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The potential of fermentation on nutritional and technological improvement of cereal and legume flours: a review

Garrido-Galand, S., Asensio-Grau, A., Calvo-Lerma, J., Heredia, A., Andrés, A.

Instituto Universitario de Ingeniería de Alimentos para el Desarrollo (IU-IAD). Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia.

Abstract

Nowadays there is an increasing demand for vegetable protein sources as an alternative to that of animal origin, not only for its greater environmental sustainability but also for its relationship with lower risk of suffering cardiovascular diseases. Legumes, cereals and seeds are seen as a good proteinaceous source providing as well dietetic fiber and phytochemicals with antioxidant properties. However, their digestibility and bioavailability are limited by the presence of anti-nutritional factors (ANFs) but susceptible of being improved by soaking, cooking or fermentation. The objective of this work is to review the solid-state and submerged fermentation effect on nutritional and functional properties of legumes, cereals and seeds. The microorganisms involved (bacteria, fungus and yeasts) are able to produce enzymes that degrade ANFs giving rise to more digestible flours with a more interesting nutritional, sensorial and technological profile. Solid-state fermentation is more commonly used for its higher efficiency, accepting agro-industrial residues as substrates and its lower volume of effluents. Fermented legumes had their technological properties enhanced while an increment in antioxidant properties was characteristic of cereals. The present review highlights fermentation of cereals and legumes mainly as a key process that at industrial scale could generate new products with enhanced nutritional and technological properties.

Key words: legumes, cereals, seeds, antinutritional factors, solid-state fermentation, submerged fermentation, flour.

Abbreviations:

ACE	Angiotensin converting enzyme
AoxA	Antioxidant activity
ANFs	Antinutritional factors
EAA	Essential amino acids
FAA	Free amino acids
FPC	Free phenolic compounds
GAE	Gallic Acid Equivalent
IVPD	*In-vitro* protein digestibility
LAB	Lactic acid bacteria
OBC	Oil binding capacity
SmF	Submerged fermentation
SSF	Solid-state fermentation
TFC	Total flavonoids content
TPC	Total phenolic content
WHC	Water holding capacity

1. Legumes, grains and seeds as sustainable source of protein

It is undoubtable that over the 50 past years, we have witnessed a sharp increase in the consumption of animal-origin products, representing nowadays more than half of the protein supply per capita/day (58%) (Bonnet *et al.,* 2020). Meat and derivatives are the main source contributing to protein intake (33.14%), closely followed by cereal products (17.38%) and milk and dairy products (17.17%). However, other important sources of animal protein should also be considered such as fish and seafood (10.63%), eggs (4.68%) or plant-based products (e.g., legumes) (3.32%), whose consumption is significantly low (ANIBES study, 2013; Bonnet *et al.,* 2020). Good quality protein intake is especially crucial in growth periods for physiological functions and organs development (Boye *et al.,* 2012). The Food and Agriculture Organization (FAO) estimates that by 2050, global population will reach 9 billion (FAO, 2012). Thereby, if these population was to keep up with such high meat intake as today, the production would need to rise by 200 million tonnes, which is environmentally unsustainable (FAO, 2009). For these reasons, there is a compelling need in seeking alternative and sustainable sources of protein to ensure an adequate protein intake to the world population, and also because protein-energy malnutrition (PEM) is currently a major public health problem (Bessada *et al.,* 2019; Khattab *et al.,* 2009). Among currently available alternatives, *in vitro* meat is becoming a trend with the intention of dealing with livestock discomfort and slaughtering. It is believed that artificial meat could help to reduce carbon footprint of meat production and provide good protein intake. However, the biggest challenge is reproducing the muscular tissue present in animal meat (Hocquette *et al.*, 2016). In this sense, new advances have been done using gelatine microstructured films in order to obtain parallel alignments of fibres.

However, more research is needed as conventional meat presented more muscle and mature fibres (MacQueen *et al.,* 2019). On the other hand, insect protein should also be mentioned as an alternative protein source, not only because of its high nutritional value but also for being breed in environmentally friendly conditions (Montowska, *et al.,* 2019). However, its consumption remains controversial and far from being commonly accepted by everyone.

In this context, promoting the presence of plant-based foods in the diet, in which legumes, cereals and seeds should be predominant, seems to be a sustainable alternative to protein intake from an environmental point of view: they present lower greenhouse gas emissions and are a highly valuable source of protein and other nutrients (Fasolin, *et al.,* 2019). Furthermore, legumes are not only able to enhance system productivity by helping to the diversification of crop rotations, but they can also restore soil nitrogen without using fertilizers (Margier *et al.,* 2018). Apart from the beneficial environmental impact, vegetal sources of proteins exhibit good nutritional profile since they are also rich in unsaturated fats, and as plant-origin products, they present phytochemicals with antioxidant properties and dietary fibre (Leitzmann, 2005). Legumes present high protein content that can range from 20% in peas or common beans *(Phaseolus vulgaris L.)* to 40% in lupin or soybean, 14-33% of dietary fibre contributing to lower the glycaemic index, 1-2% of total fat (except for oilseeds like soybean), vitamins and minerals including folic acid, thiamine, riboflavin, iron, zinc and calcium (Delgado-Andrade *et al.,* 2016). Cereals such as oats or barley contain a wide range of phenolic compounds with antioxidant activity as well as considerable high amounts of carbohydrates and protein, especially in sorghum comparing to other cereals (Đorđević *et al.,* 2010; Wu *et al.,* 2018; Wang *et al.,* 2019;

Pranoto *et al.,* 2013). In particular, cereal grains (e.g., wheat, corn) and legumes (e.g., beans, peas), present a majoritarian amount of starch apart from protein (Marshall & Chrastil, 1992). It is important to stand out that there is scientific evidence suggesting the existence of a relationship between botanic origin of starch and size (from 2 up to 100 µm), shape (oval, polygon, circular, elongated) and morphology of native granules (LeCorre *et al.,* 2011). Starch is made up of α-D-glucose units resulting in a homopolysaccharide that can be found in two forms: as transient starch and as storage starch. The former one is accumulated as a semi crystalline structure in leaf chloroplasts whereas the latter is present as granules in plant tissues and is mainly composed by amylose and amylopectin which are two glucosidic macromolecules (Gismondi *et al*., 2019). Storage starch is especially genus-specific and is characterized by successive crystalline regions and amorphous structures. In this sense, amylose can be found either in the amorphous region as in wheat starch or distributed with amylopectin clusters in the amorphous and crystalline regions as found in maize starch (LeCorre *et al.,* 2011). Differences in size can be associated to starches from different botanic origin. For instance, wheat starch nanoparticles can be twice as big as in maize (LeCorre *et al.,* 2011). Similar results were encountered by Gismondi *et al.* (2019) indicating that *T. aestivum* (wheat) starch granules exhibited a length ranging from 6 to 21 µm and a width between 5-20 µm. On the contrary, *Z. mays* (maize) starch granules were smaller (7-15 µm for length and 5-15 µm for width). Species can as well be differentiated by some particular configurations on the grains such as the presence of fissures (Torrence et al., 2004). In this sense, legumes such as *C. arietinum* (chickpeas) or *L. culinaris* (lentils) present mesial longitudinal clefts whereas cereals such as O. sativa (rice) or T. aestivum (wheat) don't present fissures (Gismondi et al., 2019). However, treatments applied such as drying or grinding can as well produce fissures (Torrence et al., 2004). Different rheological properties can be encountered in the final product depending on the plant crop employed and its size and shape of starch granules (LeCorre *et al.,* 2011).

Moreover, starch plays a crucial role as it can form interactions between proteins and lipids in the food matrix (Zhang *et al.,* 2014). Protein-starch systems not only affect the functional properties but also the quality and the nutritional value (e.g., texture, flavour, shelf life, digestibility) of the final food products (Marshall & Chrastil, 1992; Wang *et al.,* 2020). At low temperatures, this interaction is possible due to the opposite charged surfaces of starch and proteins, being the former negatively charged and the latter positively, causing, therefore, a pH-dependent interaction (Marshall & Chrastil, 1992). However, when plant crops are processed through thermal treatments, gelatinization of starch takes place which implies that starch granules do not dissolve entirely and remain as fragile and amorphous structures known as "ghosts" (Debet & Gidley, 2007; Zhang *et al.,* 2014; Wang *et al.,* 2020). As a result, proteins can as well exhibit cross-linking and generate a network which in contact with starch results in a stable protein-starch matrix (Marshall & Chrastil, 1992). On the other hand, seeds such as *Salvia hispanica*, also known as chia, offers high contents of dietary fibre, polyunsaturated fatty acids (α-linolenic acid mainly), high protein content (18-24% of their mass) and mineral supply (Kulczyński *et al.,* 2019).

Altogether, plant-based diets have been positively associated with a healthy lifestyle. There is scientific evidence indicating that legumes might reduce the risk of suffering cardiovascular disease (CVD) (Patel *et al.,* 2017), metabolic syndrome and type 2 diabetes, while they provide substantial benefits in terms of weight control and gastrointestinal health (Delgado-Andrade *et al.,* 2016). On the contrary, red and processed meat intake has directly been correlated with higher cancers prevalence (colorectal, pancreatic and prostate cancer). Moreover, processed meat consumption is as well a major factor in weight gain since it contains cholesterol and saturated fatty acids (Bonnet *et al.,* 2020). In this context, Harvard Health Publishing and nutrition experts suggested the "Healthy Eating Plate" as a guideline in order to create balanced meals. Protein intake should represent ¼ of our plates, where legumes and pulses, must be included since they are considered healthy and versatile protein sources. Furthermore, red meats are meant to be limited and processed meats avoided (Harvard School of Public Health, 2011). Later on, 2016 was declared by the United Nations as The International Year of the Pulse in order to promote grain legumes consumption for being highly nutritious and sustainable (Margier *et al.,* 2018). In terms of protein requirements, the value accepted for a safe level of intake is 0.8 g/kg body weight/day regardless of the age (WHO, 2007; Lonnie *et al.,* 2018).

