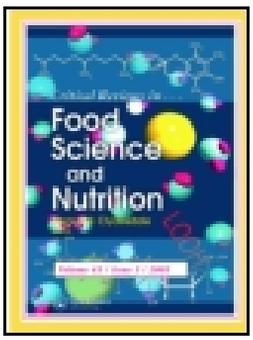

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REVIEW

Molecular techniques reveal more secrets of fermented foods

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ABSTRACT

Fermented foods were likely to have been the first among all types of processed foods consumed by human beings. The role that fermented food plays is not only related to the development of civilizations and cultural relationships between countries but also related to the nutritional importance of its population. Of course, the early manufacturers of fermented foods didn't take into account the advantages of modern sciences, because enzymes and microorganisms were discovered just 150-200 years ago. For that reason, we can conclude why the ancient fermentation techniques were known to philosophers and alchemists, but not to biologists. It demonstrated that the fermentation mechanisms involved many secrets still undiscovered. Recently, applications of molecular techniques for analyzing and study the fermented foods have been explored. In this review, we provide answers with a critical vision to many questions for understanding the role of molecular techniques to discover the secrets of fermented foods such as how to evaluate the traditional fermented foods? Why using molecular techniques to study the fermented foods not else? Is the future will carry to us a boom in molecular technologies contribute to the detection of more secrets of the fermented food?

KEYWORDS

Fermented foods; positive reflections; adverse effects; fermenting microflora; microbial characterizing approaches; molecular techniques applications

Introduction

Fermented food is a broad term which includes several concepts (Katongole 2008; Ray and Joshi 2014; Chilton, Burton, and Reid 2015; Bevilacqua et al. 2016; El Sheikha 2018b), for example:

- Fermented foods as those products that have been suboriented to the effect of microorganisms or enzymes that have been caused desirable biochemical changes.
- That food has at least one of its constituents targeted by microorganisms to get significant modifications in physicochemical, sensory and enzymatic characteristics in the final products.
- Savory and nourishing foods prepared from raw or processed materials by microbial action.
- Fermented foods as traditional foods are the best example of human innovation in the preparation of delicious food using microbes, without much of appreciation or an understanding of their underlying microbial flora, until recently.

Fermented food is one of the principal sources used to feed the populations, about one-third of the diet worldwide (Campbell-Platt 1994). These types of foods play an essential role to improve the social well-being of the people living in marginalized and vulnerable society through the provision of food "secure and safe" (El Sheikha and Montet 2014a).

It is only recently that the fermentation processes has been a development in the understanding of mechanisms and their adaptation for commercialization, even though fermentation of foods has been in use for thousands of years. There are the enormous scope and the potential for the use of microorganisms towards meeting the growing world demand for food (e.g., fermented foods), through efficient utilization of available natural food and feedstock and the transformation of waste substances (FAO 2013; El Sheikha 2018b).

Nowadays, there is an increasing focusing on fermented foods, especially the ones containing probiotics, are supposed to be healthy for the host (i.e., intestinal- and immune-related diseases). Their beneficial outcomes could be significantly enhanced by incorporating probiotic microorganisms; those have advantages for host health when consumed in a suitable viable number in food products. Probiotic products have stepped to the market and are being commercially produced under various brand names. Also, these products are legislatively obliged to be labeled for the microbial contained (El Sheikha 2018a, 2018b). Therefore, identification of their microorganisms is a cause of concern. Many culture-dependent methods have been introduced and used to distinguish the microorganisms, in which the scientists have experienced multiple obstacles and difficulties. Thereby, molecular technologies were present as an alternative, offering advantages such as accuracy, specificity, sensitivity, and speed. This review provides the answers with

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a critical vision to many questions, such as how to evaluate the traditional fermented foods? Why using molecular techniques to study the fermented foods not else? Is the future will carry to us a boom in molecular technologies contribute to the detection of more secrets of the fermented food?

The fermented food is an extraordinary ambassador of different civilizations since dawn of history

Our ancestors already consumed food products that had been subjected to food fermentation; they did not know the role of microorganisms, but they recognized the preservative and nutritional qualities of fermented foods, as well as their palatability. We do not know when humans began to use fermentation intentionally, but archaeological data suggest an intentional fermentation of honey, fruits, and rice about 10000 years ago. Moreover, we have some evidence of bread and cheese making, as well as of grape fermentation, around 6000–7000 BC (Bevilacqua et al. 2016; El Sheikha 2018b).

It is also known that food is a reflection of a people's origin, their culture and traditions. Therefore, societies that have the ability to preserve their traditional foods have thus preserved their history and cultural heritage (Botangen, Vodanovich, and Yu 2017). Traditional fermented foods are the products of biotechnological processes those are produced by taking advantages of the natural microflora associated with raw materials from plant or animal sources or by adding starter cultures that contain microorganisms. Using native knowledge of locally available, people across the globe produce this type of foods (Zulu, Dillon, and Owens 1997; Tamang 2010d; Ray, El Sheikha, and Kumar 2014). Over the years, traditional fermentation became part of the cultural norm among the indigenous communities in most developing countries (Chelule et al. 2010; El Sheikha 2018b). All around the world, traditional fermented foods are a significant part of the human diet, particularly in Latin America, Asia and Africa (LeBlanc et al. 2013; El Sheikha and Montet 2014a, 2014b; Ray, El Sheikha, and Kumar 2014; El Sheikha 2018a, 2018b). In some regions, fermented foods make up a minor 5% of daily intake, while in others their role can be as substantial as 40% (Tamang 2010d).

How to evaluate the traditional fermented foods?

This question concerns us all as consumers, producers, traders and authorities. However, we maybe do not share the same view concerning the evaluation of the fermented food products. Collectively, **Figure 1** shows the complete answer to this question from all points of view, ***which we will put a spotlight on them individually as follows.***

Consumers' acceptability point of view

The indigenous fermentation techniques making the food highly regionalized with unique characteristics. Therefore, the traditional fermented foods add pleasure and bring variety to monotonous diets through the improved organoleptic properties. The development of these properties during the fermentation makes unappealing foods more acceptable; thereby increases the consumption (Sankaran 1998).

In regards to consumers' acceptability, the advantages of traditional fermented foods are:

- Destroys undesirable sensory characteristics;
- Adds flavors and odors;
- Improves texture;
- Produces desirable colors.

Even though, several studies detailed about the beneficial effects of fermented. The overall consumers' satisfaction about fermented foods may vary on the type and composition of product, and frequency of usage. For example, the results of the survey of consumers' acceptability about consumption of fermented plant beverages in Thailand indicated that the general acceptability of fermented plant beverages consumption by the surveyed consumers was "very good" (Chaiyasut et al. 2017). **Table 1** gives some examples of sensory acceptance of fermented foods.

Nutritional point of view

The fermentation process enhances the digestibility of proteins and carbohydrates and the bioavailability of vitamins

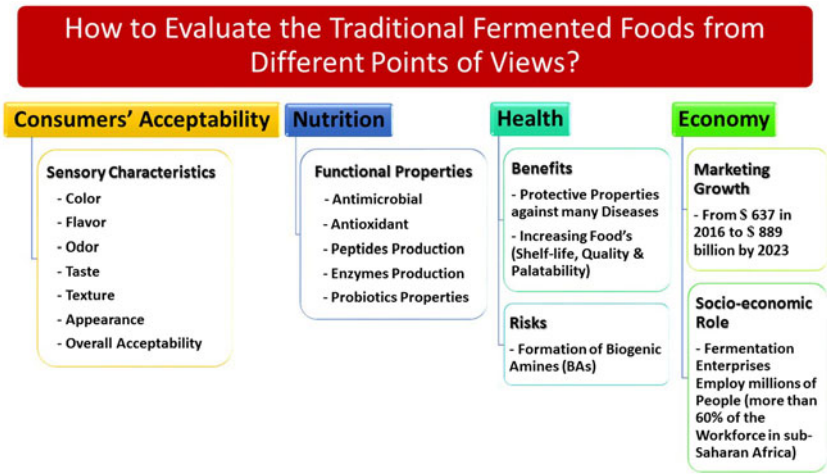

Figure 1. How to evaluate the traditional fermented foods from different points of views?

Table 1. Sensory acceptability of some fermented foods.

