UNIVERSITAT
POLITÈCNICA
DE VALÈNCIA

IIAD
INSTITUTO DE INGENIERÍA DE
ALIMENTOS PARA EL DESARROLLO

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

POTENTIAL OF FERMENTATION PROCESSES FOR NUTRITIONAL AND IMPROVEMENT OF CEREALS, LEGUMES AND SEEDS FLOURS: A REVIEW

TRABAJO FIN DE MÁSTER UNIVERSITARIO EN CIENCIA E
INGENIERÍA DE ALIMENTOS

ALUMNA: Sara Garrido Galand

DIRECTORAS ACADÉMICAS: Ana María Andrés Grau
Ana Belén Heredia Gutiérrez
TUTORES: Joaquín Calvo Lerma
Andrea Asensio Grau

Curso Académico: 2019-2020

VALENCIA, 11 de septiembre 2020

POTENCIAL DE LOS PROCESOS DE FERMENTACIÓN PARA LA MEJORA NUTRICIONAL Y FUNCIONAL DE HARINAS DE LEGUMBRES, GRANOS Y SEMILLAS: REVISIÓN BIBLIOGRÁFICA

Garrido-Galand, S., Asensio-Grau, A.[1], Calvo-Lerma, J.[1], Heredia, A.[1], Andrés, A. [1]

RESUMEN

Actualmente existe una creciente demanda por las fuentes de proteína vegetal como alternativa a la de origen animal por su mayor sostenibilidad ambiental y su relación con un menor riesgo de sufrir enfermedades cardiovasculares. Las legumbres, cereales y semillas se presentan como una buena fuente proteica aportando además, fibra dietética y fitoquímicos con propiedades antioxidantes. Sin embargo, la digestibilidad y biodisponibilidad de estos nutrientes en estas fuentes está limitada por la presencia de factores anti nutricionales (FAN), pudiendo ser mejoradas mediante procesos como el remojo, la cocción o la fermentación. Por ello, el objetivo de este trabajo es abordar una revisión del efecto que tiene la fermentación en estado sólido y en sumergido en las propiedades nutricionales y funcionales de legumbres, cereales y semillas. Los microorganismos utilizados en la fermentación (bacterias, hongos y levaduras) son capaces de generar enzimas que degraden los FAN dando lugar a harinas más digeribles y con un perfil nutricional, sensorial y tecnológico mejorado. La fermentación en estado sólido es más comúnmente utilizada por su mayor rendimiento, por aceptar residuos agroindustriales como sustratos y por su menor volumen de efluentes. Las legumbres fermentadas destacan por sus propiedades tecnológicas mejoradas mientras que un aumento en el contenido de compuestos fenólicos y de propiedades antioxidantes es más característico de cereales. En el caso de las semillas, las propiedades reológicas se ven mejoradas para su incorporación en nuevas formulaciones de productos a base de cereales. El presente trabajo muestra evidencias sobre el uso de la fermentación en legumbres, granos y semillas como proceso clave que a nivel industrial podría generar productos con un perfil nutricional mejorado y con nuevas propiedades tecnológicas.

PALABRAS CLAVE: legumbres, cereales, semillas, factores anti nutricionales, fermentación en estado sólido, fermentación en estado sumergido, harina.

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

---

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

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 is limited by the presence of anti-nutritional factors (ANFs) but susceptible of being improved by techniques such as soaking, cooking or fermentation. Therefore, the objective of this work is a review of 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. In the case of seeds, rheological properties were improved for its addition to cereal-based products. The present work highlights fermentation of the three studied substrates 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.

RESÚM:

Actualment existeix una creixent demanda de fonts de proteïna vegetal com alternativa a la d’origen animal, per associar-se a una major sostenibilitat ambiental i un menor risc de patir malalties cardiovasculars. Les llegums, cereals i llavors es presenten com una bona font proteica, aportant a més a més, fibra dietètica i fitoquímics amb propietats antioxidants. No obstant, la digestibilitat i biodisponibilitat d’estos nutrients està limitada per la presència de factors anti-nutricionals (FAN), les quals poden ser millorades mitjançant processos com el remull, la cocció o la fermentació. Així doncs, l’objectiu d’este treball és fer una revisió de l’efecte que té la fermentació en estat sòlid i submergit en les propietats nutricionals i funcionals de farines de llegums, cereals i llavors. Els microorganismes emprats per a la fermentació (bactèries, fongs i rents) són capaços de generar enzims que degraden els FAN, originant farines més digeribles i amb un perfil nutricional, sensorial i tecnològic més interessant. La fermentació en estat sòlid és la més comunament emprada pel seu rendiment, acceptar residus agroalimentaris com a substrats i pel menor volum d’efluents. Les llegums fermentades destaquen per les propietats tecnològiques millorades, mentre que un increment del contingut de compostos fenòlics i propietats antioxidants és característic dels cereals. El present treball mostra la fermentació dels tres substrats estudiats com un procés clau que a nivell industrial podria generar productes amb un perfil nutricional millorat i amb noves propietats tecnològiques.

PARAULES CLAU: llegums, cereals, llavors, factors antinutricionals, fermentació en estat sòlid, fermentació en estat submergit, farina.

GLOSSARY

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). 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). 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 aminoacid 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-flavors, 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 45-65ºC 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 99ºC (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 120-250ºC. 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).

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)**

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. 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).