Despite the above-cited benefits, legumes, cereals and seeds also exhibit some nutritional deficiencies. While legumes present a great content of essential amino acids (EAA) such as lysine (64 mg/g of protein) and threonine (38 mg/g of protein), they lack of sulphur-containing (S-C) amino acids (methionine, cysteine and tryptophan) (Havemeier *et al.,* 2017). In this sense, essential amino acids are considered critical amino acids that humans are not able to synthesize *de novo* and that are required for proteins formation. There are nine of them, among which lysine, methionine and tryptophan are limiting in plants. This can be explained by the fact that their synthesis in plant tissues is controlled by regulatory factors trough inhibition loops, as an accumulation of amino acids alters the normal biosynthesis of enzymes. Moreover, trough the catabolism of amino acids, generation of products serving for plants growth and development is enabled as well as cellular energy production (Galili *et al.,* 2016). For that reason, legumes such as pulses are usually characterized by being an incomplete source of protein, whereas animal proteins (meat, fish, eggs or milk) are nutritionally complete, since they present higher levels of EAA (Vaclavik & Christian, 2014). Even though, legumes can be combined with cereals such as wheat and rice, as they present complementary amino acid profiles (good source of S-C amino acids but deficient in lysine) improving hence the quality of the ingested protein (El Youssef *et al.*, 2020). Besides, legumes also contain anti-nutritional compounds, (ANFs), which at high concentrations, can drastically reduce the bioaccessibility of many nutrients, and thus interfere with their absorption (Robinson *et al.,* 2019). Scientific evidence has showed the ambivalent effect of ANFs as they also exhibit antioxidant and prebiotic activity at low concentrations (e.g., phytates) (Margier *et al.,* 2018) and hypolipidemic and antitumoral properties (e.g., lectins, protease inhibitors) (Bessada *et al.,* 2019). Their biological function, therefore, remains not fully understood, but evidence is certain that they are produced by the plant as secondary metabolites to exert a protective effect against attacks by microorganisms or insects (Belitz *et al.,* 2009).

ANFs can be classified into proteinaceous compounds (lectins, protease inhibitors such as trypsin and chymotrypsin inhibitors) and non-protein factors (phytic acid, phenolic compounds (tannins and saponins), α-galactosides and alkaloids) (Bessada *et al.,* 2019; Khattab & Arntifeld, 2009) (**Table 1**). Protease inhibitors are frequently found in legumes such as soybeans (20 g/kg) white beans (3.6 g/kg) and chickpeas (1.5 g/kg) (Belitz *et al.,* 2009) and are responsible of decreasing protein digestibility, since trypsin and chymotrypsin are hydrolases that break down dietary protein (Robinson *et al.,* 2019). Lectins are glycoproteins found in cereals and pulses with sugar-binding activity, and therefore with capacity to interfere in the normal nutrient absorption. At certain doses, they can exhibit agglutination activity in blood cells (hemagglutination) (Rehman *et al.,* 2014; Bessada *et al.,* 2019). Phytic acid forms complexes with dietary minerals such as calcium, zinc, iron and magnesium, and therefore decreases bioavailability and mineral absorption (Rosa-Sibakov *et al.,* 2018; Parca *et al.,* 2018). In addition, it can also bind to other nutrients like protein and digestive enzymes (proteases and amylases) resulting in lower protein solubility and proteolysis inhibition (Parca *et al.,* 2018). Phenolic compounds such as tannins are well-known for precipitating proteins, decreasing protein digestibility and amino acid availability (Robinson *et al.,* 2019). In addition, astringent properties are also popular among tannins as they can form complexes with salivary glycoproteins resulting in a reduction in palatability, causing hence a sensory limitation (Bessada *et al.,* 2019). On the other hand, saponins are frequently found in lupins, lentils, chickpeas and in some beans and peas. They are able to form large size micelles by the interaction with bile acid and cholesterol, resulting in poor absorption of cholesterol and free fatty acids. They are also responsible for sensorial rejection due to its bitter taste and foam formation capacity (Bessada *et al.,* 2019). For their part, α-galactosides such as raffinose, stachyose and verbascose, which are often found in legumes, are oligosaccharides composed by sucrose with an α-1-6-galactosyl residue joined to the molecule end (Thirunathan & Manickavasagan, 2018). These compounds are responsible for flatulence and gut gas production caused by microorganisms' fermentation (Rehman *et al.,* 2014). Alkaloids are typically found in lupins and are undesirable for human and animal consumption due to unpalatability, bitter taste and they cause toxicity of the seeds (Kasprowicz-Potocka *et al.,* 2018). Moreover, other sensorial limitations include off-flavours, due to the presence of aldehydes such as hexanal in pea proteins, resulting in an undesirable aroma (El Youssef *et al.*, 2020).

Despite the negative effects of the ANFs, their presence can be reduced, and subsequently nutrients bioavailability can be increased, by applying traditional household techniques such as soaking, cooking, roasting or germination before consumption of legumes, cereals and seeds (Bessada *et al.,* 2019) (**Table 1**). Soaking consists in exposing seeds to water and salt solutions, usually overnight (15-20h) or for a shorter time (15-20 min) (Vashishth *et al.,* 2017). To promote the hydrolysis of phytic acid, according to recent studies, soaking should be conducted at optimal conditions of temperature of 45-65 ºC and a pH range of 5-6 (Vashishth *et al.,* 2017; Samtiya *et al.,* 2020). Soaking can also enhance removal of water-soluble compounds such as phytates, in legumes and cereals (Rehman *et al.,* 2014). For instance, a reduction of 8.26% of phytic acid was reported in chickpea after 12h of soaking. However, other water-soluble components such as phytochemicals, vitamins and minerals in cereals could be also reduced (Samtiya *et al.,* 2020). Cooking involves boiling food in water at 99 ºC (Thirunathan & Manickavasagan, 2018) and it usually follows a previous soaking in the case of legumes (because it reduces cooking time and softens the texture) or a physical treatment such as dehulling (as the seed tegument is not permeable to water). Hull removal does not only improve water absorption but also palatability, as bitterness is reduced, while on the other hand it implies a loss of the nutrients that are present in the hull. In addition, during cooking, thermo-labile compounds such as enzymatic inhibitors and lectins may be inactivated, but others that are thermo-resistant such as tannins, phytic acid or saponins remain unaltered (Bessada *et al.,* 2019). Another cooking method, as roasting, is performed with dry heat at 120-250 ºC. It is considered as a thermal degradation process that can reduce α-galactosides such as raffinose, but at the expense of the reduction of other nutritional compounds in pulses such as proteins, starch or vitamins and minerals (Thirunathan & Manickavasagan, 2018). Besides, roasting technique has as well reported to decrease trypsin inhibitor activity in soybean (Samtiya *et al.,* 2020). Another commonly used technique prior consumption is germination, which is considered as the sprout of the seeds. Germination has been attributed to improve protein digestibility of beans, probably due to phytate degradation by native phytases (Rehman *et al.,* 2014; Thirunathan & Manickavasagan, 2018). In addition, reduction of the presence of tannins in germinated cereals has been documented, resulting in an increase of mineral bioavailability and hence of nutritional value (Samtiya *et al.,* 2020).

Apart from the inhibitory effect of ANFs on protein digestibility, plant protein is less digestible than animal protein. Plant-based proteins are mainly found in β-sheet conformation whereas animal protein has higher α-helix proportion. β-sheet structure is associated with a particular resistance to denaturation and hence with higher resistance properties towards protein breakdown in the gastrointestinal process (Bessada *et al.,* 2019; Aryee & Boye, 2016). Furthermore, plant-based proteins usually contain fibres that hinder the access of proteases, which therefore decreases protein digestibility (Berrazaga *et al.,* 2019).

Finally, plant-based proteins exhibit functional properties that make them suitable for food formulation, as for example in gluten free or protein-enriched products, or in bio-fortification of cereal-based products (Mora-Uzeta *et al.,* 2020). Some of these properties include water holding and oil binding capacity, bulk density, gelation ability, foaming capacity or emulsifying activity among others. These properties are highly dependent on protein and peptide structure, as well as on the interaction with other compounds such as carbohydrates, lipids, other proteins or water. However, since many ANFs can form complexes that decrease protein solubility and availability, these functional properties may be strongly affected (Bessada *et al.,* 2019).

2. Searching tools and inclusion criteria performed

In order to identify papers dealing with flour fermentation of legumes, cereals and seeds, we carried out an extensive literature search. Searches were mainly carried out in two databases considered as reliable and abundant source of research (Web of Science and Scopus). The following items were introduced each time but varying the substrate in order to refine the search: *Years*, from "all years" to "present"; *Key words*, "legumes/cereals/seeds" and "flours" and "fermentation"; type of document: "paper" or "review". Then, some subject areas that were not related to the field of food science were excluded such as "social sciences", "business, management and accounting", "environmental science"; "chemical engineering", "biochemistry, genetics and molecular biology", "chemistry" among others. Finally, some journal titles were as well excluded, for instance Animal, Journal of Animal Science, Poultry Science or Clinical Immunology. Taking into account all of these restrictions, 32 research articles related to studies in legumes, cereals and seeds flour's fermentation were included in the review (13 papers for legumes, 13 papers for cereals and 6 papers for seeds). **Figure 1** shows a diagram explaining the search criteria carried out for inclusion of articles.

3. Fermentation technology as a biotechnological opportunity for improving nutritional and functional properties of foods

Biotechnological techniques have been usually applied in agriculture for increasing production yield, improving pest resistance or enhancing nutritional quality and healthy benefits of food products (Niba, 2003; Datta & Bouis, 2000). In order to deal with global food production, poverty, environmental issues and also nutritional and health problems, biotechnology presents as a powerful tool for developing new sustainable foods with increased nutritional value (Xing *et al.,* 2020). For instance, an example of sustainable biotechnology for plant proteins production is aquaponics. This growing technology combines hydroponics and elements of recirculating aquaculture allowing aquatic organisms and plants to grow symbiotically (Jena *et al.,* 2017). In this sense, water from fish tanks is enriched with nutrients coming from their urine and faeces, and serves as liquid fertiliser for plant growth (Goddek *et al.,* 2015). Nitrifying bacteria from gravel are in charge of converting ammonia from fish waste into nitrate, the form of nitrogen that plants can utilize. It seems a solution towards unsustainability as environmental pollution is lower and there is no need of artificial fertilizers (Jena *et al.,* 2017). However, the main challenge would be the complexity of the system design and the need of standardization in order to be economically viable and easy to handle (Goddek *et al.,* 2015).

