney bean, and fava bean at 25°C during 72h increased 23%, 110%, and 110% in IVPD, respectively (Chitra *et al.*, 1996; Alonso *et al.*, 2000). On the other hand, Setia *et al.,* (2019) showed that germinated yellow pea and fava bean for 72h only increase by 1.3% and 2.4% the IVPD, respectively. To this end, is obvious that to improve even more the IVPD in pulse, the cooking process is advisable.

b. Food process impacts on starch digestibility (545)

Three structural factors are well-known to contribute to the enzymatic resistance of native starch from pulses including the compactness of the granular structure (low porosity), the specific branch-chain distribution of amylopectin, and the high amylose content favoring retrogradation (Ren *et al.*, 2021). With a sufficient amount of heat and water excess, starch will undergo an irreversible phase transition, namely gelatinization. Such order-disorder transition associate with the granular diffusion of water, amorphous background region water uptake, granule hydration and swelling, heat uptake, crystalline order loss, and structural granular disorganization leads to amylose leaching (Hoover *et al.*, 2010). As earlier illustrated by Giraldo Toro *et al.,* (2015), the gelatinization process also leads to a significant reduction of the resistant starch fraction with an increase in the food product digestibility. The degree of starch gelatinization, thus the starch digestibility can be modulated according to the processing conditions used. Physical, enzymatic and, chemical processing are known to change the structure and digestibility of starch and starch-based products (Hoover *et al.*, 2010; Singh *et al.*, 2010; Tiwari *et al.*, 2011; Magallanes-Cruz *et al.*, 2017). Consequently, the nutritional intake and the health benefits will be also modulated. Specific unit operations usually applied to cereals and legumes, such as soaking and germination were revealed to significantly increase the *in vitro* digestibility of pulse starch, with a significant reduction of the RS fraction (Bishnoi & Khetarpaul, 1993; Bravo *et al.*, 1998; Alonso *et al.*, 2000; Erba *et al.*, 2019). In particular, the sprouting process applied to chickpeas and green peas kept their well-preserved and ordered cell wall structure, with native and swelled granules (Erba *et al,* 2019). No significant variation in the SDS fraction (55 vs 62%, 39 vs 49%, respectively) was highlighted between raw and germinated seeds of both species. If the grinding process induced a major modification of the structure of the seeds with a huge reduction of the SDS fraction (lowered between 10 and 12%), the germination process did not induce a significant modification of the remaining SDS fraction in sprout and unsprouted porridge made of chickpea and green pea flours. Thus, the complementary investigation (Setia *et al.*, 2019) done to investigate the impact of short-term germination of fava bean and yellow pea flours, confirmed that the resistant starch fraction was relatively low in native flours (26 and 14%, respectively), probably due to grinding. If the soaking process did not induce any significant modification of starch digestibility, a germination duration of 24 to 72h induced a significant reduction of the RS fraction for both species native flours (8 to 5% and 15 to 9%, respectively). Moreover, as expected, the cooking process applied to the flours (10 min) induced a major rise in the starch digestibility, with a RS

fraction from 3 to 1% in fava bean, and from 7 to 1% in yellow pea after soaking and 72h of germination. Thus, considering that many raw pulse starches exhibit a high RS fraction in the 59 to 81% range and below 11% when cooked (Ma *et al.*, 2017), the last three studies illustrate that the cooking process is effectively needed to get a high starch digestible fraction for human consumption, even in the cases where soaking and germinating unit operations are also used to lower the antinutritional compounds of pulses.

c. Food process impact on B vitamins content (332)

Soaking and cooking can decrease the amount of the vitamins in pulse due to chemical destruction and leaching out into the medium due to their water-soluble characteristics. For example, soaking cowpea at 30 °C for 14 h decreased 13% of the net total folate content. On the other hand, cooking cowpea at 95 °C for 2 h reduced 50% of total folates (Coffigniez *et al.*, 2019b). Cooking chickpea at 100°C for 90 minutes retained only 34% and 48% of thiamin and riboflavin, respectively (El-Adawy, 2002). These results show that the major loss of B group vitamins is mainly due to leaching into the soaking water medium and not to oxidation, or other degradation pathways. Therefore, when the cooking medium is consumed as soup, no losses in B vitamins content need to be accounted for (Delchier *et al.*, 2012; Hefni & Witthöft, 2013; Coffigniez *et al.*, 2019b). As shown in table IX, germination can present more satisfactory results by increasing the content of vitamins in the seed. For example, sprouting of chickpea at 25°C during 3 days in the dark retained 63% and 16% of thiamin and riboflavin content, respectively (El-Adawy, 2002). Additionally, sprouting of lentils at room temperature during 6 days retained between 72-89% of thiamin and 138-420% of riboflavin content, depending on the variety (Prodanov *et al.*, 1997). It is interesting to mention that these last studies mentioned the term “germination process” but they were referring to the “post-germination process”. In this case, it is not possible to mention the positive impacts of the germination process for the reduction of these compounds in pulse, and further studies are needed for a better conclusion. Germination of chickpea at 25°C for 72h, 48h, and 24h increased of 142%, 39% and 65% in folate content, respectively (Hefni & Witthöft, 2013). Regarding these studies, it is possible to conclude that the sprouting process is the best treatment to increase the thiamin and riboflavin content and the germination process has advantages to increase folate content.