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. lactic acid bacteria (LAB), *Lactobacillus*, *Bifidobacterium* and *Streptococcus)* and yeasts (e.g. *Saccharomyces cerevisiae*, *S. carlsbergensis*) (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).

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 wastewater 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). 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).

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.

3. 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.

3.1 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). Furthermore, due to the action of a tannase that this fungus may contain, a reduction of tannins content was reported. Since tannins are known to bind proteins by forming tannin-protein complexes, protein availability and digestibility may be increased. 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 *Lactobacillus 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 *Lactobacillus 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). A 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 as well an important role: 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 hydroxymethylfurfuraldehyde 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.

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) Legume Tepary bean (Phaseolus acutifolius)	↑ protein, dietary fibre in kidney beans, ↓ lipids, dietary fibre in black beans ↑ 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 *Lactobacillus 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 *Lactobacillus plantarum VTT E-78076* L. *plantarum ATCC 14917L.* *plantarum CECT 748*	Faba bean flour Bean flour (Phaselous vulgaris L.) Cowpea flour (Vigna sinensis L) Lentil flour (Lens culinaris L.) Grass pea flour (Lathyrus sativus "Krab")	↑ FAA, TPC, p-hydroxybenzoic acid, isoflavones in aglycone form, ↓quercetin glycosides, trans-p-coumaric acid 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-flavors 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.

3.2 Impact of fermentation on functionality: technological, 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-flavors 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 flavors 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).

4. 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.

4.1 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 36 h 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 of 175%, 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 avenan thramides 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).

4.2 Impact of fermentation on functionality: technological, 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 (88ºC) and the fermented cereal (79ºC), 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 *Lactobacillus* *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.

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* *Agaricusbisporus 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, avena thramides, ↓ 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 *Rhizopus oryzae + Lactobacillus 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.

5. FERMENTATION OF SEEDS

The present section summarises the most recent evidences regarding changes in nutrient profile and properties of fermented seeds (**Table 4**). The natural fermentation with autochthonous microorganisms that are present in the substrate itself was found to be the most common in studies of seeds fermentation.

5.1 Impact of fermentation on nutrient profile of seeds

Regarding the impact on nutrients, fermentation of seeds supposed a higher increase of protein content in comparison with legumes fermentation but less significant that in fermented cereals. In this sense, natural fermented pumpkin and sesame seeds flours exhibited a similar increase in protein content, 40.7% and 34.4% respectively (Onimawo *et al.,* 2003; Olagunju & Ifesan, 2013). Enhancement of protein was attributed to enzymatic activity of microorganisms as previously discussed in legumes and cereals, but also to a loss of dry matter content as a consequence of carbohydrates and lipids consumption during fermentation of sesame seeds.

When it comes to amino acids content, in comparison with improvements in fermented legumes, methionine and cysteine did not suffer a significant increase after seeds fermentation. While raw legumes such as black beans and kidney beans are known to present low amount of SC-aminoacids (20.44 mg/g and 21.29 mg/g respectively) (Espinosa-Páez *et al.,* 2017), raw sesame seeds presented higher content of this compound (34.3 mg/g). Therefore, current evidence suggests that improvement in sulphur amino acids is predominantly associated with legumes fermentation. Moreover, provided that essential amino acids (e.g. arginine, histidine, leucine, methionine, phenylalanine and threonine) that are present in fermented sesame seeds flour meet the recommended daily allowance, it could be a considered as a potential ingredient for infant formula and weaning food (Olagunju & Ifesan, 2013).

Reduction in the lipid content of previously discussed findings in fermented legumes (e.g. soybean and tepary bean flours) and fermented cereals (e.g. corn, rice and wheat) was more significant in both cases than in seeds. In this sense, reduction of 8.3% was reported in fermented pumpkin seeds flour and to a lower extent in sesame seeds (Onimawo *et al.,* 2003; Olagunju & Ifesan, 2013). Differences cannot be attributed to the type of microorganism since pumpkin and sesame seeds flour were submitted to natural fermentation, which means that the involved microorganisms remain unknown. However, of note both legumes and cereals were fermented in solid-state conditions, whereas for pumpkin and sesame seeds flour, fermentation was carried out in liquid culture. Hence, these findings suggest that either the substrate, the involved microorganisms or the way fermentation is performed are determinant in the reduction rate.

Results in carbohydrates content of seeds were similar to previous findings obtained after cereals fermentation, in which the substrate, especially its starch structure, determined the carbohydrate final content. In this sense, carbohydrates content revealed an increase of 77.45% in fermented pumpkin flour (Onimawo *et al.,* 2003) but decreased in millet and pigeon pea flour (Onweluzo & Nwabugwu, 2009).

When it comes to mineral profile, findings suggest that improvements in its bioavailability are dependent on the strain used in the fermentation process. In this sense, solid-state fermentation of flaxseed oil cake was performed by two different strains of *Rhizopus*: *R. oligosporus* DSM 1964 and *R. oligosporus* ATCC 64063 (Dulinski *et al.,* 2017). *R. oligosporus* DSM 1964 improved greatly calcium bioavailability (by 15.5%) and slightly magnesium bioavailability (by 3.3%). Regarding phosphorous, the strain *R. oligosporus* DSM 1964 was able to increase by 3.8% its bioavailability whereas *R. oligosporus* ATCC 64063 just did it by 1.6%. This increase in mineral bioavailability is attributed to the reduction of antinutritional phytate and the resulting break down of the complexes formed with minerals.

