

Review

Microbial Fermentation for Improving the Sensory, Nutritional and Functional Attributes of Legumes

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Abstract: A rapidly growing population, resource scarcity, and the future sustainability of our food supply are among the major concerns of today's food industry. The importance of resilient food crops that will sustain in the future is imperative, and legumes are ideal future food crops owing to their rich nutrient profile, cost-effective production and resource usage efficiency. Furthermore, they have the potential to meet the protein needs of the future. There are however several limitations associated with legumes in terms of their sensory, nutritional, and functional properties, which make them challenging for the food industry to use. In this review, these challenges are discussed in detail with particular reference to fermentation as a strategy for overcoming them. A major focus is on examining the potential application of fermentation for modifying techno-functional properties, such as foaming and emulsifying properties, solubility, and water and oil binding capacities of legume substrates. In many studies, fermentation has been demonstrated to enhance the techno-functional, sensory and nutritional attributes of various legume substrates. Future studies must focus on developing scalable fermentation processes to utilize the technology for improving the techno-functional and sensory properties of legume-based ingredients at industrial scale.



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1. Introduction

A growing global population, increased incomes in emerging economies, urbanization, dietary requirements of an aging population, socio-demographic factors [1,2] and increasing awareness of the benefits of having a balanced diet will increase the demand for food in all forms. Economic models predict that the total food demand will rise by 30–62% between 2010 and 2050 [3]. Conversely, the supply of food, will be constrained because of increased pressure on land and water, and the effects of climate change [4]. For instance, the amount of land available for agriculture will be 593 million hectares less in 2050 than in 2010, which is an area almost twice the size of India [5]. It is expected that the challenges of ensuring food security, reducing carbon emissions, and meeting the increasing energy demand will grow in importance over the coming decades. In this regard, it is imperative to develop strategies to ensure food security in the future. Efforts must be aligned with the goals of creating more productive, sustainable, and resilient food systems that are beneficial to people and the environment.

In this context, legumes could play a major role in ensuring food security and sustainability, as they require fewer inputs in farming systems, lead to lower greenhouse gas emissions, and contribute to greater socioeconomic well-being [6–8]. In addition, legumes have the potential to serve as a major protein source in the future, where they complement the limited protein content of roots, tubers, and cereals [9]. Changes in lifestyles and

consumption patterns resulting from veganism and vegetarianism are also increasing plant protein consumption, which is projected to reach a USD 290 billion market by 2035 with a market penetration of 10% to 22% [10]. As a consequence of economics, ethics, health, compassion for animals, personal responsibility, and hedonic reasons [11], plant protein products including legume proteins are becoming mainstream, not just niche products [12]. Legume proteins are also healthier alternatives for consumers suffering from allergies to traditional protein sources such as milk proteins [13–15], and red meat proteins [16]. In addition to proteins, legumes also provide a variety of other nutrients, including carbohydrates (digestible and non-digestible), lipids, micronutrients, and bioactive compounds [9,17]. Several studies also indicate that regular consumption of legume proteins has several health benefits, including the reduction of hypertension, cardiovascular diseases, cancer, and weight management without compromising the quality of the diet [18–25].

To make legumes a more attractive food source, efforts need to be focused on improving their sensory properties, including flavor and texture, as well as reducing antinutritional factors (ANF) and allergens [26]. Legumes can be processed and formulated into various products that mimic the sensory characteristics of popular animal-based products, such as burgers, sausages, or nuggets [27]. This allows consumers to enjoy the familiarity of traditional animal-based dishes while benefiting from the nutritional advantages of legumes.

Among the various food processing technologies such as extrusion, high-pressure processing, and ultrasonication, controlled fermentation with defined bacterial cultures stands out with a high potential to improve the various properties of legumes [28–30]. It offers the advantage of reducing the need for energy-intensive processing and additives, distinguishing it from other food processing techniques. In addition, fermentation is considered to be a “natural” process [31] and as such enables the manufacture of foods with desired quality and pleasant taste with a “clean label” [32,33]. During fermentation, microbial metabolic activities and related physicochemical processes transform food components into unique and wholesome products by enhancing their techno-functional (emulsification, foaming, gelling, solubility, viscosity, flavor binding and film formation), sensorial, and nutritional attributes [34–37]. It has been known for centuries that fermentation extends the shelf-life of foods and enhances their organoleptic properties [38]. The overall effect of fermentation on legumes is summarized in Figure 1.

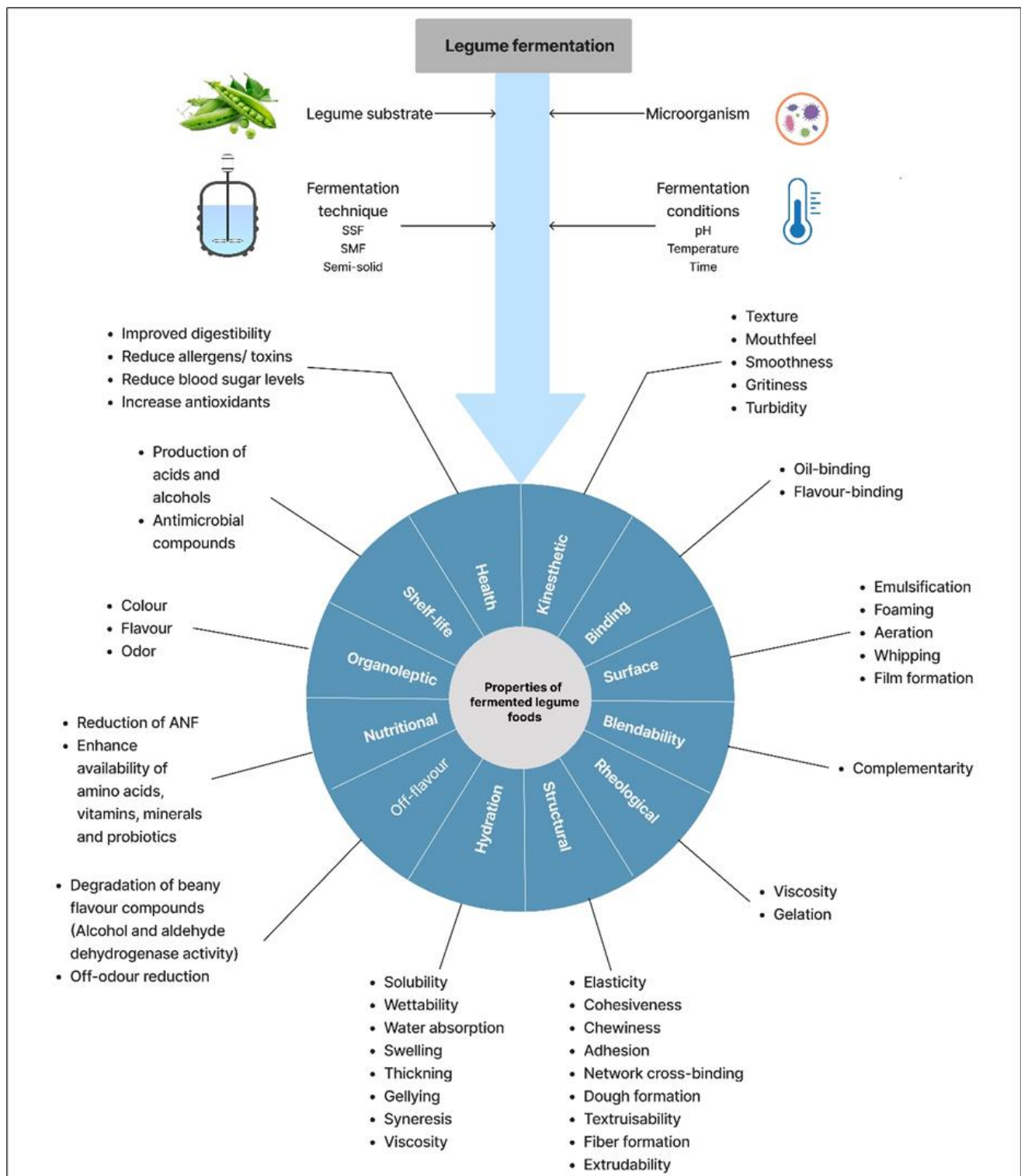


Figure 1. Summary of the overall effect of fermentation on different attributes of legumes. Sources: [22,38–40].