Type of fermented food	Sensory evaluation	References
Fermented cupuassu beverage	Lower acceptance of texture, flavor, sweetness and overall in the probiotic samples	Pereira et al. (2017)
Fermented coconut water	Moderate acceptance, presenting sour flavor and fermented odor after 48 h of fermentation	Kantachote et al. (2017)
Fermented pineapple juice	Sweetened juice received a higher preference than un-sweetened juice due to post-acidification of fermented pineapple juice	Costa et al. (2013)
Fermented olive	Mean scores of bitter, acid, hardness and crunchiness in olives fermented	Argyri et al. (2014)
Fermented emmer beverage	Fermented emmer beverage showed more acidic taste and after-taste with more intense flavor than control samples	Coda et al. (2011)
Fermented beverage made from oats, barely or malt	Mean score between 2.71 and 5.33 of consumers' acceptance in a 9 hedonic scale study	Salmerón, Keith, and Pandiella (2015)
Fermented mutton sausages	Better texture and reduced typical smell of mutton	Hoobin et al. (2013)
Dry fermented salami	Mean score of 7.04-7.34 for appearnce, flavor, texture and overall acceptance	Muthukumarasamy and holley (2006)

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and minerals (Altay et al. 2013; Hwang et al. 2017). Because of these beneficial effects, fermented foods have been an indispensable part of the human diet since ancient times and they remain important in many developing countries where are an integral part of local cultures and traditions (Borresen et al. 2012; Chilton, Burton, and Reid 2015; Ansorena and Astiasarán 2016; Kanwar and Keshani 2016; Narzary et al. 2016). The rural folk has come to prefer fermented over the unfermented foods. As one of the main pillars of the fermentation process, microorganisms play a pivotal role in transforming raw materials into edible products (nutritionally) acceptable to both the consumers and producers. For example, the fermentation process of tempe increased its content of vitamins such as niacin and riboflavin by using the starter culture *Rhizopus oligosporus* (Tamang 2010a; Astuti 2015). Similarly, in many *Bacillus*-fermented Asian fermented foods, riboflavin and niacin contents are increased (Sarkar et al. 1998; Kim and Hahm 2002; Nagai 2015). In addition, some fermentation microbes are responsible for degrading the anti-nutritive substances and thereby converting them into consumable products (Nout 1994; Tamang 2015). This is what happens during fermentation of idli (a fermented cereal food of India), whereas the phytic acid content is reduced (Reddy and Salunkhe 1980) that leads to increase the bioavailability of minerals, proteins and sugars (Sripriya, Antony, and Chandra 1997; Chelule et al. 2010; El Sheikha 2018a).

Fermented foods as functional foods

Indigenous fermented foods have unique functional properties imparting some benefits to consumers that possess antimicrobial, antioxidant, peptides production, enzymes production, probiotics properties, etc. (Tamang et al. 2016). One of the implications of the fermentation process on the quality of the food product is to increase the concentration of antimicrobial agents like bacteriocins, hydrogen peroxide and organic acids (El Sheikha 2018b). In addition, the concentration of total polyphenolic compounds and antioxidant activity was highest for fermented pomegranate juice who is responsible for inhibiting the growth of K562 tumor cells (Filannino et al. 2013). Additionally, the acidic medium provided by the fermentation process as well as the prepared thermal range (22–25 °C) are the catalysts to increase the productivity and activity of microbial enzymes (Mokoena, Chelule, and Gqaleni 2005), which include amylases, proteases, phytases and lipases, modify the primary food products through hydrolysis of polysaccharides, proteins, phytates and lipids respectively (Demirci, Izmirlioglu, and Ercan 2014). Also, bioactive peptides are formed during food fermentation by microbial enzymatic hydrolysis of proteins, which claim to have functional properties (Nagai and Tamang 2010). Furthermore, the fermented foods products are the most traditional source of probiotic microorganisms (Ray, El Sheikha, and Kumar 2014, Tamang et al. 2016). As reported by several studies, the beneficial nutritional effects of probiotic foods are constantly increasing (de LeBlanc, Matar, and Perdigón 2007; Monteagudo-Mera et al. 2012).

Healthy point of view

Ethnic foods including fermented ones have in-built systems both as food to meet up hungry and medication cures many diseases (Shin and Jeong 2015; Thapa and Tamang 2015).

Fermentation as food guard

Fermented foods achieved a promising record in terms of food safety even in the countries where household practices are common to produce the traditional fermented foods (Quevedo 1997; Yigzaw et al. 2004). Fermentation can enhance food safety through inhibition of pathogenic microbes' growth, toxin degradation and improve the shelf-life and digestibility of raw foods (Holzapfel, Geisen, and Schillinger 1995; Motarjemi 2002; Holzapfel 2002). Whereas, the main aim of lactic fermentation is to preserve food, increase its shelf-life and improve food quality and palatability (Ray and Sivakumar 2009; El Sheikha 2018a). Chiu et al. (2008) demonstrated that the antagonistic effect of *Lactobacillus plantarum* and *Pediococcus pentosaceus* strains (isolated from various pickled vegetables) against Salmonella invasion. Generally, food is susceptible to many sources of toxin contamination either naturally or through microorganisms (fungi, bacteria, viruses), e.g., fungal toxins (mycotoxins). It has been proved beyond a shadow of a doubt that LAB fermentation can completely eliminate the mycotoxins. The

detoxifying effect is believed to be through toxin binding (Haskard et al. 2001; El-Nezami et al. 2002; Turbic, Ahokas, and Haskard 2002; El Sheikha 2018b).

Fermented food as a protector from diseases

Recent human clinical studies on fermented foods support this possibility (Marco et al. 2017). Fermented foods exhibit protective properties against many diseases like hypertension, heart diseases (Anderson 2003), diabetes, osteoporosis (Yanagisawa and Sumi 2005; Shin et al. 2011; Tolhurst et al. 2012), alleviation of lactose malabsorption (Shah, da Cruz, and Faria 2013), obesity, allergies and atherosclerosis (Tamang and Kailasapathy, 2010). Moreover, the consumption of fermented foods reducing the blood cholesterol levels (Şanlier, Gökcen, and Sezgin 2017) and fighting carcinogenesis (Chandan and Kilara 2013; Mohania et al. 2013; Kwak et al. 2014).

Is the consumption of fermented foods risky for our health?

Taking the fermentation process out of control may pose health threats (Cocolin et al. 2016). Microbial decarboxylation of amino acids results in the formation of biogenic amines that can be found in fermented foods, e.g., sauerkraut, cheese, wine, beer, etc. (Halász et al. 1994; Suzzi and Gardini 2003; Spano et al. 2010; Visciano et al. 2014). The occurrence and hazard levels of biogenic amines, such as histamine and tyramine, in the fermented vegetable matrix, is becoming an economic problem directly linked to the influence of these compounds on health, i.e., can be toxic (García-Ruiz et al. 2011; Marcobal et al. 2012; Sahu, Panda, and Paramithiotis 2016). A maximum limit of 100 mg/kg of histamine in food indicates a safe level of consumption (Halász et al. 1994). Lee at al. (2012) reported that kimchi and soybean pastes are risk factors of gastric cancer and that salt or some chemicals within these foods would play important roles in the carcinogenesis of gastric cancer.

Numerous research papers regarding traditional fermented food products highlighted that positive effects are much stronger than a possibility of risks determined by epidemiological studies.

Economic point of view

Consumers are showing great interests towards products made from fermented ingredients since the 1970s because they consider these foods to be healthy and natural (Giraffa 2004). The fermented foods market is expected to grow from $636.89 billion in the year 2016 to $888.76 by 2023, registering more than 7% CAGR in terms of value from 2017 to 2023 (BISRESEARCH 2017; Persistence Market Research 2017). Increasing request for probiotic products, coupled with a rising preference for nutrition-rich food, are expected to be among key factors driving the growth of global fermented food products market (Persistence Market Research 2017).

