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*Review*

# Research Progress of Fermented Functional Foods and Protein Factory-Microbial Fermentation Technology

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**Abstract:** Fermentation has been used for ages as a safe technique for food preservation, and it uses minimal resources. Fermentation is related to a wide range of catabolic biochemical procedures in both eukaryotes and prokaryotes. Yeasts are eukaryotes; they can use oxygen while also having the ability to live without oxygen. The lactate fermentation process consists of glycolysis and some alternative steps. A review of the literature was done using keywords in main indexing systems, including PubMed/MEDLINE, Scopus, the search engine of the Institute for Scientific Web of Science and Google Scholar. The keywords reviewed were fermentation technologies, protein mass expression, health benefits of functional foods, microbial fermentation technology, anaerobic respiration, fermentation in eukaryotes, fermentation in prokaryotes, solid state fermentation and submerged fermentation. This research was carried out to highlight the importance of fermentation technology and to introduce and survey the technology and its relationship with functional foods. Research progress in the area of protein factory-microbial fermentation technology was also investigated and inspected.

**Keywords:** fermentation; protein mass expression; microbial fermentation technology; solid state fermentation; submerged fermentation

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## 1. Introduction

Fermentation is one of the earliest biotechnological methods of food preservation and processing to be extensively applied in the world [1]: foods (fermented food, food additives, functional materials and live probiotics); intestines (aids digestion and promotes absorption, synthetic bioactive substances, inhibits harmful bacteria, diabetes, cardiopathy and allergy); and industry (energy, soil transformation and sewage treatment) [1]. The current trends in fermented-based vegetable foods are growing and will likely continue into the next decade [2–7].

The first documents to report on fermented foods date back to 13,000 BC and are primarily mediated by spontaneous fermentation by autochthonous microorganisms in raw material [8]. Fermentation is defined as the chemical transformation of any organic matter through microbial metabolism and is mediated by myriad enzymes [1]. The key advantages of engineering microbial fermentation over multicellular (higher eukaryotic) tissue culture are threefold: less fastidious growth requirements; significantly faster growth cycles; and less ethical controversy and market resistance in biomedical and food applications [9–16]. Anaerobic fermentation can produce important chemicals from food waste, such as lactic acid, butyric acid and ethanol [2]. Bacterial and fungal communities are altered during sea bass fermentation, and changes in bacteria and fungi lead to differential metabolite production [17–19]. Diversity also relates to the choice of the fermentation substrates, which consist of maize, wheat, sorghum, millet and teff, and to the fermentation processes that are applied in food production [20]. It has been reported that the

production of fermented foods is based on the use of starter cultures, for instance lactic bacteria that launch rapid acidification of the raw material [21]. Organisms applied in solid state fermentation (SSF) are: (1) the microbiological parameters of SSF can be found as pure, single species, mixed distinct cultures or as totally diverse indigenous microorganisms; (2) SSF processes, e.g., tempeh, tempeh and ontjom production, may need the specific growth of organisms, like molds, which demands low levels of moisture to perform fermentation utilizing extracellular enzymes produced by microorganisms that ferment; and (3) however, bacteria and yeasts, which require more moisture for effective fermentation, can be utilized to create SSF, however, with lower yield. Solid state fermentation steps are: (1) pre-treatment of substrate raw materials either by mechanical, chemical or biochemical processing to boost the availability of the bound nutrients; (2) hydrolysis of primarily polymeric substrates, e.g., polysaccharides and proteins; (3) hydrolysis products; and (4) separation and purification of end products [22–25]. The most important benefits of SSF are products produced in high volume, higher productivity level of products, higher stability of products, absence of catabolic repression, tolerance to high substrate concentration, natural complex raw materials often provide a complete medium, absence of rigorous control of the fermentation process, easier aeration and low water demand [26–33]. The downsides of solid state fermentation are: (1) the production of heat; (2) monitoring in detail on SSF (e.g., $CO_2$ and $O_2$ levels and moisture content) is not possible; (3) the microorganisms, which can tolerate moderate moisture levels, are able to be applied; and (4) the organisms are slow to grow, which leads to significant restriction in the production of new products [34–37]. The major functions of bioreactor for SSF are: (1) to contain the substrate; (2) to contain the process microorganism; (3) to protect the process microorganism against contamination; and (4) to control environmental conditions to optimize growth and product formation [38–41]. The most important health benefits of fermented foods are shown in Figure 1.

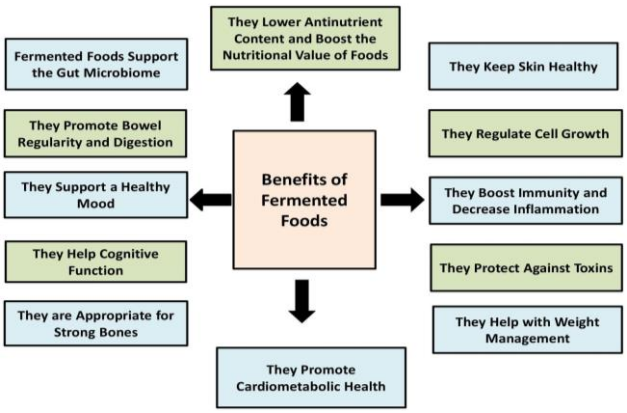


**Figure 1.** The most notable health benefits of fermented foods.

Various bioreactor kinds are: (1) Bench Scale Petri dish Erlenmeyer flask; (2) Tray Bioreactor; (3) Packed-bed Bioreactor; (4) Zymotis Bioreactor; (5) Rotating-drum Bioreactor; (6) Fluidized-bed Bioreactor; and (7) Spouted-bed Bioreactor [42,43]. Submerged fermentation has been illustrated as fermentation in the presence of excess water [44–47]. The aim of industrial-scale submerged fermentation techniques is to get a pure product with a high concentration, which is obtained by regulating oxygen, pH, temperature and other measurable and variable elements at optimal levels [48–52]. Lactic acid fermentation is applied to produce foods that cannot be produced through other methodologies, and the most commercially important genus of lactic acid-fermentation bacteria is *Lactobacillus* [53]. Alcoholic fermentation is the best known of the fermentation techniques and is involved in several important transformation, stabilization and conservation procedures for sugar-rich substrates, such as fruit and vegetable and fruit juices, and alcoholic fermenta-

tion is carried out by yeasts and some other bacteria and fungi [54–56]. In alcoholic fermentation, where yeast transforms glucose and fructose in grape juice to mainly ethanol, $CO_2$ and heat, a wide range of other compounds are also being produced [57–59]. Yeast is the most important part of the brewing fermentation procedure, and it converts sugar to alcohol, carbon dioxide and other constituents that influence the flavor and aroma of beer [60,61]. Yeasts are capable of facing the stresses of the gastrointestinal tract, such as bile salts, enzymes, organic acids and considerable changes of pH and temperature [62–64]. Yeasts can also produce nutraceuticals for the development of functional foods and for protection from cardiovascular disease [65–69]. The advantages of solid state fermentation are presented in Figure 2.