Food fermentation dates back to many centuries ago and is considered a food preserving technique, a way of obtaining traditional and nutritive foods, and also, a tool for obtaining new flavours, aromas and textures and foster gastronomic pleasure (Xu *et al.,* 2019). Fermentation of foods are considered as artisanal practices in origin (nowadays industrialized fermentations are optimised processes) and fermented products are commonly present in our daily diet. Globally, fermented foods include soy sauce, tempeh, miso and kombucha from East and Southeast Asia; yogurt, cheese, salami, kefir and quark from Europe and hot pepper sauce among other products from Africa (Xiang *et al.,* 2019). Other traditional fermented foods produced worldwide include beverages such as beer, coffee, tea, wine and cider; bread resulting from cereals fermentation; and pickles or olives from fermented fruits and vegetables (Campbell-Platt, 1994). Among the microorganisms used in fermentation of foods and production of beverages, we can mention molds or fungus (e.g., *Aspergillus spp., Mucor spp.,* and *Rhizopus spp.*), bacteria (e.g., *Lactobacillus spp.*, *Bifidobacterium spp.* and *Streptococcus spp.)* and yeasts (e.g., *Saccharomyces spp.*) (Borresen *et al.,* 2012).

Fermentation can be defined as a biological process in which microorganisms convert substrates into new products, such as enzymes, biomass and primary and secondary metabolites (Adebo *et al.,* 2017). This technique has been largely used by the industry for conservation and sensory purposes in dairy products (e.g., ripened cheese) or in the wine industry. However, other applications of fermentation include increasing the commercial value of food products. Especially regarding the latter, fermentation has proven to have the ability of improving food properties, because the microorganisms involved can generate enzymes that degrade antinutritional factors (Đorđević *et al.,* 2010; Thirunathan & Manickavasagan, 2018). As discussed in the section above, the resulting products do not only present increased nutritional value and are more digestible, but they also present different texture and flavour compared to raw materials, which makes them more palatable (Adebo *et al.,* 2017; Saharan *et* *al.,* 2017). In addition, fermentation contributes to improve food safety, as the growth of pathogenic microorganisms is prevented (Xiang *et al.,* 2019). Therefore, fermented products are meant to be key ingredients for novel foods development with enhanced properties in a wide range of areas of the food industry (food supplements, soup condiments or seasonings (Onweluzo & Nwabugwu, 2009; Onimawo *et al.,* 2003), infant food formulation (Olagunju & Ifesan, 2013) or fortified cereal-based products (e.g., sour-dough breads) (Xing *et al*., 2020)).

Fermentation can be performed either in solid-state (SSF) or by the submerged method (SmF). SmF involves the growth of microorganisms in a liquid culture containing nutrients, high content of free water and oxygen concentration where substrates are rapidly consumed (Liu & Kokare, 2017; Subramaniyam & Vimala, 2012). On the other hand, SSF enables the growth of microorganisms on solid substrates surrounded by a continuous gaseous phase. Despite of the presence of some droplets of water between the inter-particle spaces, quantity of free water is scarce (or non-existent) and spaces are filled by gas, which promotes the growth of microorganisms (Pandey, 2003). In 1940, SSF had a great impact due to the production of antibiotics such as penicillin, being this period named as the “Golden Era” of the industrial fermentation (Krishna, 2008). However, difficulties encountered in controlling SSF process (Mitchell *et al.,* 2006) enhanced SmF employment. Some of the obvious advantages of SmF include: i) good control of fermentation parameters (temperature, moisture, monitoring pH or aeration due to the facility of homogenizing the liquid culture) (Domínguez-Espinosa & Webb, 2002), ii) reduction in the risk of desiccation of the fungal hyphae when using molds (Mitchell *et al.,* 2006), iii) lower limitations of requirements when scaling-up the process (Manan & Webb, 2017) and

iv) less restrictions in the types of microorganisms able to grow in the culture (Krishna, 2008) **(Figure 2)**.

In the sixties and seventies, however, the interest in SSF suddenly reappeared mainly because the technique allowed for ferment cheap substrates like agro-industrial residues, being an environmental solution for dealing with solid pollutant wastes (Pandey, 2003; Mitchell *et al.,* 2006). Additionally, this technique accepted a broad range of matrices as substrates (Krishna, 2008). Moreover, practical, economic and environmental advantages of SSF over SmF have been highlighted: i) higher concentration of final products since substrate inhibition is scarce (SSF would convert 20-30% of the substrate whereas in SmF the maximum amount is around 5% (Liu & Kokare, 2017), ii) environmentally-friendlier due to low water consumption and waste water generation, iii) reduced water activity, making it less susceptible to contaminations, iv) higher volumetric productivity due to a compacted bioreactor and a lower level of moisture, and finally v) less expensive and simpler downstream processes in case of the product needs extraction (Soccol *et al.,*2017).

In terms of inoculum used in each technique, SSF is ideal for the development of fungus, as the process conditions are similar to the natural environment where these microorganisms are usually adapted to grow (Manan & Webb, 2017). Moreover, fungal hyphae have the specific capacity of growing in interspaces of solid particulate substrates (Mora-Uzeta *et al.,* 2020). On the other hand, while content of moisture is in the range of 12-70% in SSF (at a lower level of moisture the biological activities are halted (Krishna, 2008)), in SmF the medium is 100% liquid. As a result, this condition makes SmF more suitable for bacteria cultivation, due to the high requirement of high-water activity (Subramaniyam & Vimala, 2012; Manan & Webb, 2017). In this sense, one of the main problems associated with conventional submerged fermentation is viscosity of the broth due to the molds growth; they produce a mycelium that can interfere in the driving forces and thus in oxygen diffusion (Liu & Kokare, 2017).

Fermentation is usually performed with the substrate already milled in order to increase the contact area between substrate and microorganism (Olukomaiya *et al.,* 2020; Starzynska-Janiszewska & Stodolak, 2011). However, other process designs can include the milling state after the substrate has been fermented and dried. Milling can be defined as a process in which the grain is grinded into flour or meal with the objective of reducing the particle size and destroying the cellular structure (Oghbaei & Prakash, 2016). As a result, the surface area of the grain is increased and access of enzymes for ANFs degradation is promoted. Enhancement of compounds' bioavailability and protein digestibility is hence boosted (Nkhata *et al.,* 2018). Moreover, due to the starch content present in cereals mentioned before, the milling process is susceptible of changing starch structures resulting in a disruption of the crystalline form and degradation of starch molecules. These changes would particularly affect the functional properties of the resulting flour (e.g.; pasting and swelling properties). In this sense, the degree of starch damage is important as a mild milling is favourable leading to an increase of the loaf-volume of bread but conversely, a severe treatment would rather be negative towards bread quality (Li *et al.,* 2014). A posterior drying treatment is usually applied in order to remove the moisture and stabilise the fermented flour. It is considered as one of the final procedures before packaging or storage of powders and flours (Khan *et al.,* 2016). Drying seems to influence sensory profile of flours, especially regarding colour changes, as Maillard reactions may take place. In addition, new compounds can be generated as well, contributing to antioxidant activity enhancement. However, the optimal temperature is challenging since some bioactive compounds can experiment heat damage with the consequent loss of antioxidant properties (Stoffel *et al.*, 2019).

Not only are the characteristics of the substrate employed important (whether the substrate is milled before or after fermentation) for obtaining nutritional and technological improvements, but also the right selection of starter cultures according to their characteristics and traits. Identifying the most suitable starter culture is advantageous as for example, the growth of undesired microorganisms can be prevented by competitiveness of starters and their metabolites (FAO, 1999). In this sense, cereals fermentations are usually performed without previous pasteurization of the raw material as starch would be at the risk of suffering gelatinization and endogenous enzymes present in the cereal and required during fermentation, may be inactivated (Brandt, 2014). Therefore, when fermenting cereals, some starter cultures may show high competitiveness along with short lag phases, which indicates that the strains are able to rapidly grow in sugar enriched materials (FAO, 1999). Regarding legumes, diverse technological properties are targeted when selecting starter cultures. Since legumes present high protein content, enzymatic activities such as proteolytic are not only important for the release of bioactive compounds and essential amino acids producing desired flavors but also for the development of starters (Sáez *et al.,* 2017). As a result, high growth rates and high proteolytic activity are desired when fermenting legumes. A significant synergy between enzymes from the substrate and from the microorganisms has been observed, supporting the selection of the most accurate starter culture (FAO, 1999). Furthermore, as before mentioned, since legumes contain ANF's, strains with enzymes able to remove tannins should be selected. For instance, *Lactiplantibacillus plantarum* has been identified as a LAB strain with ability of metabolizing tannins (Sáez *et al.,* 2017). Other environmental and stressful factors that starter cultures must deal with are the increase in temperature and decrease in pH, as in the case of making bread dough.

In this context, the present review aims at compiling the most relevant scientific information about the application of fermentation in legumes, cereals and seeds in order to improve their nutritional and functional properties, with the ultimate goal of highlighting its potential in developing of new foods and ingredients.

4. FERMENTATION OF LEGUMES

Assuring a proper intake of macronutrients is crucial for a nutritive and balanced diet. However, as mentioned before, some antinutritional factors or interactions between compounds can reduce the bioavailability of nutritional compounds in legumes. The present section summarises the most recent findings regarding the application of fermentation to improve nutritional profile and functionality in legumes.

4.1 Impact of fermentation on nutrient profile of legumes

The most relevant information obtained from fermentation studies carried out using legumes as substrate are gathered in **Table 2**. Legume fermentation with fungus is more often performed in solid-state conditions while most of the SmF studies of legumes are carried out with bacteria. One of the main effects of fermentation of legumes is found in terms of protein changes. In this sense, fermentation with *Pleurotus ostreatus*, has shown to allow an increase in the protein content of kidney beans and black beans (*Phaselous vulgaris)* of 13% and 6% respectively, as a result of its ability to synthetize amino acids during fermentation (Espinosa-Páez *et al.,* 2017). An even higher increase in the protein content (18.5%) was found in fermented lentils using *P. ostreatus* as well, but at the expense of carbohydrates reduction (6%). This protein increment could be explained as during fermentation, carbohydrates serve as energy source for fungus growth and some of them may have been bioconverted into protein (Asensio-Grau *et al.,* 2020). Furthermore, due to the action of a tannase that this fungus may contain, a reduction of tannins content was reported (Espinosa-Páez *et al.,* 2017). Since tannins are known to bind proteins by forming tannin-protein complexes, protein availability and digestibility may be increased. Likewise, Asensio-Grau *et al.* (2020) reported an enhanced hydrolysed protein fraction after lentils fermentation with *P. ostreatus*, similar to the one occurring during the gastrointestinal digestion and hence contributing to a higher digestibility in the resulting flour. Similar results were found by Mora-Uzeta *et al*. (2020), in which protein content increased in tepary beans (*Phaselous acutifolius*) (+35%) fermented by *Rhizopus oligosporus.* Besides, when inoculation is carried out with co-culture, protein content seems to suffer a larger increase in comparison with individual strain fermentation as observed in lupin flour fermented by *Aspergillus sojae* and *Aspergillus ficuum*, (Olukomaiya *et al.,* 2020). Conversely, when bacteria are used in SSF, Li *et al*. (2020) reported a protein increment (+14.45%) in whole soybean flour using *Lacticaseibacillus casei,* which is similar to the previous results obtained with fungus. Therefore, the synergy between microorganisms seems to promote higher changes in the nutrient profile than individual fermentation.