Socio-economic role of traditional fermented foods

It should be noted that the indigenous fermentation techniques underpin cultural sustainability and also facilitating the development of nutritious foods which can not only cope with the difficult environmental conditions, e.g., Himalaya areas (Roy et al. 2004). Fermentation as a project promotes the preservation of cultural traditions related to food security in many countries. Hence, traditional fermented food products play a significant socio-economic role in the developing world (Chelule et al. 2010; El Sheikha et al. 2014; El Sheikha and Montet 2014a, 2014b). Fermentation enterprises that are considered to be industrial setups process, prepare, package, market and in some cases brand products and employ many millions of people, especially in traditional fermentation enterprises. For example, in sub-Saharan Africa, more than 60% of the workforce is employed in the small-scale food processing sector (Marshall and Mejia 2011).

Microbial analyzing techniques used in fermented foods

Fermenting microbial flora

The indigenous natural fermentation takes place in a mixed colony of microorganisms such as bacteria, yeasts and molds (Antony and Chandra 1997).

In many fermented foods and beverages, mainly lactic ones, lactic acid bacteria (LAB) genera are widely present including *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus*, *Alkalibacterium*, *Aerococcus*, *Carnobacterium*, *Enterococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* (Stiles and Holzapfel 1997; Blandino et al. 2003; Salminen, Wright, and Ouwehand 2004; Tamang 2010c; Axelsson et al. 2012; El Sheikha and Montet 2014a; Holzapfel and Wood 2014; El Sheikha 2018a).

As for yeast species isolated from fermented foods, they will not be excluded from the following species: *Saccharomyces, Debaryomyces, Dekkera, Galactomyces, Hansenula, Brettanomyces, Candida, Kluyveromyces, Metschnikowia, Pichia*, *Rhodotorula*, *Torulopsis*, *Hanseniaspora*, *Hyphopichia*, *Trichosporon*, *Yarrowia*, *Metschnikowia*, *Saccharomycodes*, *Saccharomycopsis*, *Schizosaccharomyces*, *Sporobolomyces*, *Torulaspora*, *Geotrichium Issatchenkia*, *Kazachstania*, *Cryptococcus* and *Zygosaccharomyces* (Wouters et al. 2002; Omemu, Oyewole, and Bankole 2007; Watanabe et al. 2008; Tamang and Fleet 2009; Lv et al. 2013; El Sheikha and Montet 2014a; El Sheikha 2015).

Regarding molds, the main role of the filamentous molds in fermented foods is the production of enzymes and the degradation of anti-nutritive factors, which used in fermented dairy products and alcoholic beverages (Aidoo and Nout 2010). As follows the molds that are reported for several fermented foods: *Penicillium*, *Rhizopus*, *Aspergillus*, *Actinomucor*, *Amylomyces*, *Monascus*, *Mucor*, *Neurospora*, *Parcilomyces* and *Ustilago* (Steinkraus 1998; Nout and Aidoo 2002; Wouters et al. 2002; Varga et al. 2005; Chen, Wu, and Xu 2014).

Emergency needs to characterize the fermenting microflora by molecular techniques

Why do we need to characterize the fermenting microbes? This is a very important question and its importance stems from the pivotal role of the fermenting microorganisms that play in the improvement of both food quality (through the development of physicochemical and sensory properties) and also food safety (by limiting the growth of pathogenic and spoilage microorganisms) (Caplice and Fitzgerald 1999; El Sheikha 2018b). Hence, it is essential to be able to characterize the fermenting microbes, in order to improve quality and safety controls of the end products. Used for this purpose numerous methods are either traditional or molecular. Where traditional or descriptive culture methods (phenotypic techniques) have been used, and remain the most employed to determine the presence/absence of colonies (i.e., cultivable cells) and their numbers (Davey 2011). Traditional biochemical and physiological methods have some limitations in the differentiation of a vast number of microbial isolates showing similar physiological characteristics (Berthier and Ehrlich 1999). In contrast, molecular methods (genotypic techniques) provide a very delicate way to explore the microbial diversity and discover the dynamics of microbial communities (El Sheikha 2018a). The emergence of molecular techniques has opened new opportunities to characterize the microbiota in fermented products at molecular levels, which are now being targeted through the quantification of biomarkers, rather than just growth/no growth quantifications (Sieuwerts et al. 2008; de Vos 2011; El Sheikha 2018a). The advantages and drawbacks of molecular identification methods of the microorganisms associated with fermented foods are given in **Table 2**.

Approaches used for characterizing the microbiota in fermented products

Several and different techniques have been applied for microbial analyzing of fermented food products which are mainly grouped into two categories: Phenotypic and genotypic (molecular) techniques. Molecular methods encompass proteomics, metabolomics and genomics-based technologies. These approaches have been illustrated in **Figure 2**. Molecular ones will be discussed in more details later based on their applications to fermented food groups analysis, i.e., cereal, root and tuber-based fermented foods, fermented fruits and vegetables, fermented dairy products, fermented meat products, fermented fish products, and alcoholic and non-alcoholic beverages.

What are the advantages of molecular methods?

There is a significant problem in assessing the microbial diversity of fermented food. It is often difficult to cultivate viable microbes in vitro using known media, as part of them might not be cultivable. Also, some species are outcompeted by numerically more abundant microbial species (Dolci et al. 2015). Moreover, traditional biochemical and physiological methods have some limitations in the differentiation of a vast number of isolates showing similar physiological characteristics (Berthier and Ehrlich 1999). In contrast, molecular methods provide a very delicate way to explore the microbial diversity and discover the dynamics of microbial communities (El Sheikha 2018a).

The evaluation of microbiota isolated from fermented food matrices by both phenotypic and genotypic is still occupied the great importance. However, the selection of new starter cultures with a desirable competitive ability and metabolic properties is of paramount importance and starts from the screening of hundreds of isolates obtained from fermented food. This has led to the development of molecular-based methods for strain identification.

Procedural mechanisms of molecular techniques commonly used in fermented foods

Simply, the molecular approaches provide efficient solutions for microbial identification in fermented foods of which for example nucleic acid probe, species-specific PCR, Rep-PCR, multiplex PCR, 16S rDNA sequencing, DGGE, and TTGE are used to analyze the microbial flora of fermented food products (Torriani, Felis, and Dellaglio 2001; Elegado et al. 2004; Kim and and Chun, 2005; Miyamoto et al. 2005; Pulido et al. 2005; Abriouel et al. 2008; di Cagno et al. 2008; Panagou et al. 2008; Cho et al. 2009; de Bellis et al. 2010; Paramithiotis, Hondrodimou, and Drosinos 2010; Botta and Cocolin 2012; Sulistiani et al. 2014). Moreover, DNA restriction fragment analysis (RFLP, RAPD), ribotyping, pulsed-field gel electrophoresis (PFGE) and Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used as primary molecular tools (Nguyen et al. 2013; El Sheikha 2018a). Comparison of procedural steps for each molecular technique provides a better understanding of their principles (**Figure 3**).

Molecular techniques applications used for cereal, root and tuber-based fermented foods

Fermented rice is a common food in most Asian countries where fermentation is done either by using mixed-cultures into alcoholic beverages or by using food beverages (Tamang 2010b). As for the fermentation of most other grains such as wheat, rye and barley and corn, it is either natural fermentation or by adding the commercial baker's yeast to the dough, and this is the practice in Europe, America and Australia (Guyot 2010). Fermented cereal foods are traditionally used as staples, complementary and weaning foods for infants and young children in Africa (Tou et al. 2007).

Cassava root (*Manihot esculenta*) is traditionally fermented staple foods such as fufu of Togo, Burkina Faso, Benin, Nigeria and Ghana, gari of Nigeria, agbelima of Ghana, chikawgue of Zaire, kivunde of Tanzania, and kocho of Ethiopia (Franz et al. 2014). Also fermented cassava root is also traditionally used into a sweet dessert such as tapé in Indonesia (Ardhana and Fleet 1989).

Table 2. Advantages and drawbacks of different microbial analyzing molecular techniques used in fermented foods.