As the food industry seeks ways to meet the expectations of consumers and to responsibly increase the availability of sustainable food supply, it is necessary to pay attention to the inherent sensory and techno-functional limitations, and nutritional deficiencies of legumes. This review examines the challenges of using legumes as a future protein crop, and the potential applications of fermentation to overcome some of these challenges and

produce quality proteins. Recently, Emkani, et al. [41] and Cichonska and Ziarno [42] reviewed the literature on the impact of lactic acid bacterial (LAB) fermentation on the nutritional and technological properties of legumes whereas Adebo, et al. [43] reviewed the impact of fermentation on the nutritional quality of legumes and cereals. The objective of this review is (i) to provide an overview of the underlying biochemical and structural changes that occur during fermentation of legumes as the basis for the beneficial effects of fermentation on the sensorial, techno-functional and nutritional quality attributes and (ii) highlight fermentation as a powerful tool for transforming legumes into legume-based food ingredients with desirable quality attributes for applications in a broad range of food products and (iii) inspire further research and development on the rational design of cost-effective and scalable fermentation processes amenable for industrial scale production of fermented legume-based food ingredients with target functional attributes making use of (1) genomics and other omic tools for rational selection of starter cultures for predictable outcomes and (2) high throughput analytic techniques such as GC-MS and LC-MS for better understanding of changes in the substrate compositional profile during fermentation to guide selection of optimal fermentation conditions for the desired target.

2. Challenges of Using Legumes as a Future Protein Crop

Legumes belong to the Fabaceae family and fall into 11 main categories: chickpeas, cowpeas, bambara beans, beans, fava beans, lentils, lupins, peas, pigeon peas, vetches, and minor pulses [44]. The most consumed legumes today include soybeans, peas, chickpeas, lentils, and beans [45,46].

In comparison to cereals, legume seeds contain a high level of protein, ranging from 20% to 45%, while cereals generally contain around 6% to 15% protein [47,48]. In addition, legumes contain dietary fiber (5–37%) [44], and carbohydrates (6–62%) [49]. As an example, lupine, an underutilized legume crop, contains 34 g of protein per 100 g of dry matter, while soybeans and fava beans contain 36.5 g and 27.2 g of protein respectively. The carbohydrate content of these legumes is 9.5 g/100 g, 30.2 g, and 46.5 g, respectively [50]. Legumes generally contain healthy fats and have no cholesterol [44], and they are also rich in essential minerals particularly Ca, K, P, Cu, Fe and Zn [51,52], and antioxidants, oligosaccharides, polyphenols and bioactive compounds [53]. Legumes provide a significant amount of folate and thiamine as well [51,54,55]. It is reported that, legumes typically contain 380–660 mg/100 g of arginine, 650–820 mg/100 g of aspartic acid, and 975–1150 mg/100 g of glutamic acid [50] which are the most abundant amino acids in legumes. These compositional attributes highlight the potential use of legumes as functional food ingredients.

Despite the many benefits that legumes provide, they have some qualitative limitations that negatively affect their sensory profile, techno-functionality, and nutritional quality [56–58] and consequently their use in a variety of food products. Table 1 presents an overview of the benefits and limitations associated with legumes.

Flavor (aroma and taste) is one of the main factors that determine the acceptance of food products. It is determined by the chemical composition of the food matrix and the structure related to the release of flavor [59]. Legumes are often associated with unpleasant aroma compounds (e.g., green, earthy, grassy, leafy, astringent, and metallic) [60–62]. These are derived from volatile (aldehydes such as acetaldehyde, decanal, hexanal; esters such as acetate, butyrate, caproate; alcohols such as butanol, methylbutanol; and ketones such as acetone, acetophenone) and non-volatile compounds (isoflavones, saponins, phenolic compounds and peptides) [62,63]. In addition, beany flavor is a common off flavor associated with legumes, which intensifies during harvesting, storage and processing [64]. Beany flavor compounds are formed through the lipoxygenase catalyzed oxidation [65] of unsaturated fatty acids such as linoleic acid, linolenic acid and oleic acid, which produce volatile aroma compounds [66]. The intensity of beany flavor is highly species dependent, for instance, soybean has a higher concentration of off-flavor volatiles than lupin [67]. Other factors such as geographical origin, agronomical practice, processing method, stor-

age and packaging conditions also influence the generation and intensity of beany flavor in legumes [68].

Similarly, bitterness is a common off flavor associated with legumes, particularly due to the presence of low molecular weight peptides that contain hydrophobic amino acids such as leucine, proline, phenylalanine, and tyrosine [69]. Non-volatiles derived from saponins, such as triterpenoids and saponins A, B, E, and DDMP saponins, also contribute to bitterness [68,70]. The degree of bitterness is dependent on the type and concentration of saponins. The bitterness of DDMP saponin is significantly higher than that of saponin B, for example [70].

Furthermore, ANF compounds present in legumes inhibit the bioavailability of many nutrients, including proteins, carbohydrates, vitamins, and minerals, which is a major challenge [54,71,72]. Among the ANF found in legumes are tannins, trypsin inhibitors, phytic acid, hemagglutinins [54,73,74] and non-digestible carbohydrates such as stachyose, raffinose and verbascose (raffinose family oligosaccharides) [75]. The phytate content in common beans is reported to be 0.6–0.8 mg/g, while it is 1.2 mg/g in peas [76]. Similarly, the tannin content of common beans and peas is reported to be 6–28.4 mg/g and 27.8–30.9 mg/g respectively [76]. There is evidence that multiple ANF work synergistically. For instance, tannins and other polyphenols, oxalates, and fibers synergistically act with phytic acid to inhibit the bioavailability of minerals [77]. ANF can disrupt protein digestion and utilization, mineral absorption and metabolism and cause gastrointestinal discomfort such as bloating and nausea. All of these contribute to the reduction of legumes’ nutritional value [71,72].

In terms of allergenicity, compared to other legumes, allergic reactions to soybeans and peanuts are relatively common and the allergic reaction can be severe [78,79]. Lentils, chickpeas, peas, mung beans, and red grams have also been reported to have allergenic potential [80,81]. What is most challenging is the fact that legumes exhibit immunological cross-reactivity among themselves as well as with other sources, increasing the severity of an allergenic response, as reported with peanut and lupin [81,82]. For instance, patients who are allergic to peanuts develop allergies to lupin, demonstrating the ability of cross-reactivity to trigger an immune response to molecules found in closely related species [82].

In general, legume proteins have inferior techno-functional properties compared to animal proteins (Table 1). However, the magnitude of the difference between the two can vary depending on multiple factors, such as the specific type of legume and animal protein being compared (including source, structure, and molecular weight), as well as the processing methods utilized [83,84]. Rahmati, et al. [85] investigated the feasibility of using soymilk as an alternative to egg yolk in mayonnaise formulation. The study revealed that mayonnaise prepared using egg yolk exhibited greater emulsion stability in comparison to those containing soy milk [85].

Table 1. An overview of the benefits and limitations associated with legumes.