d. Food process impact on antinutritional factors content (1302)

Soaking, germination, and cooking process can induce leaching into the soak water, and thermal and/or chemical changes (degradation, formation, and/or conversion) of antinutritional factors. It can thus reduce the content of condensed tannins, phytic acid, α-galactosides, lectins and protease, and α-amylase inhibitors in pulse (Njoumi *et al.*, 2019; Coffigniez *et al.*, 2018; Alonso *et al.*, 2000; Khandelwal *et al.*, 2010). Table X shows the impact of soaking, cooking, and germination on antinutritional content in pulse.

The effect of antinutritional factors loss during soaking depends on the cultivar, the soaking solution, the period, the temperature, the variety, and the enzymatic activity of each pulse, but in general, satisfactory results are not obtained with just the soaking process (Shi *et al.*, 2004). During the soaking process, the reduction of antinutritional compounds may be due to leaching into the soaking water or by enzymatic activity. Phytic acid, lectins, protease inhibitor, and α-amylase inhibitor may be reduced by diffusion to the soaking water (Egli *et al.*, 2002; Shi *et al.*, 2017; Shi *et al.*, 2018). However, soaked pulses had very little influence to reduce these antinutritional compounds. For example, Egli *et al.*, (2002) showed that soaking lentils, cowpea, and chickpea at 25°C for 16h, the phytic acid content remained 87%, 92%, and 85% of the initial values, respectively. Shi *et al.* (2018) showed that pea, lentil, fava bean, chickpea

and navy bean soaked for 4h at room temperature only had a lectin reduction range from 0.5 - 3%. Similarly, Shi *et al.,* (2017) showed that α-amylase inhibitors in soaked *Phaseolus genus* at room temperature for 4 h reduce between 4-10%. These results may be explained by the low enzymatic activity at these soaking conditions and by the cellular structure of the intact seed that could limit the removal of antinutritional factors by leaching out into the soaking water. It is noteworthy, that soaked water analyses in these conditions would be needed to confirm this hypothesis (Shi *et al.,* 2017). Coffigniez *et al.,* (2018) showed that the diffusion and the enzymatic reaction of α-galactosidase depend on the soaking conditions. For example, soaking cowpea at 30° reduces α-galactosides by enzymatic degradation and not by leaching out into the soaking medium. Soaked cowpea during 38h at this temperature, decreased by 35% the stachyose concentration. However, with an increase in temperature (60°C) the α-galactosides are not enzymatic degraded, and the losses are transferred to the soaking water. When cowpea is soaked for 14h in this condition, the concentration of verbascose, stachyose, and raffinose was reduced by 63%, 76%, and 74% of their respective initial values (Coffigniez *et al.,* 2018). The loss of tannins may result from leaching into the soak water or by an enzymatic activity of polyphenol oxidase (Khandelwal *et al.,* 2010). Further analyses for the soaking medium and/or for the polyphenol oxidase activity are needed to better explain tannin losses.

Increasing process temperature (cooking) and/or doing a biological process (germination), can weaken the cellular structure, facilitating the diffusion to the soak water, and can activate the metabolism, thus increasing enzymatic activity. To this end, it is interesting to invest in these food processes to reduce to a greater extent the antinutritional content in pulse. Cooking can reduce the antinutritional factors contents in pulse, by leaching into the soaking water or by the heat-labile of the compound. This last may result in a breakdown of their structures into subunits or other unknown changes in conformational structure (Shi *et al.,* 2004). Diffusion into the soaking water is responsible to reduce tannins and α-galactosides. For example, cooking pigeon pea, chickpea, and lentils under pressure at 121°C, can reduce tannins content up to 27%, 35%, and 36%, respectively (Khandelwal *et al.* 2010). Cooking at 95°C for 3h reduces the concentration of verbascose, stachyose, and raffinose in cowpea seeds of 69%, 61%, and 63%, respectively (Coffigniez *et al.* 2018). It is noteworthy that in these cases if the cooking water is consumed, there is no decrease in tannins and α-galactosides content. Phytic acid, lectins, and protease inhibitors are known for their heat-labile nature (Shi *et al.,* 2004; Shi *et al.* 2017; Shi *et al.,* 2018). Cooking under pressure at 121°C for different periods (10, 20, 40, 60 and 90 minutes) and 128°C for 20 minutes, phytic acid contents in black gram, chickpea, lentil, red kidney bean, and navy bean reduced between 28 and 52% (Rehman & Shah, 2005). Cooking for 1h at 95°C reduced up to 94–99% lectins content in different varieties of chickpea, bean, fava bean, lentils, and pea. Grant *et al.,* (1995) showed that α-amylase inhibitors in kidney beans present a large thermal resistance, and processing at 80°C for 30 minutes did not significantly reduce the level of the compound. On the other hand, cooking kidney beans at 100°C for 30 minutes removed completely α-amylase inhibitor activity (Grant *et al.,* 1995). Other studies were also in agreement with this result (Shekib *et al.,* 1988; Alonso *et al.,* 2000; Shi *et al.,* 2017). To this end, cooking has been reported to be effective to inactivate antinutritional factors in several food legumes (Udensi *et al.,* 2007). However, excessive heating reduces the nutritive value of legume proteins and vitamins. For instance, some limiting essential amino acid of pulses, such as methionine, has been reported to undergo nutritional damage when heated. Therefore, it is important to establish the optimum heat condition or to combine different processing (*e.g.* germination and cooking) to best benefit the nutritional qualities of pulse (Singh, 1988).