Molecular approach	Advantages	Drawbacks
Proteomics techniques		
RP-HPLC	• Rapid detection • Accurate • High sensitivity • Tolerable cost	• High consume of solvents and reagents • Complex sample preparation • Labor intensive • Often require statistical analysis
MALDI-TOF	• Accurate and sensitive • High throughput • Fast	• High initial cost of the MALDI-TOF equipment
SDS-PAGE	• Simple and easy • Sensitive • Less-costly	• Degradation profile of peptide marker • Need reference sample preparation • Non-quantitative
2-DE	• Simplistic • Robust	• Involve large amount of sample • Low throughput • High inter-gel variability • Poor recovery of hydrophobic proteins
Metabolomics techniques		
FTIR	• Relatively fast and simple to use: • Sensitive method that requires very little sample • Non-destructive • Universal method • Qualitative as well as quantitative analysis • Relatively less expensive	• Environmental conditions around the FT-IR instrument can cause variations in the spectra • Complex samples like mixtures of microbiota produce overlapping spectra • A complete library of spectra is recommended to facilitate detection • Require standardization, rigorous data collection, and expertise
Genomics techniques		
I. Culture dependent techniques		
Ribotyping	• High discrimination level • Easy interpretation • High reproducibility • Automated platforms available	• Cumbersome • Time-consuming • Expensive
ARDRA	• Rapidity • High reproducibility • Easy to use and interpret • Low or moderate costs	• Lesser discriminatory power than ribotyping, RAPD and PFGE
AFLP	• Safe, efficient, convenient • Good reproducible • Little DNA template	• High cost • High requirements of DNA quality and enzyme purity
RFLP	• Mature technology • Low detection limit • Good repeatability	• Vulnerable to individuals mutations and false negative
PFGE	• High discriminatory power • High repeatability	• Time and labor intensive • Strain-dependent specificity
MLST	• Differentiation of highly related genotypes • Excellent discrimination	• Requirement for specialized equipment • Trained personnel • Expensive reagents
RAPD	• Fast, easy to operate • Little sample	• Poor stability and repeatability • Method not standardized • Less information
AP-PCR	• Ease of its application • No time consuming	• No standardization
Rep-PCR	• Ease of its application • Good for studying a large number of isolates • High capacity for differentiation	• Less discrimination power than PFGE
II. Culture independent techniques		
ARISA	• Inexpensive • High detection capacity for highly complex samples	• It seemed to underestimate sample richness
DGGE/ TGGE	• Rapid estimation of diversity • Clearly represent the evolution and dynamics of microbial populations in fermentation and spoilage processes • Simultaneous analysis of multiple samples • Low-cost equipment	• Similar migration characteristics of heterologous sequences • Overestimation of microbial diversity because of heteroduplexes
T-RFLP	• Determination of suitable differences in genotypes • Comparative community analysis • Good sensitivity • High-throughput	• The sequences must be known for enzyme selection • The same length of the fragment for many species • Qualitative • Require clone library for identification • Expensive equipment
qPCR	• Highly stable DNA biomarkers • Highly sensitive and can be used with degraded sample • Robust, reproducible and efficient	• Expensive and needs expertise
FCM	• Investigation of samples quantitatively and qualitatively • Estimation of viability of microorganisms	• Labor intensive of species level

(*continued*)

Table 2. Continued.

Molecular approach	Advantages	Drawbacks
FISH	• Visualization, identification, enumeration and localization of individual microbial cells	• Require probe design • Labor intensive • Unable to study simultaneously large numbers of different targets
Pyrosequencing	• High sensitivity • Exhaustive profiling of microbial communities due to the sequencing of thousands to billions of raw DNA fragment reads • Enables phylogeny-based diversity	• Need to develop new bioinformatic algorithms to manage large amounts of data
LH-PCR	• Gives a broad view of the dynamics of the whole community of microbial cells	• Low sensitivity • Biases inherent to the PCR process
SSCP-PCR	• No need for gradient gels • Performed using an automated sequencer	• Dependent on the availability of a reliable database

Acronyms legend: RP-HPLC: Reversed phase-high performance liquid chromatography; **MALDI-TOF:** Matrix-assisted laser desorption ionization time-of-flight; **SDS-PAGE:** Sodium dodecyl sulfate polyacrylamide gel electrophoresis; **2-DE:** Two-dimensional gel electrophoresis; **FTIR:** Fourier tansform infrared; **ARDRA:** Amplification Ribosomal DNA Restriction Analysis; **AFLP:** Amplified fragment length polymorphism; **RFLP:** Restriction fragment length polymorphism; **PFGE:** Pulsed field gel electrophoresis; **MLST:** Multi-locus sequence typing; **RAPD:** Random amplified polymorphic DNA; **AP-PCR:** Arbitrarily primed-polymerase chain reaction; **Rep-PCR:** Repetitive extragenic palindromic-polymerase chain reaction; **ARISA:** Automated ribosomal intergenic spacer analysis; **DGGE/ TGGE:** Denaturing/ Temperature gradient gel electrophoresis; **T-RFLP:** Terminal-restriction fragment length polymorphism; **qPCR:** Quantitative polymerase chain reaction; **FCM:** Flow cytometry; **FISH:** Fluorescent *in situ* hybridization; **LH-PCR:** Length heterogeneity-polymerase chain reaction; **SSCP-PCR:** Single-stranded conformation polymorphism-polymerase chain reaction.

***Sources*:** Giraffa and Carminati (2008), Davis and Mauer (2010), Dolci et al. (2015), Vieira et al. (2017), El Sheikha (2018a), El Sheikha and Xu (2018).

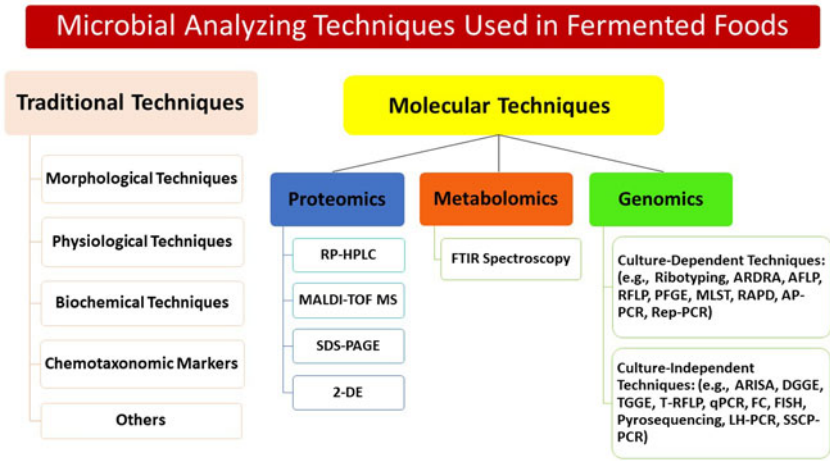


Figure 2. Microbial analyzing techniques used in fermented foods.

To amplify the growth of a selected freeze-dried LAB starter culture during cassava fermentation for gari production, many molecular fingerprinting techniques were used like random amplified polymorphic DNA with polymerase chain reaction (RAPD-PCR) and pulsed-field gel electrophoresis (PFGE) (Huch et al. 2008; Oguntoyinbo and Dodd 2010). Also, Abriouel and others (2007) have conducted the studies to evaluate different DNA-based methods [Repetitive element PCR analysis (Rep-PCR), 16S rDNA sequencing, multiplex real-time PCR) to characterize *Bacillus cereus* group isolates from two traditional cereal-based fermented foods of Burkina Faso and Republic of Congo, i.e., poto poto and dégué respectively.

Effective tools of next-generation sequencing (NGS) such as metagenomics, phylobiomics and metatranscriptomics are nowadays applied for documentation of cultures in traditionally fermented products (Mozzi et al. 2013; van Hijum, Vaughan, and Vogel 2013). Interestingly, the first study suggesting metagenomic approaches investigated microbial diversity in poto poto, a maize dough used as weaning food, and in dégué, a millet-based paste, by PCR-TGGE and by comparing DNA extraction techniques (Abriouel et al. 2006). To provide information on microbial communities of different fermented foods, it has been applied metagenomics by using parallel pyrosequencing of tagged 16S rRNA gene amplicons as profiled in nukadoko, a fermented rice bran of Japan (Sakamoto et al. 2011), cassava gari production (Oguntoyinbo and Dodd 2010), ben-saalga, a traditional gruel of pearl millet of Burkina Faso (Humblot and Guyot 2009) and ogi, a fermented cereal pudding from West Africa, typically made from maize, sorghum, or millet (Adeyemo and Onilude 2014).

Identification of sourdough microflora using molecular methods

Sourdough is a mixture of wheat and/or rye flour and water, that is fermented with a combination of lactic acid bacteria (LAB) and yeasts, and which is used as inoculum for the production of sourdough bread and other cereal baked products (Gobbetti 1998; Vogel et al. 1999, 2011; Brandt 2007; de Vuyst et al. 2009).

