fermentation

Review

Spontaneous Food Fermentations and Potential Risks for Human Health

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Abstract: Fermented foods and beverages are a heterogeneous class of products with a relevant worldwide significance for human economy, nutrition and health for millennia. A huge diversity of microorganisms is associated with the enormous variety in terms of raw materials, fermentative behavior and obtained products. In this wide microbiodiversity it is possible that the presence of microbial pathogens and toxic by-products of microbial origin, including mycotoxins, ethyl carbamate and biogenic amines, are aspects liable to reduce the safety of the consumed product. Together with other approaches (e.g., use of preservatives, respect of specific physico-chemical parameters), starter cultures technology has been conceived to successfully dominate indigenous microflora and to drive fermentation to foresee the desired attributes of the matrix, assuring quality and safety. Recent trends indicate a general return to spontaneous food fermentation. In this review, we point out the potential risks for human health associated with uncontrolled (uninoculated) food fermentation and we discuss biotechnological approaches susceptible to conciliate fermented food safety, with instances of an enhanced contribution of microbes associated to spontaneous fermentation.

Keywords: fermented food; fermentation; beverage; safety; risks; spontaneous fermentation; starter cultures; spoilage microbes; pathogens; contaminant

1. Fermented Foods and Beverages: Scientific Dimension, Social Relevance, and Economic Significance

A large basket of food and beverages is obtained from a microbial-based transformation of food raw materials. Different classes of microorganisms can be involved, mainly yeasts and bacteria, with a certain role of molds. The obtained fermented foods and beverages have ben staple foods for millennia, with a considerable importance in the human diet for reasons of generally enhanced shelf-life, palatability, safety and nutritional quality [1]. In fact, the desired fermentation process consists of protechnological microbial development in the given matrix, with direct and indirect effects of primary and secondary microbial metabolism. Protechnological microorganisms, in order to obtain energy and to sustain their anabolic processes, reduce the content of carbohydrates and other macromolecules available in the raw matrix, accumulating catabolic products (e.g., lactic acid, ethanol). These biological dynamics, together with the possible release of antimicrobial compounds [2], reduce the risks of undesired microbial developments (thus increasing product shelf-life and safety level). On the other hand, both primary and secondary metabolites strongly influence palatability and sensory attributes of the fermented matrices [3]. Finally, these modifications performed by microorganisms radically change the nutritional quality of the food/beverage, also because of the production of biomolecules of nutritional interest synthetized by fermenting cells [4]. Certainly, this is just a partial brief overview on the impact of fermenting microbes on the global (e.g., hygienic, sensory, nutritional, functional) quality of fermented foods/beverages, a topic that will be partially deepened in the next section. What is important to remark is the importance of the so-called "omics" approaches (metagenomics, transcriptomics, proteomics, metabolomics, volatomics) to offer new perspectives in the understanding of microbial contributions to the global quality of fermented foods [5–9].

From a geographical perspective, several scientific studies elucidate the microbiological and nutritional significance of fermented foods and beverages in the different continents: Asia (e.g., [10,11]), Africa (e.g., [11,12]), America (e.g., [11,13]), Australia (e.g., [11,14]), and Europe (e.g., [11,15]). On the other hand, as a function of the nature of the matrices (raw material) subjected to fermentation, recent literature suggests categorization of worldwide fermented foods and beverages into nine principal classes: (a) fermented cereals; (b) fermented vegetables and bamboo shoots; (c) fermented legumes; (d) fermented roots/tubers; (e) fermented milk products; (f) fermented and preserved meat products; (g) fermented, dried and smoked fish products; (h) miscellaneous fermented products; and (i) alcoholic beverages ([16]). Several productions can be considered worldwide diffused or with a national/continental presence, while considerable diversity in terms of matrices and of kind of fermentations has a regional diffusion. The widespread geographical diffusion ([11,16]) and the relevance in terms of consumption across populations make fermented foods and beverage a global sector, with an increasing relevance in human nutrition and economy. The significance in actual human nutrition framework is also well testified by the direct and indirect inclusion in the dietary guidelines of several countries (e.g., the Indian pyramid explicitly promotes the consumption of fermented foods) [17,18]. In order to underline the economic/social importance of fermentations, we have to remember that the so-called "ancient biotechnology" was empirically discovered and replicated as a food preservation technique [19].

2. The Microbiology of Fermented Foods and Beverages: "Microbiodiversity", Impact on Food Qualities and on Human Health

A wide diversity of microorganisms is associated with the huge diversity in terms of raw materials, fermentative behavior and obtained products [16]. Table 1 reports just a few exemplificative cases of fermented products belonging to the nine categories proposed by Tamang et al. [16]. This brief overview also helps to provide an idea of the dimension of the microbial diversity associate to food fermentations: fermentative processes associated with the production of these twenty fermented products involved more than fifty microbial genera and hundreds of different species of bacteria, yeasts, and filamentous fungi. In addition, (i) if we consider that behind each species there is a consistent intraspecific diversity and that (ii) often desired and undesired microbial features associated with food fermentations are often strain-dependent characters, the potentially articulate impact of this diversity on global food quality appears clear.