The decrease of antinutritional factors in pulse during a complex biological process, such as germination, may be due to the activation of metabolic pathways inducing an increase in enzymatic activity, and thus degrading the antinutritional compounds. For example, during germination, tannins may be reduced by the activation of polyphenol oxidase activity and other catabolic enzymes. Germination at room temperature for 24h reduces up to 43% in the tannin amount for chickpea and was not detected in pigeon pea and lentils. This represents the greatest loss of tannins when compared to pressure-cooked and soaked pulses (Khandelwal *et al.*, 2010). In the same way, seeds use phytate as a source of inorganic phosphate and increase the phytase activity during the germination process (Reddy *et al.*, 1982). After 6 days of post-germination process, the amount of IP_4 and IP_3 increased, thus indicating a high enzymatic activity (Vidal-Valverde *et al.*, 2002). After 72 h of germination, the phytic acid content decrease by 66%, 61%, and 30% for pigeon pea, fava bean, and common bean, respectively (Chitra *et al.*, 1996; Alonso *et al.*, 2000). Another study shows that after 72h of germination at 25°C, white bean, chickpea, lentils, and pea had a reduction of just 24%, 14%, 5%, and 2% in phytic acid content, respectively. In the case of cowpea, the phytic acid content increase during germination (Egli *et al.*, 2002). Germination has also the advantage to reduce the content of α-galactosides (Vidal-Valverde *et al.*, 2002). After 24h of germination at room temperature, the stachyose and raffinose content reduced by 100% in cowpea (Ibrahim *et al.*, 2002). However, another study with cowpea did not show the same results under the same condition, where verbascose and raffinose were eliminated only after 48h and stachyose after 72h (Akinlosotu & Akinyele, 1991). Alonso *et al.*, (2000) showed that germination can further reduce the α -amylase inhibitors in comparison to soaking experiments. For instance, kidney beans and fava beans germinated at 25°C for 72h reduced at 34% and 37%, respectively. Neither soaking nor germination process influenced to reduce the level of lectins in kidney bean and fava bean (Alonso *et al.*, 2000). The final result for the antinutritional amount in germinated pulse depends either on the conditions of germination, such as temperature, time, oxygen availability, moisture, light, seed variety, and difference in analysis methods used that may vary (Nelson *et al.*, 2013).

5. Conclusion (404)

Pulses have a major impact on livelihoods, as they provide an affordable economic source, contribute to soil fertility, and are great house food security. Incorporating pulse into the diet reduces malnutrition in both underdeveloped and developed countries, especially for children, pregnant women, the elderly, and the obese. Pulses are considered to be rich in complex carbohydrates, protein, vitamins, minerals, and fiber with a very low glycemic index (GI). However, pulses are known for the presence of antinutritional factors, such as polyphenolic compounds, phytic acid, α-galactosides, lectins, protease, and α-amylase inhibitors, which can impact their nutritional value. There are responsible for the reduction of protein, starch, minerals, and vitamins bioavailability for human consumption. Pulses are usually consumed after soaked or cooked. However, germination is also a traditional process that usually takes place at home and is a re-emerging trend in healthy foods. The germination process is a natural and complex process in which seeds pass through major metabolic changes and activates pathways for the synthesis of necessary nutrients for seedling growth. During this process, seeds synthesize B-vitamins, change in amino acids composition, and decrease phytic acid content. For a better benefit of nutritional aspects in germinated pulses, further research is needed to optimize germination conditions and reduce microbiological risks without affecting nutrient content. Additionally, in the scientific community is a misunderstanding in germination

terminology, making it difficult to interpret its actual impact on the pulse nutritional value. To this end, it is important that literature clarifies and agrees with the germination terminology to give more comparable results by analysing the same seed properties. Furthermore, it is interesting to mix physical (soaking and cooking) and biological (germination) food processes to increases the nutritional benefit from pulse consumption. Besides the advantages of germination process, soaking process is responsible to decrease the α-galactosides content, and cooking will increase protein/starch digestibility, decrease polyphenolic compounds, lectins, and protease/α-amylase inhibitor content in pulse. This can result in a final plant-based product or an ingredient in the food industry, rich in B vitamins, protein, starch, and low in antinutrient compounds. In conclusion, the combination of processes, such as soaking, germination, and cooking pulses, allows versatility in formulation to produce novel food products and/or ingredients with a better nutritional content into the final product. This can encourage the food industry to invest in research and development to increase plant-based food, that can replace meat, produce gluten-free products, and enhance the food nutrient content.