Molecular methods applicable to sourdough LAB include plasmid profiling, the analyses of fragment length polymorphisms

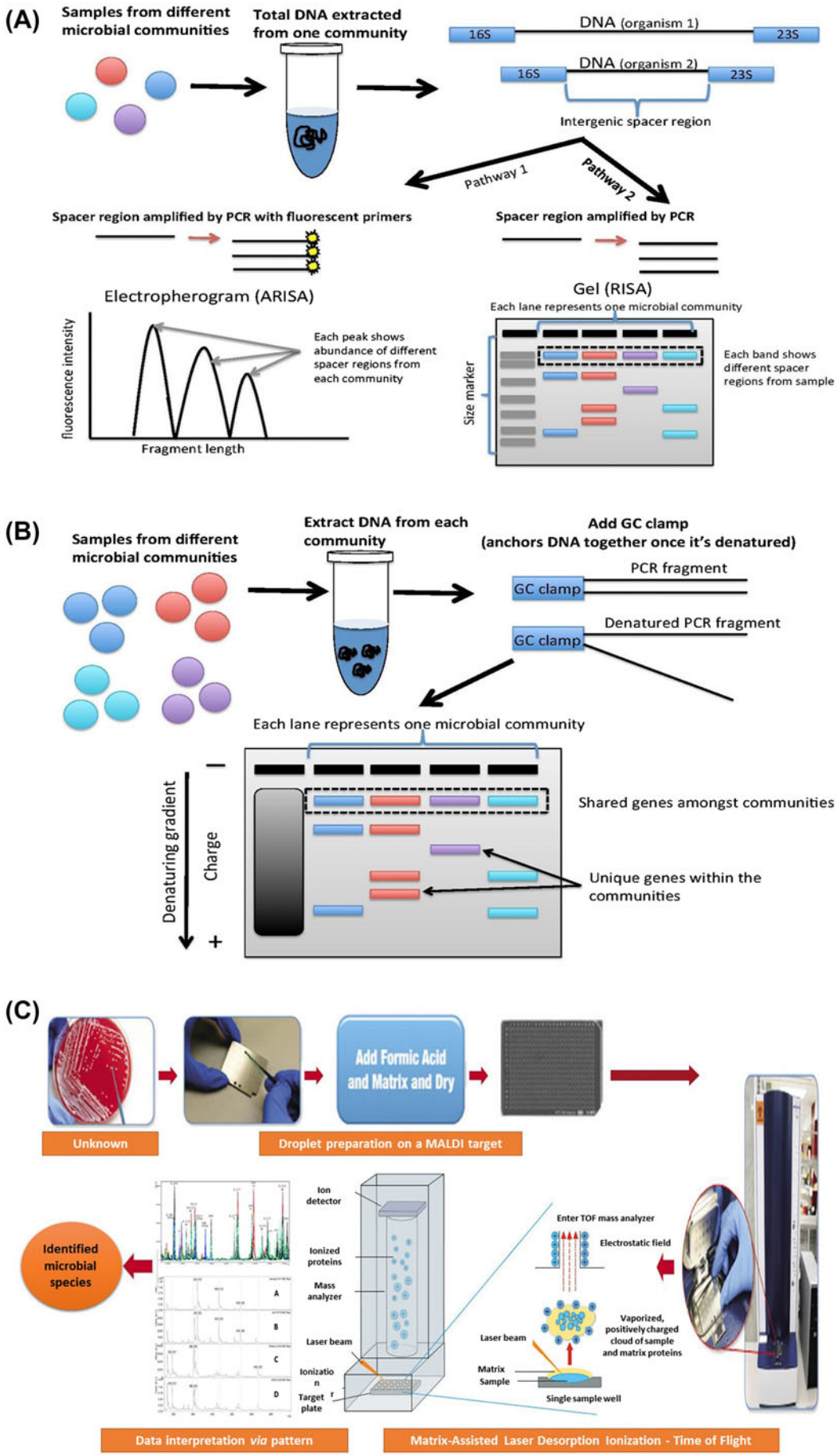
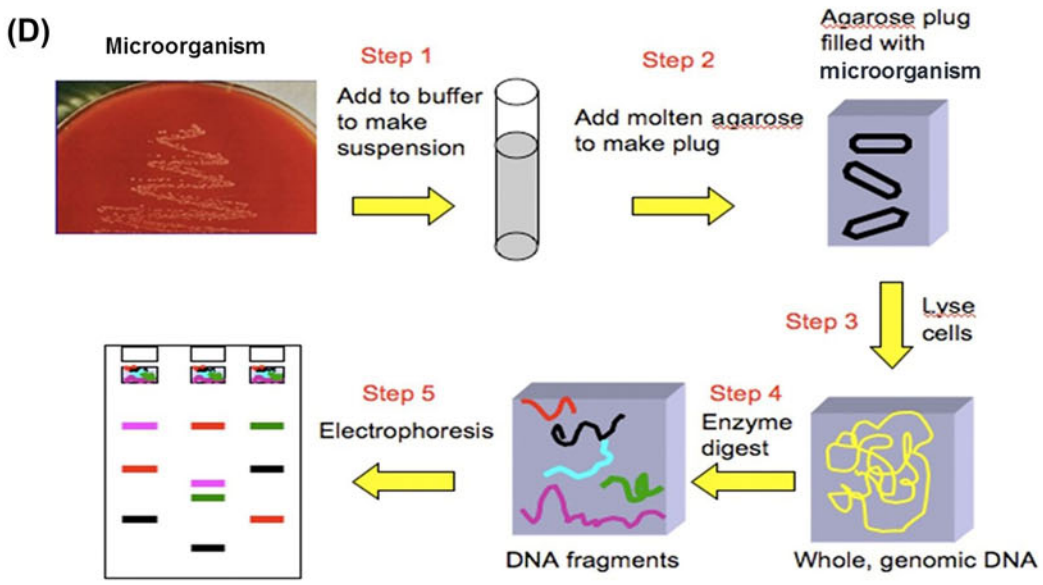
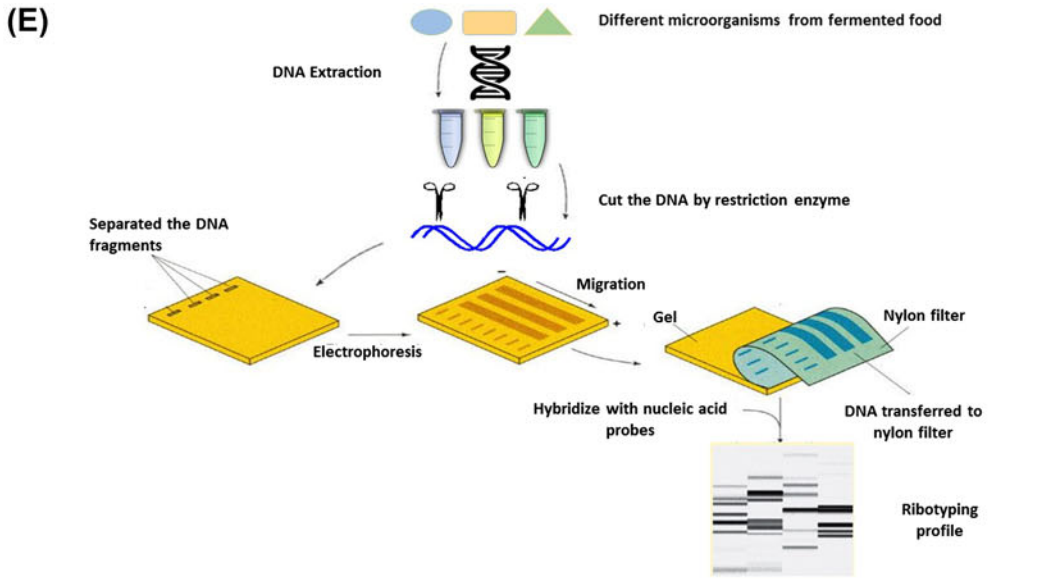


Figure 3. Procedural flowcharts for the molecular methods commonly used in fermented foods. (A): ARISA; (B): DGGE; (C): MALDI-TOF MS; (D): PFGE; (E): Ribotyping; (F): RAPD; (G): rep-PCR; (H): DNA Sequencing. Licensed under CC BY-SA 3.0 and Reproduced with permission of John Wiley & Sons.