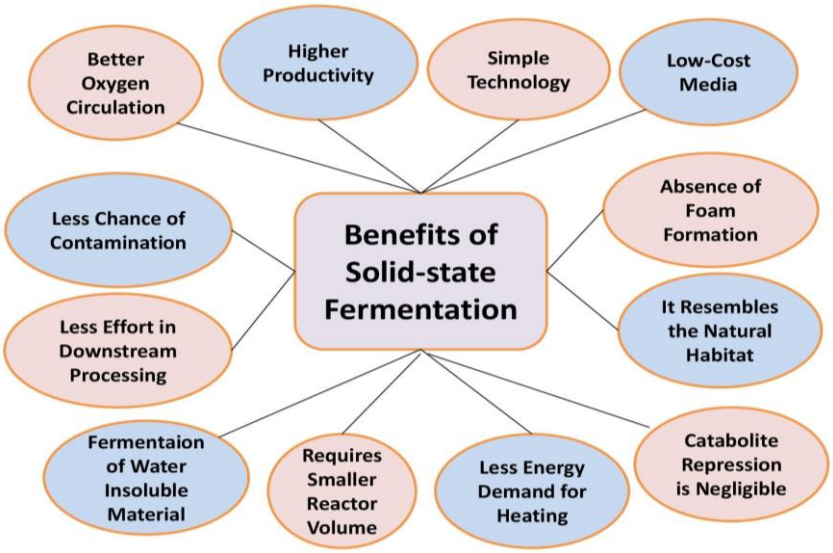


**Figure 2.** The benefits of Solid-state fermentation.

The most common fermentations are alcohol fermentation, lactic acid (homofermentation), lactic acid (heterofermentation), butyric acid, mixed acid, propionic acid and acetic acid [70,71]. Yeasts are involved in alcohol fermentation; lactic acid bacteria (*Lactobacillus* spp. etc), *Clostridium* spp., *Butyrivibrio* spp., *Bacillus* spp. and other anaerobes are involved in butyric acid; Enterobacteriaceae (*Escherichia* spp., *Enterobacter* spp., *Salmonella* spp., *Klebsiella* spp., *Shigella* spp. etc.) are involved in mixed acid; *Propionibacterium* spp., *Veillonella* spp., *Bacteroides* spp., some *Clostridia* spp., are involved in Propionic acid; *Acetobacter* spp., *Gluconobacter* spp., and *Bacillus subtilis* are involved in acetic acid [71,72]. Alcohol fermentation has been used for wine, beer and sourdough; lactic acid (homofermentation and heterofermentation) has been applied for dairy products, fermented meats and fermented vegetables, etc.; butyric acid has been used for marsh sediments and sewage systems; mixed acid has been applied for the human and animal digestive tract and for fresh water; propionic acid has been utilized for dairy products; and acetic acid has been used for the acetic acid industry [71]. Bacterial diversity (Genus) in fermentation pathways are *Acetobacter*, *Gluconacetobacter*, *Halomonas*, *Hafnia*, *Tatumella*, *Zymomonas*, *Brachybacterium*, *Microbacterium*, *Brevibacterium*, *Corynebacterium*, *Micrococcus*, *Kocuria*, *Arthrobacter*, *Streptomyces*, *Propionibacterium*, *Bifidobacterium*, *Bacillus*, *Gemella*, *Jeotgalicoccus*, *Enterococcus*, *Carnobacterium*, *Tetragenococcus*, *Vagococcus*, *Weissela*, *Leuconostoc*, *Oenococcus*, *Lactococcus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus* and *Pediococcus* [71]. The polyphasic technique applied to evaluate microbial content and dynamics in fermented foods are Denaturing gradient gel electrophoresis (thermal gradient gel electrophoresis) (DGGR) (or TGGE) + plating (for evaluation of the compositional pattern of the dominant populations); Fluorescence in situ hybridization (FISH) (or Direct epifluorescence technique (DEFT)) + plat-

ing (for quantification of non-cultivable populations); DGGE + Reverse transcriptase-polymerase chain reaction (RT-PCR); rRNA quantitative hybridization (for semi-quantification of metabolically active groups); Analysis of sugars and fermentation end products accumulation (for evaluation and monitoring of the fermentation process); Quantitative-polymerase chain reaction (q-PCR), Competitive-polymerase chain reaction (c-PCR) Flow cytometry (for detection and quantification of non-dominant species/strains, evaluation of the physiological status (viability), stress response and survival of LAB starters and probiotics in foods); Microarray-based rRNA detection without amplification (for semi quantification and dynamics of both dominant and non-dominant microbial populations) [73]. Various categories of fermentation, according to the end product formed, are presented in Figure 3.

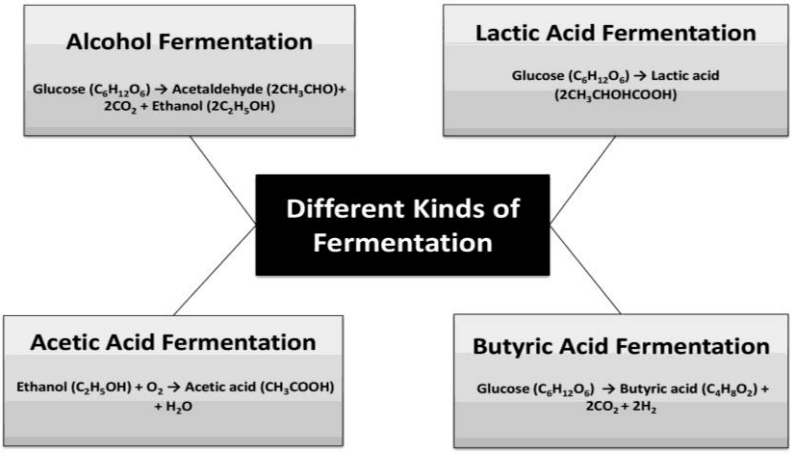


**Figure 3.** Different types of Fermentation on the basis of the end product formed.

The biosynthetic pathway of lactic is sugars being converted into lactic acid; the biosynthetic pathway of acetic is several substrates being converted into acetic acid; the biosynthetic pathway of alcoholic is sugars being converted to alcohols and $CO_2$; and the biosynthetic pathway of alkali is proteins being converted into amino acids, peptides and ammonia [74]. Solid state fermentation is a microbial fermentation process through which selected microorganisms (bacteria, fungi and yeasts) are cultivated on a moist, solid, non-soluble organic material [75,76] in the absence or near absence of free-flowing water, and the enzymes produced from agro-industrial wastes with solid state fermentation using microbial biotechnology are $\alpha$-amylse, $\alpha$-Galactosidase, β-fructofuranosidase, Cellulase, Gellulase, Glucoamylase, Inulinase, Lipase, Pectinase, Pectin esterase, Protease, β-amylase, Fibrinolytic enzyme and Laccase [77]. The goals of the manuscript are both to introduce and survey the fermentation technology and its relationship with functional foods and to investigate and inspect different research progress in protein factory-microbial fermentation technology.

## 2. Materials and Methods

The manuscript contains review articles, randomized control experiments, observations and analytical studies that have been gathered from different sources, such as Scopus, Science Direct, PubMed and Google Scholar. A review of the literature was carried out using the following keywords: fermentation technologies, protein mass expression, health benefits of functional foods, microbial fermentation technology, solid state fermentation and submerged fermentation.

## 3. Fermentation

Fermentation has been used for ages as a safe technique for food preservation, and it uses minimal resources [2]. Some essential commercial enzymes used in fermented

foods/beverages are: Protease, Catalase and Lactase for dairy; Amylase, Protease, Glucose oxidase, Pentosanase, Phytase, Pullulanase, Xylanase, Lipases, B-glucanase, Amyloglucosidase, A-acetolactate-decarboxylase, Cellulase and Pectinase for cereals; Glucose oxidase and Tannase for beverages; and Papain and Protease for meat [78–81]. Microorganisms involved in the fermentation of dairy products are *Lactobacillus bulgaricus*, *Lactococcus lactis*, *L. acidophilus*, *L. cremoris*, *L. thermophilus*, *L. casei*, *L. paracasei*, *L. kefiri*, *L. caucasicus*, *Penicillium camembreti*, *Acetobacter lovaniensis*, *P. roqueforti*, *Kluyveromyces lactis* and *Saccharomyces cerevisiae* [80]. Microorganisms involved in the fermentation of vegetable products are *Leuconostoc mesenteroides*, *Aspergillus* sp., *Rhizopus oligosporus*, *R. oryzae*, *L. sakei*, *L. plantarum*, *Thermotoga* sp., *L. hokkaidonensis*, *L. rhamnosus*, *Rhodotorula rubra*, *Leuconostoc carnosum*, *Bifidobacterium dentium*, *Weissella confusa*, *Enterococcus faecalis* and *Candida sake* [80].

Microorganisms involved in the fermentation of cereals are L. pantheris, L. plantarum, Penicillium sp., S. cerevisiae, L. mesenteroides, E. faecalis, Trichosporon pullulans, Pediococcus acidilactici, P. cerevisiae, Delbrueckii hansenii and Deb. tamari [80]. Microorganisms involved in the fermentation of beverages are Aspergillus oryzae, Zygosaccharomyces bailii, S. cerevisiae, Acetobacter pasteurianus, Acetobacter xylinus, Gluconacetobacter and Komagataeibacter xylinus [80]. Microorganisms involved in the fermentation of meat products are L. sakei, L. curvatus, L. plantarum, Leuconostoc carnosum, Leuconostoc gelidium, B. licheniformis, E. durans, E. faecalis, E. hirae, Bacillus subtilis, L. divergens, L. carnis, E. cecorum and B. lentus [80,82]. Fermentation can increase phenolic content and antioxidant capacity in the majority of foods [83]. The microorganisms used in the production of fermented foods and beverages include bacteria (e.g., lactic acid bacteria (LAB) such as Lactobacillus, Enterococcus, Streptococcus, Lactococcus and Bifidobacterium); molds (e.g., Aspergillus sojae, Penicillium roqueforti, Aspergillus oryzae and Penicillium chrysogenum); and yeasts (e.g., Saccharomyces cerevisiae, Candida krusei and Candida humilis) [84,85].