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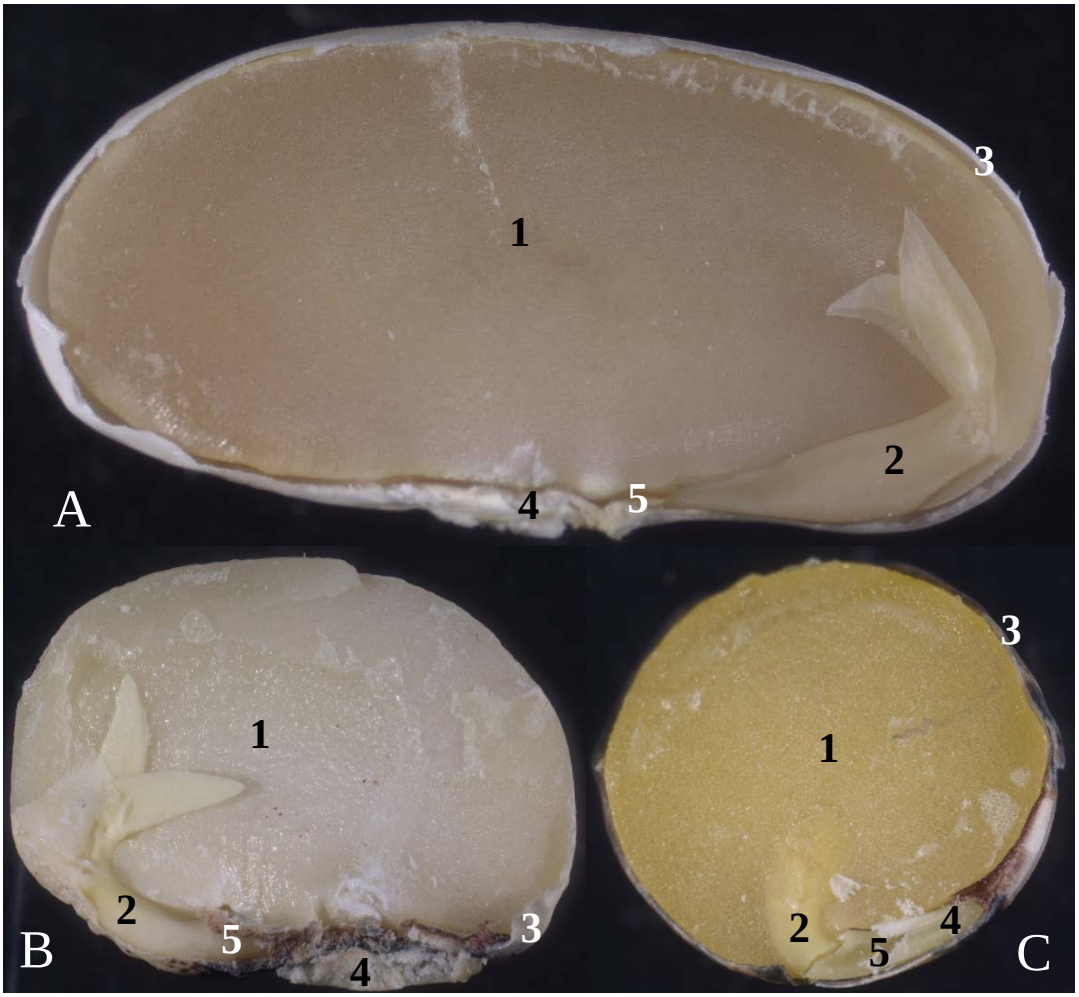

Figure I. Symmetric longitudinal seed sections of *Phaseolus vulgaris* (A), *Vigna unguiculata* (B), and *Lens culinaris* (C). The structure comprises the cotyledon (1), the embryonic axis (2), the testa (3), the hilum (4), and the micropyle (5).