Attribute	Pros	Cons	Reference
Flavor and taste			
Beany flavor	• Add depth and complexity to dishes, especially in soups, stews, and curries. • Versatility: Complement a variety of other flavors, such as herbs, spices, and vegetables. • Cultural significance: Beany flavor is often associated with traditional dishes and culinary traditions.	• Legumes can cause digestive issues such as bloating and gas due to complex sugars and fibers. • Legumes require a longer cooking time to become tender and flavorful, and overcooking can intensify the beany flavor. • In some cultures, legumes are not traditionally consumed, and the beany flavor may be unfamiliar or unappetizing, making it difficult to incorporate into diets.	[86–89]

Table 1. Cont.

Attribute	Pros	Cons	Reference
Bitter flavor	Bitterness can indicate the presence of certain phytochemicals with potential health benefits. (e.g., Flavonoids in chickpeas and lentils are known to have antioxidant and anti-inflammatory properties)	Some bitter compounds in legumes, such as phytohemagglutinin in kidney beans, can be toxic if consumed in large quantities or if the beans are not properly cooked.	[90,91]
Texture	- Legumes are versatile in texture, from smooth and creamy to firm and chewy. This makes legumes a valuable ingredient in many plant-based meat substitutes, dairy alternatives, and other food products. - Legume-based products can be customized to create specific textures and mouthfeel by adding different legume flours or proteins.	- Some legumes, such as chickpeas and beans, can be hard and tough when cooked, making them challenging to use in certain food products or less enjoyable to eat. - Legume-based products such as flours, protein isolates, or fibers may not fully dissolve or disperse in liquids, resulting in particle sedimentation and an unpleasant gritty or sandy texture (e.g., in beverages).	[92–95]
Allergens and ANF			
ANF	- ANF, such as phytic acid, can act as natural preservatives, helping to extend shelf life. - ANF such as lectins slows down the digestion and absorption of carbohydrates, which may be beneficial for individuals with diabetes and may also help in weight management. - Oligosaccharides (raffinose, stachyose, verbascose) and resistant starches, can act as prebiotics, promoting the growth of beneficial bacteria in the gut and improving overall gut health.	- Phytic acid, tannins, and oxalates can bind to minerals and prevent their absorption in the gut leading to deficiencies in essential nutrients such as Fe, Zn, and Ca. - Oligosaccharides such as raffinose can cause digestive issues such as flatulence, bloating, and diarrhea in some individuals, particularly if consumed in large quantities. - Trypsin inhibitors, can inhibit the digestion of proteins, reducing the bioavailability of amino acids. - High levels of ANF compounds in early childhood can impair growth and development.	[96–99]
Allergens	Most of the allergens are proteins with nutritional benefits to non-sensitive individuals.	- Certain types of proteins in legumes, such as profilins, prolamins, and cupins can cause adverse health effects in some individuals with allergies or sensitivities. Symptoms can range from mild to severe. - Some consumers perceive legumes as unhealthy or unsafe, even if they are not allergic or sensitive to them.	[72,100–103]
Nutritional quality			
Proteins	Contain a fair amount of arginine, aspartic acid, glutamic acid, leucine and lysine.	Lower levels of certain essential amino acids such as methionine, tryptophan and cysteine.	[50]
Carbohydrates	Complex carbohydrates in legumes provide a slow and sustained release of energy, which helps in maintaining blood glucose levels and reducing the risk of developing type 2 diabetes.	Legume carbohydrates have a higher content of non-starch polysaccharides, such as cellulose and hemicellulose, which can be difficult to digest for some people, leading to digestive issues.	[104,105]
Techno-functionality			
Emulsification	- Legumes such as chickpeas and soybeans contain natural emulsifiers such as lecithin. - Compared to cereals (e.g., wheat, barley, oats) legumes (e.g., chickpea, pea, soybean, lentil, fava bean) exhibit better emulsification activity and stability.	- Due to larger molecular size and structural limitations, legume proteins tend to create a thicker layer at the oil/water interface in comparison to dairy proteins. This can reduce emulsion stability. - The low emulsification capacity of legumes can make it challenging to achieve a smooth and creamy texture and increase the risk of sedimentation and instability in the emulsion	[85,106–109]

Table 1. Cont.

Attribute	Pros	Cons	Reference
Gelation	Potential to develop plant-based gelling agents with pectin and carrageenan.	- Plant globulins pose difficulty in gelation due to their limited solubility and tendency to aggregate during extraction. - Legumes with poor gelation properties make it challenging to create gels and other semi-solid textures.	[93,110,111]
Foaming	- Legume foams are low in fat content, making them a healthier option. - Legume flours have relatively higher foaming properties compared to cereal flours due to their higher levels of albumins and globulins.	Legumes with limited foaming properties make it difficult to create light and fluffy products such as meringues or whipped cream.	[107,112,113]
Solubility	Legume proteins offer the flexibility of enhancing their solubility through various methods, such as physical, chemical, or enzymatic approaches.	Legumes with lower solubility may create a gritty or chalky texture or cause a product to be too thick or viscous, which can negatively affect other properties such as emulsification, foaming, and gelation.	[107,114,115]

The limitations explained above can be effectively addressed with fermentation, which simultaneously enhances nutritional value, improves flavor and taste, reduces allergenic and antinutritional factors, and promotes the production of bioactive compounds while producing unique properties [116]. Fermentation's multifaceted benefits make it a particularly attractive approach for effectively overcoming the challenges associated with legumes.

3. Microbial Fermentation of Legumes

Microbial fermentation is a sustainable method of producing wholesome, nutritious, aesthetically pleasing, and high-quality food products. This technology has been employed for millennia to enhance the functional properties of food, as well as its shelf life, safety, transportability, palatability, and organoleptic properties, including texture, color, aroma, mouthfeel and overall acceptability and also for the reduction of ANF such as phytic acid, tannins, and protease inhibitors [72,117–119]. Fermentation can also enhance the protein quality of legumes (seeds, flour, protein concentrates, and protein isolates) by increasing the content of essential amino acids, such as lysine and methionine, and promoting the synthesis of bioactive peptides with antioxidant, anti-inflammatory, and antimicrobial properties [120–122]. The process of fermentation can be broadly categorized into traditional and modern fermentation methods [123] which will be discussed in the next sections.

3.1. Traditional Fermentation of Legumes

Traditional fermentation of legumes and other substances is a complex process involving several microorganisms at different stages of the process. Many of these fermentation processes are not well investigated. The main microorganisms that are involved in the traditional fermentation of legumes are fungi such as *Aspergillus* spp. and *Rhizopus* spp. and bacteria such as *Bacillus* spp. and LAB. The LAB that are predominant in such processes are *Leuconostoc*, *Lactiplantibacillus*, *Streptococcus*, and *Pediococcus* species. Traditional fermented legume products, the predominant microbial strains involved in their fermentation and the fermentation processes are summarized in Table 2.

Traditional legume fermentation involves back slopping, i.e., using a portion of the previously fermented material as a fermentation starter inoculant in subsequent fermentation. The microbial profile and inoculum concentration vary depending on the fermentation technique, type of legume, temperature, pH, moisture, oxygen availability, season and region. For instance, traditional soybean fermentation is dominated by fungi such as *Aspergillus* and *Rhizopus* while the traditional fermentation of African locust bean is dominated by *Bacillus* species [124,125]. In addition, nutrient availability affects the composition of the microbial community, resulting in a change in the dominant microorganism over time. Furthermore,

fermentation conditions are not well defined and optimized in traditional fermentation, but rather approximate conditions are used. As a consequence, the products manufactured by this process may vary in terms of their techno-functional properties, nutritional profile, and sensory characteristics [32,42,126]. Apart from product consistency, safety concerns such as the presence of biogenic amines, mycotoxins and pathogenic organisms is another major issue with traditional fermented legume and other food products. Liu, et al. [127] noted that there is a significant heterogeneity in the quality of traditional fermented soy products such as soy sauce, douchi, sufu and dajiang.