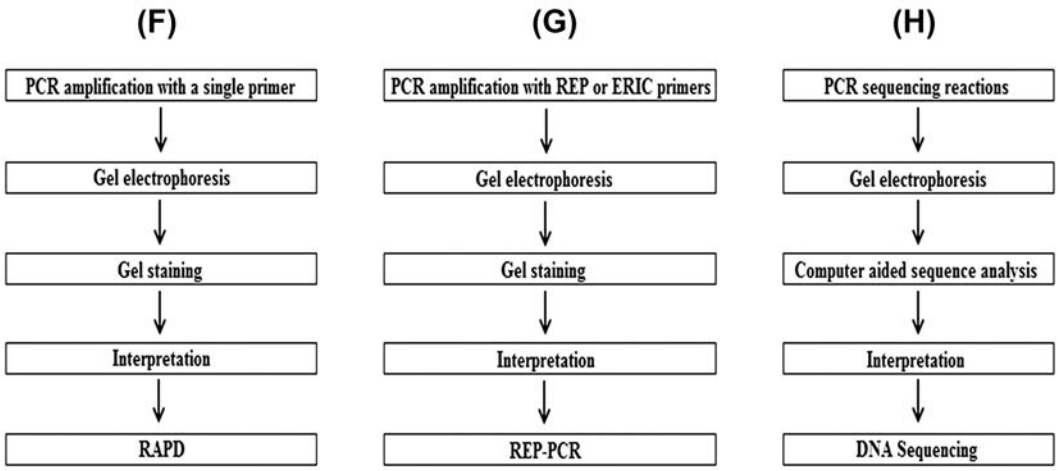


Figure 3. Continued

(AFLP, REA, RFLP, ribotyping), pulse-field gel electrophoresis (PFGE) and RAPD (Stahl and Molin 1994; Duffner and O'Connell 1995; Ahrné and Molin 1997; Kunene et al. 2000; Ehrmann and Vogel 2001; Pepe et al. 2004; Catzeddu et al. 2006; Vogel and Ehrmann 2008; Vogelmann and Hertel 2011).

Repetitive chromosomal elements randomly distributed in microbial genomes are the target of rep-PCR. This technique has been applied to characterize *Lactobacillus sanfranciscensis* isolates from Italian sourdoughs (De Angelis et al. 2007).

During the past two decades, denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) have received great attention for profiling microbial communities (Dolci et al. 2015). The PCR-DGGE approach used for monitoring the bacterial population dynamics in different sourdoughs fermentation processes (Meroth et al. 2003; Randazzo et al. 2005). Identification of LAB in rye sourdoughs from four bakeries with different propagation parameters was performed using plating, DGGE, and pyrosequencing of 16S rRNA gene amplicons (Viiard et al. 2016).

Species-specific PCR technique is widely applied in the identification of LAB isolated from fermented foods, e.g., French wheat sourdough (Robert, Gabriel, and Fontagné-Faucher 2009).

Recently, Kogno and colleagues (Kogno et al. 2017) used molecular methods (PCR, sequencing, and bioinformatics) for genotypic characterization of lactic acid bacteria involved in the Togolese traditional fermented cereal foods, i.e., mawè (fermented maize dough), epoma (fermented sorghum dough), emakumè (fermented dough cooked maize), kom (fermented dough baked and packaged maize), egblin (fermented dough baked and packaged maize), akpan (fermented dough baked and packaged maize) and elimawè (millet fermented dough).

Molecular methods (karyotyping, PFGE, PCR-RFLP, RAPD), as well as comparative sequence analysis, proved high potential when classifying sourdough yeasts.

Markers of interest are rRNA genes, internal transcribed spacer (ITS), elongation factor 3 (EF-3) or others (Pulvirenti et al. 2001; Pulvirenti et al. 2004; Vogel and Ehrmann 2008; Vogelmann and Hertel 2011). A fungi-specific PCR-DGGE approach was established to monitor the development of the yeast biota in fermentation processes of sourdoughs (Meroth, Hammes, and Hertel 2003).

Molecular techniques applications used for fermented vegetables and fruits

People eat plants, both domesticated and wild, preparing them according to a variety of recipes. Using the indigenous knowledge of biopreservation, the perishable and seasonal leafy vegetables, radish, cucumber and young edible tender bamboo shoots could be traditionally fermented into edible products (Watanabe et al. 2009, Savadogo et al. 2011; El Sheikha 2018a). Some fermented fruit pickles are atchara (green unripe papaya), burong mangga (green unripe mango), burong prutas (local fruits) of the Philippines, achar of India and Nepal, and so on. Ca muoi is a fermented fruit of Vietnam, and tempoyak is a fermented durian fruit of Malaysia (Tamang et al. 2015).

The applications of molecular methods to characterize the lactic acid bacteria and yeasts isolated from traditionally fermented vegetables and fruits [e.g., mesu, soidon, soibum, soijim (fermented tender bamboo shoot), gundruk (fermented and acidic vegetable), sinki (fermented radish tap root), khalpi (fermented cucumber), sayur asin (fermented mustard), kimchi (fermented vegetable), fermented table olive and probiotic table olives] by genotyping using ribotyping, RAPD-PCR, rep-PCR, species-specific PCR, 16S rRNA gene sequencing, DGGE/TTGE, multiplex PCR and DNA-DNA hybridization techniques (Breidt and Fleming 1996; Tamang et al. 2005, 2008; Abriouel et al. 2008; De Bellis et al. 2010; Botta and Cocolin 2012; Sulistiani et al. 2014).

The metagenomic approaches using pyrosequencing of 16S rRNA gene could provide information on bacterial communities as profiled in kimchi, a naturally fermented vegetable product of Korea (Jung et al. 2011; Park et al. 2012).

PFGE has been used to follow and characterize LAB communities during treated green olives inoculation (Saravanos et al. 2008) and black olives packed with different compositions of gas and storage times (Doulgeraki et al. 2012).

Also, PCR-DGGE approach carried out on Aloreña table olives during fermentation revealed higher differences in microbial diversity, i.e., yeasts, molds and LAB (Abriouel et al. 2011). The same technique followed by sequencing of the 16S rRNA gene and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to monitor changes in the bacterial microflora of kimchi and salted Chinese cabbage as the main component of kimchi (Hong et al. 2013, 2014).

A proteomics identification method based on protein profiling using matrix-assisted laser desorption ionizing-time of flight mass spectrometry (MALDI-TOF MS) has been used to identify species of *Bacillus* in bikalga and soumbala, condiments of traditional fermented legumes from Burkina Faso (Savadogo et al. 2011) and detailed description of the LAB diversity associated with the production of assorted Vietnamese fermented vegetables and soy sauce (Nguyen et al. 2013; Kuda et al. 2014).

Molecular techniques applications used for fermented dairy products

The past twenty years have seen the revivification and globalization of several traditional fermented dairy products as consumers seek novel taste experiences. This trend has been accelerated by raising awareness about the health benefits of these products. There are two approaches to fermentation in dairy products: firstly, natural fermentation approach as the oldest methods of milk processing using raw and boiled milk to ferment spontaneously. The second, the back-slopping method where a part of the previous batch of a fermented product is used to inoculate the new batch (Holzapfel 2002; Josephsen and Jespersen 2004). Cheese is considered one of the major fermented dairy

products importance nutritionally and commercially throughout the world (Chandan et al. 2006).

The diversity and dynamics of microbial populations in dairy products (e.g., cheese, yoghurt) and during manufacturing have been profiled by means of PCR-based methods, i.e., PCR-TTGE, PCR-DGGE, RAPD-PCR, PCR-RFLP, terminal restriction fragment length polymorphism (T-RFLP), single-strand conformation polymorphism (SSCP-PCR), length heterogeneity (LH-PCR), rep-PCR, quantitative PCR (qPCR), automated ribosomal intergenic spacer analysis (ARISA) by different authors (Coppola et al. 2001; Ercolini, Hill, and Dodd 2003; Rademaker et al. 2005; Belén Flòrez and Mayo 2006; Randazzo, Vaughan, and Caggia 2006; Bonetta et al. 2008; Coppola, Blaiotta, and Ercolini 2008; Dolci et al. 2008; Gala et al. 2008; Gatti et al. 2008; Nikolic et al. 2008; Alegrìa et al. 2009; Casalta et al. 2009; Mounier et al. 2009; Arteau, Labrie, and Roy 2010; Carraro et al. 2011; Arcuri et al. 2013; Rychlik et al. 2017).