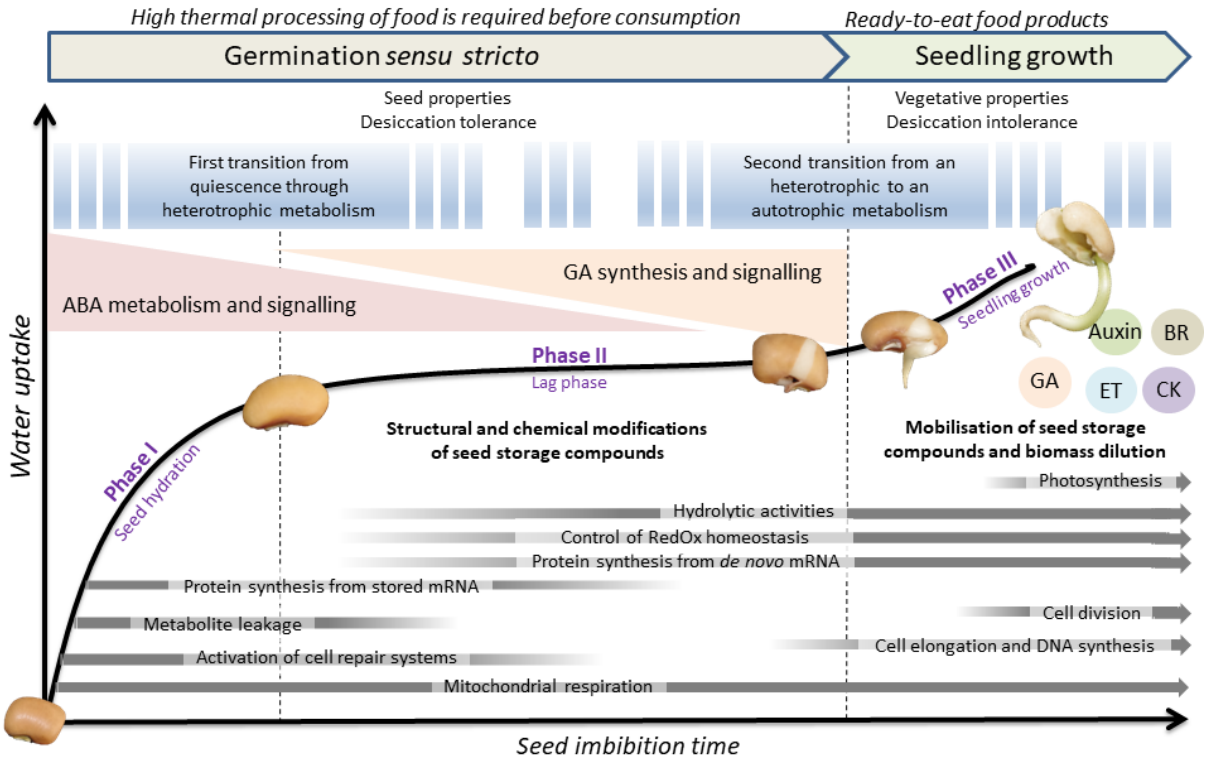

Figure II. Seed water uptake associated with major events during germination *sensu stricto* and seedling growth. Phase I has strong water activity and ABA metabolism and signalling. Phase II (lag phase) represents structural and chemical modifications of seed storage compounds. These phases are related to germination *sensu stricto* process. Phase III represents the seedling growth process with some hormone activation, such as gibberellins (GA), ethylene (ET), brassinosteroids (BR), auxin, and cytokinin (CK).

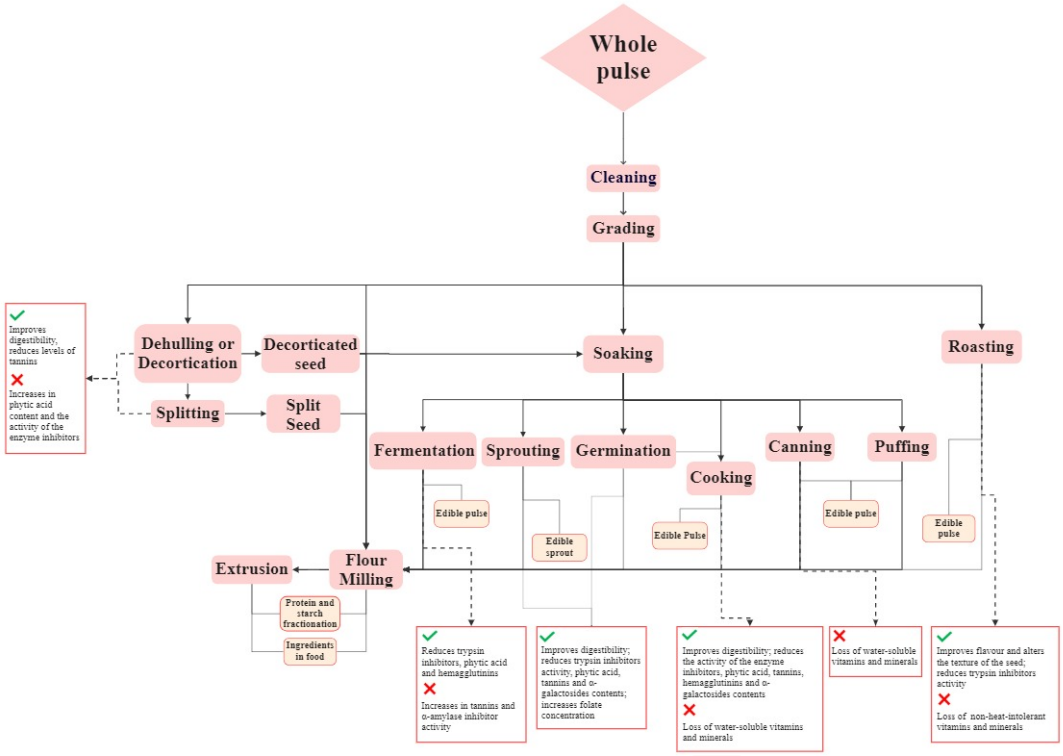

Figure III. Flowchart of Pulse food processing. The ▭ indicates different food processing to obtain the final product indicated in ▭. The ▭ indicates the advantages and disadvantages of each process. The germination process needs a further high thermal process (such as cooking) before consumption and the sprouting process gives an edible sprout as a ready-to-eat food product.