While the effects on total protein content have been defined, the impact of fermentation on amino acid profile remains uncertain since, the effect might be different depending on the type of microorganism, the strain and the substrate used (Mora-Uzeta *et al.,* 2020). The content of most of the essential amino acids (EAA) such as threonine, leucine, isoleucine, phenylalanine, methionine and valine was increased by fungus in kidney, black and tepary beans. Furthermore, SC-amino acids (methionine + cysteine) usually present in low quantities in legumes increased 9.83%, 2.72% in black beans and kidney beans fermented by *P. ostreatus* and 16.46% in tepary beans fermented by *R. oligosporus* (Espinosa-Páez *et al.,* 2017; Mora-Uzeta *et al.,* 2020). As a result, the quality of the protein in the resulting legume flour was boosted. However, regarding basic amino acid such as lysine and arginine, a reduction was observed, probably due to the acidic conditions (pH<4) during fermentation, leading to amino acid destabilization (Espinosa-Páez *et al.,* 2017). In those studies of bacterial fermentation, total EAA content exhibited a great increment as well (+10.25%) after 72h fermentation in whole soybean. Free amino acid content (FAA) can also be affected by other factors such the addition of exogenous phytase in submerged fermentation as reported by Rosa-Sibakov *et al.* (2018). The ability of the enzyme to perform dephosphorylation of phytic acid improve protein digestibility and availability of minerals. This is in agreement with findings reported by Bautista-Expósito *et al.* (2018), where addition of hydrolytic enzymes such as savinase to the fermentation with *Lactiplantibacillus plantarum*, increased the release of peptides with potential biological activity. As a result, it has been considered as an efficient tool for production of lentil flour with enhanced health-promoting properties.

Another significant change observed in fermented legumes is the reduction in lipids and fat content; for example, a reduction of 22.16%was found in whole soybean fermented by *L. casei* and also in tepary bean flour fermented by *R. oligosporus* (47.45%) (Li *et al*., 2020; Mora-Uzeta *et al.,* 2020). This reduction is more than doubled in fermented tepary beans by *R. oligosporus* being again higher in comparison with the other mentioned studies. This is likely due to the ability of some fungus such as *R. oligosporus*, to produce lipases and to its capacity of obtaining energy from released fatty acids (Mora-Uzeta *et al.,* 2020). In addition, reduction of saturated fatty acids such as palmitic and stearic was encountered with microbial fermentation(-32.6% and -16% respectively), whereas ω-3 fatty acids (e.g., α-linolenic) were increased (+15%) in whole soybean, enhancing the nutritional quality of the resulting flour (Li *et al*., 2020).Unsaturated fatty acids tend to be oxidised by lipoxygenases, resulting in the generation of off-flavours, so provided that fermentation reduces lipoxygenases activity, this problem would be avoided. In this sense, fermentation by bacteria has shown to allow a reduction of undesirable enzyme activities and thereby improve the sensorial profile of the resulting flours.

One special aspect to consider is hardness of legumes and grain hulls, which exhibit a protective function towards microbial attacks and will determine the extent to which microorganisms will have access to their substrates. In this sense, harder hulls will prevent against lignin and cellulose degradation, resulting in less reduction of fibre content. Hence, it can be found that dietary fibre can either remain unaltered or reduced, according to the hardness of the hull. In this sense, dietary fibre exhibited a decrease of 59% in fermented black beans by *P. ostreatus* due to the lower hardness index of the grain hull. Released lignin and cellulose by cellulases, xylanases and laccases, are used by the fungus as nutrients for its growth (Espinosa-Páez *et al.,* 2017). Similarly, crude fibre content also decreased in whole soybean flour (-38.6%) during lactic fermentation by *L. casei,* as bacteria are able to synthetize cellulolytic and hemicellulolytic enzymes (Li *et al*., 2020). Contrarily, substrates with harder hull such as kidney beans even exhibited an increase in dietary fibre content (+16%) after fermentation by *P. ostreatus* (Espinosa-Páez *et al.,* 2017). One possible reason could be that some fungus are able to use carbohydrates and fats as substrates and produce an enriched fibre mycelium. *Rhizopus* has been identified as a polysaccharides producer, including cellulose and chitin, resulting in a higher dietary fibre content in fermented substrates such as tepary beans (+86%) as seen by Mora-Uzeta *et al.* (2020).

It is well known that minerals and vitamins bioaccessibility may be compromised by the formation of complexes with ANF's, and this is another aspect that can be improved by SSF fermentation. An increase of iron and zinc content as well as an improvement of its bioavailability was observed in black eyed peas fermented by *Aspergillus oryzae* as a result of a decrease in ANFs and toxic factors (Chawla *et al.,* 2017). Another example is the increase of calcium and phosphorous content observed in solid-state co-fermented lupin flour with *Aspergillus sojae* and *Aspergillus ficuum.* These results may be explained considering the fermentation's ability of degrading phytic acid. Thereby, minerals forming complexes with phytic acid are released resulting in a lupin flour with mineral concentration increased (Olukomaiya *et al*., 2020).

Other antinutritional compounds are oligosaccharides such as raffinose, well-known for their excessive ability of gas formation and gastrointestinal discomfort. They seem to be reduced after 48h fermentation in fermented lupin meal. Especially, *K. lactis* was able to reduce total oligosaccharides by 63% while *S. cerevisiae* by 81% and *C. utilis* by 100%. Available oligosaccharides were used up by yeasts justifying its reduction after fermentation. Thereby, depending on the yeast strain the content of oligosaccharides was affected differently (Kasprowicz-Potocka *et al.*, 2018).

Impact of fermentation processes in phenolics content of legumes has also been studied. *P. ostreatus* is able to excrete phenol oxidases such as laccases that may depolymerize conjugated phenolic compounds from legume substrates and the mycelium can additionally synthetize phenols, this contributing to the overall increase in TPC (Espinosa-Páez *et al.,* 2017). This increase was of 26.3% in SSF of black beans but absent in kidney beans. As a result, antioxidant activity (AoxA) was also affected: since kidney beans presented a harder hull, phenol oxidases had limited access for degrading bound phenolic compounds and AoxA did not increase. Contrarily, AoxA increased in fermented black beans (+39.5%) due to the higher permeability of its hull (Espinosa-Páez *et al.,* 2017). Similarly, a higher increase in the polyphenol content was reported in fermented lentils with *P. ostreatus* (+53%) as a result of the hydrolysis of bound phenols and the rise in phenylalanine and tyrosine precursors (Asensio-Grau *et al.,* 2020). An even greater increase of TPC was found in SSF of tepary beans by *R. oligosporus* (+196.7%); together with FAA and peptides, they were associated with the observed increase of AoxA (+116%) (Mora-Uzeta *et al.,* 2020).

When fermentation is performed under submerged conditions, some differences can be encountered. TPC decreased after processing lentil flour with *L. plantarum* (-31.5%), the reduction being attributed to a decrease in flavan-3-ols content. Since flavan-3-ol monomers are sensitive to pH above 6, and fermentation was performed at pH= 6.8, their stability was compromised and hence the compounds degraded. This suggests that pH is a key factor in these processes (Bautista-Expósito *et al.*, 2018). Moreover, the effect of fermentation by *L. plantarum* in combination with enzymatic hydrolysis of savinase have been studied (Bautista-Expósito *et al.*, 2018). Phenolic compounds linked to the cell wall can be released due to protease and esterase activity of savinase but also to extracellular esterase of *L. plantarum*, resulting in increased p-hydroxybenzoic acid and flavonols contents. Therefore, it could be said that combining fermentation with hydrolytic enzymes, may have a positive effect in the release of phenolic compounds. Other SmF studies in which *L. plantarum* has been used, reported a decrease in the conjugated forms for ferulolyl derivates (-21.9%) and p-coumaric derivatives (-23.7%) suggesting that the strain may have a phenolic acid decarboxylase (PAD) activity. Moreover, new phenolic compounds that are not present in unfermented flour, can be produced such as tirosol and quercetin, the latter as a result of the hydrolysis of quercetin glycosides (quercetin 3-*O*-glucoside and 3-*O*-galactoside) (Dueñas *et al.*, 2005).

In general terms, variables such as processing conditions or fermentation time are key factors to optimize and control the process. Fermentation time plays an important role as well: Kasprowicz-Potocka *et al.* (2018) reported a greater improvement in the protein content with 72h fermentation (+12.6%) in comparison with 48h fermentation (+6.8%) in lupin meal fermented by *Candida utilis*. As a result, the duration of the fermentation process is as well an important parameter, since longer time fermentation tends to highly improve nutrients bioavailability in comparison with shorter time fermentation.

Additionally, post-treatments after fermentation may be effective in improving functionality of the resulting product; AoxA was increased after heating fermented cowpea flour (Dueñas *et al*., 2005). A possible explanation for this finding is that heat driven reactions produce new phenolic compounds with high antioxidant activity, such as hydroxymethylfurfur aldehyde after Maillard's reaction. On the other hand, Mora-Uzeta *et al.* (2020) concluded that in vitro protein digestibility (IVPD) increased (+17.54%) in fermented tepary beans, in part due to the applied pre-treatments: bean cotyledons were cooked (90ºC, 30 min) before inoculation with *R. oligosporus*. As a result, proteins are likely to have been denaturalized and the access for hydrolysis, enhanced.

4.2 Impact of fermentation on functional, sensorial and healthy properties of legumes

Properties with technological functionality such as water holding and oil binding capacity, bulk density or emulsifying and foaming properties, have a fundamental role in processing and development of new food products.

WHC was increased in black eyed pea flour (from 0.69 to 1.33 g water/g dry powder), as fermentation causes protein denaturalization and exposure of hydrophilic amino acid residues (Chawla *et al.,*2017). This result was in accordance with findings in fermented chickpea flour, where WHC improved from 1.1 to 1.7 g water/g dry powder (+54.5%) (Xing *et al.,* 2020). Contrarily, WHC decreased in fermented lupin flour as a result of a lack of hydrophilic groups, well-known by their ability of forming bonds with water molecules. Hence, a fermented flour with low WHC is indicated for gruels production (Olukomaiya *et al.*, 2020).