By PFGE, Bouton et al. (2002) exhibited good discrimination between homo-fermentative lactobacilli, playing the role in Comté cheese ripening. Additionally, capillary zone electrophoresis (CZE) was applied to detect chemical components in fermented milk products (Ligor et al. 2008).

Fluorescence *in situ* hybridization (FISH) is a non-PCR technique based on fluorescence-labeled oligonucleotide probes targeting specific DNA sequences. The interest in this technique is based on the possibility of observing target cells within their native environment, and it is often associated with PCR-based methods for a complete overview of the product analyzed (Dolci et al. 2015). Mounier et al. (2009) studied the microbiota of Livarot cheese; FISH probes targeting the dominant yeasts present in the cheese, namely, *Candida catenulata*, *Candida intermedia*, *Geotrichum* sp. and *Yarrowia* lipolytica were designed and allowed the detection of these yeasts directly in cheese.

A new tool in the study of fermented food is represented by pyrosequencing, which allows one to sequence thousands to billions of raw DNA fragment reads in a single run, leading to a massive amount of information and to a more exhaustive profiling of microbial communities (Dolci et al. 2015). Because of that, pyrosequencing has already been applied for the study of microbial successions in various fermented foods. Dairy products have been objects of study by numerous authors (Alegría et al. 2012; Ercolini et al. 2012; Masoud et al. 2011, 2012; Quigley et al. 2012).

Molecular techniques applications used for fermented meat and fish products

Fermented meats are unique products that are often represented as elements of culinary heritage and identity (El Sheikha and Bakar 2014). Traditionally preserved and fermented meat products of many countries are the salami of Europe (Toldrá 2007), alheira of Portugal (Albano et al. 2009), androlla of Spain (García Fontán et al. 2007), nham of Tailand (Chokesajjawatee et al. 2009), kargyong, satchu, and suka ko masu of India and Nepal (Rai, Palni, and Tamang 2009, 2010), arjia, chartayshya and jamma of India (Oki et al. 2011), and nem chua of Vietnam (Khanh et al. 2011; Nguyen et al. 2011).

PCR-DGGE was used to monitor the dynamics of bacteria in Argentinean dry fermented Sausages (Fontana, Cocconcelli, and Vignolo 2005; Fontana, Vignolo, and Cocconcelli 2005). Cocolin et al. (2007) applied both PCR-DGGE and FISH techniques to fresh and fermented meats and their products.

From few years ago, researchers have applied PFGE in the study of the biodiversity of strains belonging to microbial groups or genera recurring in fermented foods, such as staphylococci isolated from naturally fermented dry sausages (Leroy et al. 2010), fermented meat products (Marty et al. 2012).

Among PCR-based methods, RAPD-PCR has been the most popular technique applied to food ecosystems. In precedent years, it has been successfully applied to detect the presence, the succession, and the persistence of LAB starter cultures inoculated in meats, as well as to determine the variations of microbial populations in naturally fermented meat products (Urso, Comi, and Cocolin 2006).

The fishery product that has subjected changes through microbiological or enzymatic activities in the presence or absence of salt is called "the fermented fish product" (Zakhia and Cuq 1993). Some ethnic fermented fish products of the world are hentak, ngari, and tungtap, bordia, karati, and lashim of India (Thapa, Pal, and Tamang 2004; Thapa, Pal, and Tamang 2007; Thapa 2016), jeotgal or jeot or saeu-jeot of Korea (Guan, Cho, and Lee 2011; Jung et al. 2013), plaa-som of Tailand (Saithong et al. 2010), shiokara of Japan (Fujii et al. 1999), patis of the Philippines (Steinkraus 1996), surströmming of Sweden (Kobayashi, Kimura, and Fujii 2000), salanga of Chad, adjuevan from Ivory Coast (El Sheikha and Montet 2014b).

Regarding the applications of molecular techniques used for fermented fish products, pyrosequencing of 16S rRNA gene as one of the metagenomic approaches was applied on fermented salted fish to analyze and profile the microbial communities (Kiyohara et al. 2012). The ecology of lactic acid bacteria of Ivorian fermented fish "adjuevan" was studied using culture-dependent methods and culture-independent methods (DGGE analysis), which allowed to describe its biodiversity (Kouakou et al. 2012).

Molecular techniques applications used for alcoholic and non-alcoholic beverages

Fermented beverages, i.e., alcoholic (wine, beer, bouza, cider, pito, tchapalo, koumiss, etc.) and non-alcoholic (kvass, keribo, ayran, hardaliye, salgam juice, gilaburu juice, kombucha, etc.) are culturally and socially accepted products for consumption, drinking, entertainment, customary practices, and religious purposes worldwide (Darby 1979; El Sheikha and Montet 2014a; Baschali et al. 2017).

Several PCR-based techniques are used to identify strains of yeast in wine. The most commonly used is random amplified polymorphic DNA-PCR or RAPD-PCR (Urso et al. 2008; Ivey and Phister 2011). Real-time PCR (qPCR)

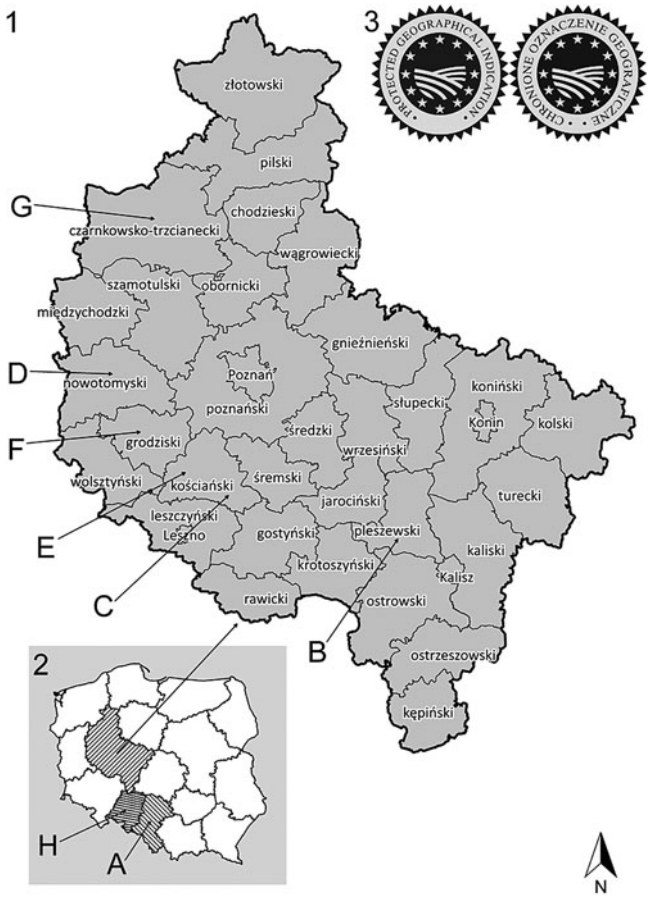


Figure 4. Geo-origins of the fried cheese samples. 1: Wielkopolska Voivodeship; 2: Poland (A: Samples from Silesia; B, C, D, E, F, G: Samples from Wielkopolska; H: Samples from Opole Voivodeship); 3: Grayscale reproduction of the Protected Geographical Indication (PGI) logo in English and Polish version. Reproduced with permission of Elsevier.

offers a faster and reliable technique to identify and quantify yeast during fermentation. The method is based on the amplification of a DNA target which is linked to a fluorescence reporter molecule. There are several reporters that can be used, however, SYBR Green is the most common one used for detection of wine-related microorganisms (Díaz et al. 2013). In the most comprehensive use of DGGE to date, Renouf, Strehaiano, and Lonvaud-Funel (2007) monitored the microbial population through the entire winemaking process from berry to wine. AFLP and SSR markers are techniques most often used in relation to wine for the genotyping of grapes and molds (Ergül et al. 2006, 2011). The PCR-based methods used so far in beer fermentations are PCR-RFLP, TTGE/DGGE-PCR, RAPD-PCR and microsatellites analysis (Juvonen and Satokari 1999; Manzano et al. 2005; Giusto, et al. 2005, 2006; Rainieri et al. 2006).