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Table I. Nutritional content in raw pulses (g/100g of dry matter)

	Protein	**Carbohydrates**	**Fat**	**Fiber**	**Ash Content**
			g/100g		
Vigna genus [a,b,d,e]	17-28	54-63	1-6.5	3.9–6.2	2.9-5
Cajanus genus[d]	19	63	2.0	6.4	-
Phaseolus genus [a]	19-27	67-75	2.0	14-25	4-4.9
Cicer genus [a,c,d]	20	61	5.3	16	1.8 - 3.5
Lens genus [a,c,d]	25	59	0.7	19	2.1 - 3.2
Pisum genus [a]	14-31	55-72	1-4	3-20	2.3 - 3.7

[a]Source: (Hall *et al.*, 2017)
[b]Source: (Gqaleni, 2014)
[c]Source : (Perez-Hidalgo *et al.*, 1997)
[d]Source: (Singh & Singh, 1992)
[e]Source: (Harms *et al.*, 1987)

Table II. Amino acid composition of pulse (% of total protein)

	Essential Amino Acids								
	Lysine	**Isoleucine**	**Leucine**	**Methionine**	**Phenylalanine**	**Valine**	**Threonine**	**Histidine**	**Tryptophan**
Vigna genus[b]	7.5	4.5	7.7	2.2	7.5	5.0	3.8	3.1	0.7
Cajanus genus[a]	1.4	1.2	1.5	0.2	1.7	1.0	0.7	-	0.1
Phaseolus genus[a,c]	1.6	1.1–1.3	1.9	0.2-0.3	1.2-1.3	1.3	1.0	0.7	02
Cicer genus[b]	7.2	4.8	8.7	1.1	5.5	4.6	3.1	3.0	0.9
Lens genus[b]	7,0	4.1	7.8	0.8	5	5	3.5	2.2	0.7
Psium genus[b]	8.1	4.5	7.4	1.1	5.2	5.0	3.8	2.4	0.8

	Non-Essential Amino Acids				Conditionally Essential Amino Acids				
	Alanine	**Aspartic acid and Asparagine**	**Glutamic acid**	**Serine**	**Arginine**	**Cysteine**	**Glycine**	**Proline**	**Tyrosine**
Vigna genus[b]	4.2	11	17	4.5	7.5	5.0	3.8	4.0	3
Cajanus genus[a]	-	-	-	-	1.5	0.3	-	-	0.6
Phaseolus genus[a,c]	1.0	2.8	3.9	1.4	1.5	0.2	1.0	0.9	0.8
Cicer genus[b]	5.0	11	17	3.7	8.3	0.6	3.7	3.8	2.8
Lens genus[b]	4.2	12	22	5.2	7.8	0.9	3.6	3.5	32

Psium genus [b]	5.2	11	18	5.1	7.2	1.7	4.5	3.8	3.7

[a]Source: (Tiwari *et al.*, 2011)
[b]Source: (Iqbal *et al.*, 2006)
[c]Source: (Carbonaro *et al.*, 1997)

Table III. *In vitro* protein digestibility (IVPD) and Protein Digestibility Corrected Amino Acid Score (PDCAAS) in raw pulses

	IVPD (%)	**PDCAAS (%)**
Vigna genus [a,c]	72-75	56-80
Cajanus genus[d]	69	-
Phaseolus genus[a,c]	71-78	28
Vicia genus [b]	-	56
Cicer genus [a,c,d]	34-76	69*
Lens genus [a]	76-77	-
Pisum genus [a,b,c]	78-80	59-62

*Values for *Cicer genus* flour
[a]Source: (Bessada *et al.*, 2019)
[b]Source: (Setia *et al.*, 2019)
[c] Source: (Boye et al., 2012)
[d]Source: (Chitra *et al.*, 1996)

Table IV. Protein fractions of pulses

	Proteins fractions (%)			
	Globulins	**Albumins**	**Glutelins**	**Prolamins**
Vigna genus [a,b,c]	50-70	11-25	15-24	0.2-5
Phaseolus genus [a]	45-80	10-30	-	-
Vicia genus[a]	70-78	22-30	0	0
Cicer genus [a]	53-60	8-12	19-25	3-7
Lens genus [a]	49	17	11	3
Pisum genus[a]	50-85	15-25	-	-

[a]Source: (Bessada *et al.*, 2019)
[b]Source: (Vasconcelos *et al.*, 2010)
[c]Source: (Gupta *et al.*, 2010)

Table V. Starch fractions (%RDS, %SDS, and %RS), hydrolysis index (HI), extimated glycemic index (eGI) and the content of Soluble (SDF), Insoluble (IDF) and Total Dietary Fiber (TDF) (g/100g dry matter)

RDS (%)	**SDS (%)**	**RS (%)**	**HI**	**eGI**	**Soluble Dietary**	**Insoluble Dietary**	**Total Dietary**