In terms of phenolic compounds content, a greater enhancement was found in fermented cereals when compared to fermented seeds. For instance, fermentation of chia sourdough by autochthonous LAB and inoculation of *L. plantarum*, allowed an increase in TPC content of 40%, from 14.90 mg GAE/g to 20.80 mg GAE/g (Bustos *et al.,* 2017). As a result, some phenolic compounds increased (e.g. coumaric, benzoic, caffeic, ferulic) and new ones were generated such as chlorogenic acid. It remains important to mention that *L. plantarum* is the most used LAB when phenolic compounds are desired to be metabolized. In this sense, esterase activity of LAB during fermentation of chia sourdough is considered as the main reason of phenolic compounds release into free forms of phenolic acids.

5.2 Impact of fermentation on functionality: technological, sensorial and healthy properties of seeds

As it has been said above, assays of functional properties are essential in order to put new food products in the market.

Increase in the exposure of hydrophilic amino acid residues with affinity with the aqueous medium not only was observed in fermented legumes but also in seeds. As a result, WHC raised in processed peanuts oil cake (+100%), which was similar to the aforementioned increase in fermented black eyed pea flour (+92.75%) (Sadh *et al.,* 2018; Chawla *et al.,* 2017). However, a decrease of WHC in fermented pigeon pea and millet flours was found and similar to the one reported before in fermented lupin meal. In both cases, low WHC was desirable for thin gruels production aimed for infants foods (Onweluzo & Nwabugwu, 2009; Olukomaiya *et al.*, 2020).

Conversion of complex carbohydrates with high molecular weight into simpler compounds during fermentation, supposes an increase in water solubility index (WSI): fermented millet and pigeon pea exhibited an increase in WSI of 15% and 24% respectively, being desirable for further extrudates production (Onweluzo & Nwabugwu, 2009).

When the substrate comes from oil extraction processes, pre-treatments applied (e.g. extraction method) have a great influence in structural characteristics (e.g. porosity and particle size) of the resulting oil cake which impact the results of the flours obtained, particularly the OBC. In this sense, OBC was enhanced in fermented peanut oil cake by 38%, but to a less extent than in black eyed peas flour (+97.8%) (Sadh *et al.,* 2018) indicating that fat retention during storage would be easier in the latter.

When it comes to emulsifying properties, the same tendency observed before in black eyed peas was noted in SSF of peanuts oil cake, using in both cases the fungus *A. oryzae* (Sadh *et al.,* 2018; Chawla *et al.*, 2017). However, improvements were higher when using legume as substrate: emulsion activity and stability increased by 50% and 36% respectively in black eyed peas whereas in peanuts oil cake the raise was about 23% and 9% for the same variables. Considering that both fermentation were performed in SSF conditions and by the same fungus, differences may be attributed to the type of substrate.

Furthermore, apparent viscosity is bound to suffer a reduction after fermentation as a result of the hydrolysis of long chains of polysaccharides and proteins into dextrin and peptides. This reduction found in fermented millet and pigeon pea flours, is considered as positive since higher amount of solid can be added, making the product more nutritious but less pasty and viscous (Onweluzo & Nwabugwu, 2009). High nutrient density products are suitable for producing infant and baby foods.

In the same tendency, least gelation concentration of both substrates, millet and pigeon pea flours, increased after fermentation (62.5% and 66.6%) as a result of hydrolysis of carbohydrates and proteins as well. It seems interesting to obtain flours forming gels at high concentrations in order to avoid adding great amounts of water for improving digestibility. Thereby, the resulting product would be less diluted and present more energy density (Onweluzo & Nwabugwu, 2009).

In terms of microstructural properties, fermentation can lead to a wide open structure, more porous and spongy, where water absorption is promoted as seen in fermented pumpkin seeds flour (Onimawo *et al.,* 2003). On the other hand, as the particle size is reduced during fermentation, the smoothness of the sample's surface can be increased as reported by Sadh *et al.* (2018) in peanuts oil cake.

Moreover, analysis of rheological properties suggest that after seeds fermentation, samples are less consistent but more viscous in comparison with unfermented samples. This is the case of chia sourdough fermentation by *L. plantarum C8* where the increment of viscosity is justified by the proteolytic activity during fermentation (Bustos *et al.,* 2017). In addition, one of the purposes of making chia sourdough, is for wheat bread production with increased phenolic compounds content and enhanced antioxidant activity. By adding chia flour to wheat flour, the volume of bread's loaves suffers a reduction since the formulation presents less quantity of gluten for air bubbles retention (Bustos *et al.,* 2017). In addition, firmness and chewiness are as well reduced after fermentation in comparison with traditional wheat bread which can be justified by the results mentioned above about rheological properties. However, when adding chia flour or chia sourdough, cohesiveness, which is a parameter that affects the texture of the product, was improved. Hence, using chia sourdough supposed an improvement of physical and antioxidant properties of the resulting bread.