Oil binding capacity (OBC), defined as the quantity of oil that can be absorbed by 1 g of protein is relevant for food development since industrial processing, shelf-life and sensory quality of products (for example flavour retention) is greatly affected by this property (Bessada *et al.,* 2019). The increase of OBC observed in fermented black-eyed peas and lupin flour could be associated with exposure of nonpolar amino acids or oil entrapment in the surface after flour fermentation (Chawla *et al.,* 2017; Olukomaiya *et al.*, 2020).

On the other hand, fungal proteases able to hydrolyse large-size peptides into lower molecular peptides lead to an improvement of emulsifying properties in black eyed peas. The resulting short chained peptides can easily migrate into the interface between immiscible liquids like oil and water and form emulsions. Moreover, hydrolysis enabled the exposure of hydrophobic groups producing a shift in the hydrophilic-lipophilic balance contributing as well to the increase in emulsifying properties (Chawla *et al.,* 2017; Bessada *et al.,* 2019).

Some other properties such as bulk density of flours, refers to flowability and its ability to be compacted under pressure. It indicates the amount of flour that can be packed per unit area and is associated with texture, mouthfeel and the amount and strength of packaging material. In this sense, fermentation of black-eyed pea resulted in a reduction of bulk density (0.31 g/cm^3) (Chawla *et al.,* 2017). Variation of bulk density is commonly associated with variation in the content of starch: higher starch content supposes increment in bulk density and higher bulk density needs denser packaging material. As a result, low bulk density flour not only makes easier food packaging but it is as well advantageous for formulation of infant and weaning foods of high nutrient density (Awuchi *et al*., 2019).

Furthermore, regarding foaming properties it can be distinguished the foaming capacity as the volume of air that the protein is able to incorporate and the foaming stability as the time that this foam remains stable (Bessada *et al.,* 2019). Effect of fermentation on these properties remains unclear since in black eyed peas flour fermented by *A. oryzae,* foaming capacity increased whereas in fermented chickpea flour by LAB it decreased. Increase in foaming capacity in black eyed peas could be explained by enhanced WHC previously mentioned (Chawla *et al.,* 2017). Besides, fermentation generates electrostatic changes in macromolecules such as proteins, which are able to form thick films around each air bubble and thereby to reduce the surface tension increasing foam capacity and stability. As a result, the combination of an increment in electrostatic charges and WHC led to increased foam stability. Good foaming capacity and stability are desirable in flours in order to produce baked products but also to be used as additives in food formulation (Awuchi *et al.*, 2019). Contrarily, foaming capacity decreased in chickpea fermented flour (-50%) as a consequence of partial proteolysis during fermentation (Xing *et al.,* 2020). Protein is the main responsible of maintaining the suspension of air bubbles, hence if proteins are hydrolysed, their capacity of foam formation is reduced (Awuchi *et al.*, 2019).

Depending on the substrate, findings may be particularly specific for some legumes as there will be mentioned below.

Off-flavours associated with beany, green and leguminous attributes have been reported in pea proteins (El Youssef *et al.*, 2020). Sensory properties are essential when it comes to new foods production in order to have consumer’s acceptability. In this sense, by using microbial co-culture of LAB and yeasts, not only are aldehydes, ketones and alcohols reduced, but also new compounds such as esters are generated. As a result, beer and yeasts attributes arose and mitigated pea-protein off flavours improving its sensory profile (El Youssef *et al.*, 2020). LAB ensure the obtention of an appropriate gel by pH reduction but they are not able to decrease negative attributes by themselves, that is why fermentation is combined with yeasts such as *Saccharomyces* and *Kluyveromyces* as they exhibit aldehyde and dehydrogenase activity. Furthermore, co-inoculum of LAB of the gender of *Pediococcus*, has allowed as well a reduction of beany smells present in chickpeas (Xing *et al.,* 2020). By SSF, the resulting sourdoughs presented a milder and acidic odour, which was positively appreciated. Besides, unfermented sourdoughs exhibited an early darkening in comparison with fermented doughs, presumably due to pH and moisture stabilization during fermentation. This shows that by the synergistic action of yeasts and bacteria or the mixture of different LAB strains, sensory properties can be enhanced in fermented legumes.

On the other hand, alkaloids are commonly found in lupin seeds and are important poisonous compounds produced by the plant. Kasprowicz-Potocka *et al.* (2018) concluded that its reduction depended on factors such as the type of microorganism used or the particle size of the substrate. In their studies of fermented lupin meal by yeasts, reduction was only about 5-16% while in other findings using fungus the reduction amounted to 90%. In addition, lupin seeds in this case, were in the meal form instead of flour, meaning that the particle size is finer in the latter which may explain the differences encountered (Kasprowicz-Potocka *et al.* (2018). In fact, a larger surface area to volume ratio allows an easier access to enzymes and a higher contact between microorganisms and nutrients (Gowthaman *et al.,* 2001) which may be favourable.

Finally, an endogenous neurotoxic and non-proteinaceous amino acid (β-ODAP) is typically found in grass peas. If its consumption is extended over time, it can degenerate the motor neuron leading to a disease also known as lathyrism. LAB like

L. plantarum are believed to reduce free amino acid (FAA) content, therefore β-ODAP may be used as a source of carbon and nitrogen resulting in a decrease in its content as reported by Starzynska-Janiszewska & Stodolak (2011).

Regarding the impact on properties related to health, some enzymes such as β-glucosidases responsible for hydrolysing the glycosidic bond of isoflavones glucosides are activated during fermentation by pH reduction. This has been observed in fermented grass pea by *L. plantarum*, but also in whole soybean by *L. casei* and tepary beans by fungus (Starzynska-Janiszewska & Stodolak, 2011; Li *et al.,* 2020; Mora-Uzeta *et al.,* 2020). As a result, the aglycone form is released and has been associated with powerful antioxidant activity for the ease of absorption by the organism and its contribution to risk reduction of suffering cancer (Li *et al.,* 2020). Moreover, submerged fermentation of lentil flour exhibited an enhanced inhibitory activity of the angiotensin I converting enzyme (ACE) (Bautista-Expósito *et al.,* 2018; Bessada *et al.,* 2019). This enzyme converts inactive decapeptide angiotensin I into octapeptide angiotensin II, the latter being responsible of increasing blood pressure for its great vasoconstrictor properties. Since hypertension affects nowadays a wide range of population and is the main cause of cardiovascular diseases, fermented lentils by *L. plantarum* are seen as suitable ingredients to be add to new food products for people with metabolic syndrome. Specially, small-size peptides (2-12 amino acids) containing aromatic amino acids (proline or hydroxyproline residues) in C-terminal are believed to have a greater effect for being good substrates for ACE (Bessada *et al.,* 2019).

Besides, flavonols such as kaempferol and quercetin glucosides may be able to inhibit α-glucosidases (Bautista-Expósito *et al.,* 2018). α-Glucosidase enables glucose absorption as the enzyme is responsible of breaking down the glycosidic bond of disaccharides into more simple sugars, ready to be absorbed (Samtiya *et al.,* 2020). Thereby, by inhibition of this enzyme, hydrolysis of carbohydrates is reduced. As a result, fermented lentil flour is as well considered as suitable for obtaining products for patients suffering hyperglycaemia and hence for type-2 diabetes prevention (Bautista-Expósito *et al.,* 2018).

5. FERMENTATION OF CEREALS

The present section summarises the main findings regarding changes in nutrient profile and functional properties of cereals resulting from fermentation processes. Similarly to legumes, solid-state fermentation is predominantly performed with fungus; while bacteria or yeasts are most employed in submerged one. The potentiality of using co-inoculum, compared to single fermentation, to enhance resistance to contamination by altering microorganisms and to increase adaptability to the growing medium, have been also reported in literature (Tesfaw & Assefa, 2014). Nevertheless, biomass growth yield and their synergic metabolic response is highly dependent on the substrate and fermentation conditions (**Table 3**). On the other hand, it is important to point out that studies carried out on cereals were more focused on the impact of fermentation on phenolic compounds and antioxidant properties; whereas improvements in technological properties were more studied in fermented legumes than in fermented cereals.

5.1 Impact of fermentation on nutrient profile of cereals

As it has been discussed for legumes, fermentation in cereals mainly aims at increasing protein content and/or their digestibility. Thus, many studies analyse the impact of fermentation, submerged or solid-state, and their variables onto this macronutrient. In this sense, Wu *et al*. (2018) compared of single and co-inoculum for solid-state fermentation of oat. Concretely, greater increase of soluble protein was obtained with the fungus strain of *R. oryzae* (+104.7%) in comparison with the joint use of *R. oryzae* and *L. plantarum* (+44.8%). These results could be explained by the synergetic action of both microorganisms: more soluble protein was produced with *R. oryzae* due to its fungal enzymatic activity, but the resulting protein was consumed by LAB in order to survive (Wu *et al.*, 2018). In this sense, LAB are rarely used alone in SSF due to their annoying requirements of moisture and nutrition, especially of nitrogen. In fact, LABs are co-cultured with fungus such as *R. oryzae* as the latter is able to convert polymers into simpler forms that are a source of nutrients and energy for LAB (Wang *et al*., 2019; Wu *et al.*, 2018).

Within the studies carried out with fungus, Xu *et al.* (2019) reported an increase of protein content in fermented quinoa by three medicinal mushrooms. The highest increase was obtained by *Agaricus bisporus* (+133.6%) followed by *Helvella lacunose* (+90%) and *Fomitiporia yanbeinsis* (+58.8%). SSF of rice, wheat and corn with three macro fungi *Agaricus blazei*, *Auricularia fuscosuccinea* and *Pleurotus albidus* resulted in protein enhancement but in a lower extent (+30%, +19% and +46%, respectively) (Stoffel *et al.*, 2019). Protein content increased as well after fermentation with yeasts such as *S. cerevisiae* of rice-black gram mixed flour. Cell yeasts have been reported to contain 10% protein on the dry basis justifying the increase of protein (Rani *et al.*, 2018). Comparing protein results of fermented legumes and cereals, the increase of protein seems to be higher in cereals, since the highest rate in legumes was reported in tepary bean (+35%) against +133.6% in quinoa (Mora-Uzeta *et al*., 2020).