Some of the most important samples of global fermented food products are grain-based; vegetable-based; fruit-based; fish-based; honey-based; dairy-based; meat-based; rice-, maize- and barley-based; soy-based; and tea-based [85]. The compounds generated during fermentation are volatile compounds, carbonyl compounds, alcohols, acids, esters, sulfur compounds, amino acids, lactones, peptides and fatty acids [85,86]. A nonexhaustive list of recent studies on the development of fermentation-enabled wellness foods are an addition to a microbial generation of bioactive compounds, probiotic microbes, additional of nonmicrobial ingredients and removal of undesired compounds [86]. Lactic acid fermentation boosts the functional traits of fruit beverages, and nanotechnology is an innovative technique for the design of new fermented beverages [87]. During food fermentation, insoluble biomass polysaccharides, such as amylose, cellulose and pectin, can first be degraded by various CAZyme-producing microbiotas to form products with better availability, for example, oligosaccharides, soluble polysaccharides and monosaccharides [88].

Fermented foods are a solution to health problems associated to the modern diet [89]. Both botanical source and particle size affected in vitro fermentation outcomes and fermentability associated to both particle nature and chemical composition [90]. Fermentation can facilitate the extraction of bioactive constituents from seaweeds, and products of seaweed fermentation indicated improved bioactive and sensory profiles [91]. Lactic acid (LA), a versatile platform molecule, can be fermented from organic wastes, such as food waste and waste-activated sludge [92,93]. The yield of lactic acid was enhanced via the addition of copper [94]. Lactic acid bacteria (LAB) starters are an important parameter contributing to fermented food quality [95–98]. Some of the most important health advantages of lactic acid bacteria fermentates are improved gluten-associated disorders; modulate mucosal immune system and improved gut disorders; elicited reduced influx of gliadin peptides into cells; induced mucosal immune system; reduced severity of an

infection in the offspring of lactating mice; excluded pathogens ameliorated enteral nutrition; and effective use against several pathogens, including *Salmonella typhimurium*, *Listeria monocytogenes* and *Cronobacter sakazakii* [99]. Archaea are prokaryotic organisms dissimilar from bacteria in the structural and molecular biological sense, and these microorganisms are known to thrive mainly at extreme environments [100]. Numerous commercial enzymes applied in fermentation procedures for foods and beverages are presented in Table 1.

**Table 1.** Commercial enzymes applied in different fermentation processes.

| Types | Commercial Enzymes Used in Fermentation Process | References |
|---|---|---|
| Dairy Products | *Lactobacillus bulgaricus* | [80] |
| | *Lactococcus lactis* | [80] |
| | *L. acidophilus* | [80] |
| | *L. cremoris* | [80] |
| | *L. thermophilus* | [80] |
| | *L. casei* | [80] |
| | *L. paracasei* | [80] |
| | *L. kefiri* | [80] |
| | *L. caucasicus* | [80] |
| | *Penicillium camembreti* | [80] |
| | *Acetobacter lovaniensis* | [80] |
| | *P. roqueforti* | [80] |
| | *Kluyveromyces lactis* | [80] |
| | *Saccharomyces cerevisiae* | [80] |
| Cereals | *L. pantheris* | [80] |
| | *L. plantarum* | [80] |
| | *Penicillium sp.* | [80] |
| | *S. cerevisiae* | [80] |
| | *L. mesenteroides* | [80] |
| | *E. faecalis* | [80] |
| | *Trichosporon pullulans* | [80] |
| | *Pediococcus acidilactici* | [80] |
| | *P. cerevisiae* | [80] |
| | *Delbrueckii hansenii* | [80] |
| | *Deb. tamari* | [80] |
| Beverages | *Aspergillus oryzae* | [80] |
| | *Zygosaccharomyces bailii* | [80] |
| | *S. cerevisiae* | [80] |
| | *Acetobacter pasteurianus* | [80] |
| | *Acetobacter xylinus* | [80] |
| | *Gluconacetobacter* | [80] |
| | *Komagataeibacter xylinus* | [80] |
| Meat products | *L. sakei* | [80,82] |
| | *L. curvatus* | [80,82] |
| | *L. plantarum* | [80,82] |
| | *Leuconostoc carnosum* | [80,82] |
| | *Leuconostoc gelidium* | [80,82] |
| | *B. licheniformis* | [80,82] |
| | *E. durans* | [80,82] |
| | *E. hirae* | [80,82] |
| | *Bacillus subtilis* | [80,82] |

| | |
|---|---|
| *L. divergens* | [80,82] |
| *L. carnis* | [80,82] |
| *E. cecorum* | [80,82] |
| *B. lentus* | [80,82] |
| *E. faecalis* | [80,82] |

## 4. Functional Foods

During the fermentation of cruciferous vegetables, complete fermentation of glucosinolates occurs; fermentation decreases the content of complex polyphenols while improving the content of polyphenols in free form, and carotenoid constituents decrease during the fermentation of cruciferous vegetables [101,102]. Carbohydrate was the main substrate for lactic acid fermentation [103]. Fermented broccoli stalk provided a functional novel product, and the *Latilactobacillus sakei* subsp. *carnosus* is in the principal LAB in fermented broccoli stalk; furthermore, mustard dressing increases the nutritional value by its high content in phenolic compounds [103]. *Lactobacillus casei* PRA205 overcame *Lactobacillus rhamnosus* PRA331 in viability during yogurt refrigeration; yogurt with PRA205 had notable effect on angiotensin-converting enzyme (ACE) inhibitory antioxidant activities; and yogurt with PRA205 contains tri-peptides Valine–Proline–Proline (VPP) and Isoleucine–Proline–Proline (IPP) during refrigeration [104]. *Lactobacillus plantarum* LUHS135 and *Lactobacillus paracasei* LUHS244 from fermented cereals ferment a broad spectrum of carbohydrates and restrain pathogenic bacteria, and both are good candidates for the reduction of mycotoxins [105].

The phenolics and antioxidant activity were increased in fermented *Artemisia argyi* tea, and the fermentation process decreased the anti-lipoxygenase activity of *Artemisia argyi* tea [106]. Fermentation type, time and the blanching operation increased the food application of Cardaba banana [107]. Fermentation of aqueous extracts of chickpea flour with lactic acid bacteria can be targeted strategically to boost antioxidant and anti-hyperglycemic relevant functional qualities in select chickpea varieties [108]. *Lactiplantibacillus plantarum* WLPL01 fermentation improved the bioactive compounds of *Artemisia argyi* [109]. *Lactiplantibacillus plantarum* X7021 is a novel strain originated from the brine of stinky tofu, and it is a prospect starter candidate for the fermentation of plant foodstuffs [110]. The entire fermentation process of traditional Chinese broad bean paste with chili comprises three individual stages: *Tianbanzi*, chili *pei* and paste fermentation (*Tianbanzi*-chili *pei* mixture), and three (*Lactobacillus*, *Tetragenococcus* and *Pseudomonas*), four (*Tetragenococcus*, *Bacillus*, *Lactobacillus* and *Pseudomonas*) and five (*Tetragenococcus*, *Lactobacillus*, *Pseudomonas*, *Bacillus* and *Pediococcus*) genera are considered the core functional bacteria of *Tianbanzi*, chili *pei* and paste fermentation, respectively [111]. Cocoa fermentation is a traditional, spontaneous, on-farm process, chiefly carried out by yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB); however, cocoa fermentation processes inoculated with a *Saccharomyces cerevisiae* strain, increased flavor production during the fermentation and drying steps, which was reflected in richer and more reproductive aroma profiles of the cocoa liquors and chocolates [112].

*Bacillus amyloliquefaciens* produced a new fermented soybean food with needed red color and multiple bioactivities, which provided a simple and new technique for enhancing the functionality of soybean, and fermented soybean is plentiful in nutrients and bioactive ingredients [113]. It has been reported that the optimum fermentation condition for pearl millet flour was obtained with 2% baker's yeast at 30 °C for 18 h, and fermentation at this condition significantly increased the phytochemical constituents of pearl millet flour [114]. Fermented locust bean is a principal, culinary preparation used to increase flavor and meatiness of sauces, soups and other food products, and conventional fermentation procedure is often based on natural contamination; however, *Bacillus subtilis* are the key bacterial strains involved [115]. *Lactobacillus kefri* fermentation components (LAF) in-

dicate excellent antioxidant effect *in vitro*, and LAF can regulate the expression of oxidative stress, autophagy and aging-related genes in damaged cells treated with $H_2O_2$, thereby delaying cell senescence [116]. Brewer's spent grain (BSG) fermentation boosted its soluble sugars greatly, and ultrasonic pretreatment was beneficial to protease secretion but not to cellulase [117]. *Bacillus velezensis* improved *Levilactobacillus brevis* growth during BSG fermentation, and hydrolase activities improved to make more soluble sugars but not protein [118].