In the traditional fermentation processes, it takes a relatively long time to stabilize microbial populations. During this time, there is a risk of contamination by other microorganisms, which can lead to product spoilage. In addition to affecting the safety of the final product, competition for nutrients in the substrate under such conditions might change the outcome of fermentation from a quality perspective.

The pre-treatment processes before fermentation might also affect the techno-functional properties of the final product. Conducting the soaking stage of soybeans under controlled conditions, for instance, can lead to a more predictable acidification result [128]. Depending on the soaking temperature, the resultant product might have different characteristics, since the dominant microorganism varies [129]. Pre-treatments facilitate the activation of enzymes, therefore expediting the breakdown process resulting in accelerated fermentation [97]. Establishing standards and using defined pre-treatment and fermentation methodologies in traditional fermentation are essential for improving the quality and safety of traditional fermented foods.

Some of the ways that can improve traditional fermentation include: (i) selecting substrate varieties suitable for fermentation, (ii) isolating, selecting and preserving cultures with desirable traits for use as starter cultures, (iii) developing optimized fermentation processes with appropriate process and quality control for faster production, safe and consistent product and scale of operation. In general, the use of well-defined starter cultures, optimized fermentation conditions and a better understanding of the substrate structure-functionality relationship results in more consistent products at the industrial scale, although that potentially comes with some loss of characteristic sensory attributes [37].

During traditional fermentation, microbial growth, metabolism, and different types of interactions among the microbial consortia (mutualism, favorism, competition) result in the production of unique flavor attributes and a rich nutrient profile [130], which are difficult to reproduce in industrial fermentation processes employing defined starter culture(s). Furthermore, following fermentation, legume products often do not undergo subsequent cooking/heat treatment procedures. As a result, such products are a source of viable microorganisms, including lactic acid bacteria, which are known for their potential probiotic and other health benefits [131] and can be considered to be synbiotic products considering the relatively high concentration of prebiotic resistant starch and oligosaccharides in legumes [42].

The quality of traditionally fermented legumes is a result of the combined effects and interactions among multiple microorganisms involved in the fermentation process, rather than a single species or strain. The science behind traditional fermentation is not well understood and has an untapped potential to emulate and beneficially use microbial interactions such as symbiotic co-existence [132] to create unique techno-functional, organoleptic, nutritional and health attributes and enable biotechnological innovation.

Table 2. Traditional fermented products are consumed around the world and their fermentation conditions. Preprocessed seeds are used as substrates in these processes unless is specified.

Product, Region of Origin and Substrate	Fermentation Conditions and Microorganism(s)	Fermentation Changes, Attributes of Product and Use	References
Product: Adai Origin: South India Substrate: Legume seeds and cereal grains	Fermentation: Soak rice, black gram and dhal for 2 h, add sweeteners and condiments and ferment Microorganism(s): LAB (<i>Streptococcus</i> spp., <i>Pediococcus</i> spp., and <i>Leuconostoc</i> spp.)	Fermentation changes: Increase nutrient bioavailability and digestibility, increase vitamin and mineral availability Nature of the product: Savoury pancake with a soft center and crispy edge	[133,134]
Product: Dawadawa Origin: West Africa Substrate: African locust bean	Fermentation: Boil cotyledons, ferment for 84 h at 35 °C Microorganism(s): <i>Bacillus subtilis</i> , <i>Leuconostoc mesenteroides</i> and <i>Leuconostoc dextranicus</i>	Fermentation changes: Better functional properties (emulsification) and increased mineral availability Attributes: A flavoring agent with black color medium size balls with a strong pungent smell	[135–137]
Product: Dhokla Origin: India Substrate: Bengal gram, split beans	Fermentation: Soak Bengal gram, rice and split beans, and ferment at 32 °C for 18 h Microorganism(s): <i>Leuconostoc mesenteroides</i> , <i>Lactobacillus fermentum</i> , <i>Pichia silvicola</i> , <i>Candida</i> sp., <i>Streptococcus faecalis</i> , <i>Torulopsis candida</i> , <i>Torulopsis pullulans</i>	Fermentation changes: Increase vitamin and mineral availability (Ca, K, Fe) Attributes: Savoury light yellow color dish with a soft and spongy texture	[134,138,139]
Product: Douchi Origin: China and Taiwan Substrate: Soybean, salt	Fermentation: Soak black soybeans and ferment at 27–32 °C for about 72 h Microorganism(s): Primarily <i>Aspergillus oryzae</i> ; also <i>Pichia</i> , <i>Bacillus</i> , <i>Staphylococcus</i> and <i>Pediococcus</i>	Fermentation changes: Improved nutrient bioavailability, increased protein digestibility, improved flavor and aroma, and potential probiotic effects Attributes: Small balls with aromatic, pungent, earthy and salty flavor	[140–143]
Product: Kinema Origin: India, Nepal and Bhutan Substrate: Soybean	Fermentation: Crack soybeans, soak overnight, ferment for 2–3 d at 25–40 °C Microorganism(s): <i>Bacillus cereus</i> , <i>Bacillus subtilis</i> , <i>Bacillus licheniformis</i> , <i>Bacillus circulans</i> , <i>Bacillus thuringiensis</i> and <i>Bacillus sphaericus</i>	Fermentation changes: Production of bioactive substances such as phenolics and production of exopolysaccharides Attributes: Alkaline and sticky paste with an ammoniacal odor	[144–147]
Product: Idli Origin: India Substrate: Blackgram and rice	Fermentation: Mix parboiled rice and decorticated black gram (3:1), soak for 3–4 h, ferment for 16–18 h at ambient temperature Microorganism(s): Yeast, <i>Leuconostoc mesenteroides</i>	Fermentation changes: Increase availability of vitamins (A, B1, B2, B12), increase levels of essential amino acids (lysine, cysteine and methionine) Attributes: Savoury cake with a soft and spongy texture	[134,148,149]
Product: Maseura Origin: Nepal Substrate: Blackgram or greengram	Fermentation: Make a paste from soaked seeds, ferment for 2–3 days in an open room, sun-dry for 3–5 days Microorganism(s): Bacteria and mold	Fermentation changes: Increase protein solubility and vitamin availability Attributes: Cake-like dried balls	[150–152]
Product: Meju Origin: Korea Substrate: Soybean	Fermentation: Make a paste from soaked boiled soybean, compress into a cube, dry until firm, tie with rice straw, ferment for 12 days at 28 °C Microorganism(s): Fungi and <i>Bacillus</i> spp.	Fermentation changes: Increase bioactive peptides, decreased the total amount of isoflavonoids Attributes: Brick like appearance	[153–155]
Product: Natto Origin: Japan, Korea Substrate: Soybean	Fermentation: Cooked black soybeans fermented for 48 h at 37 °C Microorganism(s): <i>Bacillus</i> spp.	Fermentation changes: Increase amino acids and peptide content Attributes: Sticky outlook, sour aroma, nutty flavor, slippery texture	[156–158]

Table 2. Cont.