Antinutritional compounds such as phytates are as well reduced during seeds fermentation. Both strains, *R. oligosporus* DSM 1964 and *R. oligosporus* ATCC 64063 decreased phytates content of flaxseed flour by 44% and 35% respectively (Dulinski *et al.,* 2017). The same trend was as well observed in fermented millet and pigeon pea flours, obtaining the highest reduction rate with higher time fermentation (reduction of 59% for millet flour and 79.7% for pigeon pea flour) (Olagunju & Ifesan, 2013). Therefore, mineral bioavailability can increase as a result of fungus metabolic activity which facilitates the release of low molecular compounds. For instance, in the case of phosphorous, phytases hydrolyses phytates into inositol and phosphoric acid. In fact, some of those inositol phosphates have been associated with healthy effects. Fermentation of flaxseed oil cake by *R. oligosporus* DM 1964 gave rise to a more interesting profile of inositol phosphates (InsP_3)= isomers, and particularly $\mathrm{Ins}(1,2,6)/(1,4,5)\mathrm{P}_3$ and $\mathrm{Ins}(2,4,6)\mathrm{P}_3$ predominant (Dulinski *et al.,* 2017). However, fermentation by the other strain, *R. oligosporus* ATCC 64603, released different products of phytate degradation, InsP_5 (18-22%) and $\mathrm{InsP}_{1\text{-}2}$ (17-28%), which shows different phytase activity of both strains. While $\mathrm{InsP}_{6\text{-}5}$ are components with strong capacity of mineral chelation, a profile with low inositol phosphates is preferable. Specially, an important amount of inositol triphosphates has been attributed with antioxidant and anti-carcinogenic properties (Dulinski *et al.,* 2017).

TABLE 4. 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.

6. 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 *Lactobacillus 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 as well improvements 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.

7. REFERENCES

Adebo, O.A., Njobeh, P.B., Adebiyi, J.A., Gbashi, F., Phoku J.Z. & Kayitesi, E. (2017). Fermented Pulse-Based Food Products in Developing Nations as Functional Foods and Ingredients. In: Chavarri Hueda, M. (ed). Functional Food – Improve Health through adequate food. doi:10.5772/intechopen.69170.

Aryee, A.N.A. & Boye, J.I. (2016). Improving the Digestibility of Lentil Flours and Protein Isolate and Characterization of Their Enzymatically Prepared Hydrolysates. *International Journal of Food Properties*, 19(12): 2649-2665. doi: 10.1080/10942912.2015.1123269.

Awuchi, C., Victory, I. & Echeta, C. (2019). The Functional Properties of Foods and Flours. *International Journal of Advanced Academic Research*, 5(11): 139-160.

Ayyash, M., Johnson, S.K., Liu, S., Al-Mheiri, A. & Abushelaibi, A. (2018). Cytotoxicity, antihypertensive, antidiabetic and antioxidant activities of solid-state fermented lupin, quinoa and wheat by Bifidobacterium species: In-vitro investigations. *Food Science & Technology*, 95: 295-302. doi: 10.1016/j.lwt.2018.04.099.

Bautista-Expósito, S., Martínez-Villaluenga, C., Dueñas, M., Silván, J.M., Frias, J. & Peñas, E. (2018). Combination of pH-controlled fermentation in mild acidic conditions and enzymatic hydrolysis by Savinase to improve metabolic health-promoting properties of lentil. *Journal of Functional Foods*, 48: 9-18. doi: 10.1016/j.jff.2018.06.019.

Beltiz, H.D., Grosch, W. & Schieberle, P. (2009). Legumes. In: Food Chemistry, Springer, Berlin, 746-768.

Berrazaga, I., Micard, V., Gueugneau, M. & Walrand, S. (2019). The Role of the Anabolic Properties of Plant- versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review. *Nutrients*, 11(8): 1825. doi: 10.3390/nu11081825.

Bessada, S.M.F., Barreira, J.C.M. & Oliveira, M. Beatriz P. P. (2019). Pulses and food security: Dietary protein, digestibility, bioactive and functional properties. *Trends in Food Science & Technology*, 93: 53-68. doi: 10.1016/j.tifs.2019.08.022.

Bonnet, C., Bouamra-Mechemache, Z., Réquillart, V. & Treich, N. (2020). Viewpoint: Regulating meat consumption to improve health, the environment and animal welfare. *Food Policy*: 101847. doi: 10.1016/j.foodpol.2020.101847.

Borresen, E.C., Henderson, A.J., Kumar, A., Weir, T.L. & Ryan, E.P. (2012). Fermented foods: patented approaches and formulations for nutritional supplementation and health promotion. *Recent Patents on Food, Nutrition & Agriculture*, 4(2): 134-140. doi: 10.2174/2212798411204020134.

Boye, J., Wijesinha-Bettoni, R. & Burlingame, B. (2012). Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. *British Journal of Nutrition*, 108(2): 183-211. doi: 10.1017/S0007114512002309.

Bustos, A.Y., Gerez, C.L., Mohtar Mohtar, L.G., Paz Zanini, V.I., Nazareno, M.A., Taranto, M.P. & Iturriaga, L.B. (2017). Lactic Acid Fermentation Improved Textural Behaviour, Phenolic Compounds and Antioxidant Activity of Chia (Salvia hispanica L.) Dough. *Food Technology and Biotechnology*, 55(3): 381-389. doi: 10.17113/ftb.55.03.17.5133.

Campbell-Platt, G. (1994). Fermented foods — a world perspective. *Food Research International*, 27(3): 253-257. doi: 10.1016/0963-9969(94)90093-0.

Chawla, P., Bhandari, L., Sadh, P.K. & Kaushik, R. (2017). Impact of Solid-State Fermentation (Aspergillus oryzae) on Functional Properties and Mineral Bioavailability of Black-Eyed Pea (Vigna unguiculata) Seed Flour. *Cereal Chemistry*, 94(3): 437-442. doi: 10.1094/cchem-05-16-0128-r.