Fermentation can increase the anti-inflammatory impacts of cattle bile, and cattle bile has an anti-inflammatory impact by inhibiting the NLRP3 inflammasome pathway, which can expand the clinical application of cattle bile and develop novel concepts and methodologies for the utilization of cattle bile [119]. Co-fermentation of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* influenced their growth kinetics; co-fermentation varied the acidity and bitterness of cider and improved the intensity and diversity of the aroma of cider [120]. *Lactobacillus*, *Acetobacter* and *Clostridium* were the major bacterial genera in *Hongqu* aromatic vinegar (HAV) fermentation, and *Alternaria*, *Aspergillus, Candida* and *Issatchenkia* were the principal fungal genera in HAV fermentation [121]. *Bacillus amyloliquefaciens* 20,029 revealed better fermentation performance upon sonication, and sonication caused impacts of different degrees to varying fermentation substrates [121]. *Saccharomyces cerevisiae* does not always dominate sequential fermentations with *Torulaspora delbrueckii*; adding micronutrients increases sequential fermentations after removing *T. delbrueckii*; and thiamine, zinc and amino acids are important for *S. cerevisiae* after *T. delbrueckii* [122]. Fermentation significantly altered the structural characteristics of polysaccharides in longan vinegar or wine, and the polysaccharide in longan vinegar had higher immunomodulatory activity [123]. The application of ultrasound treatment intensified the fermentation process of soya press cake (SPC); sonicated and fermented SPC additive influenced bread properties [124].

The antibacterial constituents were initially separated from the fermentation of *Paecilomyces cicadae*, and the fermentation compounds could bind with cellular DNA and influenced the expression or related genes [125]. A new *biopesticide Trichoderma* Brev T069 was produced using cassava peels substrate; a new fermentation bioreactor system was improved to produce 250 kg of biopesticide; and biopesticide *T. Brev* T069 had a 64.65% biocontrol efficiency on banana fusarium wilt [126]. Products of pea protein flour fermentation were evaluated using solid phase microextraction (SPME)-gas chromatography mass spectrometry (GCMS), and fermentation of pea protein enriched flour (PP) by *Lactobacillus plantarum* also resulted in the formation of hexamine, which is a known food preservation agent [127]. Cocoa bean fermentation determines the formation of flavor constituents and bioactive peptides, and cocoa bean peptides are released by autolysis, hydrolysis with exogenous enzymes and fermentation [128]. It has been suggested that the yeast fermentation extraction of *Lycium barbarum* polysaccharide (LBP) produces better antioxidant and anti-aging impacts than those obtained with the traditional hot water extraction, which is more appropriate for obtaining raw materials with anti-aging functions that can potentially be applied in the food and cosmetic industries [129]. Both microbial fermentation and plant ingredients are the sources of the constituents that contribute most significantly to Sichuan paocai aroma, flavor and taste, and the cysteine and methionine metabolism aspartate and the glutamate biosynthesis and metabolism and pyruvate metabolism were responsible for the major flavors formation [130]. Organic matter type and abundance increased in the combined fermentation liquid, and *Methanosarcina* were enriched in the integrated fermentation liquid [131]. *Bacillus clausii*-fermented spent coffee grounds (SCG) improved protein hydrolysates content, and peptides with potential biological activity were accelerated in *B. clausii*-fermented SCG [132].

Alcoholic fermentation with *S. cerevisiae* is a promising methodology to mitigate mycotoxin without the magnetic field (MF) application, indicated by the altered profile of the yeast-synthesized oxireductant molecules [133]. The fermentation of cereal vinegar by manual operation (MO) and mechanical operation (TO) were compared, and the more

productive and heat transfer were accountable for the higher metabolic activity in TO than in MO; acetic acid bacterial and lactic acid bacterial are mainly responsible for those differences [134]. Lactic acid bacteria were used to influence the sensory quality of lupine production, and fermentations improved sourness and vinegar odor in the samples; fermentations increased lactic acid and volatile acetic and hexanoic acid constituents, and it reduced the contents of hexanal, a candidate for green note in lupine (*Lupinus angustifolius* L.) [135]. Lactic acid bacteria dominated sorghum and corn kernels silage fermentation; amylases and a longer storage decreased the starch content and increased dry matter loss [136].

Fermentation of browning pear juice by *Lactobacillus acidophilus* CH-2 boosted the color, and *Lactobacillus acidophilus* CH-2 played a function of detoxication by glutathione metabolism and related metabolism [137]. Fecal fermentation of raspberry-enriched ileal fluid improves phenolic content; raspberry ileal fluid fermentates; phenolic acids decrease DNA damage in colonocytes; and the cytoprotective Nrf2-ARE pathway, stimulated by ileal fluid, fermentates and yields phenolic acids [138]. It has been reported that the upregulated glycolysis, fermentation, TCA and energy metabolism might stimulate sucrose decomposition and thus sugar recession of longan pulp [139]. The fermentation products played an important function in the antidiabetic effect of fermented bread with sourdough (FMP) [140]. Fermentation profile connects with metabolic profile; esters, peptides and tributyrin may affect the growth of *Lactococcus lactis* strains [141]. Inoculated fermentation affected bacterial diversity and metabolites [142]. A solid state fermentation with *Cordyceps militaris* was utilized to produce fermented strawberry, and the fermented strawberry might be applied as a functional and nutraceutical food to give anti-adipogenesis activity [143].

## 5. Health Promotion and Fermentation

Fermentation is the second oldest known methodology applied for food preservation after drying and is used for food processing a vast array of meats as fish, dairy, cereal grains, legumes, vegetables and fruits, as well as by-products of each of these groups [144]. Fermented foods consist of beneficial microbes, especially lactic acid bacteria, some strains of which may be potential probiotics and which, in general, are responsible for flavor, texture and appropriate preservation of fermented foods [145]. Top 5 health advantages of fermenting are: (1) source of beneficial lactic acid bacteria; (2) easier to digest; (3) increases the availability of nutrients; (4) may boost mood and behavior; and (5) may support heart health [146,147]. Health-promoting activities of fermented milks are anti-hypertensive activity, antioxidant activity, increase of vitamin content, alleviation of lactose intolerance, improvement of protein digestibility and probiotic activity [148,149]. Health-promoting activities in fermented, grain-based products are antioxidant activity, vitamin content, anti-hypertensive activity, anti-diabetic properties and protein hydrolysis [149].

Health-promoting activities in fermented vegetables and fruit are antioxidant activity, protein hydrolysis and vitamin content, and health-promoting activity in fermented meat and fish is antioxidant activity [149]. Fermentation is a traditional preservation methodology that also boosts the sensorial and health benefits of fruits and vegetables by the enzymatic machinery of the microorganisms, creating a high added-value product. Improved antioxidant capacity, production of health-related metabolites, incorporation of probiotic properties and vitamin production are some of the benefits found in fermented vegetables and fruits beverages. Health benefits of food-associated *Lpb*. *plantarum* strains are antimicrobial activity, antimutagenic activity, antigenotoxic and bile salt hydrolase activity, antioxidant properties and immune modulation [150]. Gallic acid (GA)–g–carboxymethyl chitosan (CMCS) fermentation stimulated the production of short-chain fatty acids, and the digestive stability and prebiotic impacts of CMCS were improved by grafting with GA [151]. Vanillic acid was the most bioaccessible constituent in tomato and husk tomato, and 3-Hydroxyphenylacetic acid was the main metabolite found after 48-hour fermentation [152]. *Klebsiella*, *Paenibacillus*, *Pantoea*, *Cohnella*, *Aspergillus* and *Cyberlindnera*

were major microbial genera in Qingzhuan tea (QZR) fermentation; most of the bacteria and fungi in QZT fermentation were synergistic rather than antagonistic, and *Aspergillus* genus promoted to the aroma quality of QZT through pile fermentation. Grzelak-Blaszczyk et al. [153] reported that the fermentation efficiency was associated with the bacterial strain, cultivar and pre-treatment method. *Lactobacillus plantarum* DMS 20,174 of ASF could change its structure to increase the bioactive properties, implying the probable application of ferment *Asparagus sprengeri* fructan (F-ASF) in the medical field [154].

It has been found that the low temperature (37°C) most likely promotes more to the formation of important flavor constituents during the fermentation process and production of short-chain fatty acids during storage [155]. Fermentation of wheat and rice bran increases water quality and growth performance in juveniles in the biofloc system; digestive enzymes activity and body composition were altered by fermented and non-fermented treatments; and fermented treatments increased intestine mucosal layer characteristics [156]. Solid state fermentation of by-products to upgrade their nutritional value is suggested [157]. Ultrasound treatment during the lag phase of lactic acid bacteria shortened the fermentation time, and ultrasound stimulated the rate of lactose hydrolysis by up to 49.2% [158]. Onion cell walls (OCW) composition and architecture each influence both fermentation and microbiota shifts [159]. Fermentation could be a promising access to improve jujube phenolic quality [160]. Fermentation time may increase physicochemical properties and antioxidant activity of barley β-glucan [161]. *Cyprinus carpio* XMX-1 stabilized fermentation product improved the integrity of the gut barrier of common carp and overall gut health, and it can sustain the fermentation product of improved overall liver health of carp [162].