Product, Region of Origin and Substrate	Fermentation Conditions and Microorganism(s)	Fermentation Changes, Attributes of Product and Use	References
Product: Oncom Origin: Indonesia Substrate: Peanut meal	Fermentation: Soak peanut meal, steam for 60 min, ferment for 48 h at 25–30 °C under anaerobic conditions Microorganism(s): <i>Rigidoporus oligosporus</i> , <i>Neurospora sitophila</i> , <i>N. crassa</i>	Fermentation changes: Increased water absorption and decreased bulk density, increased protein availability and minerals Attributes: Distinctive red or black colored powder	[141,159,160]
Product: Sufu Origin: China Substrate: Soybean curd (tofu)	Fermentation: Ferment in a brine solution for 40–60 days Microorganism(s): <i>Bacillus cereus</i>	Fermentation changes: Production of flavor compounds Attributes: Soft texture with a cheese like appearance	[141,161]
Product: Temph Origin: East Java, Indonesia Substrate: Soybean	Fermentation: Soak, dehull and cook whole soybeans, drain excess water and ferment for 1–2 days by inoculating with a piece of tempe or culture Microorganism(s): <i>Rhizopus</i> spp.	Fermentation changes: Degradation of ANF, Increase solubility of proteins and peptides Attributes: Compact cake with umami taste	[157,162,163]

3.2. Modern Fermentation

Even though traditional fermentation has been practiced for thousands of years, it is still a very uncontrolled process susceptible to variable and inconsistent outcomes. Modern advances in microbiology and processing technology have presented possibilities for more precise and controlled fermentation processes. By controlling the type of starter culture and dosage and environmental factors such as temperature, pH, and nutrient levels, modern industrialized fermentation can produce more consistent and predictable outcomes than traditional methods. Furthermore, the use of specific starter cultures that have been optimized for certain food products or food ingredients allows for greater control over the fermentation process and can lead to the development of new and innovative functional food products. These developments have already led to the industrialization of the manufacture of traditional fermented products such as beer, spirits, cheese and yogurt. More recently, kefir and kombucha have also become mainstream products manufactured at a commercial scale in response to consumers' renewed interest in fermented foods for health and wellness [37]. There are also several research and development efforts both by industry and academia to modernize the fermentation of legumes and other substrates with the use of defined starter cultures. Fermented soymilk and other plant-based yogurts incorporating pea and fava bean protein are already available in the market as dairy alternatives. There are also a few fermented soybeans (e.g., JeollaNamado fermented soybean powder, Kalustyan's Mejugaru fermented soybean powder) and pea powders (e.g., Phytopea SF sprouted fermented pea protein powder, Nutrasumma fermented pea powder) in the market for use as condiments in Asian cooking or as dietary supplements. However, these products are cost-prohibitive for use as bulk food ingredients. Thus, there is a need for the development of cost-effective and scalable processes for the manufacture of bulk fermented legume ingredients with defined functionality to use as food ingredients in a broad range of food products, making use of the versatility of fermentation as a tool to improve the nutritional, sensorial and functional properties of legumes. Tables 3 and 4 provide a summary of studies that investigated the effects of modern fermentation processes at laboratory scale on various quality attributes of legume-based products.

3.2.1. Effect of Fermentation on Flavor Profile

Fermentation with appropriate starter culture can be used to substantially improve the flavor profile of legumes. For instance, fermentation of soy drink with *Lycoperdon pyriforme* fungi for 28 h incubation period at 24 °C under dark conditions while being agitated on a rotary shaker operating at 150 rpm resulted in a noteworthy decline of "green" odor elements derived from hexanal, (E)-2-nonenal, and (E,E)-2,4-decadienal, and a reduction in

the sensory strength of “green odor” in comparison to unfermented soy milk [164]. It is worth highlighting that, following 28 h of fermentation, 60% of the sensory panelists were unable to detect off-notes [164]. This is further supported by a study conducted with pea protein isolates [165].

When fermentation was carried out with LAB and yeasts (*Kluyveromyces lactis*, *Kluyveromyces marxianus*, or *Torulaspora delbrueckii*) as starters, the levels of hexanal, butanal, and nonanal in pea protein decreased to below the limit of detection. Additionally, the most predominant volatile, 2-pentylfuran, which has been characterized as “earthy/musty”, “green”, and “floral”, was reduced to one-sixth of the concentration found in the non-fermented samples while yeast triggered the generation of esters which improve appealing flavours [165]. Similarly, fermenting chickpea fortified bread with *Lactiplantibacillus plantarum* M8d [166], and fava beans with *Lactiplantibacillus plantarum* DPPMAB24W resulted in an enhanced flavor profile and reduced off-flavors, similar to what was observed in the fermentation of pea protein isolates [167].

Flavor development during fermentation is a result of a sequence of biochemical processes where the starter culture produces enzymes and other metabolites that modulate the flavor profile of the product. During the early stages of fermentation, enzymes produced by the microorganisms play a crucial role in breaking down complex nutrients into simpler compounds [168]. Proteases break down proteins into peptides and amino acids, while lipases break down fats into fatty acids and glycerol. Amylases break down starches into simple sugars such as glucose and maltose, and galactosidases breakdown lactose into glucose and galactose. These simpler compounds act as precursors for the development of flavor and aroma compounds later on in the fermentation process [169]. As the fermentation progresses, the microorganisms convert these simple compounds into a variety of compounds, including organic acids, alcohols, esters, and ketones, which contribute to the unique flavor and aroma of the final product [127,169]. Among the various biochemical processes in LAB fermentation, it has been found that the major contributing pathway for flavour formation is proteolysis [169].

It is important to note that the improvement in flavor profile and reduction of off-flavors after fermentation is not universal across all fermented products (Table 3). The changes in flavor and off-flavors can vary depending on the specific product, the microbial strains used for fermentation, and the fermentation conditions. Therefore, it is important to evaluate the flavor and sensory attributes of each fermented product individually and optimize the fermentation condition to achieve the target flavor profile.

Table 3. Effect of LAB fermentation of legumes and legume containing formulations on different quality attributes.

Substrate Form and Characteristics (Substrate), Microorganism(s) Involved in Fermentation (Microorganism(s)), Fermentation Conditions (Conditions) and Product Type (Product)	Product Properties after Fermentation	References
<i>Cicer arietinum</i> (Chickpea)		
Substrate: Black chickpea flour made into semi liquid dough after mixing flour with water Microorganism(s): <i>Lactiplantibacillus plantarum</i> T0A10 Conditions: 30 °C for 24 h Product: Fortified semolina pasta containing 15% fermented chickpea flour	- Higher in-vitro protein digestibility (up to 38%) - Lower starch hydrolysis rate - Inhibition of linolenic acid peroxidation, related to organoleptic properties - Increased hardness compared to the control	[170]
Substrate: Chickpea flour made into dough Microorganism(s): <i>Lactiplantibacillus plantarum</i> CRL2211 <i>Weissella paramesenteroides</i> CRL2182 Conditions: 37 °C for 24 h Product: Crackers and cookies	- Fermented cookies were more acidic than unfermented controls - Fermented cookies showed increased moisture content (1.9% compared to control) - Increased antioxidant activity (61%) - Increased total phenolic content	[171]

Table 3. Cont.