Datta, S. & Bouis, H.E. (2000). Application of Biotechnology to Improving the Nutritional Quality of Rice. *Food and Nutrition Bulletin*, 21(4): 451-456. doi: 10.1177/156482650002100421.

Delgado-Andrade, C., Olías, R., Jiménez-López, J.C. & Clemente, A. (2016). Aspectos de las legumbres nutricionales y beneficiosos para la salud humana. *Arbor*, 192(779): 313. doi: 10.3989/arbor.2016.779n3003.

Domínguez-Espinosa, R.M. & Webb, C. (2003). Submerged fermentation in wheat substrates for production of *Monascus* pigments. *World Journal of Microbiology and Biotechnology* 19: 329–336. doi: 1023609427750

Đorđević, T.M., Šiler-Marinković, S.S. & Dimitrijević-Branković, S.I. (2010). Effect of fermentation on antioxidant properties of some cereals and pseudo cereals. *Food Chemistry*, 119(3): 957-963. doi: 10.1016/j.foodchem.2009.07.049.

Dueñas, M., Fernández, D., Hernández, T., Estrella, I. & Muñoz, R. (2005). Bioactive phenolic compounds of cowpeas (Vigna sinensis L). Modifications by fermentation with natural microflora and with Lactobacillus plantarum ATCC 14917. *Journal of the Science of Food and Agriculture*, 85(2): 297-304. doi: 10.1002/jsfa.1924.

Duliński, R., Stodolak, B., Byczyński, Ł, Poreda, A., Starzyńska-Janiszewska, A. & Żyła, K. (2017). Solid-State Fermentation Reduces Phytic Acid Level, Improves the Profile of Myo- Inositol Phosphates and Enhances the Availability of Selected Minerals in Flaxseed Oil Cake. *Food Technology and Biotechnology*, 55(3): 413-419. doi: 10.17113/ftb.55.03.17.4981.

Egounlety, M. & Aworh, O.C. (2003). Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (Glycine max Merr.), cowpea (Vigna unguiculata L. Walp) and groundbean (Macrotyloma geocarpa Harms). *Journal of Food Engineering*, 56(2-3): 249-254. doi: 10.1016/s0260-8774(02)00262-5.

El Youssef, C., Bonnarme, P., Fraud, S., Péron, A., Helinck, S. & Landaud, S. (2020). Sensory Improvement of a Pea Protein-Based Product Using Microbial Co-Cultures of Lactic Acid Bacteria and Yeasts. *Foods*, 9(3): 349. doi: 10.3390/foods9030349.

Espinosa-Páez, E., Alanis-Guzmán, M.G., Hernández-Luna, C.E., Báez-González, J.G., Amaya-Guerra, C.A. & Andrés-Grau, A.M. (2017). Increasing Antioxidant Activity and Protein Digestibility in Phaseolus vulgaris and Avena sativa by Fermentation with the *Pleurotus ostreatus* Fungus. *Molecules (Basel, Switzerland)*, 22(12) doi: 10.3390/molecules22122275.

Estudio científico ANIBES, 7 (2013). Distribución de macronutrientes y fuentes alimentarias en la población española: resultados obtenidos del estudio científico ANIBES. *Fundación Española de la Nutrición.* <http://www.fen.org.es/anibes/archivos/documentos/ANIBES_numero_1.pdf>.

FAO (2009). How to feed the world in 2050. <http://www.fao.org/fileadmin/templates/wsfs/docs/expert_paper/How_to_Feed_the_World_in_2050.pdf>. Accessed date: 4 August 2020.

FAO (2012). World Agriculture towards 2030/2050. <http://www.fao.org/3/a-ap106e.pdf>. Accessed date: 4 August 2020.

Fasolin, L.H., Pereira, R.N., Pinheiro, A.C., Martins, J.T., Andrade, C.C.P., Ramos, O.L. & Vicente, A.A. (2019). Emergent food proteins – Towards sustainability, health and innovation. *Food Research International*, 125: 108586. doi: 10.1016/j.foodres.2019.108586.

Frias, J., Vidal-Valverde, C., Sotomayor, C., Diaz-Pollan, C. & Urbano, G. (2000). Influence of processing on available carbohydrate content and antinutritional factors of chickpeas. *European Food Research and Technology*, 210(5): 340-345. doi: 10.1007/s002170050560.

Gibson, R.S., Perlas, L. & Hotz, C. (2006). Improving the bioavailability of nutrients in plant foods at the household level. *Proceedings of the Nutrition Society*, 65(2): 160-168. doi: 10.1079/PNS2006489.

Gowthaman, M.K., Krishna, C. & Moo-Young, M. (2001). Fungal solid state fermentation — an overview. In: *Applied Mycology and Biotechnology, 1:* 305-352. doi:10.1016/S1874-5334(01)80014-9.

Havemeier, S., Erickson, J. & Slavin, J. (2017). Dietary guidance for pulses: the challenge and opportunity to be part of both the vegetable and protein food groups. *Annals of the New York Academy of Sciences*, 1392(1): 58-66. doi: 10.1111/nyas.13308.

Healthy Eating Plate. Harvard Health Publishing and Nutrition (2011). <https://www.hsph.harvard.edu/nutritionsource/healthy-eating-plate/>. [Accessed date: 3 Aug.2020].