Table 1. Non-exhaustive list of fermented foods belonging to the main categories of global fermented foods. Information reported in accordance to Tamang et al. [16] (to Petruzzi et al. [20] for wine and Greppi et al. [21] for ogi and for tchoukoutou).

Major Groups Global Fermented Foods	Fermented Product (Raw Material, Geographical Diffusion): Microorganisms Involved in the Fermentation Process
(a) Fermented cereals	Sourdough (Rye, wheat; America, Europe, Australia): *Lb. sanfranciscensis*, *Lb. alimentarius*, *Lb. buchneri*, *Lb. casei*, *Lb. delbrueckii*, *Lb. fructivorans*, *Lb. plantarum*, *Lb. reuteri*, *Lb. johnsonii*, *Cand. humili*, *Issatchenkia orientalis*
	Ogi (Maize, sorghum, millet; Nigeria): *Lb. plantarum*, *Lb. pantheris*, *Lb. vaccinostercus*, *Corynebacterium* sp., *Aerobacter* sp., *Candida krusei*, *Clavispora lusitaniae*, *Sacch. cerevisiae*, *Rhodotorula* sp., *Cephalosporium* sp., *Fusarium* sp., *Aspergillus* sp., *Penicillium* sp.
	Idli (Rice, black gram or other dehusked pulses; India, Sri Lanka, Malaysia, Singapore): *Leuc. mesenteroides*, *Lb. delbrueckii*, *Lb. fermenti*, *Lb. coryniformis*, *Ped. acidilactis*, *Ped. cerevisae*, *Streptococcus* sp., *Ent. faecalis*, *Lact. lactis*, *B. amyloliquefaciens*, *Cand. cacaoi*, *Cand. fragicola*, *Cand. glabrata*, *Cand. kefyr*, *Cand. pseudotropicalis*, *Cand. sake*, *Cand. tropicalis*, *Deb. hansenii*, *Deb. tamarii*, *Issatchenkia terricola*, *Rhiz. graminis*, *Sacch. cerevisiae*, *Tor. candida*, *Tor. holmii*
(b) Fermented vegetables and bamboo shoots	Table Olives (Olive; USA, Spain, Portugal, Italy, Greece, Peru, Chile): *Leuc. mesenteroides*, *Ped. pentosaceus; Lb. plantarum Lb. pentosus/Lb. plantarum*, *Lb. paracollinoides*, *Lb. vaccinostercus/Lb. suebicus* and *Pediococcus* sp. non-lactics (*Gordonia* sp./*Pseudomonas* sp., *Halorubrum orientalis*, *Halosarcina pallid*, *Sphingomonas* sp./*Sphingobium* sp./*Sphingopyxis* sp., *Thalassomonas agarivorans*) and yeasts (*Candida* cf. *apicola*, *Pichia* sp., *Pic. manshurica/Pic. galeiformis*, *Sacch. cerevisiae*)
	Kimchi (Cabbage, green onion, hot pepper, ginger; Korea): *Leuc. mesenteroides*, *Leuc. citreum*, *Leuc. gasicomitatum*, *Leuc. kimchii*, *Leuc. inhae*, *W. koreensis*, *W. kimchii*, *W. cibaria*, *Lb. plantarum*, *Lb. sakei*, *Lb. delbrueckii*, *Lb. buchneri*, *Lb. brevis*, *Lb. fermentum*, *Ped. acidilactici*, *Ped. pentosaceus*, *Lc. lactis*, yeasts species of *Candida*, *Halococcus*, *Haloterrigena*, *Kluyveromyces*, *Lodderomyces*, *Natrialba*, *Natronococcus*, *Pichia*, *Saccharomyces*, *Sporisorium*, *Trichosporon*
	Soibum (Bamboo shoot; India): *Lb. plantarum*, *Lb. brevis*, *Lb. coryniformis*, *Lb. delbrueckii*, *Leuc. fallax*, *Leuc. Lact. lactis*, *Leuc. mesenteroides*, *Ent. durans*, *Strep. lactis*, *B. subtilis*, *B. lichniformis*, *B. coagulans*, *B. cereus*, *B. pumilus*, *Pseudomonas fluorescens*, *Saccharomyces* sp., *Torulopsis* sp.
(c) Fermented legumes	Tempeh (Soybean; Indonesia, The Netherlands, Japan, USA): *Rhiz. oligisporus*, *Rhiz. arrhizus*, *Rhiz. oryzae*, *Rhiz. stolonifer*, *Asp. niger*, *Citrobacter freundii*, *Enterobacter cloacae*, *K. pneumoniae*, *K. pneumoniae subsp. ozaenae*, *Pseudomas fluorescens*, *Lb. fermentum*, *Lb. lactis*, *Lb. plantarum*, *Lb. reuteri*
	Dawadawa (Locust bean; Ghana, Nigeria): *B. pumilus*, *B. licheniformis*, *B. subtilis*, *B. firmus*, *B. atrophaeus*, *B. amyloliquefaciens*, *B. mojavensis*, *Lysininbacillus sphaericus*.
(d) Fermented roots/tubers	Fufu (Cassava; West Africa): *Bacillus* sp., *Lb. plantarum*, *Leuc. mesenteroides*, *Lb. cellobiosus*, *Lb. brevis; Lb. coprophilus*, *Lc. lactis; Leuc. lactis*, *Lb. bulgaricus*, *Klebsiella* sp., *Leuconostoc* sp., *Corynebacterium* sp., *Candida* sp.
	Tapé (Cassava; Indonesia): *Streptococcus* sp., *Rhizopus* sp., *Saccharomycopsis fibuligera*
(e) Fermented milk products	Cheese (Animal milk; Worldwide): *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis*, *Lb. delbrueckii* subsp. *delbrueckii*, *Lb. delbrueckii* subsp. *lactis*, *Lb. helveticus*, *Lb. casei*, *Lb. plantarum*, *Lb. salivarius*, *Leuconostoc* spp., *Strep. thermophilus*, *Ent. durans*, *Ent. faecium*, and *Staphylococcus* spp., *Brevibacterium linens*, *Propionibacterium freudenreichii*, *Debaryomyces hansenii*, *Geotrichum candidum*, *Penicillium camemberti*, *P. roqueforti*
	Kefir (Goat, sheep, cow; Russia): *Lb. brevis*, *Lb. caucasicus*, *Lb. kefiri*, *Strep. thermophilus*, *Lb. bulgaricus*, *Lb. plantarum*, *Lb. casei*, *Lb. brevis*, *Tor. holmii*, *Tor. delbruechii*
(f) Fermented and preserved meat products	Chorizo (Pork; Spain): *Lb. sake*, *Lb. curvatus*, *Lb. plantarum*
	Nem-chua (Pork, salt, cooked rice; Vietnam): *Lb. pentosus*, *Lb. plantarum*, *Lb. brevis*, *Lb. paracasei*, *Lb. fermentum*, *Lb. acidipiscis*, *Lb. farciminis*, *Lb. rossiae*, *Lb. fuchuensis*, *Lb. namurensis*, *Lc. lactis*, *Leuc. citreum*, *Leuc. fallax*, *Ped. acidilactici*, *Ped. pentosaceus*, *Ped. stilesii*, *Weissella cibaria*, *W. paramesenteroides*