Substrate Form and Characteristics (Substrate), Microorganism(s) Involved in Fermentation (Microorganism(s)), Fermentation Conditions (Conditions) and Product Type (Product)	Product Properties after Fermentation	References
Substrate: Garbanzo chickpeas were soaked, blended and extracted with water Microorganism(s): <i>Streptococcus thermophilus</i> , <i>Lactobacillus bulgaricus</i> , <i>Lactobacillus acidophilus</i> Conditions: 42 °C for 16 h Product: Beverage from blended chickpea	- Compared to the control (soymilk), chickpea beverage contained a lower amount of protein, fat, and sugar - Significantly increased syneresis - Weaker protein network led to starch retrogradation, and appearance scored lower values compared to the control	[172]
Substrate: Chickpea flour made into liquid sourdough Microorganism(s): <i>Lactiplantibacillus plantarum</i> M8d Conditions: 30 °C for 6 h, stirred at 100 rpm; inoculated by back-slopping Product: Bread fortified with chickpea flour (0, 5, 10, 20%)	- Improved texture and flavor - Reduced content of raffinose family oligosaccharides by 63.22% - Significantly increased free amino acid, lysine and total phenolic content	[166]
Substrate: Coarsely mill seeds first and then milled into flour, mix with water to form dough for fermentation Microorganism(s): <i>Pediococcus pentosaceus</i> , <i>Pediococcus acidilactici</i> Conditions: 37 °C for 72 h, SSF Product: Protein and starch enriched concentrates	- Increased water holding capacity (WHC) - Increased polyphenols - Decreased raffinose, stachyose and phytic acid concentrations and off-flavours	[33]
<i>Lens culinaris</i> (Lentil)		
Substrate: Lentil flour added to yogurt; flour addition rates of 0%, 1%, 2%, 3% and 4% Microorganism(s): <i>Streptococcus thermophilus</i> , <i>Lactobacillus dulbrueckii</i> subsp. <i>Bulgaricus</i> Conditions: 42–45 °C, fermented until pH 4.6 was reached Product: Lentil yogurt	- Increased syneresis, water holding capacity and viscosity - Increase resistance to changes in pH, and acidity - Increased nutritional profile, mineral content, and protein compared to the control yogurt made with buffalo milk	[173]
Substrate: Over night soaked and blended lentil grains Microorganism(s): <i>Lactobacillus</i> strains Conditions: Proteolytic pre-enzyme treatment, then fermentation at 37 °C for 48 h Product: Lentil beverage	- Reduced phytic acid and raffinose family oligosaccharides - Reduced proteins and peptides - Increased free amino acids slightly	[174]
<i>Lupinus</i> spp. (Lupin)		
Substrate: Hydrolyzed upin protein isolates Microorganism(s): <i>Lactobacillus sakei</i> ssp. <i>Carnosus</i> , <i>Lactobacillus amylolyticus</i> <i>Lactobacillus helveticus</i> Conditions: 37 °C or 42 °C for 24 h (depending on the strain) Product: Protein isolates	- Increased foaming activity - Increased protein solubility at acidic conditions - Decrease in size of large polypeptides forming lower molecule polypeptides - Balanced aroma and taste profile	[175]
Substrate: Pea protein suspension Microorganism(s): <i>Lactiplantibacillus plantarum</i> Conditions: 37 °C for 25 h Product: Pea protein hydrolysate	- Decreased water-soluble protein content with increased fermentation time - Reduced off-flavors and aldehydes and ketones - Improved aroma profile	[167]
<i>Vicia fava</i> (Fava bean)		
Substrate: Dough prepared by mixing 40 g fava bean flour with 60 mL distilled water Microorganism(s): <i>Leuconostoc</i> spp. and <i>Weissella</i> spp. Conditions: 30 °C for 24 h; Sucrose enriched or without sucrose (control) Product: Different clean label products	- Polymers produced by <i>Leuconostoc pseudomesenteroides</i> DSM 20193 showed the highest gelling and thickening capability - Decreased raffinose family oligosaccharides - Sucrose addition strongly induced the production of dextran and glucan	[176]

3.2.2. Effect of Fermentation on Techno-Functional Properties

Food matrix interactions are disrupted during fermentation, allowing nutrients and phytochemicals such as polyphenols to be released in more bioactive forms [37,122]. Concurrently, carbohydrates, fats, and proteins undergo several biochemical processes that alter their functional properties. It is these functional properties that determine the use of food ingredients in final products. As an example, legume ingredients that have increased solubility are suitable for preparing protein-based beverages [107] such as plant-based

milk, while legume ingredients that are good at water and oil absorption are suitable for meat-like products. There are several studies where fermentation has been investigated for modifying the techno-functional and other quality attributes of legume flours alone or mixed with other grains as presented in Tables 3 and 4.

Table 4. Effect of non-LAB fermentation of legumes on different food quality attributes.

Substrate Form and Characteristics (Substrate), Microorganism(s) Involved in Fermentation (Microorganism(s)), Fermentation Conditions (Conditions), and Product Type (Product)	Product Properties after Fermentation	References
<i>Cicer arietinum</i> (Chickpea)		
Substrate: Soak seeds with water overnight, decant water and sterilize; cool before fermentation Microorganism(s): <i>Cordyceps militaris</i> SN-18 Conditions: 25 °C for 7 days, SSF Product: Flour	- Increased water holding capacity (WHC), in vitro protein digestibility, fat absorption capacity, and emulsifying properties - Increased crude protein, and essential amino acid contents	[177]
Substrate form and characteristics: Soaked chickpeas Microorganism(s): <i>Bacillus subtilis</i> Fermentation conditions: 37 °C for 72 h, SSF, agitate every 6 h Product: Flour	- Increased small peptides (hydrophilic) with potential bio/functional properties - Decreased large peptides (hydrophobic) - After 48 h—Reported maximum value of protease activity, thus the increased release of peptides, soluble proteins, and free amino nitrogen	[178]
<i>Glycine max</i> (Soy bean)		
Substrate: Overnight soaked soybeans made into milk (soy:distilled water = 1:5) Microorganism(s): <i>Lycoperdon pyriforme</i> Conditions: 24 °C, for 28 h on a rotary shaker (150 rpm), dark conditions Product: Soy drink	- Reduced green, beany off flavor - Reduced hexanal, (E,E)-2,4-decadienal, and (E)-2-nonenal content - Changed color profile to the redder and bluer range, but hardly distinguishable by the human eye	[164]
<i>Pisum sativum</i> (Pea)		
Substrate: Pea protein enriched flour Microorganism(s): <i>Aspergillus oryzae</i> NRRL 5590, <i>Aspergillus niger</i> NRRL 334 Conditions: 40 °C for 6 h, SSF Product: Pea protein enriched flour	- Increased WHC and oil-binding capacity - Increased surface charge - Decreased surface hydrophobicity - Decreased solubility - Decreased zeta potential, and nitrogen solubility - Negative effects on foaming properties - Decreased lipid content Increased protein content	[179]
Substrate: 4% thermally treated pea protein isolate made with osmotic water and 3% sucrose Microorganism(s): LAB and yeast co-cultures Conditions: Start with an initial pH of 7 and stopped fermentation at pH 4.55 Product: Pea protein based product	- Reduced hexanal, butanal, and nonanal content - Significant reduction in the green/leguminous attributes - Increased production of esters	[165]
<i>Vigna unguiculata</i> (Black eyed pea)		
Substrate: Ground and soaked black eyed peas Microorganism(s): <i>Aspergillus oryzae</i> Conditions: 30 °C for 48, 72, and 96 h, SSF Product: Flour	- Increased WHC, oil binding capacity, foaming capacity, emulsifying activity, emulsion stability, and foam stability - Reduced bulk density	[180]

SSF: Solid State Fermentation, SMF: Submerged-state Fermentation.

The major storage carbohydrate in legumes is starch, which accounts for 40–50% of their total weight, followed by dietary fibre, oligosaccharides, and simple sugars [21,181]. During fermentation, carbohydrates are transformed into monosaccharides or disaccharides, ethanol and pyruvate by enzymes produced by the fermenting microorganisms. Legumes contain semicrystalline granules of starch that are composed of two principal polysaccharides: amylose and amylopectin [182]. They are polymers of α -D-glucoses that are linked together in two different configurations, with their structure influencing the gelatinisation, dextrinisation and swelling capacity [182]. Starch granule swelling capacity, one of the carbohydrate related functional property, is shown to be improved by

fermentation, as reported for chickpeas, pigeon peas and soybeans [40,183]. In contrast, a study involving fermented bambara flour and wheat for croissant production revealed a decrease in swelling capacity (0.17–0.4%) as the proportion of bambara flour in the mixture increased [184]. During fermentation, the decrease in swelling power of starch granules can be attributed to the hydrolysis of starch molecules into smaller molecules such as dextrin and oligosaccharides in the presence of organic acids and amylase. The swelling capacity of starch granules is dependent on various factors such as the substrate's original particle size, the proportion of α -amylose and amylopectin, and fermentation conditions, with starches containing higher levels of branched amylopectin having higher swelling capacity due to spatial bonding with more water molecules [185,186].