Mixed fermented blueberry pomace by probiotics increases the content of the total phenols, flavonoids and inoxidizability; it has significant impact on cholesterol clearance; and it shows great influence on anti-fatigue capability on weight-loaded swimming in mice [163]. *Lactobacillus plantarum* POM1 and C2 were suitable starters for pomegranate juice fermentation; lactic acid fermentation improved the concentration of ellagic acid; and the fermented pomegranate juice increased health-promoting sensory and preservative features [164].

## 6. The Method of Protein Mass Expression

Fermentation now spans industrial chemistry, therapeutics, biomaterials, medicine, fuels and advanced food components. The suite of tools developed through fermentation's evolution is now poised to revolutionize the food section by escalating the rise of substitute proteins. The advances in the fermentation industry are biomass, traditional and precision; the organisms in the fermentation industry are fungal mycelium, east+single-cell fungi, micro-algae, bacteria and Protists+other microbes; and the feedstocks in the fermentation industry are Agro-industrial side streams, sugar, $CO_2$ or methane, food waste, sunlight, wood and other biomass. Different services in the fermentation industry are bioprocess, host strain development, purification and target molecules; the production methods in the fermentation industry are liquid state and solid state; the product types in the fermentation industry are seafood, tempeh+novel categories, ground meats, whole-cut meats, collagen+gelatin, milk+cheese, fats+oils, functional ingredients and egg whites; and the business strategies in the fermentation industry are B2C end products, B2B ingredients, B2C ingredients, mixed+hybrid and B2B equipment+services. The protein industry utilizes fermentation in three primary ways: (1) Traditional fermentation applies intact live microorganisms to regulate and adjust plant-derived ingredients, promoting products with unique flavor and nutritional profiles and altered texture, for instance, use of the fungus *Rhizopus* to ferment soybeans into tempeh and different lactic acid bacteria to produce yogurt and cheese, as well as more modern renditions of this approach, such as Myco Technology's fermentation of plant-based proteins to ameliorate flavor and functionality. (2) Biomass fermentation leverages the fast growth and high protein constituent

of various microorganisms to effectively produce large quantities of protein. The microbial biomass itself presents as an ingredient with the cells intact or minimally processed, for instance, with the cells broken open to boost digestibility or to enrich for even higher protein content, similar to processing plant flours into protein concentrates and isolates. This biomass acts as the major ingredient of a food product or as one of certain primary ingredients in a blend. Samples of biomass fermentation are Quron's and Meati's of filamentous fungi as the base for their meat analogs. (3) Precision fermentation uses microbial hosts as cell factories for producing specific functional ingredients that generally need greater purity than the primary protein ingredients and are incorporated at lower levels. These functional constituents are dominant enablers of improved sensory properties and functional characteristics of plant-based products or cultivated meat.

Samples are proteins, such as Clara Foods' egg proteins, Perfect Day's dairy proteins and Impossible Foods' heme protein; fats; enzymes; flavoring agents; natural pigments; and vitamins. To regulate, traditional fermentation used intact live microorganisms and the procedure of plant-derived ingredients. Biomass fermentation leverages the fast growth and high protein constituents of different microorganisms to efficacious production of large quantities of protein. Precision fermentation uses microbial hosts as cell factories for creating the specific functional ingredients. Appropriate choices for advancing fermentation can be categorized into five main areas spanning the value chain: target selection and design; strain development; bioprocess design; feedstock optimization; and end-product formulation and manufacturing. Target selection and design is the beginning point for the procedure of precision fermentation. The molecule or molecules of choice are related to as the target. The target can be a protein, a lipid, a pigment, a flavor constituent, a growth factor, a fragrance, an enzyme or another class of molecule. Fermentation-derived ingredients are already widely utilized across the food industry. Precision fermentation targets specific molecules. Fermentation results in a decoupling of the original concept of a target molecule and its production technique, and this decoupling greatly develops the search landscape for biomolecules with valuable and unique functions—from cloning and transformation through fermentation, downstream purification and final product testing, microbial protein expression and manufacturing needs; host cell lines; chemically defined media and vectors; high resolution, high capacity, salt-tolerant resins for polish chromatography; validated and automated rapid contaminant and impurity trials. P64k is a *Neisseria meningitidis* high molecular weight protein in meningococcal vaccine preparations, for the KLa/k scale-up fermentation criterion; the methodology described, which allowed the P64k protein at 50 l scale and the P64k protein total production at the 50 l culture scale to be obtained, was 546 mg $l^{-1}$ compared to the 284 mg $l^{-1}$ obtained at 1.5 l bench scale [165]. Methods for overcoming problems during recombinant protein expression in *Escherichia coli* are no or low expression (possible explanations are protein may be toxic before induction, protein may be toxic after induction, Codon bias); inclusion body formation (possible explanations are incorrect disulfide bond formation, incorrect folding, low solubility of the protein, an important post translational and modification is required); and protein inactivity (incomplete folding, mutations in cDNA) [166].

Protein fermentation by gut microbiota provides significantly to the metabolite pool in the large intestine and may lead to host amino acid balance [167]. Proteolytic fermentation is a highly networked procedure that can apply numerous impacts on the host [167], and the alterations in proteolytic fermentation on the basis of fiber availability indicate that examining the function of protein fermentation on health must also consider the carbohydrate requirement of the gut microbiota [168]. Recombinant protein production includes upstream and downstream: upstream processes are including construction of plasmid, transformation into host, selection of positive colony and induction for production; downstream techniques contain a collection of target protein, purification of produced protein and characterization of target protein [169]. *Escherichia coli* expression systems are often applied for producing exogenous protein on laboratory and industrial scales. The

benefits are rapid expression, high yield, ease of culture and genome modifications, affordability and rapid mass production; the disadvantages are that proteins with disulfide bonds are difficult to express, acetate formation results in cell toxicity, production of unglycosylated proteins, proteins with endotoxins are produced and proteins produced as inclusion bodies are inactive; thus, refolding is needed [169]. Yeasts, the single-celled eukaryotic fungal cells, are also utilized for the development of recombinant proteins that are not well developed in *E. coil*, and its advantages are high yield, stable production strains, persistence, cost-effectiveness, high-density growth, high proficiency, relevance for isotopically labeled protein production, rapid growth in chemically defined media, product processing akin to mammalian cells, ability to handle S-S-rich proteins, ability to benefit in protein folding and ability to glycosylate proteins. The downsides are N or O-linked glycosylation pattern (different from higher eukaryote), proteolytic degradation and hypermannosylation [169].

Protein concentrate (WPC) hydrolysis by *Streptococcus thermophilus* strains and WPC fermented with *S. thermophilus* RBC06 indicated the highest bioactivities because the main of bioactive peptides were anti-hypertensive and anti-diabetic peptides and RBC06 strain released the highest amount of anti-hypertensive lactotripeptides [170]. PE-2 strain of *Saccharomyces cerevisiae* could be utilized in fermentation procedure for ethanol production and for managing recombinant proteins simultaneously; recombinant CaneCPI-1 expressed in PE-2 was capable of inhibiting the papain activity, showing that protein is functional, and the probability of producing recombinant proteins with biotechnological operations during the ethanol fermentation process has been demonstrated [171].

## 7. Fermentation Technologies

### *7.1. Solid State Fermentation (SSF)*

Solid state fermentation (SSF) is a fermentation technique performed by different industries like the pharmaceuticals, textile, food, etc., to produce metabolite microorganisms using solid support in place of the liquid medium [172,173]. Compared with submerged fermentation (SmF), SSF has different benefits like direct use of agricultural and industrial residues as carbon sources and leading in affordable cost; however, systematic analysis of genome-wide gene expression in filamentous fungi under various cultivation conditions, namely SSF and SmF, is scarce [174–176]. The microbiological components of SSF can happen as single pure cultures, mixed identifiable cultures or totally integrated indigenous microorganisms; some SSF technologies, e.g., tempeh and oncom production, need the selective growth of organisms such as molds that need low moisture levels to carry out fermentation with the assistance of extracellular enzymes secreted by fermenting microorganisms [177,178]. However, bacteria and yeasts, which need higher moisture content for effective fermentation, can also be used for SSF, but with a lower yield [179]. The most important advantages of solid state fermentation are: (1) it produces a minimum amount of waste and liquid effluent, thus it is not very damaging to the environment; (2) solid substrate fermentation employs simple natural solids as the media; (3) low technology and low energy expenditure require less capital investment; (4) no need for sterilization, less microbial contamination and easy downstream processing; (5) the utilization of agro-industrial residues as substrates in SSF processes provides an alternative avenue and value-addition to these otherwise under- or non-utilized residues; (6) the yield of the products is reasonably high; (7) bioreactor design, aeration process and effluent treatment are quite simple; and (8) many domestic, industrial and agricultural wastes can be fruitfully used in SSF. The limitations of solid state fermentation are: (1) microorganisms that tolerate only low moisture can be used; (2) precise monitoring of SSF (e.g., $O_2$ and $CO_2$ levels, moisture content) is not possible; (3) organisms grow slowly, and consequently, there is a limitation in product formation; and (4) heat production creates problems, and it is very difficult to regulate the growth environment [180–183]. The variety of enzymes produced in SSF are: Naringinase (Orange and grapefruit rind), Polygalacturonase (Apple