Table 1. *Cont.*

Major Groups Global Fermented Foods	Fermented Product (Raw Material, Geographical Diffusion): Microorganisms Involved in the Fermentation Process
(g) Fermented, dried and smoked fish products	Ngari (Fish, salt; India): *Lact. lactis*, *Lb. plantarum*, *Lb. pobuzihii*, *Lb. fructosus*, *Lb. amylophilus*, *Lb. coryniformis*, *Ent. faecium*, *B. subtilis*, *B. pumilus*, *B indicus*, *Micrococcus* sp., *Staphy. cohnii* subsp. *cohnii*, *Staphy. carnosus*, *Tetragenococcus halophilus* subsp. *flandriensis*, *Clostridium irregular*, *Azorhizobium caulinodans*, *Candida* sp., *Saccharomycopsis* sp.
	Surströmming (Fish; Sweden): *Haloanaerobium praevalens*
(h) Miscellaneous fermented products	Balsamic Vinegar (Grape must; Italy): *Acetobacter aceti* subsp. *aceti*, *Acetobacter pasteurianus*, *Acetobacter polyxygenes*, *Acetobacter xylinum*, *Acetobacter malorum*, *Acetobacter pomorum*, *Candida lactis-condensi*, *Candida stellata*, *Hanseniaspora valbyensis*, *Hanseniaspora osmophila*, *Saccharomycodes ludwigii*, *Sacch. cerevisiae*, *Zygosaccharomyces bailii*, *Zygosaccharomyces bisporus*, *Zygosaccharomyces lentus*, *Zygosaccharomyces mellis*, *Zygosaccharomyces Pseudorouxii*, *Zygosaccharomyces Rouxii*
	Pidan (duck eggs; Chinese): *B. cereus*, *B. macerans*, *Staph. cohnii*, *Staph. epidermidis*, *Staph. Haemolyticus*, *Staph. warneri*
(i) Alcoholic beverages	Wine (Grape must; Worldwide): *Saccharomyces* and non-*Saccharomyces* (so-called "wild") yeasts (e.g., *Candida colliculosa*, *C. stellata*, *Hanseniaspora uvarum*, *Kloeckera apiculata*, *Kl. thermotolerans*, *Torulaspora delbrueckii*, *Metschnikowia pulcherrima*, *Pichia fermentans*, *Schizosaccharomyces pombe*, *Hanseniaspora uvarum*); bacteria (*Oenococcus oeni*, *Lactobacillus plantatum*)
	Pulque (cactus (Agave) plant of Mexico): LAB (*Lc. lactis* subsp. *lactis*, *Lb. acetotolerans*, *Lb. acidophilus*, *Lb. hilgardii*, *Lb. kefir*, *Lb. plantarum*, *Leuc. citreum*, *Leuc. kimchi*, *Leuc. mesenteroides*, *Leuc. pseudomesenteroides*), the γ-Proteobacteria (*Erwinia rhapontici*, *Enterobacter* spp., and *Acinetobacter radioresistens*, several α-Proteobacteria), *Zymomonas mobilis*, *Acetobacter malorum*, *A. pomorium*, *Microbacterium arborescens*, *Flavobacterium johnsoniae*, *Gluconobacter oxydans*, *Hafnia alvei*
	Tchoukoutou (spontaneously fermented beer) (sorghum, Benin): *S. cerevisiae*, *Candida krusei*, *Clavispora lusitaniae*, *Candida rugosa*