Legumes are renowned for their high protein content, which varies significantly across different species. For example, common beans contain 16.7 to 27.2 g/100 g protein, whereas cowpea's protein content ranges from 20.9 to 24.7 g/100 g [182,187]. During fermentation, the activity of microbial proteases hydrolyse legume proteins into peptides and free amino acids resulting in changes in protein properties such as hydration, surface activity (charge distribution and hydrophobicity) and protein structure (primary, secondary, tertiary and quaternary structure) [188]. LAB decomposes food proteins through proteolysis, which degrades proteins into oligopeptides. Afterward, dipeptides, tripeptides, and oligopeptides are formed and as a final step, these dipeptides, tripeptides, and oligopeptides are hydrolyzed into amino acids, which includes deamination and decarboxylation [168,189]. In this process, endopeptidases, aminopeptidases, dipeptidases, tripeptidases, and proline-specific peptidases produced by the fermentation microorganisms degrade the peptides [190].

Surface changes in protein could lead to the exposure of hydrophobic groups, cause a change in surface charge, and promote protein-protein interactions that influence techno-functional properties [191]. Functional properties such as solubility, aggregation, wettability, ability to form foams and emulsions, and rheological properties such as viscosity, elasticity, and adhesiveness are affected by changes in surface charge. For example, proteolysis of legume proteins by microbial proteases during fermentation changes the structure of the protein, exposing more hydrophilic sites, therefore allowing for more interactions between the protein and water [33,38,192]. As fermentation changes the protein structure, resulting in smaller nitrogenous molecules, it imparts better foaming stability with the effect on foaming capacity dependent on pH and duration of fermentation [33].

The effect of fermentation on the water-holding capacity of legumes is variable and influenced by protein content [193]. For instance, some research studies have reported an increase in the water binding capacity of chickpea-based sourdough after fermentation [33,177]. This was attributed to protein hydrolysis which makes more hydrophilic groups exposed [33]. The results were similar to the findings of another study conducted on chickpeas and pigeon peas, which found that microbial protease enzymes break down peptide linkages leading to increased water binding capacities [40]. In contrast, a decrease in water binding capacity after fermentation was reported with pigeon peas [194]. This may be explained by the microbial strains used in the fermentation process, the type of substrate, and the conditions under which the fermentation was conducted.

Several studies have shown that fermentation can enhance protein solubility in legumes. For example, the fermentation of lupin and pea protein isolates with *Lactobacillus* spp. has been found to increase protein solubility [175]. Other studies have reported similar findings on the fermentation of soybeans, lentils, and other legumes. This increased solubility is thought to be due to the breakdown of protein aggregates or complexes, as well as the production of proteases by the fermenting microorganisms [191]. However, depending on the pH of the food matrix, protein solubility may vary. This was observed for pea protein, whose solubility increased at pH 5 and decreased at pH 3 and 7, as discussed in an earlier section [191].

Fermentation is also accompanied by a change in the fatty acid profile, since fermenting organisms may produce lipases that catalyze the hydrolysis of lipids into free fatty acids.

Concomitantly, the degradation of proteins into low molecular weight peptides occurs. The peptides can migrate easily to the oil-water interface, increasing in emulsion stability and emulsifying capacity [40,167]. The emulsifying capacity and emulsion stability increased by 30–37% and 15–30% respectively, following fermentation by *Rhizopus oligosporus* of chickpeas, pigeon peas and soybeans [40]. This was attributed to protein hydrolysis, which leads to the exposure of hydrophilic and/or hydrophobic regions resulting in higher surface activity [40]. Fermentation of chickpea flour with *Cordyceps militaris* SN-18 revealed that proteolytic activity during fermentation unmasks hydrophilic and/or hydrophobic regions of proteins, resulting in a greater surface activity [177].

3.2.3. Effect of Fermentation on Nutritional Profile

Through fermentation, starch-hydrolyzing enzymes such as amylase and glucoamylase, which are secreted extracellularly by the fermenting microorganisms, degrade starch into monosaccharides and disaccharides [195]. As expected, the fermentation of legumes results in a degradation of carbohydrates while increasing the concentration of soluble carbohydrates that are more easily absorbed by the gut [43,196]. When chickpea was fermented with *Cordyceps militaris* SN-18 there was a 6.7% reduction in total carbohydrate content [177]. The secretion of microbial enzymes such as glucosidases and amylases hydrolyze carbohydrates in the substrate into monosaccharides, which are taken up by the microorganisms as a source of energy for the bioconversion of carbohydrates into microbial proteins [40,43].

Fermentation generally increases the concentration of amino acids due to the degradation of proteins as well as a microbial synthesis of amino acids and proteins. For example, protein content of pigeon peas, chickpeas and red beans was reported to be increased through fermentation due to the microbial synthesis of enzymes such as lipases and proteases [40]. A similar study on solid state fermentation of chickpeas reported an increment in true protein content (19.9%), crude protein content (19.4%) and ash content (6.15%) [177]. During fermentation, proteinase and peptidase activities of LAB may release peptides and free amino acids coupled with higher antioxidant capacity [174]. On the other hand, changes in protein structure and hydrolysis of proteins during fermentation can decrease protein content as reported for fava bean flour fermented with different LAB species *Weissella confusa* VTT E-143403 and *Leuconostoc pseudomesenteroides* DSM 20193) [197]. Similar results were observed in terms of the reduction of free amino acids when fermenting peas, lupins, and bean grains with *Lactobacillus* and *Limosilactobacillus* strains to produce fermented water extracts. In the study it was reported that those bacterial strains predominantly consumed the amino acids cysteine and tyrosine in the substrates [198].

Fermentation may also enhance the vitamin content and antioxidant capacity of legumes. Numerous studies suggest that fermenting legumes is an effective method for producing functional legume-based foods that are rich in antioxidants and vitamins [43,199]. For instance, fermentation of idli batter containing black gram and rice by *Saccharomyces boulardii* SAA655 and *Lactococcus lactis* N8 increased folate levels by 40–90%, demonstrating the ability of the starter microorganisms to synthesize B group vitamins [200]. Similarly, fermentation of lentil flour with *Aspergillus oryzae* increased the antioxidant activity by 107%, while fermentation with *Aspergillus niger* showed an increase of 81% [118].

Microorganisms also produce a range of polyunsaturated fatty acids (PUFA) such as arachidonic acid, docosahexaenoic acid, γ -linolenic acid, and eicosapentaenoic acid during legume fermentation [201]. For example, fermented desi chickpeas showed a 119% increase in PUFA content with mainly linoleic acid followed by α -linolenic acid [40]. These PUFAs have significant health benefits and are usually involved in the maintenance of cell membrane fluidity, inhibition of inflammatory processes and the reduction of triglyceride synthesis in the liver [202].

3.2.4. Effect of Fermentation on Antinutritional Factors (ANF) and Toxins

During microbial fermentations, ANF is transformed into complex macronutrients which are organoleptically and biochemically useful products (e.g., short-chain fatty acids, peptides, organic acids) as a result of enzymatic and non-enzymatic microbial reactions [71,72,122]. Fermentation with *Lactiplantibacillus plantarum* significantly reduced the tannins and phytate content of soybean, indicating that fermentation is a suitable strategy for reducing antinutrients [203].