bagasse and wheat bran), α-Amylase (Rice husk, banana husk, millet, water melon husk, lentil bran, wheat bran and maize oil cake), Manganese peroxidase (Pineapple leaf), Lipase (Sunflower seed and sugarcane bagasse), Protease (Wheat bran and soybean meal), Cellulase and hemicellulase (Corn straw, rice husk, grass powder, sugarcane barbojo and sugarcane bagasse), Ellagitannase (Sugarcane bagasse, corn cobs, coconut husk and candelilla stalks), Phytase (Wheat bran) and Laccase (Poplar sawdust) [184]. Lipids produced in SSF are: γ-Linolenic acid (*Mortierella isabellina*), Gamma linolenic acid (*Mucor rouxii*), Oleic acid and Palmitic acid (*Mortierella isabellina*), Lipids (*A. oryzae*), Oleic acid and Palmitic acid and Linoleic acid (*Mortierella isabellina*), Lipids (*Mortierella isabellina*) and Lipids (*Aspergillus tubingensis* TSIP9) [184].

Organic acids produced in SSF are Citric acid (*Aspergillus niger* DS 1, *Aspergillus niger* CECT-2090, *Aspergillus niger* PTCC-5010), Lactic acid (*Lactobacillus delbrueckii*, Lactobacillus casei, Lactobacillus amylophilus GV6), Gluconic acid (Aspergillus niger ARNU-4, *Aspergillus niger*) and Ellagic acid (*Aspergillus niger*, *Aspergillus niger* GH1) [184]. Cashew and guava byproducts were successfully subjected to solid state fermentation for protein enrichment through single-cell protein and then included in cereal bars for human nutrition, and the addition of protein-enriched byproducts is a substitute to add nutritional and economic value to cereal bars [185]. The addition of 0.1% and especially of 0.5% solid state fermentation product (Synergen™) could markedly improve growth performance and feed efficiency of lupin diets [186]. Fermentation of de-oiled rice bran (DORB) resulted in decreased in vitro protein digestibility; fermentation of DORB with *Rhizopus oryzae* increases the n-6 fatty acid profile; and fermentation leads to reduction in phytate and trypsin inhibitor activity of DORB [187]. Inoculation of suitable cellulolytic microbes to enrich protein content and improve in vitro digestibility of herbage with solid state fermentation for chicken feed is the prospective method for animal husbandry, agriculture and substantial management [188].

The protein constituent of fermented pangola grass increased from 5.97–6.28% to 7.09–16.96%, and the in vitro digestion increased from 4.11–4.38% to 6.08–19.89% with the inoculation of cellulolytic microbes by solid state fermentation; this procedure may enrich protein content, increase in vitro digestibility and boost the quality for animal feeding [189]. Fermentation by *Bacillus subtilis* increased the nutritional quality of soybean meal (SBM), and fermentation principally decreases trypsin inhibitor and beta-conglycinin in SBM [190]. It has been reported that the solid state fermentation of aquatic macrophytes in the production of crude protein extraction is encouraging, which makes aquatic macrophytes a potential source and thus is suitable to the long-term ecological restoration of eutrophic lakes [191]. The electronic nose (e-nose) technique was designed to monitor the SSF process of protein feed and the application of linear and non-linear algorithms in calibrating the discrimination model using e-nose data [192]. *Pleurotus ostreatus*-based solid state fermentation of mechanically managed canola meal increased its protein constituent, and fungal fermentation degraded glucosinolates and phytate up to 98.8% and 75.8%, respectively [193]. Solid state fermentation increased protein and amino acid constituents of soybean meal (SBM), and *B. subtilis* brought about a greater impact to increase protein and AA than *A. oryzae* [194].

Solid state fermentation with *Rhizophus obligosporus*, according to nitrogen compounds balance, helped to increase the nutritional value of the grains and the digestibility of its protein in lupin [195]. Solid state fermentation revealed better enzyme activity than submerged fermentation for both raw and processed canola meal [196]. Solid state fermentation of pineapple peels with *Trichoderma viride* ATCC 36,316 resulted in protein production, and protein enriched peels from an on-farm fermenter had higher protein content than the conical flask experiment's product, 16 and 14.89%, respectively [197]. Solid state fermentation enriches fruit and vegetable discards in protein and amino acid profile, highly improving their suitability as animal feed, and *Rhizopus* fermentation of fruit and vegetable leachate leads to a 31% protein biomass, being a valuable alternative protein [198]. SSF involved the consumption of mainly amylopectin instead of amylose and non-

resistant starch instead of resistant starch irrespective of the Australian sorghum variety, and all fermented samples were found to have increased protein content [199]. A novel solid state fermentation with *Bacillus subtilis* was applied to produce fermented chickpeas, and chickpea proteins were degraded to low molecular weight peptides during fermentation [200]. Fermentation-assisted hydrolysis increased the protein quality of soybean mean, and fermentation-assisted hydrolysis decreased the potential antigenicity of soybean meal [201]. Solid state fermentation was conductive to boosting drumstick (*Moringa oleifera* Lam.) leaf nutritional value, and protein content was also increased [202]. It has been reported that solid state fermentation leads to an effective approach to increasing the quality of proteins sources, such as rapeseed cake, as well as increasing the enzyme activity of endoglucanase, acid protease, xylanase and phytase [203]. It was found that SSF decreased the organic matter and reduced the sugar content of the fermented product, while crude protein and fiber fractions were improved; SFF led to a stabilized feed ingredient enriched in protein but at the expense of digestibility reduction [204].

### *7.2. Submerged Fermentation (SmF)*

SmF is a procedure in which the growth of microorganisms happens in a liquid broth medium, which is escalated with mandatory nutrient to have a better cultivation of microorganisms, and this consists of accurately growing the selected microorganisms in closed reactors with medium fermentation and a high concentration of oxygen [205,206]. Bacteria are usually utilized as a source in this procedure as it needs high moisture content [207]. Submerged fermentation, using *Trichoderma viride* ATCC 36,316 on cassava peel, particularly on unpretreated cassava peel for 3 to 4 days, improved crude protein content of cassava peel 8-fold and true protein constituent 22-fold [208]. Although submerged fermentation (SmF) is responsible for the majority of current enzyme industries, it has been reported that solid state fermentation (SSF) can produce higher enzyme yields in laboratory scale. The non-enzyme proteins in SSF were active in fungal mycelia growth and condition, while those in SmF were more associated to stress tolerance and glycometabolism [209]. The solid state fermentation step improved the protein content in waste bread by 161%, and the fermented product has potency to be applied as nutrient rich feed [210]. Production in solid state fermentation was two times higher than submerged liquid fermentation, and this significant difference in yields of hydrophobins underlines the appropriateness of solid substrate fermentation procedure along with the addition of oil cakes to boost the yields [211]. Sustainable production of mycoproteins and surface-active proteins can be progressed by growing a marine fungal strain for shedding light on the potentiality of an integrated methodology that promotes the circular economy [212]. A novel magnetic field technology aid for submerged fermentation was performed; the morphology of mycelium was altered significantly after magnetic field treatment; the scale-up magnetic field fermentation notably enhanced mycelium biomass; and the magnetic field increased fermentation by stimulating the expression of genes [213]. Cellulase activities of micoorganisms changed according to various conditions, and solid state fermentation indicated better enzyme activity than submerged fermentation [213]. An isolate of *Aspergillus niger* was assessed for citric acid production and enriched protein mycelium using molasses and whey for the fermentation medium, and utilizing industrial wastes of cheese whey fortified with beet molasses increased the consistent, economical, large-scale yield of citric acid by protein enriched *A. niger* [214]. Among different microorganisms, *Fusarium venenatum* is the most prevalent species to be successfully utilized in food industry, and it has been applied to produce mycoprotein as food being under the trade name Quorn, and mycoprotein indicates satiation characteristics which can be a solution for obesity by enabling people to obtain a healthier diet with low fat and high fiber content [215]. It has been reported that *Vitreoscilla hemoglobin* has profitable advantages on improving total protein secretion and cellulase activity of *Trichoderma reesei* in submerged fermentation [216]. Benefits and disadvantages of Solid State Fermentation and Submerged Fermentation are presented in Table 2.