The microbiota associated with the fermenting matrix is in a strong relationship, in a sort of continuous dichotomy, with the acceptance of the final products: it is liable to positively and negatively affect the main quality and safety attributes. In fact, microorganisms may improve or depreciate (i) the safety of the foodstuffs, modulating the content of biological and chemical contaminants (e.g., [22]); (ii) the palatability and the aroma, releasing volatile organic compounds and influencing the taste and the texture (e.g., [23]); (iii) the nutritional quality, modifying the quantity of macro- and micro-nutrients and their digestibility and bioavailability (e.g., [24]); (iv) the presence (in quality and quantity) of biological and/or chemical entities susceptible to maximize the consumer health (out of the benefits associated to the nutritional contribute) (e.g., [1,25]). Other case studies are reported in Table 2 to better exemplify how microbes can affect the global quality of fermented foods and beverages, encompassing applications such as toxic compound degradation, texturizing properties, bio-fortifications, and addition of functional ingredients [26–29].

Table 2. Some examples about the impact of microorganisms associated with fermentations on the main aspect of global quality of fermented products.

Global Quality	Positive Effect of Fermentations	References
hygienic quality	Pyrazines biodegradation	[30]
	Toxins biodegradation	[26,31]
	Biogenic amines biodegradation	[32]
sensory quality	Flavor improvement	[33,34]
	Texturizing properties	[29,35]
nutritional quality	Vitamin bio-fortification	[4,27]
	Increased bioaccessibility of minerals	[36]
	Reduction of antinutritional properties	[37]
	Reduction of lactose	[38]
functional quality	Antioxidant activity enhancement	[35]
	Probiotic properties of selected strains	[28,39]
	Bio-fortification in microbial β-glucans	[40]
	Gluten degradation	[41]

In particular, the benefits for human health are of outstanding relevance: transformation of food constituents, biosynthesis of compounds with nutritional and/or functional importance, delivery of commensal microbes to the human gastro-intestinal tract, and delivery of probiotic strains [1]. Naturally, the presence of several possible benefits led us to focus on minimizing the risks for products safety in order to assure consumers health.

3. Risks for Human Health Associated with Fermentations

Just as for fresh or alternatively processed foods, there is a certain risk of contaminants that pose hazard to human health associated with fermented foods. We have to separate (micro) biological risks from chemical risks of microbiological origin. Cases of microbial pathogens have been reported in association with several fermented foods, such as cheese, sausages, fermented fish and fermented cereals [41]. On the other hand, we have to consider toxic by-products of microbial origin, including mycotoxins, ethyl carbamate and biogenic amines [41] (Table 3). As it is possible to notice, in only a few examples, we report different relevant pathogens (*Bacillus cereus*, *Escherichia coli*, *Salmonella* sp., *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Vibrio cholera*, *Listeria monocytogenes*, *Aeromonas*, *Klebsiella*, *Campylobacter* and *Shigella* sp.), potent neurotoxin (Ochratoxin A), and several molecules belonging to the class of biogenic amines [42–45]. All cases that testify well the relevance of risks of microbial origin associated with this considerable class of foodstuffs.

Table 3. Examples of the presence of contaminants of microbial origin hazardous for human health in fermented matrices.

Safety Issue	Evidence	Reference
Pathogens	Some fermented foods (ready-to-eat) were found positive for the presence of *Listeria monocytogenes* in a global survey on several types of food products on sale in Portugal	[42]
	The most commonly encountered pathogens in African fermented foods include *Bacillus cereus*, *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus*, *Vibrio cholera*, *Aeromonas*, *Klebsiella*, *Campylobacter* and *Shigella* sp.	[43]
	51 *doenjang* samples have been found broadly contaminated with *Bacillus cereus*; while, only one sample was positive for *Bacillus thuringiensis*. All *B. cereus* isolates from doenjang were positive for diarrheal toxin genes.	[46]
	Epidemiologic investigation linked Escherichia coli O157:H7 infection with consumption of a commercial dry-cured salami product distributed in several western U.S. states.	[47]
	An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider.	[48]
	Two commonly consumed traditional condiments (*iru* and *ogiri*) and their respective raw seeds (locust bean and melon) were found contaminated with potentially pathogenic species such as *Alcaligenes faecalis*, *Bacillus anthracis*, *Proteus mirabilis* and *Staphylococcus sciuri* subsp. *sciuri* occurred in the samples.	[49]
	Consumption of fermented raw pork sausage was associated with infection *Salmonella enterica* serovar *Bovismorbificans*.	[50]
Mycotoxin	Dietary sources of Ochratoxin A including fermented foods.	[44]
	Fermentation influences contamination of cocoa beans by Ochratoxin A.	[51]
Biogenic amines	Bioproduction of putrescine, histamine, tyramine and cadaverine in wine is a bacterial strain-dependent character.	[52]
	Bioproduction of tyramine in cheese up to considerable levels (e.g., 2010 mg/kg in Egyptian blue cheese).	[53]
	In fermented sausages, biogenic amines are mainly produced by fermentative microbial population.	[45]
Ethyl carbamate	Ethyl carbamate produced by selected yeasts and lactic acid bacteria in red wine.	[54]