Fermentation by LAB strains of chickpea sourdough reduced the concentration of α -galactosides, which can cause flatulence, bloating, and digestive discomfort. More specifically, raffinose and stachyose contents were reduced by 88.3–92.3%, and 97.7–99.1% respectively [33]. Similarly, a 17% reduction of phytic acid was observed in chickpeas sourdough fermented by *Pediococcus pentosaceus* and *Pediococcus acidilactici*. The stachyose and raffinose concentrations were reduced by 88.3–99.1% while verbascose amounts became undetectable [33]. Similar results were observed with chickpeas, kidney beans, peas, lentils and grass beans where tannin and raffinose levels decreased following fermentation with starter cultures consisting of *Lactobacillus brevis* and *Lactiplantibacillus plantarum* [204].

Some legumes (e.g., pigeon peas) contain cyanogenic glycosides (4.5 to 5.5 mg/g) which are converted to the toxin hydrogen cyanide through the activity of endogenous enzymes. This may result in cyanide poisoning after consumption [205]. Fermentation by bacteria such as *Lactiplantibacillus plantarum* is capable of degrading cyanogenic glycosides to non-harmful substances [206]. Moreover, during fermentation, microorganisms can produce various metabolites, such as organic acids and bacteriocins, which can inhibit the growth of undesirable microorganisms that may produce toxic compounds [207]. Figure 2 illustrates the mechanisms by which fermentation reduces ANFs in legumes.

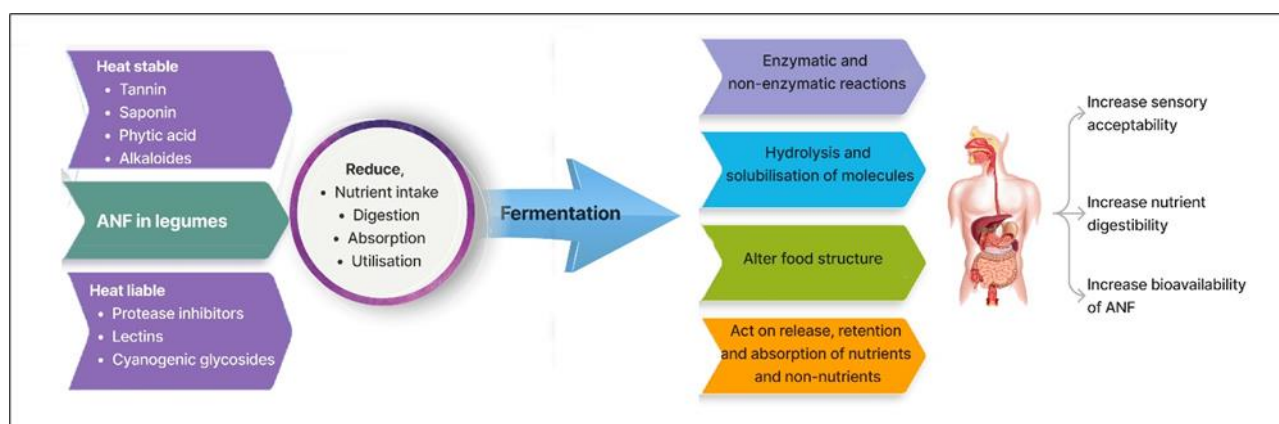


Figure 2. How fermentation reduces antinutritional factors associated with legumes, Sources: [22,122,203].

3.2.5. Effect of Fermentation on Allergenic Profile

Fermentation has been demonstrated to reduce or eliminate allergens with minimal detrimental outcomes. For instance, the allergenic sequences were hydrolyzed and the binding capacity of IgE was reduced upon fermentation of soybean meal with *Lactobacillus casei*, yeast, and *Bacillus subtilis* as fermenting organisms [208].

One of the main mechanisms of reduction of allergens during fermentation is enzymatic hydrolysis, where enzymes produced by microorganisms break down the allergenic proteins into smaller peptides and amino acids, rendering them less allergenic or non-allergenic [209]. In addition, microbial fermentation can alter the pH and redox potential of the food matrix, which can further contribute to the breakdown of allergenic proteins [210]. For instance, the acidic conditions produced during fermentation can cause conformational changes in the allergenic proteins, which can lead to their denaturation and subsequent loss of allergenicity [211].

4. Conclusions

Legumes play a vital role in many traditional diets throughout the world and have great potential as a future protein crop due to their high protein content and quality nutritional profile, as well as their low input farming requirements and low carbon footprint. Legumes exhibit a range of health benefits including the reduction of risk of cardiovascular disease, weight management, and the prevention of certain types of cancer. In addition, legume proteins offer effective solutions to allergies associated with animal protein products, such as lactose intolerance, red meat allergies, and milk protein allergies. However, there are several qualitative limitations associated with legumes, such as the presence of antinutritional factors, which limit the bioavailability of nutrients, characteristic off-flavors, such as beany flavor and unpleasant aroma, and inferior functional properties (low solubility, poor emulsification, lower water binding and oil binding capacities, poor foaming properties) compared to animal protein products. These characteristics of legumes hinder their application in a wide range of food formulations and applications. Because of these reasons, the full potential of legumes as a source of quality protein is yet to be realized, especially in the context of dietary habits shifting from animal proteins to alternative protein sources.

Microbial fermentation has been shown to be a promising approach for enhancing the sensory, functional, and nutritional properties of legumes. Fermentation can improve the texture, flavor, and aroma of legumes while reducing off-flavors and allergenic compounds. It can also increase the digestibility and bioavailability of nutrients in legumes, making them more nutritious. Moreover, fermentation can increase protein solubility, water holding capacity, and viscosity of legume products, enhancing their techno-functional properties. The use of selected microorganisms and optimization of fermentation conditions can further improve the quality of fermented legume products. Therefore, microbial fermentation holds great potential for the development of healthy, sustainable, and tasty legume-based food products.

Although fermentation is known to improve the functional, nutritional, and sensory properties of legumes, the results of different studies vary and are inconsistent depending on a variety of other factors. These include substrate characteristics (composition, state), fermentation conditions (temperature, length of fermentation, osmotic potential in the fermentation medium, pH), characteristics of starter culture (species, strain, inoculum concentration, use of single/mixed cultures), and fermentation method (solid-state fermentation/semi-solid-state fermentation/liquid state fermentation). Furthermore, most data are from laboratory scale studies, so there is little information about the scalability and commercial feasibility of these fermentation processes. Current legume fermentation processes at a commercial scale are limited to the manufacture of soy-based yogurt and similar products containing legume proteins, and fermented legume powders for condiments and nutraceutical applications. Further research and development effort needs to focus on the development of cost-effective and scalable fermentation processes for the fermentative transformation of various legume substrates to bulk functional ingredients amenable for use in a broad range of food products.

During the development of fermentation processes for legumes and any food substrates for that matter, the following aspects need to be considered.

1. The composition of the substrate including antinutritional factors
2. Selection of appropriate starter culture with the desired metabolic activity to achieve target functionality
3. Potential unintended consequences on other functional attributes
4. The scalability and the cost of the process

The development in omics including genomics, transcriptomics, proteomics and metabolomics in recent times is making the selection of starter cultures rationalized enabling selection based on target functions. Advances in high throughput analytic techniques such as LC-MS and GC-MS are improving our understanding of the compositional profile of

food substrates and the complex metabolic changes that occur during both traditional spontaneous and modern fermentation processes with defined starter cultures. Further studies need to make use of these advanced tools for rational design and optimization of fermentation processes with predictable outcomes for the transformation of legumes into functional food ingredients with desirable sensory, techno-functional and nutritional attributes.

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