Hocquette, J. (2016). Is in vitro meat the solution for the future? *Meat Science*, 120: 167-176. doi: 10.1016/j.meatsci.2016.04.036.

Jiménez-Martínez, C., Hernández-Sánchez, H. & Dávila-Ortiz, G. (2007). Diminution of quinolizidine alkaloids, oligosaccharides and phenolic compounds from two species of Lupinus and soybean seeds by the effect of Rhizopus oligosporus. *Journal of the Science of Food and Agriculture*, 87(7): 1315-1322. doi: 10.1002/jsfa.2851.

Kasprowicz-Potocka, M., Zaworska, A., Gulewicz, P., Nowak, P. & Frankiewicz, A. (2018). The effect of fermentation of high alkaloid seeds of Lupinus angustifolius var. Karo by Saccharomyces cerevisieae, Kluyveromyces lactis, and Candida utilis on the chemical and microbial composition of products. *Journal of Food Processing and Preservation*, 42(2): e13487-n/a. doi: 10.1111/jfpp.13487.

Khan, M.A., Bala, L. & Husain, S.A. (2016). To study the effect of drying methods on physic-chemical characteristics of fermented soybean (hawaijar). *International Journal of Scientific Research in Science, Engineering and Technology*, 2(4): 840-845.

Khattab, R.Y., Arntfield, S.D. & Nyachoti, C.M. (2009). Nutritional quality of legume seeds as affected by some physical treatments, Part 1: Protein quality evaluation. *Food Science & Technology*, 42(6): 1107-1112. doi: 10.1016/j.lwt.2009.02.008.

Khattab, R.Y. & Arntfield, S.D. (2009). Nutritional quality of legume seeds as affected by some physical treatments, Part 2: Antinutritional factors. *Food Science & Technology*, 42(6): 1113-1118. doi: 10.1016/j.lwt.2009.02.004.

Krishna, C. (2008). Solid-State Fermentation Systems—An Overview. *Critical Reviews in Biotechnology*, 25(1-2): 1-30. doi: 10.1080/07388550590925383.

Kulczyński, B., Kobus-Cisowska, J., Taczanowski, M., Kmiecik, D. & Gramza-Michałowska, A. (2019). The Chemical Composition and Nutritional Value of Chia Seeds—Current State of Knowledge. *Nutrients*, 11(6): 1242. doi: 10.3390/nu11061242.

Leitzmann, C. (2005). Vegetarian diets: What are the advantages? In: Eldmadfa, I. (ed). Diet diversification and Health Promotion. Forum Nutr., Basel, Karger, Switzerland, 147-156. doi:10.1159/000083787.

Li, S., Jin, Z., Hu, D., Yang, W., Yan, Y., Nie, X., Lin, J., Zhang, Q., Gai, D., Ji, Y. & Chen, X. (2020). Effect of solid-state fermentation with Lactobacillus casei on the nutritional value, isoflavones, phenolic acids and antioxidant activity of whole soybean flour. *Lwt*, 125: 109264. doi: 10.1016/j.lwt.2020.109264.

Liu, X. & Kokare, C. (2017). Microbial Enzymes of Use in Industry. In: Brahmachari, G., Demain, A.L. & Adro, J.L. Biotechnology of Microbial Enzymes. Elsevier Inc, 267-298. doi:10.1016/B978-0-12-803725-6.00011-X.

Lonnie, M., Hooker, E., Brunstrom, J., Corfe, B., Green, M., Watson, A., Williams, E., Stevenson, E., Penson, S. & Johnstone, A. (2018). Protein for Life: Review of Optimal Protein Intake, Sustainable Dietary Sources and the Effect on Appetite in Ageing Adults. *Nutrients*, 10(3): 360. doi: 10.3390/nu10030360.

MacQueen, L.A., Alver, C.G., Chantre, C.O., Ahn, S., Cera, L., Gonzalez, G.M., O'Connor, B.B., Drennan, D.J., Peters, M.M., Motta, S.E., Zimmerman, J.F. & Parker, K.K. (2019). Muscle tissue engineering in fibrous gelatin: implications for meat analogs. *Npj Science of Food*, 3(1): 1-12. doi: 10.1038/s41538-019-0054-8.

Manan, M.A. & Webb, C. (2017). Design aspects of solid state fermentation as applied to microbial bioprocessing. *Journal of Applied Biotechnology & Bioengineering*, 4(1): 511-532. doi:10.15406/jabb.2017.04.00094

Margier, M., Georgé, S., Hafnaoui, N., Remond, D., Nowicki, M., Du Chaffaut, L., Amiot, M. & Reboul, E. (2018). Nutritional Composition and Bioactive Content of Legumes: Characterization of Pulses Frequently Consumed in France and Effect of the Cooking Method. *Nutrients*, 10(11): 1668. doi: 10.3390/nu10111668.

Millar, K.A., Gallagher, E., Burke, R., McCarthy, S. & Barry-Ryan, C. (2019). Proximate composition and anti-nutritional factors of fava-bean (Vicia faba), green-pea and yellow-pea (Pisum sativum) flour. *Journal of Food Composition and Analysis*, 82: 103233. doi: 10.1016/j.jfca.2019.103233.

Mitchell, D.A., Krieger, N. & Berovic, M. (2006). Solid-state fermentation bioreactor fundamentals: introduction and overview. In: Solid-state fermentation bioreactors, Springer ed, Berlin, 1-12.