Naturally, we have to remember the risk of the presence of biological, chemical and physical contaminants that do not deal with the microbiological dimension (e.g., insects, pesticides, glass sliver). However, the treatment of these categories of contaminants is out of the scope of this review, especially if we consider that their presence can be generally considered as unaffected in case of both spontaneous fermentation and inoculation with starter cultures.

Coming back to microbial associated hazards for human health, it is essential to separate (i) risks associated with microbial genera/species usually not found in association with fermented matrices, and (ii) genera/species detected in the monitoring of spontaneous fermentation. The first is the case of pathogens, while to the second class often belong to producers of mycotoxins, ethyl carbamate and biogenic amines. Hence, the second class is more insidious, considering that also in the same species we can find strains of protechnological interest and strains liable to produce toxic compounds. With this concern, in Table 4, it is possible to find a (non-exhaustive) list of microbial species associated with fermented matrices (in accordance with data reported in Table 1), for which the selection of strains liable to produce compounds toxic (to different extents) for human health has been reported in literature.

Table 4. Presence of at least one strain belonging to the genera/species reported in Table 1 (thus associated with fermented matrices) and for which has been reported in literature a concern of safety nature.

Safety Issue	Strain	Reference
MIC [1]	*Aspergillus* sp.	[55]
MIC	*Cephalosporium* sp.	[56]
BA [2]	*Enterococcus durans*	[57]
BA	*Enterococcus faecalis*	[57]
BA	*Enterococcus faecium*	[58]
MIC	*Fusarium* sp.	[55]
BA	*Issatchenkia terricola*	[59]
BA	*Lactobacillus buchneri*	[60]
BA	*Lactobacillus brevis*	[60]
BA	*Lactobacillus curvatus*	[61]
BA	*Lactobacillus hilgardii*	[60]
EC [3]	*Lactobacillus hilgardii*	[62]
BA	*Lactobacillus mali*	[60]
BA	*Lactobacillus plantarum*	[63]
BA	*Lactobacillus reuteri*	[61]
BA	*Leuconostoc mesenteroides*	[60]
BA	*Metschnikowia pulcherrima*	[59]
BA	*Micrococcus spp.*	[61]
BA	*Oenococcus oeni*	[60]
MIC	*Penicillium* sp.	[55]
BA	*Pichia manshurica*	[59]
BA	*Staphylococcus carnosus*	[61]

[1] mycotoxins, [2] biogenic amines, [3] ethyl carbamate precursors.

Moreover, we have to consider that not all the species reported by Tamang et al. [16] (e.g., those reported in Table 1) in association with worldwide food fermentations are (i) recognized as safe for human use in the framework of the principal national legislative frameworks (e.g., Generally Recognized as Safe (GRAS) by U.S. Food and Drug Administration; Qualified Presumption of Safety (QPS) by the European Food Safety Authority) [64], and/or (ii) included in the "inventory of microbial species used in food fermentations" proposed by the European Food and Feed Cultures Association ([65]). Moreover, from a biological point of view, we have to stress two other crucial concerns that deal with the safety of strains associated with food/beverages fermentations: (i) the occurrence of virulence traits; and (ii) the transfer of antibiotic resistance determinants [64,66,67].

All the mentioned aspects underline the presence of a situation that is particularly complex considering that in the case of fermented food a microbial development is desired, and thus is more difficult to limit the multiplication of undesired microbes. In addition, we have to consider recent tendencies such as the coming back to "natural" processing that in some cases increase food safety risks of microbial origin. It was the case of *E. coli* O157:H7 organisms found to survive for 20 days in unpreserved refrigerated apple cider artisanally produced (apples were not washed, cider was not pasteurized, and no preservatives were added) [48]. Finally, we have to underline that in specific clinical cases, also food-delivering microorganisms generally recognized as safe, such as lactic acid bacteria of protechnological interest and/or probiotic strains, can cause illness [68].