						Fiber (SDF)	Fiber (IDF)	Fiber (TDF)
Vigna genus [b]	9.5	30	61	17	49	-	-	-
Cajanus genus [c]	4.2	17	79	8.2	44	-	-	-
Phaseolus genus[a,e]	8.2	32	59	-	-	5.9	20	26
Cicer genus [c,e]	22-30	46-58	8.1-18	70-74	69-72	5.0	13	18
Lens genus [c,e]	16-17	58-62	13	67	66	1.8	17	19
Pisum genus [c,d]	18-24	54-59	8.1-13	69-72	68-70	5.6	9.7	15

[a]Source: (Chung *et al.*, 2010)
[b]Source : (Chung *et al.*, 2008b)
[c]Source : (Sandhu & Lim, 2008)
[d]Source : (Martín-Cabrejas *et al.*, 2003)
[e]Source : (Perez-Hidalgo *et al.*, 1997)

Table VI. Mineral content in pulses

	Chromium	Copper	Iron	Magnesium	Manganese	Zinc	Sodium	Potassium	Phosphorus	Calcium
	µg/g						mg/100g			
Vigna genus [b]	-	97	26	48	17	51	102	1280	303	176
Phaseolus genus[a,c]	0.08-0.2	3-3.5	62-67	1166	12	39-50	-	-	-	-
Cicer genus [a,b,c]	0.09-0.3	3-5	65-70	1176	26	37-43	101	1155	251	197
Lens genus [a,b,c]	0.12-0.6	2-3	65-75	922	13	45-70	79	874	394	120
Pisum genus [a,b]	0.05-0.1	1.5-2	19-26	42	22	33-40	111	1021	283	110

[a]Source: (Cabrera *et al.*, 2003)
[b]Source: (Iqbal *et al.*, 2006)
[c]Source: (Ramírez-Ojeda *et al.*, 2018)

Table VII. Vitamins contents in pulse (µg/100g) and their respective daily intake percentage for 100g of raw pulses (%)

	Thiamine	Riboflavin	Folate
Vigna genus[a,f]	771 (70)	250 (23)	420 (105)
Cajanus genus[e]	-	-	174 (43)
Phaseolus genus[d,e]	750 (68)	280 (26)	131-140 (35)
Cicer genus[b,c]	453 (41)	173 (16)	150 (37)
Lens genus[b]	560 (51)	280 (26)	146-290 (55)

Pisum genus[c,d]	740 (67)	150 (14)	102 (25)

[a]Source: (Coffigniez *et al.*, 2021)
[b]Source: (Hall *et al.*, 2017)
[c]Source: (Campos-Vega *et al.*, 2010)
[d]Source: (Vidal-Valverde *et al.*, 2002)
[e]Source: (Hoppner & Lampi, 1993)
[f]Source: (Uzogara *et al.*, 1991)

Table VIII. Antinutritional factors contents in raw pulse

	Tannins	Phytic acid	Oligosaccharides	Lectins	Trypsin inhibitors	α-Amylase inhibitors
	g/kg	g/kg	g/kg	HU/mg	TIU/mg	AIU/mg
Vigna genus[a,d,e,h,i,l,o]	175-590	4.5-9.8	26-47	0.01-11	1.1-30	3.8
Cajanus genus[h,n,p]	380-1710	12	-	-	8.1-12	0.02-0.03
Phaseolus genus[b,e,f,k,m]	-	6.4-19	6.1	75-89	21-25	0.8-1.4
Cicer genus[b,c,e,h,j,n,o,p]	175-590	9.2-11	25-42	2.7-6.2	15-19	2.2-8.7
Lens genus[b,c,e,g,h,k,n]	3.0	6.6-15	28-30	11	3-8	3.3
Pisum genus[b,e,k]	-	4-8.6	52	5.7	2.9-15	-

HU = hemagglutinin activity
TIU = trypsin inhibitor units
AIU = amylase inhibitor units

[a]Source: (Akissoé *et al.*, 2021)
[b]Source: (Ohanenye *et al.*, 2020)
[c]Source: (Njoumi *et al.*, 2019)
[d]Source: (Coffigniez *et al.*, 2018)
[e]Source: (Shi *et al.*, 2018)
[f]Source: (Shi *et al.*, 2017)
[g]Souce: (Khandelwal *et al.*, 2010)
[h]Source: (Jain *et al.*, 2009)
[i]Source: (Udensi *et al.*, 2007)
[j]Source: (Alajaji & El-Adawy, 2006)
[k]Source: (Vidal-Valverde *et al.*, 2002)
[l]Source: (Ibrahim *et al.*, 2002)
[m]Source: (Alonso *et al.*, 2000)
[n]Source: (Chitra *et al.*, 1996)
[o]Source: (Shekib *et al.*, 1988)
[p]Source: (Singh, 1988)

Table IX. Impacts of food treatment in B vitamins content in pulse (% of reduction)

Treatment	**Seeds**	**Time**	**Temperature (°C)**	**Thiamin retention (%)**	**Riboflavin retention (%)**	**Folate retention (%)**	**References**