Furthermore, fungus strains seem to contribute to a greater extent to this increase in comparison with yeasts or bacteria. In fact, SmF with *L. plantarum* only implied a protein increase of 12.39% in sorghum. A slightly reduction was even reported because of amino acids conversion into flavours compounds (e.g., lactate and acetate) after 36h of incubation (Pranoto *et al.,* 2013). The *in-vitro* protein digestibility (IVPD) was also evaluated in fermented sorghum by *L. plantarum*. Apparently, IVPD was notable enhanced by the proteolytic and tannase activities present in the bacterium. Thus, protein would be hydrolysed into small peptides and amino acids and on the other hand, complexes of tannins-proteins released, resulting in higher IVPD (Pranoto *et al.*, 2013). These findings were in accordance with those obtained in other legumes (Espinosa-Páez *et al.,* 2017).

With respect to the effect fermentation on other macronutrients, net variations, positive or negative, seems to be very dependent on metabolic activity of microorganisms involved. Stoffel *et al*. (2019) reported the effectiveness of the three above-mentioned macro fungi for fat content reduction and dietary fibre increase in cereals. Specially, *P. albidus* produced the highest reduction of fat in corn (89%), wheat (87%) and rice flour (83%) while a dietary fibre increase of175%, 112% and 100% was reported in rice, wheat and corn flour, respectively with the same fungus (Stoffel *et al.*, 2019). An increase of total fat content was, however, produced under SSF with *A. blazei.* Besides, it is possible to conclude that macro fungi are more efficient in cereals than in legumes for fat content reduction and dietary fibre increase. Physiochemically, there is a clear tendency of pH reduction and titratable acidity increment as long as fermentation progresses. Titratable acidity increase in fermented rice-black gram flour by fungus could be attributed to the carbohydrates conversion into fermentable sugars, and in turn into organic acids such as citric acid, lactic acid or acetic acid (Rani *et al.*, 2018). Reducing sugars content can, however, experiment an increase, as reported after co-fermentation of dehusked barley and whole grain oats by *L. plantarum* and *R. oryzae*. There is scientific evidence of the major role of *Rhizopus* in saccharification and liquefaction processes due to their amylolytic capability (Wu *et al.*, 2018). A decrease in starch content has been reported in sorghum by means of SmF by *L. plantarum* as this microorganism is considered as a proteolytic bacterium able to hydrolyse starch granules stuck within the protein. In this sense, after proteolysis, bacterial amylases allow easier access to the substrate degrading starch into simple sugars and increasing *in-vitro* starch digestibility (IVSD) (Pranoto *et al.*, 2013). These results agree with those reported by Xu *et al.* (2019) in quinoa fermented by edible fungus, in which starch content decreased as long as residual sugars increased.

As previously mentioned, polyphenols compounds are of great interest due to their antioxidant activity. They are, however, mainly found in conjugated forms that can reduce their bioavailability and compromise their healthy benefits (Rani *et al.,*2018). Moreover, their content differs from one cereal to another. For instance, quinoa contains a great amount of vitamins and minerals as well as a wide variety of antioxidants (e.g. polyphenols and flavonoids), which makes TPC values even more significant after fermentation (Xu *et al.*, 2019). Contrarily, other cereals such as rice, oat or corn do not present as much as total phenols as other cereals and no tocopherols or β-carotene after husk removed. As a consequence, they may present low TPC content and less strong antioxidant properties (Xu *et al.*, 2018).

Regardless of the TPC of cereals, their bioavailability can be significantly enhanced due to the enzymatic activity of microorganisms (e.g., amylases, xylanases and glucosidases) and its ability of releasing phenolic and bioactive compounds bound to the cell wall. In this sense, Rani *et al.* (2018) reported an increase of total phenolics content (TPC) of 0.44 mg GAE/g in fermented rice-black gram mixed flours by yeasts after 6h of solid-state fermentation. In turn, Sánchez-Magaña *et al.* (2019) observed a higher increase in TPC content in corn flour (9.93 mg GAE/g) after 108h of SSF by *R. oligosporus.* Such big differences may be attributed to the microorganism involved as well as other factors such as fermentation time, being much longer in corn flour than rice-black. Moreover, free phenolic content (FPC) was measured amounting to 2.28 mg GAE/g. Likely, a release of FPC (1.29mg GAE/g) was found in barley under co-fermentation with LAB and fungus, being phenolic acids such as esculin, caffeic acid and coumaric acid greatly boosted (Wang *et al.*, 2019). Fungal SSF increased free phenolic acids suggesting that carbohydrases release bounding phenols from carbohydrates along fermentation (Sánchez-Magaña *et al.,* 2019). Furthermore, it has been found that ferulic acid is the most predominant phenolic acid present in bioprocessed maize (~50%). TPC content was as well evaluated by Saharan *et al.* (2017) in wheat and rice after fermentation by the fungus *Aspergillus oryzae*. In the case of wheat, the increment was about 6 times (+460%) whereas in rice, the enhancement was about 9 times (+758.8%). This elucidates that antioxidant properties and bioavailability strongly depends on species, variety of the grain, cultivation characteristics and processing conditions (Sánchez-Magaña *et al.,* 2019). Furthermore, since fungi are known to be β-glucosidase producers, soluble aglycones can be released contributing to the increment of TPC content (Sánchez-Magaña *et al.,* 2019).

On the other hand, Ayyash *et al.* (2018) showed that individual bacteria strains of *Bifidobacterium* gender were as well capable of synthetizing new phenolic compounds contributing to TPC increase. In these studies, TPC content was increased for quinoa (~41 mg GAE/g) and wheat (~35 mg GAE/g) using *B. longus* (Ayyash *et al.,* 2018).

On the other hand, and comparing yeasts or lactic acid bacteria performance, the use of *L. rhamnosus* seems to be more advisable than *S. cerevisiae* with the aim of increasing TPC (Đorđević *et al.*, 2010). For instance, fermentation of buckwheat with yeast increased TPC to 53.2 mg GAE/g while LAB managed to increase the content to a greater extent amounting 59.4 mg GAE/g.

It is important to point out that the differences found among studies could be likely due to the solvent used for extraction, which makes usually difficult to compare results. In this sense, different solvents depending of its solubility and polarity can be used in order to perform extraction of antioxidants from food. Đorđević *et al.* (2010) reported that solvent extraction effectiveness was acetone>ethanol>methanol, which agrees with the findings in fermented oats with the fungus *C. militaris* (Xiao *et al.*, 2015). While water extracts exhibited the highest extraction yields in fermented oat (25.46%, w/w), acetone extract presented the highest TPC content (19.71%) followed by methanol (16.80%), ethanol (15%) and water (14.12%). In fact, total avenanthramides content exhibited higher values in inorganic solvent extracts than in water extracts, revealing that antioxidant activities are strongly dependant on the solvent used for extraction (Xiao *et al.*, 2015).

Not only are phenolic compounds but also total flavonoids content (TFC) relevant in cereals. Thus, SSF of wheat and oat with *A. oryzae* exhibits a notable increase of quercetin equivalent content, being more notable in wheat than in corn (Saharan *et al.*

(2017). Besides, similar results were obtained by Xiao *et al.* (2015) in solid-fermented oat which an increase of luteolin, apigenin and tricin (Xiao *et al.*, 2015) and in co-fermented barley by LAB and fungus (Wang *et al.*, 2019).

5.2 Impact of fermentation on functional, sensorial and healthy properties of cereals

Technological properties have not been commonly studied after cereals fermentation in a great extent; some findings, however, can be cited. Pranoto *et al.* (2013) analysed pasting properties after *L. plantarum* fermentation of sorghum. For instance, the gelatinization temperature was reduced when comparing native sorghum (88 ºC) and the fermented cereal (79 ºC), revealing that the structure was weaker after bioprocessing. As a result, starch stuck of sorghum was easily released with the consequently easier expansion during heating as a consequence of water absorption by the hydroxyl group that starch presents. Complementarily, viscosity peak increased as a result of bacteria growth and its proteolytic activity, releasing starch from the protein matrix. In fact, sorghum fermentation with *L. plantarum* was considered as a promising tool for the production of cereal-based fermented flours with enhanced technological properties as ingredient of cookies, cakes or noodles formulation.

Concerning sensorial properties, significant changes on the optical properties were reported in rice, wheat and corn flours as a consequence mycelium growth (Stoffel *et al.*, 2019). Fermentation with *P. albidus* exhibited the greatest changes in rice and wheat flours due to an important increase in luminosity (L*). In fermented corn, *A. fuscosuccinea* caused a decrease of the parameter b* leading to a less yellowish sample.

Incubation time appears to be other critical variable that can significantly influence sensory attributes of fermented cereals. Thus, while 6h was the optimal fermentation time using *S. cerevisiae* (Rani *et al.*, 2018), until 35 days of fermentation were required with filamentous fungus such as *H. lacunose, F. yanbeiensis, A. bisporus, A. blazei, A. cosuccinea or P. albidus* (Xu *et al.*, 2019; Xu *et al.*, 2018; Stoffel *et al.*, 2019). Sánchez-Magaña *et al.* (2019) revealed the longer the fermentation time (108h), the highest the undesirable off-odours in fungal fermented corn grains. In this sense, ergosterol which is a compound produced by macrofungi can be an indicator for quantification of mycelial biomass. Wang *et al.* (2019) reported an increase in ergosterol content during co-fermentation of dehusked-barley with *Rhizopus oryzae* and *Lactiplantibacillus plantarum* after 36h of fermentation. Not only is ergosterol interesting for estimating fungal biomass but it is also a bioactive compound with antioxidant properties, anti-inflammatory and anti-cancer effects (Stoffel *et al.*, 2019).

Regarding the antioxidant activity, theoretically antioxidant properties, TPC and TFC seem to be positively correlated with it (Rani *et al.*, 2018). This was confirmed by Saharan *et al.* (2017) as rice exhibited not only the second major increase in TPC but also the highest antioxidant activity assayed as DPPH radical scavenging potential. This is also in agreement with work made by Sánchez-Magaña *et al.* (2019) where positive correlations between TPC and AoxA were found in corn. In this sense, phenolic compounds are the major contributors to the antioxidant activity of cereal grains, and hence to their associated-healthy benefits such as anti-inflammatory and antibacterial properties.