4. Spontaneous Versus Induced Fermentation: Starter Cultures, Scientific Evidence and Actual Trends

From an historical perspective, the management of microbial resources performed for millennia, without any awareness of existence of microscopic organisms, can be summarized as the inoculation of the raw material with a small amount of matrix from a previous successful fermentation [69]. In other terms, this practice foresees the use the microbiota that had demonstrated efficiency the day before as inoculum for the new production. This management has been declined in the different production domains and using different languages, for example: "back-slopping" (sourdough preparation), "*sieroinnesto*" and "*lattoinnesto*" (dairy production), "*pied de cuve*" (enological productions), "inoculum enrichment". The shifting from this empirical regimen to the modern microbial cultures framework got through the discovery of microbes and the advent of microbiological disciplines, that in several cases started with studies and applications in the field of food microbiology (e.g., Pasteur′s "*Études sur le vin*"; Emil Christian Hansen′s pure-culturing techniques applied to yeasts for beer production) [69]. The use of starter cultures coincides with the industrialization of fermented productions, satisfying the exigencies of standardization associated with modern large-scale fermentations. In Table 5, we propose some recent definitions reported in the literature.

Table 5. A selection of definitions for "starter cultures" reported in recent scientific publications.

	Definition	Reference
Starter cultures	"Starter cultures" are preparations of live microorganisms or their resting forms, whose metabolic activity has desired effects in the fermentation substrate, the food. The preparations may contain unavoidable residues from the culture substrate and additives that support the vitality and technological functionality of the microorganisms (such as antifreeze or antioxidant compounds).	[70]
Microbial food cultures	"Microbial food cultures" (MFC) are live bacteria, yeasts or molds used in food production. MFC preparations are formulations, consisting of one or more microbial species and/or strains, including media components carried over from the fermentation and components which are necessary for their survival, storage, standardization, and to facilitate their application in the food production process.	[65]
Commercial starter cultures	"Commercial starter cultures" are standardized inoculum to be used for the production of fermented foods. Starter cultures are produced by specialized manufactures. Rigorous quality assurance and quality control are conducted to ensure performance, composition, and safety of the culture.	[71]

Various scientific evidence on the comparison of spontaneous versus inoculated fermentation processes (e.g., those reported in Table 6) testified the crucial importance of the starter culture technology to assure food safety worldwide, at all levels of fermented food production: household, traditional, and industrial. This desired affect is achieved by means of different biological activities such as faster acidification activity, domination of the indigenous microflora, reduction of fermentation time and reduction of undesired microbial strains/species and toxic compounds [72–75] (Table 6).

Table 6. Selected scientific evidences concerning the effect of starter cultures inoculation in comparison with spontaneous fermentation.

Matrix	Evidences	Reference
Table olives	Selected lactobacilli and yeast showed a fast acidification of brine. Olives inoculated with lactobacilli and yeast showed the lowest biodiversity and the highest appreciation for both texture profile analysis and sensory evaluation	[72]
	Inoculation of brine medium with lactic acid bacteria starters significantly influenced aroma profiles	[76]
	Autochthonous starter produced same sensory quality as natural traditional table olives in a shorter time	[77]
Fermented leek kimchi.	Leeks fermented with *Weissella confusa* LK4 showed the highest radical scavenging effects and reducing ability. Total flavonoid and poly-phenolic contents changed during fermentation and showed correlation with anti-oxidant effects	[78]
Soybean fermented product	A reduction of biogenic amines and aflatoxins has been reported in Doenjang samples fermented with various Meju as starter cultures	[73]

Table 6. *Cont.*

Matrix	Evidences	Reference
Dry-cured foal sausage	The inclusion of starter cultures contributes to improve the hygienic quality (stronger acidification and reduced count of *Enterobacteriaceae*) of foal sausages without significant effect on lipolysis, texture and appearance	[79]
Chinese fermented dry sausages	Nitrite content of all inoculated sausages declined rapidly during ripening compared to non-inoculated	[74]
Fermented sausage	Selected starter cultures improve quality, safety and sensorial properties of Dacia sausage, a traditional Romanian dry-sausage variety	[80]
	Evidences indicated that the selected *Lactobacillus plantarum* strain had a strong effect on inhibiting the production of biogenic amines	[81]
	Starter cultures minimize the formation of biogenic amines during the process of Nham fermentation	[82]
Thai fermented shrimp (Kung-Som)	Starter culture enhance GABA content, improved microbiological safety (dominated the total microflora) as well as reduced fermentation time	[75]
Wine	In cellars where biogenic amines are usually high, repeated experiments showed that in inoculated wines, biogenic amine concentrations were very low, while uninoculated control wines contained all the usual amines	[83]
	The use of a selected malolactic starter resulted in reductions in biogenic amines concentrations in wine produced by started malolactic fermentation compared with wine produced by spontaneous malolactic fermentation	[84]

Certainly, it is mandatory to underline that starter culture technology is not effective per se. The effectiveness of each tailored starter culture on a specific aspect of the inoculated food matrices is a function of the quality of biotechnological solutions conceived to cope with a given specific real problem. In general, considering the safety assurance, it is crucial the assessment of the safety of species/strains and of the quality/purity of biomass preparation [64,85]. More in depth, the scientific demonstration of efficacy regarding the specific safety target is important. For example, Van Ba et al. [86], evaluating several starter cultures and an inoculated control in sausage fermentation, found that not all the starter cultures were effective in lowering the content of different biogenic amines (in comparison with uninoculated control). We also have to remember that starter cultures have not always been found to improve a specific safety target. That was the case of Torrea et al., who found concentrations of nonvolatile amines and phenethylamine in wines from inoculated must were superior to those of the control wine (uninoculated must) [87]. On the other hand, with the concern of strain safety, is interesting to remark on the advances allowed by integrated genome-based assessments of the safety of specific microbial biotypes (e.g., [88,89]).