Montowska, M., Kowalczewski, P.Ł, Rybicka, I. & Fornal, E. (2019). Nutritional value, protein and peptide composition of edible cricket powders. *Food Chemistry*, 289: 130-138. doi: 10.1016/j.foodchem.2019.03.062.

Mora-Uzeta, C., Cuevas-Rodriguez, E., Lopez-Cervantes, J., Milán-Carrillo, J., Gutiérrez Dorado, R. & Reyes Moreno, C. (2020). Improvement nutritional/antioxidant properties of underutilized legume tepary bean (Phaseolus Acutifolius) by solid state fermentation. *Agrociencia*, 53: 987-1003.

Niba, L.L. (2003). The relevance of biotechnology in the development of functional foods for improved nutritional and health quality in developing countries. *African Journal of Biotechnology*, 2(12): 631-635. doi: 10.5897/AJB2003.000-1117.

Nkhata, S.G., Ayua, E., Kamau, E.H. y Shingiro, J. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Science & Nutrition*, 6(8): 2446-2458. doi: 10.1002/fsn3.846.

Oghbaei, M. & Prakash, J. (2016) Effect of primary processing of cereals and legumes on its nutritional quality: A comprehensive review. *Cogent Food & Agriculture*, 2 (1): 1136015. doi: 10.1080/23311932.2015.1136015

Olagunju, A.I. & Ifesan, B.O.T. (2013). Changes in nutrient and antinutritional contents of sesame seeds during fermentation. *Journal of Microbiology*, *Biotechnology and Food Sciences*, 2(6): 2407-2410.

Olukomaiya, O.O., Adiamo, O.Q., Fernando, W.C., Mereddy, R., Li, X. & Sultanbawa, Y. (2020). Effect of solid-state fermentation on proximate composition, anti-nutritional factor, microbiological and functional properties of lupin flour. *Food Chemistry*, 315: 126238. doi: 10.1016/j.foodchem.2020.126238.

Onimawo, I.A., Nmerole, E.C., Idoko, P.I. & Akubor, P.I. (2003). Effects of fermentation on nutrient content and some functional properties of pumpkin seed (Telfaria occidentalis). *Plant Foods for Human Nutrition*, 58(3): 1-9. doi: 10.1023/B:QUAL.0000040330.58205.dc.

Onweluzo, J. & Nwabugwu, C.C. (2009). Fermentation of Millet (Pennisetum americanum) and Pigeon Pea (Cajanus cajan) Seeds for Flour Production: Effects on Composition and Selected Functional Properties. *Pakistan Journal of Nutrition*, 8. doi: 10.3923/pjn.2009.737.744.

Pandey, A. (2003). Solid-state fermentation. *Biochemical Engineering Journal*, 13(2-3): 81-84. doi: 10.1016/s1369-703x(02)00121-3.

Parca, F., Koca, Y.O. & Unay, A. (2018). Nutritional and Antinutritional Factors of Some Pulses Seed and Their Effects on Human Health. *International Journal of Secondary Metabolite*, 5(4): 331-342. doi: 10.21448/ijsm.488651.

Patel, H., Chandra, S., Alexander, S., Soble, J. & Williams, K.A. (2017). Plant-Based Nutrition: An Essential Component of Cardiovascular Disease Prevention and Management. *Current Cardiology Reports*, 19(10): 1-10. doi: 10.1007/s11886-017-0909-z.

Pranoto, Y., Anggrahini, S. & Efendi, Z. (2013). Effect of natural and Lactobacillus plantarum fermentation on in-vitro protein and starch digestibilities of sorghum flour. *Food Bioscience*, 2: 46–52. doi: 10.1016/j.fbio.2013.04.001.

Rani, P., Kumar, A., Purohit, S.R. & Rao, P.S. (2018). Impact of fermentation and extrusion processing on physicochemical, sensory and bioactive properties of rice-black gram mixed flour. *LWT - Food Science and Technology*, 89: 155-163. doi: 10.1016/j.lwt.2017.10.050.

Rehman, S., Awan, J., Anjum, F. & Randhawa, M. (2014). Antinutrients and Toxicity in Plant-based Foods: Cereals and Pulses*. Practical Food Safety: Contemporary Issues and Future Directions*: 311-339. doi: 10.1002/9781118474563.ch16.

Robinson, G.H.J., Balk, J. & Domoney, C. (2019). Improving pulse crops as a source of protein, starch and micronutrients*. Nutrition Bulletin*, 44(3): 202-215. doi: 10.1111/nbu.12399.

Rosa-Sibakov, N., Re, M., Karsma, A., Laitila, A. & Nordlund, E. (2018). Phytic Acid Reduction by Bioprocessing as a Tool To Improve the In Vitro Digestibility of Faba Bean Protein*. Journal of Agricultural and Food Chemistry*, 66(40): 10394-10399. doi: 10.1021/acs.jafc.8b02948.

Sadh, P.K., Chawla, P., Bhandari, L. & Duhan, J.S. (2017). Bio-enrichment of functional properties of peanut oil cakes by solid state fermentation using Aspergillus oryzae*. Journal of Food Measurement & Characterization*, 12(1): 622-633. doi: 10.1007/s11694-017-9675-2.

Saharan, P., Sadh, P.K. & Singh Duhan, J. (2017). Comparative assessment of effect of fermentation on phenolics, flavanoids and free radical scavenging activity of commonly used cereals*. Biocatalysis and Agricultural Biotechnology*, 12: 236-240. doi: 10.1016/j.bcab.2017.10.013.