**Table 2.** Comparison of Solid State Fermentation and Submerged Fermentation.

| Types | Advantages | Disadvantages |
| --- | --- | --- |
| Solid State Fermentation | Substrates need less pretreatment in comparison with liquid media | Low moisture level can restrict the growth of microorganisms |
| | The medium is easily available, simple, and inexpensive | A problem in removing metabolic heat in large scale |
| | Forced aeration is usually easier | Problems and difficulties in monitoring the process parameters |
| | Contaminations are restricted since the moisture content is low | |
| | Simple fermentation equipment | |
| | Minimized and simplified downstream process and waste disposal | |
| | High volumetric productivity | |
| Submerged Fermentation | Simplicity of measuring process parameters | Utilization of expensive equipment and costly media |
| | Even distribution of microorganisms and nutrients | Expensive and complex downstream procedure and difficulty in the waste disposal |
| | Capability to control and monitor growth conditions | High power consumption |
| | Accessibility of high-water content for the growth of microbes | |

## 8. Lactic Acid Fermentation and Protein

Lactic acid fermentation is believed to be a simple and appropriate form of biotechnology to keep and/or increase the safety, sensory, nutritional and shelf life characteristics of fruits and vegetables [217]. Lactic acid bacteria are a group of organisms that generally ferment sugar (i.e., glucose) to lactic acid, and they are gram positive, non-sporulating rods and cocci having low guanine-cytosine content; this class of bacteria is divided into two sub-groups, homo-fermentative and hetero-fermentative [218]. Lactic acid fermentation of foods is frequent in tropical countries because the following advantages are intrinsic to this procedure: (i) An affordable method of food preservation, spoilage and pathogenic microorganisms are prevented by a combination of pH reduction, a lowering of oxidation-reduction potential, competition for important nutrients and the production of inhibitory compounds-antibiotic constituents and hydrogen peroxide; (ii) increased organoleptic qualities; and (iii) in some cases, the nutritional value or the digestibility of the raw material is boosted [219]. Fermentation of rapeseed protein concentrate clearly increased the free amino acid profile, e.g., lysine, isoleucine and methionine, which are deficient in wheat bread [220].

Protease hydrolysis of lactic acid bacteria on the fermentation caused protein molecule alterations that promoted gel formation in soybeans, and the hydrophobic peptides and hydrophobic amino acids were boosted [221]. Lactic acid fermentation of pea protein was improved to decrease off-flavors; fermentation led to the breakdown of larger peptides, resulting in lower protein solubility, and lactic acid fermentation treatment increased the taste of pea proteins, according to the descriptive sensory analysis [222]. It has been reported that lactic acid fermentation is the main methodology for the extraction of leaf proteins, and the maturity of the plants should be considered when utilized as feedstock for producing protein concentrates for animal feeding to optimize the process yields [223]. Oil-in-water emulsions made with goat milk were fermented with lactic acid bacteria, and the oil droplet size was decreased after the fermentation; caseins were the pre-

dominant protein species at the interface at the end of processing [224]. Macroalgal biomass is a possible sustainable feedstock for lactic acid production, and valorization of the immense amounts of spent macroalgal biomass residue post hydrocolloid extraction in a biorefinery is an applicable strategy for affordable lactic acid production [225]. Cooperative fermentation by yeast and lactic acid bacteria lead to structural changes in bran, gluten and starch, and produced enzymes, exopolysaccharides and organic acids of wheat flour dough system [226].

The lactic acid bacteria fermentation has the capability as a technique to promote the emulsifying properties and bioactivity of phosvitin [227]. Lacto-fermentation significantly boosted lupin protein functional components, and supplementation with fermented lupin flour improved the texture of wheat-lupine bread [228]. Lactic acid bacteria at different phases revealed different utilization ability to carbon sources, co-culture of lactic acid bacteria and yeast-improved ester formation, and restricted acids formation on the flavor of *Baijiu* [229]. Fermentation enabled higher protein and amino acid bioavailability, and increased overall nutritional quality of faba bean [230]. Lactic acid bacteria fermentation could meaningfully increase the gelling ability of soy protein isolate [231]. It has been found that fermentation led to a decline in glucose and fructose concentrations because of their consumption as a source of energy for growth and metabolism of lactic acid bacteria in rice beverages [232]. The impacts of lactic acid fermentation on legume protein are: technological properties (changes in protein solubility, modification in emulsifying and foaming properties, altering the water-holding and oil-holding capacity, adjustment of gel formation); taste and flavor (decrease in beany and bitterness, degradation of aromatic compounds relating to proteolysis, glycolysis and lipolysis, increase in sour and tangy lactic acid taste); protein composition (hydrolysis of protein, production of smaller polypeptides, production of free amino acids and bioactive peptides, improvement in the ration of essential amino acids); nutritional properties (modification in protein digestibility, reduction of ANF, enhancement in mineral and nutrient bioavailability); health and well being (microorganisms as probiotics, release of bioactive peptides, reduction of contaminants, biogenic amines, mycotoxines and decrease in allergenicity); and preservation (production of organic acids, production of antimicrobials, shelf-life extension) [233].

## 9. Alcoholic Fermentation and Protein

Alcoholic fermentation is a complicated biochemical procedure during which yeasts convert sugars to carbon dioxide, ethanol and other metabolic byproducts that promote to the chemical composition and sensorial characteristics of the fermented foodstuffs. Alcoholic fermentation is the outstanding science of the fermentation processes and is active in several chief transformation, stabilization and conservation techniques for sugar-rich substrates, such as fruit, and vegetable and fruit juices [234]. In this fermentation practice, yeast is mainly used as a bio-culture and aqueous solution of monosaccharide (raw materials) as the culture media for the production of beverages [235]. Alcoholic fermentation starts with the breakdown of sugars by yeasts to form pyruvate molecules [236,237]. The appropriate control of the dosage of the amino acid addition and the application of mixed amino acid supplementation may be a technique to adjust the fermentation kinetics and volatile compound modulation in soy whey alcohol fermentation [238]. Alcoholic fermentation is the base for the manufacturing of alcoholic beverages like beer and wine, and control of fermentation is usually considered as a prerequisite to demonstrating the quality of the final product [239–241]. Under anaerobic conditions, the pyruvate can be converted to ethanol [242–244].

## 10. Acetic Acid Fermentation and Protein

Acetic acid bacteria (AAB) are part of the family Acetobacteraceae and are gram-negative, aerobic-catalase-positive microorganisms; from glucose, AAB produce acetic acid, and their morphology may vary from spherical, swollen, club-shaped, elongated, filamen-

tous to curved rods [245–247]. Acetic acid bacteria were first microbes discovered as causing principle wine spoilage in the mid-1860s even though extensive studies consequently related them to commercial vinegar production and examined their activity on grapes, wine and must [248–250]. They are introduced in different habitats: fruit, flowers and vegetables; wine and beer as spoilage microorganisms (because their metabolites induce in unpleasant organoleptic characteristics) and vinegar as the dominant fermenters [251–253]. *Acetobacter*, *Gluconacetobacter* and *Gluconobacter* are the principle genera connected with grape and wine spoilage [254,255]. *Acetobacter* involved in winemaking are *A. aceti*, *A. liquefaciens*, *A. hansenii* and *A. pasteurianus* (*A. hansenii* and *A. liquefaciens* have lately been reassigned as *Gluconacetobacter hansenii* and *G. liquefaciens*) [256–258]. *Gluconobacter* is represented by three species *G. frateurii*, *G. asaii* and *G. oxydans*; this last one is the major specie found in connection with grapes and grape must [259–261].

## 11. Eukaryotic Microorganism Species and Fermentation Technology

Fermentation engineering, which is one of the most important components of modern biotechnology, has been extensively applied in areas including food, pharmaceutical and chemical industries, energy and environmental protection [262]. Various methods, such as microscopy, product and substrate evaluation, toxicity tests or biomass monitoring assist in generating a complete picture of the strains' characteristics and demands and enable control over precise fermentation procedures. Yeasts are eukaryotic single-cell microorganisms that act during the pulque fermentation procedure, providing appropriate aromatic constituents, proteolytic and lipolytic activities; producing carbon dioxide and ethanol; and helping bacterial growth by producing vitamins, amino acids and other metabolites [262]. Yeast fermentation procedures are alcoholic fermentations, beer fermentation, wine fermentation, cider fermentation; non-alcoholic fermentation of yeasts are coffee fermentation, bread fermentation and chocolate fermentation. Yeasts are eukaryotic, unicellular microfungi that are extensively distributed in the natural environment [263,264]. They are included in a group of organisms termed fungi, which also consists of molds and mushrooms [265,266], and they can have both negative and positive impacts on fermented products consumed by animals and humans [267–269].