Generally, the importance of the adoption of a starter culture regimen in order to minimize the risk of food-borne diseases [43] has also been confirmed by means of artificially inoculated pathogens [90].

Starter cultures exploit changes as a function of peculiarities associated with the degree of development of the different countries. In developing countries, the significance of starter cultures is strongly related to the importance of food preservation, yield increasing, and food security [91]. By contrast, in the “Western world”, starter cultures are also designed (i) to pursue personalized nutrition; (ii) to reach new health targets; and (iii) to sustainably increase shelf life, particularly of artisanal, traditional, typical, organic and biodynamic productions [64,92].

Recent social, economic and environmental trends imply a progressive return to the reliance of spontaneous fermentations in the sector of traditional, typical, and artisanal fermented foods (including Geographical Indications), but also considering organic and biodynamic productions. Increasing evidence from scientists and stakeholders from different disciplines/fields counterpoise the use of commercial starter cultures with the exploitation of spontaneous fermentation, preferring this second approach in the management of food fermentations [93]. From this point of view, the return to spontaneous fermentations represents a strategy to restore a content of tradition, typicality and artisanality already loose. The other leading idea is that the use of commercial starter cultures corresponds to a drift from the “natural” manufacture of fermented foods, with a proportion of "synthetic fertilizers" that are connected to "agricultural production" as well as "commercial microbial starter cultures" that are connected to "food fermentations" [92]. For example, winemakers are constantly searching for new techniques to modulate wine style, and the exploitation of indigenous yeasts present in grape must is re-emerging as a commercial option in several wine regions (wines made with indigenous or "wild" yeasts are perceived to be more complex by showing a greater diversity of flavors) [94,95]. It is a concept, shifting to cheese-making practice, well summarized by Piero Sardo, President of the Slow Food Foundation for Biodiversity Onlus: "With industrial starters, cheese made in the mountains will be only slightly different from cheese made in the plains [. . .]. With the industrial packets the music is already written and the cheese-maker just plays it" [64]. All these points of view are indirectly sustained by recent scientific evidence indicating the presence of a so-called "*microbial terroir*" dimension in association with specific fermented productions liable to influence perceived quality [96–100]. The recent debate on the proposal of microorganisms as a driver of sensory innovation in the gastronomy/artisanal fermented food production fields testify well to the scientific point of view about spontaneous fermentation by autochthonous microorganisms [101,102]. Relying on spontaneous uncontrolled fermentations poses serious challenges for the safety and the quality of fermented foods. In fact, spontaneous fermentation generally increases the risks of the implantation/domination of microbial strains dangerous for the human health [64]. In addition, the risk of spoiling microbial communities in food matrices is considerable.

5. How to Conciliate Fermented Food Safety with Instances of an Enhanced Contribution of Microbes Associated to Spontaneous Fermentation

In this section, we delve into the two main biotechnological solutions that could conjugate (a) safety/quality of fermented foods and (b) the adoption of a microbial regimen in food fermentations compatible with enhanced contribution of microbes associated with spontaneous fermentation: (i) the design of multi-strains starter cultures based on the selection of ecotypes from spontaneous fermentations [15,92,103] ("top-down" solution); and (ii) the application of innovative biotechnologies and microbiological methods to monitor the safety of spontaneous fermentations ("bottom-up" solution).

The design of tailored starter cultures for specific productions in such a way to mimic protechnological microbial diversity associated to spontaneous fermentation is a solution experienced worldwide (few examples in Table 7).

Table 7. Exemplificative cases of characterization of indigenous microorganisms selected from spontaneous fermentation in different Continents.

Major Groups Global Fermented Foods	Product Name	Country	Reference
(a) Fermented cereals	Ogi	Nigeria	[104]
(b) Fermented vegetables and bamboo shoots	Soidon	India	[105]
(c) Fermented legumes	Kedong sufu	China	[106]
(d) Fermented roots/tubers	Gari	Kenya	[107]
(e) Fermented milk products	Suero Costeño	Colombia	[108]
(f) Fermented and preserved meat products	Salchichón	Spain	[109]
(g) Fermented, dried and smoked fish products	Jeotgal	Korea	[110]
(h) Miscellaneous fermented products	Vinegar	Italy	[111]
(i) Alcoholic beverages	Malvar Wine	Philippines.	[112]

To better understand the dimension of the existing efforts in the design of "tailored" starter cultures for traditional, typical, and artisanal fermented foods (including geographical indications), you can find in Table 8 a non-exhaustive list of scientific works reported in the recent literature on the characterization of microbes associated with Apulian (Southern Italian region) spontaneous fermentations (carried out also to select strains of protechnological interest). Taken together, information reported in Table 7 (international interest in the topic) and Table 8 (regional interest in the topic) testifies well the dimension of a biotechnological latent potential globally characterized as a function of local productions, and that might be exploited to pursue unique sensory quality without compromising the safety of the production.