In oat grains, phenolic compounds are also the major responsible of antioxidant properties including avenanthramides, a compound typically found in oats, phenolic acids (e.g. ferulic acid, p-coumaric acid, gallic acid, caffeic acid and hydroxybenzoic acid) and flavonoids (e.g. luteolin, apigenin and tricin) (Xiao *et al.*, 2015). Similarly, Ayyash *et al.* (2018) observed that phenolic compounds in grains were able to neutralize free radicals by donating electrons and protons.

On the other hand, it is interesting to point out that fermentation with the fungus *L. rhamnosus*, resulted in an increment in DPPH scavenging activity in all cereals (buckwheat, barley, rye and wheat), buckwheat exhibiting the highest values (Đorđević *et al.*, 2010). However, a positive correlation was not found between TPC and DPPH radical scavenging activity. Similarly, Xu *et al.* (2018) found that TPC in fermented sorghum and corn was lower than in the control samples but antioxidant properties after fermentation were higher. There is not a full explanation but it is plausible that during fermentation other metabolites with antioxidant properties such as ergothioneine, an unusual thio-histidine betaine amino acid, may have been formed (Xu *et al.,*2018). Moreover, the method used for phenolic content evaluation is also a matter of concern. In their studies, Đorđević *et al.* (2010) used the Folin-Ciocalteau method which can present some limitations: since some compounds such as ascorbic acid can react with the reagent used, total phenol content can be overestimated.

These findings elucidate that AoxA and TPC are not always positively correlated; being the evaluation method, the microorganisms involved, the type of substrate and polyphenols major determinants for improving bioactive compounds in cereals.

In terms of inhibitory effects, fermentation presents the ability of inhibiting enzymatic activities such as pancreatic lipases, a part from α-glucosidases above-mentioned in legumes fermentation.

Lipases are responsible of hydrolysing triacylglycerols to glycerol and fatty acids facilitating its absorption by the small intestine. Thanks to fermentation of wheat grains by *P. albidus* and *A. fuscosuccinea*, lipase activity inhibition was improved (+413% and +40% respectively). As a result, wheat fermentation can be considered an efficient tool for controlling obesity (Stoffel *et al.*, 2019). In addition, glucose absorption can be regulated by inhibition of α-glucosidase and α-amylase as previously seen in fermented lentils (Bautista-Expósito *et al.,* 2018). In this sense, *P. albidus* and *A. blazei* exhibited the highest inhibition power (+78%) of α-glucosidase regardless of the grain used but not inhibitory effects were found for α-amylase (Stoffel *et al.*, 2019). This is in agreement with Ayyash *et al.* (2018) who reported that different strains of *Bifidobacterium* are able to manage to inhibit α-glucosidase and α-amylase activities in fermented quinoa and wheat. Therefore, fermentation of legumes and cereals as well, is confirmed to be a positive technique for controlling diabetes.

Furthermore, oat flour can be used with pharmaceutical purposes as a food supplement in order to reduce the risk of suffering oxidative diseases such as cancer, atherosclerosis or arthritis. Besides, since avenanthramides have the ability to act as metal chelators and interfere with the region sites of H_2O_2, they exhibit a protective function against DNA damage (Xiao *et al.,* 2015). Other studies involving oats, showed an enhanced ACE inhibitory activity due to proteolytic activity of *L. plantarum* and release of small peptides (Wu *et al.*, 2018). Similar results using *L. plantarum* were observed in lentils flour as previously discussed (Bautista-Expósito *et al.,* 2018). As a result, it is possible to affirm that this bacteria strain is able to produce key ingredients for production of therapeutic products enriched with probiotics. Similarly, findings by Ayyash *et al.* (2018) using *Bifidobacterium* strains in quinoa, reported an important degree of hydrolysis releasing as well small size proteins (<10 kDa) associated with antioxidant and antihypertensive properties.

6. FERMENTATION OF SEEDS

Other plant-origin materials, such as seeds, have been also used as substrates for fermentation, though to a much lesser extent than cereals or legumes. In seeds, natural fermentation with autochthonous microorganism has been found to be the most applied technique **(Table 4).** As a result of seeds fermentation, similar changes in nutrient composition compared to cereals and legumes can be found. For instance, protein increases in a similar proportion and lipid decreases, but to a lower extent (Onimawo et al., 2003; Olagunju & Ifesan, 2013). In addition, technological properties are modified when seeds are fermented, including increased WHC. Concerning emulsifying properties, the same increasing tendency observed in legumes is found in seeds, but improvement being significantly lower (Sadh *et al.,*2018; Chawla *et al.*, 2017). Finally, apparent viscosity and gelation capacity decrease because of hydrolysis of long-chain polysaccharides and proteins (Onweluzo & Nwabugwu, 2009).

7. CONCLUSIONS

The present work reveals the potential of fermenting legumes, cereals and seeds in order to obtain functional flours as key ingredients for new foods production or re-working formulations already in place. Scientific literature evidence that solid-state fermentation is more commonly performed by fungus, whereas bacteria and yeasts are more typically used in liquid culture due to moisture requirements for growth. Fermented legumes flours stand out for an increased protein content, enhanced technological properties and improved sensorial profile by off-flavours elimination especially common in peas. A rise of phenolic compounds and antioxidant properties is characteristic of fermented cereals. Especially, fermentation with the strain *Lactiplantibacillus plantarum* has showed to be an interesting tool for production of foods enriched with probiotics and antihypertensive properties as seen in fermented quinoa. In the case of seeds, fermentation is commonly performed with autochthonous microorganisms already present in the substrate, resulting in less significant nutritional changes but interesting technological and sensorial results as seen in fermented chia sourdough. Fermentation studies reveal improvements as well in healthy properties, giving rise to functional products with pharmaceutical purposes for oxidative diseases (e.g., fermented oat flour) or suitable for people with hyperglycaemia and type-2 diabetes (e.g. lentil flour). The characteristics of the fermented products depend on the following variables: i) the microorganism strain and its metabolic activity, ii) the positive synergy between microorganisms when inoculation is co-cultured, iii) the reduced particle size of the substrate and the facilitated access to enzymes and iv) the duration of fermentation, being generally longer times preferable for greater changes. However, longer times have also been associated with off-odors which makes essential the optimization of the above cited parameters in order to obtain flours with the desired improvements.

Further research is encouraged regarding innovation in fermented foods, including those products currently found in the market, the ones that were in the market but disappeared and products that remain at laboratory scale.

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COMPETING INTERESTS STATEMENT

None of the authors have any competing interest to declare.

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TABLES

Table 1. Classification of ANFs and their effect

TYPE	ANF	MAIN FOOD SOURCES	NUTRITIONAL EFFECT	PROCESSING STRATEGIES	REFERENCES
Proteinaceous	Lectins	Cereals and pulses	sugar-binding activity, interfere with nutrient absorption and hemagglutination	Cooking, soaking	**Bessada *et al.* (2019)** **Gibson *et al.* (2006)** **Samtiya *et al.* (2020)**
	Protease inhibitors	Soybeans, white beans, chickpeas	reduces protein digestibility and sulphur amino acid content	Cooking, roasting, cooking + soaking	**Bessada*et al.* (2019)** **Samtiya*et al.*(2020)** **Frias*et al.* (2000)**
Non-proteinaceous	Phytic acid	Lupins, chickpeas, corn, millet and sorghum	Forms insoluble complexes with metal ions, ↓ mineral bioavailability and absorption, proteolysis inhibition	Soaking, germination	**Vashishth *et al.* (2017)** **Rehman *et al.* (2014)** **Gibson *et al.* (2006)** **Rehman *et al.* (2014)**
	Tannins	Bean, cowpea, soybean	↓ protein digestibility and AA availability. Astringent properties and ↓palatability	Germination, soaking and dehulling	**Samtiya *et al.* (2020)** **Egounlety & Aworth (2003)**
	Saponins	Lupins, chickpeas, lentils, beans and peas	bitter taste and sensory rejection inhibit cholesterol absorption, vit A and E	Cooking	**Margier *et al.* (2018)** **Samtiya *et al.* (2020)**
	Alfa-galactosides	Legumes	flatulence and gas gut production	Roasting, soaking + dehulling + cooking	**Thirunathan & Manickavasagan, (2018)** **Frias *et al.* (2000)** **Khattab *et al.* (2009)** **Egounlety & Aworth (2003)**
	Alkaloids	Lupins	unpalatability, bitter taste and toxicity of seeds	Soaking, cooking	**Jiménez-Martínez *et al.* (2007)**

Table 2. Legume fermentation studies and the main outcomes obtained.

FERMENTATION TYPE	INOCULUM	SUBSTRATE	MAIN FINDINGS	REFERENCES
SSF	**FUNGUS** *Aspergillus oryzae* *Pleurotus ostreatus* *Rhizopus oligosporus*	Black-eyed pea seed flour Kidney beans (Phaselous vulgaris) Black beans (Phaselous vulgaris) Lentils *(Lens culinaris)* Legume Tepary bean (Phaseolus acutifolius)	↑ protein, dietary fibre in kidney beans, ↓ lipids, dietary fibre in black beans, ↓ carbohydrates in lentils ↑ EAA, FAA, TPC, isoflavones, mineral content ↑ protein digestibility, mineral bioavailability, AoxA, ↓ tannins ↑ WHC, OBC, emulsifying properties, ↓ bulk density	**Chawla *et al.* (2017)** **Espinosa-Páez *et al.* (2017)** **Mora-Uzeta *et al.* (2020)**
	BACTERIA *Lacticaseibacillus casei*	Whole soybean flour	↑ protein, fat and crude fibre, w-3 fatty acids ↑ EAA, FAA, phenolic acids, isoflavones ↑ AoxA, ↓TIA and lipoxygenase activity	**Li *et al.* (2020)**
	CO-CULTURE *Aspergillus sojae + Aspergillus ficuum* *Pediococcus pentosaceus + Pediococcus acidilactici + Pediococcus lolii*	Lupin flour Chickpea flour	↑ mineral content, TPC, ↓ pH ↑ IVPD, ↓ raffinose and stachyose, ↓ phytic acid ↑ WHC, ↓ foaming capacity, ↑ milder and acidic odours, ↓ beany smells	**Olukomaiya *et al.* (2020)** **Xing *et al.* (2020)**
SmF	**YEASTS** *Saccharomyces cerevisiae, Kluyveromyces lactis and Candida utilis*	Lupin meal	↑ crude protein ↑ EAA (glutamic acid, proline, glycine, valine and alanine), ↓ EAA (isoleucine, histidine, arginine, phenylalanine and leucine) ↓ phytates, oligosaccharides, alkaloids	**Kasprowicz-Potocka *et al.* (2018)**
	BACTERIA *Lactiplantibacillusplantarum VTT E-78076* *L. plantarum ATCC 14917* *L. plantarum CECT 748*	Faba bean flour Bean flour (Phaselous vulgaris L.) Cowpea flour (Vignasinensis L) Lentil flour (Lens culinaris L.) Grass pea flour (Lathyrus sativus "Krab")	↑ FAA, TPC, p-hydroxybenzoicacid, isoflavones in aglycone form, ↓quercetin glycosides, trans-p-coumaricacid and pH ↑ protein solubility, ↓ phytic acid, TIA, tannins, lectins and β-ODAP	**Rosa-Sibakov *et al.* (2018)** **Martín-Cabrejas *et al.* (2004)** **Dueñas *et al.* (2005)** **Bautista-Expósito *et al.* (2018)** **Starzynska-Janiszewska & Stodolak (2011)**
	CO-CULTURE Starter LAB + one of the following yeasts: *Kluyveromyces lactis, Kluyveromyces marxianus* or *Torulaspora delbruecckii*	Pea protein isolates	↑ esters and beer/yeast attributes ↓ off-flavours like green/leguminous attributes (aldehydes, ketones, furans, alcohols).	**El Youssef *et al.* (2020)**

*EAA: essential amino acids; FAA: free amino acids; TPC: total phenolics content; AoxA: antioxidant activity; WHC: water holding capacity; OBC: oil binding capacity; IVPD: *in vitro* protein digestibility; TIA: trypsin inhibitors.