Soaking	*Vigna genus*	9 -38 min	Room temperature	-	-	81	Akissoé *et al.*, 2021
		14 h	30	-	-	87	Coffigniez *et al.*, 2019
Cooking	*Vigna genus*	2 h	95	-	-	50	Coffigniez *et al.*, 2019
		45 min	100	31	-	-	Uzogara *et al.*, 1991
	Cicer genus	90 min	100	34	48	-	El-Adawy, 2002
Germination	*Vigna genus*	4 days	30	-	-	200	Coffigniez *et al.*, 2021
	Vicia genus	24 h	25	-	-	131	Hefni & Witthoft, 2013
		48 h	25	-	-	172	
		72 h	25	-	-	177	
	Cicer genus	24 h	25	-	-	165	Hefni & Witthoft, 2013
		48 h	25	-	-	139	
		72 h	25	-	-	242	
		3 days	25	63	16	-	Prodanov *et al.*, 1997
	Lens genus	6 days	Room temperature	72-89	138-420	-	Prodanov *et al.*, 1997

Table X. Impacts of food treatment in antinutrional factors content in pulse (% of reduction)

Treatment	Seeds	Time	Temperature (°C)	Polyphenolic compounds	Phytic acid	α-galactosides	Lectins	Protease inhibitors	α-amylase inhibitor	References
Soaking	*Vigna genus*	9 - 38 min	Room temperature	-	0	15-20	-	-	-	Akissoé *et al.*, 2021
		16 h	25	-	8	-	-	-	-	Egli *et al.*, 2002
		16 h	28	-	-	35-41	-	-	-	Onyenekwe *et al.*, 2000
		14 h	60	-	-	71	-	-	-	Coffigniez *et al.*, 2018
		4 h	80	-	-	83-86	-	-	-	Onyenekwe *et al.*, 2000
	Cajanus genus	12 h	25	15	-	-	-	-	-	Khandelwal *et al.*, 2010
	Phaseolus genus	12 h	30	24	6	-	0	5-15	11	Alonso *et al.*, 2000
	Vicia genus	12 h	30	48	33	-	0	4.5	15	
	Cicer genus	12 h	25	22	-	-	-	-	-	Khandelwal *et al.*, 2010
		16 h	25	-	15	40	-	-	-	Egli *et al.*, 2002 ; Njoumi *et al.*, 2019
	Lens genus	12 h	25	24	-	-	-	-	-	Khandelwal *et al.*, 2010
		16 h	25	-	13	10	-	-	-	Egli *et al.*, 2002 ; Njoumi *et al.*, 2019
	Pisum genus	16 h	25	-	1.6	-	-	-	-	Egli *et al.*, 2002
Cooking	*Vigna genus*	3 h	95	-	-	64	-	-	-	Coffigniez *et al.*, 2018
		40 min	100	-	-	38-41	-	-	-	
		60 min	100	-	-	-	76	52	-	Udensi *et al.*, 2007
		60 min	120	-	-	-	58	-	-	

	Cajanus genus	-	121	27	-	-	-	-	-	Khandelwal *et al.*, 2010
	Cicer genus	60 min	100	-	-	-	-	82	-	Alajaji & El-Adawy, 2006
		90 min	100	-	-	-	ND	-	-	Singh, 1988 ; Alajaji & El-Adawy, 2006
		35 min	121	35	-	-	ND	-	-	Singh, 1988 ; Alajaji & El-Adawy, 2006 ; Khandelwal *et al.*, 2010
	Lens genus		121	36	-	-		-	-	Khandelwal *et al.*, 2010
Germination	*Vigna genus*	24 h	Room temperature	-	-	ND	-	-	-	Ibrahim *et al.*, 2002
	Cajanus genus	24 h	Room temperature	ND	-	-	-	-	-	Khandelwal *et al.*, 2010
		72 h	Room temperature	-	66	-	-	-	-	Chitra *et al.*, 1996 ; Alonso *et al.*, 2000
	Phaseolus genus	24 h	25	43	-	-	0	7-1	24	Alonso *et al.*, 2000
		48 h	Room temperature	-	19	-	0	20-22	32	Chitra *et al.*, 1996 ; Alonso *et al.*, 2000
		72 h	25	72	2 - 30	-	0	23-29	34	Alonso *et al.*, 2000 ; Egli *et al.*, 2002
	Vicia genus	24 h	25	56	-	-	0	6-9	27	Alonso *et al.*, 2000
		48 h	Room temperature	-	59	-	0	11-16	33	Chitra *et al.*, 1996 ; Alonso *et al.*, 2000
		72 h	25	60	61	-	0	12-25	37	Alonso *et al.*, 2000
	Cicer genus	24 h	Room temperature	43	-	-	-	-	-	Khandelwal *et al.*, 2010
		48 h	Room temperature	-	64	-	-	-	-	Chitra *et al.*, 1996 ; Alonso *et al.*, 2000
		72 h	25	-	14	-	-	-	-	Egli *et al.*, 2002

	Lens genus	24 h	Room temperature	ND	-	-	-	-	-	Khandelwal *et al.*, 2010
		72 h	25	-	24	-	-	-	-	Egli *et al.*, 2002
		2 days	20	-	-	ND	-	-	-	Vidal-Valverde et al., 2002
	Pisum genus	72 h	25	-	5	-	-	-	-	Egli *et al.*, 2002
		4 days	20	-	-	ND	-	-	-	Vidal-Valverde et al., 2002