Samtiya, M., Aluko, R.E. & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview*. Food Production, Processing and Nutrition*, 2(1): 1-14. doi: 10.1186/s43014-020-0020-5.

Sánchez Magaña, L., Reyes Moreno, C., Milán-Carrillo, J., Mora Rochin, S., León-López, L., Gutiérrez Dorado, R. y Cuevas-Rodriguez, E. (2019). Influence of solid-state bioconversion by rhizopus oligosporus on antioxidant activity and phenolic compounds Of Maize (Zea Mays L.)*. Agrociencia*, 53: 45-57.

Soccol, C.R., Costa, Eduardo Scopel Ferreira da, Letti, L.A.J., Karp, S.G., Woiciechowski, A.L. & Vandenberghe, Luciana Porto de Souza (2017). Recent developments and innovations in solid state fermentation*. Biotechnology Research and Innovation*, 1(1): 52-71. doi: 10.1016/j.biori.2017.01.002.

Starzyńska–Janiszewska, A. & Stodolak, B. (2011). Effect of Inoculated Lactic Acid Fermentation on Antinutritional and Antiradical Properties of Grass Pea (Lathyrus sativus 'Krab') Flour*. Polish Journal of Food and Nutrition Sciences*, 61(4): 245-249. doi: 10.2478/v10222-011-0027-3.

Stoffel, F., Santana, W.D.O., Fontana, R.C., Gregolon, J.G.N., Kist, T.B.L., De Siqueira, F.G., Mendonça, S. & Camassola, M. (2019). Chemical features and bioactivity of grain flours colonized by macrofungi as a strategy for nutritional enrichment*. Food Chemistry*, 297: 124988. doi: 10.1016/j.foodchem.2019.124988.

Subramaniyam, R., & Vimala, R. (2012). Solid state and submerged fermentation for the production of bioactive substances: a comparative study. I*nternational Journal of Science and Nature*, 3(3): 480-486

Tesfaw, A. & Assefa, F. (2014). Co-culture: A great promising method in single cell protein production*. Biotechnology and Molecular Biology Reviews*, 9(2): 12-20. doi: 10.5897/BMBR2014.0223.

Thirunathan, P. & Manickavasagan, A. (2019). Processing methods for reducing alpha-galactosides in pulses*. Critical Reviews in Food Science and Nutrition*, 59(20): 3334-3348. doi: 10.1080/10408398.2018.1490886.

Vaclavik, V.A. & Christian, E.W. (2013)*. Essentials of Food Science.* 4th ed. Springer, New York. doi: 10.1007/978-1-4614-9138-5.

Vashishth, A., Ram, S. & Beniwal, V. (2017). Cereal phytases and their importance in improvement of micronutrients bioavailability*. 3 Biotech*, 7(1): 42-7. doi: 10.1007/s13205-017-0698-5.

Wang, K., Niu, M., Song, D., Liu, Y., Wu, Y., Zhao, J., Li, S. & Lu, B. (2019). Evaluation of biochemical and antioxidant dynamics during the co-fermentation of dehusked barley with Rhizopus oryzae and Lactobacillus plantarum*. Journal of Food Biochemistry*, 44(2): e13106-n/a. doi: 10.1111/jfbc.13106.

WHO (2007). Protein and amino acid requirements in human nutrition: report of a joint FAO/WHO/UNU expert consultation, World Health Organisation, technical report series, nº935.

Wu, H., Rui, X., Li, W., Xiao, Y., Zhou, J. & Dong, M. (2018). Whole-grain oats (Avena sativa L.) as a carrier of lactic acid bacteria and a supplement rich in angiotensin I-converting enzyme inhibitory peptides through solid-state fermentation*. Food & Function*, 9(4): 2270-2281. doi: 10.1039/c7fo01578j.

Xiao, Y., Rui, X., Xing, G., Wu, H., Li, W., Chen, X., Jiang, M. & Dong, M. (2015). Solid state fermentation with Cordyceps militaris SN-18 enhanced antioxidant capacity and DNA damage protective effect of oats (Avena sativa L.)*. Journal of Functional Foods*, 16: 58-73. doi: 10.1016/j.jff.2015.04.032.

Xiang, H., Sun-Waterhouse, D., Waterhouse, G.I.N., Cui, C. & Ruan, Z. (2019). Fermentation-enabled wellness foods: A fresh perspective*. Food Science and Human Wellness*, 8(3): 203-243. doi: 10.1016/j.fshw.2019.08.003.

Xing, Q., Dekker, S., Kyriakopoulou, K., Boom, R.M., Smid, E.J. & Schutyser, M.A.I. (2020). Enhanced nutritional value of chickpea protein concentrate by dry separation and solid state fermentation*. Innovative Food Science and Emerging Technologies*, 59: 102269. doi: 10.1016/j.ifset.2019.102269.

Xu, L.N., Guo, S. & Zhang, S.W. (2018). Effects of solid-state fermentation with three higher fungi on the total phenol contents and antioxidant properties of diverse cereal grains*. FEMS Microbiology Letters*, 365(16) doi: 10.1093/femsle/fny163.

Xu, L.N., Guo, S. & Zhang, S.W. (2019). Effects of solid-state fermentation on the nutritional components and antioxidant properties from quinoa*. Emirates Journal of Food and Agriculture*, 31(1): 39-45. doi: 10.9755/ejfa.2019.v31.i1.1898.