Table 3. Cereals fermentation studies and the main outcomes obtained.

FERMENTATION TYPE	INOCULUM	SUBSTRATE	MAIN FINDINGS	REFERENCE
SSF	**YEASTS** *Saccharomyces cerevisiae*	Rice-black gram mixed flours	↑ protein content, ↓ fermentable sugars ↑ TPC, titratable acidity, ↓ moisture content, pH ↑ AoxA ↑ texture and mouthfeel properties, optimization of processing conditions	**Rani et al. (2018)**
	FUNGUS *Helvella lacunosaX1* *Agaricus bisporus AS2796* *Fomitiporia yanbeiensis G1* *Aspergillus oryzae* *Cordyceps militaris* *Rhizopus oligosporus* *Lentinula edodes*	Oat Quinoa Wheat Rice Corn Millet Buckwheat Sorghum Brown rice	↑protein content, fat content, reducing sugars, ↓ dietary fibre ↑ TFC, phenolic acids, avenathramides, ↓ conjugated phenolic compounds ↑ protein digestibility, AoxA, phytase, endocellulase and polyphenol oxidase activity, ↓ tannins and phytic acid, lipase activity ↑ WHC, luminosity in rice and wheat, anti-obesity in vitro activity in wheat, amylase, xylanase and β-glucosidase activity in rice, DNA damage protection in oats	**Xu et al. (2019)** **Xu et al. (2018)** **Stoffel et al. (2019)** **Saharan et al. (2017)** **Xiao et al. (2015)** **Sánchez-Magaña et al. (2019)**
	BACTERIA *Bifidobacterium spp. B. animalis, B. breve and B. longum*	Quinoa and wheat flours	↑ %DH, small size peptides (< 6 kDa), TPC, ↓ pH ↑ AoxA, ACE inhibitory activity, inhibition of α-glucosidase and α-amylase activity	**Ayyash et al. (2018)**
	CO-CULTURE *Rhizopusoryzae + Lactiplantibacillus plantarum*	Dehusked barley Whole grain oat	↑ soluble protein, small size peptides, reducing sugars ↑ amino acid nitrogen, TFC, FPC, ↓ pH ↑ DPPH, ABTS radical scavenging activity, amylase and protease activity ↑ protein solubility, aroma formation, ACE inhibitory activity, enrichment of probiotics microorganisms	**Wang et al. (2019)** **Wu et al. (2018)**
SmF	**YEASTS** *Saccharomyces cerevisiae*	Buckwheat, wheat germ, barley and rye	↑ TPC content ↑ AoxA (DPPH radical scavenging activity)	**Đorđević *et al.* (2010)**
	BACTERIA Natural fermentation and *L. Plantarum L. rhamnosus*	Sorghum flour Buckwheat, wheat germ, barley and rye	↑ titratable acidity, ↓ pH ↑ IVPD and IVSD, ↓ gelatinization temperature, ↑ peak viscosity	**Pranoto et al. (2013)** **Đorđević *et al.* (2010)**

*TPC: total phenolic content; AoxA: antioxidant activity; TFC: total flavonoid content; IVPD: *in-vitro* protein digestibility; IVSD: *in-vitro* starch digestibility.

Table 3. Seeds fermentation studies and the main outcomes obtained.

FERMENTATION TYPE	INOCULUM	SUBSTRATE	MAIN FINDINGS	REFERENCE
SSF	**FUNGUS** *Rhizopus oligosporus* (DSM 1964 and ATCC 64063) *Aspergillus oryzae*	Flaxseed oil cake Peanuts oil cake	↑ crude protein content and ↓dry matter ↑ Mg, InsP3, ↓InsP5-6 and phytate content ↑ bioavailability of Ca, Mg and P ↑ WHC, OBC, emulsifying properties, smoothness in the grain surface	**Dulinski et al. (2017)** **Sadh et al. (2018)**
SmF	**NATURAL FERMENTATION**	Pumpkin seeds flour Sesame seeds Millet and Pigeon pea seeds	↑ protein, carbohydrates, ↓fat ↑mineral content and EAA ↓ phytic acid, phytin phosphorous ↑ WSI, ↓ foam and emulsion capacity and stability, apparent viscosity, WHC and reconstitution time	**Onimawo et al. (2003)** **Olagunju & Ifesan (2013)** **Onweluzo & Nwabugwu (2009)**
	CO-CULTURE Autochthonous LAB + *L. plantarum* C8	Chia dough	↑ phenolic compounds, chlorogenic acid ↑ viscosity ↓ consistency, volume of bread loves, firmness and chewiness	**Bustos et al. (2017)**

*Mg: magnesium; InsP3: inositol triphosphate; InsP6: myo-inositol hexakisphosphate; InsP5: myo-inositol pentakisphosphate; WHC: water holding capacity; OBC: oil binding capacity; WSI: water solubility index; AoxA: antioxidant activity.

FIGURES

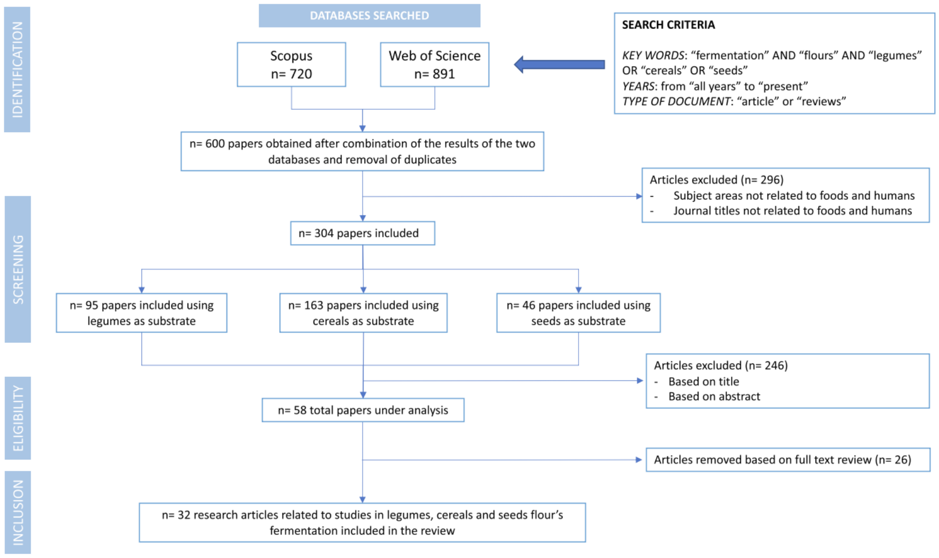

Figure 1. Flow diagram of the search criteria applied to select the papers used in this review.

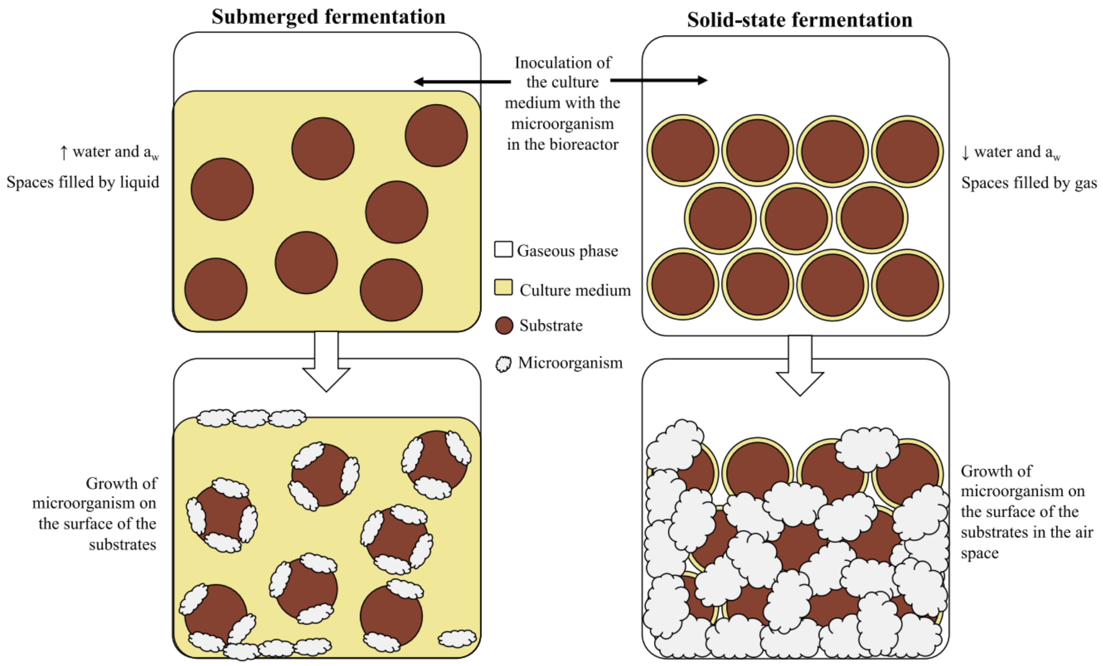

Figure 2. Comparison between submerged and solid-state fermentations.