Yeast is applied as a starter culture in bread and cheeses, as well as in beer, wine and other alcoholic fermentation products, but they can also propose spoilage in foods, such as yogurt, salads, fruit juice and mayonnaise [270–273]. In addition to being extensively applied in the production of beverages, foods and pharmaceuticals, yeasts play significant functions as model eukaryotic cells in improving our knowledge in the biomedical and biological sciences [274–277]. Processing methodology of fermented vegetables had a significant impact on eukaryotic microbial communities in comparison with the raw material and packing, and under the same process techniques, raw materials had a noticeable effect on eukaryotic microbial communities compared with packaging [278]. Omics Database of Fermentative Microbes (ODFM) is a data management system that combines comprehensive omics knowledge for fermentative microorganisms [279]. Yeast fermentation altered the volatiles of the larvae without boosting mortality, and it can also significantly improve intensity of fruity flavor volatiles [280].

Hydrocolloids supplementation led to the immobilization of yeast cells via flocculation, providing a protective impact on the physiological characterization of large yeast during high gravity brewing [281]. Low-temperature fermentation is regarded to enrich the aroma of wine; it can increase ethyl acetate, ethanol and ethyl butanoate synthesis, and it can also decrease phenylethanol, acetic acid and phenylethyl acetate synthesis [282]. Supplementation of protein hydrolysate is an important technique for boosting the salt tolerance of soy sauce aroma-producing yeast [282–284]. The application of baker's yeast in fermentation or rice bran for extraction of protein concentrate can be more effectively managed to increase the extraction yield in comparison to natural fermented and untreated rice bran [285].

## 12. Prokaryotic Microorganism Species and Fermentation Technology

Prokaryotes are typically simple, single-celled organisms; they have ribosomes to make proteins, a membrane and a cell wall to contain the contents of the cell, and their DNA is packed up in the middle of the cell [286–290]. Certain prokaryotes, consisting of some species of Archaea and bacteria, use anaerobic respiration, which can be discovered in soil and in the digestive tracts of ruminants, like cows and sheep [291–294]. Many prokaryotes can switch between aerobic respiration and fermentation, depending on the availability of oxygen [295–297]. The group of Archaea called methanogens decreases carbon dioxide to methane to oxidize NADH, and some sulfate-reducing bacteria and Archaea are anaerobic, decline sulfate to hydrogen sulfide to regenerate $NAD^+$ from NADH [298–300]. Archaea consists of an individual domain of organisms with discrete biochemical and genetic distinctions from bacteria, and methane-forming methanogens comprise the prevalent group of archaea in the human gut microbiota [301]. In anaerobic systems without inhibition by $NH_3$-N, organic acids created from acidogenesis are fermented to acetate and $H_2$, and the ordinary distribution of the electron flow to methane is 67% acetate and 33% $H_2$ [302].

Dissimilarities in the constitution and activity of the rumen microorganisms may have a role in variation in host feed adaptability through their impact on feed digestion, fermentation and $CH_4$ production [303]. Halophilic archaea consisted of 74.5% of the microbial communities in fermented fish, and archaea may have a function in both fermentation and health benefits of fermented fish [304]. Up to now, archaea have been categorized into 5 phyla, namely *Korarchaeota*, *Crenarchaeota*, *Nanoarchaeota*, *Euryarchaeota* and *Thaumarchaeota* [305–308]. IntensiCarb™ (IC) is an innovative technology that permits coinciding thickening and anaerobic fermentation in a single treatment step; IC can increase both volatile fatty acid (VFA) and hydrolysis yields compared to control fermenter, and IC produced condensate at higher quality without solids and low nutrient constituents [309–318].

## 13. Conclusions

Fermentation has been used for ages as a safe technique for food preservation, and it uses minimal resources. Most common fermentations are alcohol fermentation, lactic acid (homofermentation), lactic acid (heterofermentation), butyric acid, mixed acid, propionic acid and acetic acid. Bacterial diversity in fermentation pathways are *Acetobacter*, *Gluconacetobacter*, *Halomonas*, *Hafnia*, *Tatumella*, *Zymomonas*, *Brachybacterium*, *Microbacterium*, *Brevibacterium*, *Corynebacterium*, *Micrococcus*, *Kocuria*, *Arthrobacter*, *Streptomyces*, *Propionibacterium*, *Bifidobacterium*, *Bacillus*, *Gemella*, *Jeotgalicoccus*, *Enterococcus*, *Carnobacterium*, *Tetragenococcus*, *Vagococcus*, *Weissela*, *Leuconostoc*, *Oenococcus*, *Lactococcus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus* and *Pediococcus*. The most important health advantages of fermenting are the origin of valuable lactic acid bacteria, simple digestion, increase in the availability of nutrients, possible mood and behavior boost and possible support for heart health. Some of the most important health advantages of lactic acid bacteria are fermentates have improved gluten-associated disorders; modulated mucosal immune system and improved gut disorders; elicited reduced influx of gliadin peptides into cells; induced mucosal immune system; and reduced severity of an infection in offspring of lactating mice. These advantages excluded pathogens ameliorated enteral nutrition and effective against several pathogens including *Salmonella typhimurium*, *Cronobacter sakazakii* and *Listeria monocytogenes*. Alcoholic fermentation is the outstanding science of the fermentation processes and is active in several chief transformation, stabilization and conservation techniques for sugar-rich substrates, such as fruit, and vegetable and fruit juices. Acetic acid bacteria (AAB) are part of the family Acetobacteraceae and are Gram-negative, aerobic-catalase-positive microorganisms; from glucose, AAB produce acetic acid, and their morphology may vary from spherical, swollen, club-shaped, elongated, filamentous to curved

rods. The protein industry uses fermentation in three primary ways: (1) Traditional fermentation; (2) Biomass fermentation; and (3) Precision fermentation. Best choices for improving fermentation can be categorized into five key areas spanning the value chain: target selection and design, strain development, bioprocess design, feedstock optimization and end-product formulation and manufacturing. Solid state fermentation (SSF) is a fermentation technique performed by different sections such as food, pharmaceuticals, textile, etc., to produce metabolite microorganisms using solid support in place of the liquid medium. The most important advantages of solid state fermentation are (1) production of a minimum amount of waste and liquid effluent that is not very damaging to the environment and (2) employment of simple natural solids as the media. (3) Simple technology, low energy expenditure and less capital investment are needed, and there is (4) no requirement for sterilization, less microbial contamination and simple downstream processing. (5) Application of agro-industrial residues as substrates in SSF procedures provides a substitute avenue and value-addition to the residues; (6) the yield is significantly high; (7) bioreactor design, aeration procedure and effluent treatment are considerably simple; and (8) many domestic, agricultural, and industrial wastes can be positively used in SSF. Yeasts are eukaryotic single-cell microorganisms that act during the pulque fermentation procedure, providing appropriate aromatic constituents, proteolytic and lipolytic activities, producing carbon dioxide, and ethanol, and helping bacterial growth by producing vitamins, amino acids and other metabolites. Many prokaryotes can replace between aerobic respiration and fermentation, according to the availability of oxygen. Archaea consists of an individual domain of organisms with discrete biochemical and genetic dissimilarities from bacteria, and methane-forming methanogens, archaea, comprise the prevalent category of archaea in the human gut microbiota.

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## Abbreviations

SSF: Solid state fermentation; TGGE: Thermal gradient gel electrophoresis; FISH: Fluorescence in situ hybridization; DEFT: Direct epifluorescence technique; RT-PCR: Reverse transcriptase-polymerase chain reaction; q-PCR: Quantitative-polymerase chain reaction; c-PCR: Competitive-polymerase chain reaction; LAB: Lactic acid bacteria; LA: Lactic acid; ACE: Angiotensin-converting enzyme; VPP: Valine-Proline-Proline; IPP: Isoleucine-Proline-Proline; AAB: Acetic acid bacteria; ALF: *Lactobacillus kefri* fermentation components; BSG: Brewer's spent grain; SPC: Soya press cake; SPME: Solid phase microextraction; GCMS: Gas-Chromatography mass spectrometry; PP: Pea protein enriched flour; LBP: *Lycium barbarum* polysaccharide; SCG: Spent coffee grounds; MF: Magnetic field application; MO: Manual operation; TO: Mechanical operation; FMP: Fermented with sourdough; GA: Callic acid; CMCS: Carboxymethyl chitosan; QZR: Qingzhuan tea; F-ASF: Ferment *Asparagus sprengeri* fructan; OCW: Onion cell walls; WPC: Why protein concentrate; SSF: Solid state fermentation; Smf: Submerged fermentation; DORB: De-oiled rice bran; E-nose: Electronic nose; AAB: Acetic acid bacteria; ODFM: Omics database of fermentative microbes; IC: IntensiCarb™.

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