Table 8. Exemplificative cases of characterization of indigenous microorganisms selected from spontaneous fermentation in Apulian region (Southern Italy) in the last 15 years.

Fermented Foods/Beverages	Autochthonous Variety/Typical Product	Studied Microorganisms	Main Potential Impact on Food Quality	References
Bread	Sourdough for Altamura bread (bread)	Lactic acid bacteria	Sensory quality	[113]
Cheese	Canestrato Pugliese (cheese)	Lactobacilli and lactococci	Sensory quality	[114]
Cheese	Mozzarella cheese (cheese)	Lactobacilli, lactococci, streptococci and enterococci	Sensory quality	[115]
Cheese	Mozzarella cheese (cheese)	*Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus delbrueckii* subsp. *lactis*, *Streptococcus thermophilus*, *Enterococcus faecalis*, *Enterococcus durans*	Sensory quality	[116]
Table olives	Bella di Cerignola (Olives)	Yeasts	Sensory quality	[117]
Bread	Sourdough for Altamura bread (bread)	Yeasts	Functional quality	[118]
Table olives	Bella di Cerignola (Olives)	Lactic acid bacteria	Functional quality	[119]
Wine		Different yeast species	Hygienic quality	[59]
Cheese	Fior di Latte (cheese)	Lactic acid bacteria	Sensory quality	[120]
Table olives	Cellina di Nardò and Leccino (olives)	Yeasts and lactic acid bacteria	Sensory quality	[121]
Wine	Uva di Troia (grape)	*Oenococcus oeni*, *Saccharomyces cerevisiae*	Sensory quality	[122]
Wine	Negroamaro (grape)	*Hanseniaspora uvarum*, *Saccharomyces cerevisiae*	Sensory quality	[123]
Wine	Uva di Troia (grape)	*Oenococcus oeni, Lactobacillus plantarum*	Sensory quality	[124–126]
Wine	Uva di Troia (grape)	Non-*Saccharomyces* yeasts	Sensory quality	[127]
Wine	Negroamaro (grape)	Yeasts and lactic acid bacteria	Sensory quality	[128]
Wine	Uva di Troia (grape)	Yeasts	Sensory quality	[129]
Wine	Different autochthonous grape varieties	*Brettanomyces bruxellensis*	Sensory quality	[130,131]
Wine	Uva di Troia (grape)	*Lactobacillus plantarum*	Sensory quality	[132]
Wine	Uva di Troia (grape)	*Saccharomyces cerevisiae*	Hygienic quality	[133]

On the other hand, the opportunity exists to use an integrated approach of combined molecular and microbiological methods to assess the safety of the microbiota associated with spontaneous fermentation. The main example is the development and application of sequence-based molecular technologies (phylobiomics, metagenomics and metatranscriptomics) for examining the diversity and safety of indigenous microbiota associated with traditional fermented foods and beverages [134].

In addition, the combination of culture-independent and culture-dependent analysis might be used to verify the (legislative) safety standard compliance of dominant strains associated with spontaneous fermentation (e.g., adoption of a QPS approach to dominant LAB associated with Grana Padano cheese whey starters, as a proposed approach susceptible to be extended to other types of undefined-strain cultures [135]). In general, all these molecular and physiological approaches might help determine the presence of strains associated with the indigenous microbiota that could pose risks to human health (e.g., presence of genes involved in biogenic amines production and verification of the corresponding phenotype in vivo). Naturally, it is needed to take account of this importance to periodically perform these monitoring activities, considering the possible variability of microbial consortia associated with "inoculum enrichment" practices.

6. Conclusions

Fermented foods and beverages represent a worldwide category of edible products with a prominent significance for human economy, nutrition and health for millennia. A huge diversity of microorganisms has been detected in association with spontaneous fermentations all around the world. In the framework of this heterogeneous microbiota, it is possible to find microbial pathogens and/or strains liable to synthetize toxic by-products such as mycotoxins, ethyl carbamate and biogenic amines. These microbial contaminants can reduce the safety of the corresponding fermented product. Starter culture technology represents a cornerstone in the assurance of quality and the safety of fermentation. However, recent economic, productive and social trends have led to the rediscovery of the potential of spontaneous fermentation in improving the unique quality of fermented products. With this review, we remember the potential risks for human health associated with uncontrolled (uninoculated) food fermentations and we point out how modern microbial biotechnologies offer solutions to conciliate fermented food safety with instances of an enhanced contribution of microbes associated with spontaneous fermentation.

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