For fermented red dragon fruit (*Hylocereus polyrhizus*) beverages, the genotypic methods were used to confirm the identity of lactic acid bacteria (LAB). Using the randomly amplified polymorphic DNA method (RAPD), the LAB isolates can be clustered into two groups. Also, the nucleotide sequencing and restriction fragment length polymorphism of the 16S rRNA region was used to identify either *Enterococcus faecalis* or *Enterococcus durans* (Ong et al. 2012).

Why is considered the early detection of unwanted microorganisms extremely important in fermented foods?

The early detection of unwanted microorganisms (e.g., contaminated microbes) is crucial for main two reasons: 1) avoid process failure and costly delays in fermentation

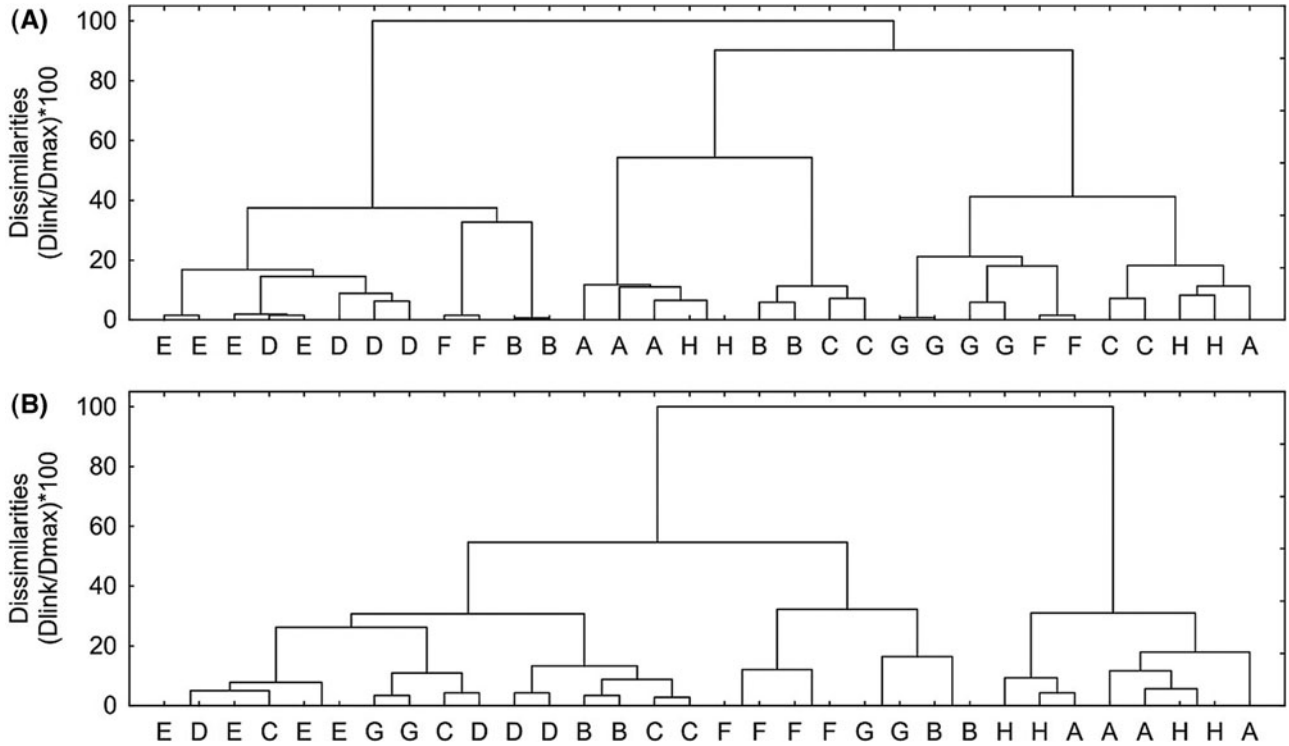


Figure 5. Cluster analysis of 26S rDNA banding profiles (R_f) (A) and the Dice similarity coefficient (S_D) matrix (B) of different origins of fried cheese samples. A: Silesia region; B, C, D, E, F, G: Wielkopolska, H: Samples from Opole Voivodeship. Reproduced with permission of Elsevier.

industries; 2) improve food safety issue. However, traditional detection methods such as plate counting and microscopy are labor-intensive, insensitive, and time-consuming. Modern techniques, i.e., molecular ones that can detect unwanted microbes rapidly and cost-effectively are therefore sought (Sue et al. 2011).

As known, the microorganisms are rapidly adjusting their metabolism in response to environmental changes such as the presence of an unwanted organism (Kell et al. 2005; Mapelli, Olsson, and Nielsen 2008). Rapidly, changes in microbial metabolism are detected through changes in their metabolic marker profile. This metabolic marker is highly specific to the genetic background of the unwanted microbial cells and the environmental conditions under which they grew (Kell et al. 2005; Villas-Bôas et al. 2006). Hence, through metabolic marker analysis, we will be able to detect and distinguish the wanted and unwanted microorganisms in the fermented food products (Kell et al. 2005; Villas-Bôas et al. 2005; Villas-Bôas et al. 2006). Metabolomics as molecular techniques, therefore, have the potential to reveal the presence of undesired microbes. Gas chromatography-mass spectrometry (GC-MS) is the most commonly used molecular approach to obtain comprehensive metabolite profiles of biological samples (Kell et al. 2005; Villas-Bôas et al. 2011). Sue et al. (2011) proved the applicability of GC-MS-based metabolic marker analysis as a rapid and reliable method for the detection of microbial contamination as unwanted microbes in fermented foods.

Could molecular techniques use to reveal the origin of the fermented foods?

The answer to this question will involve the answer to the following one: *Are the ferment microorganisms indicated the geo-origins of fermented foods?*

Infographic 1. Using molecular techniques, more secrets about fermented food can be unraveled … Is it possible?!!!

The fermented foods are produced in many places around the world, making it possible to link patterns in microbial diversity from place to place to the diversity fermented foods' characteristics which called "microbial biogeography". The validity of the hypothesis is already has demonstrated by several studies applied to many different fermented foods such as wines and cheeses (Renouf et al. 2006; Fierer 2008; Ciani et al. 2010; Arcuri et al. 2013; Bokulich et al. 2013; Gilbert, van der Lelie, and Zarraonaindia 2014; Montel et al. 2014; Rychlik et al. 2017).

The revolution in the applications of molecular technologies over the last decade particularly for microbial characterization of foods, which contributed significantly to the development of biogeography research. The study conducted by El Sheikha (2010) proved that the DNA fingerprinting of microorganisms on fruits from the specific geographic area using DGGE approach could be used as a "biological barcode" of this food. Another molecular tool using a high-throughput, short-amplicon sequencing technique applied on wine grapes provided the evidence for a link between microbiota and regional areas (Bokulich et al. 2013).

Recently, Rychlik et al. (2017) used 26S rDNA profiles generated by PCR-DGGE to the assess the variation the fungal communities of traditional fried cheese (Wielkopolska) from eight different locations (**Figure 4**). Cluster analysis was a useful statistical tool to organize different objects into groups, considering the variation in DGGE fingerprint patterns. The analysis of the hierarchical tree shows that the eukaryotes profiles of samples A and H were similar to those of B, C, F and G, and these samples formed one cluster (**Figure 5**).

Infographic: molecular techniques & fermented foods

Scientifically reliable information helps us make the best choices for foods. Yet it can be surprisingly difficult to get straight answers on fermented foods. Thanks to the application of molecular methods allowing precise imaging of microbial ecology, the field of food fermentation has experienced exponential growth in the last few years. This has resulted in the availability of new information on the quality, safety, healthy and traceability of fermented foods (see Infographic 1).

Future directions

Next-generation DNA sequencing such as pyrosequencing allows high throughput, is less labor-expensive and should be a powerful culture-independent method for fermented food samples in the future. This approach eliminates the need for cloning and culture-dependent methods, thereby avoiding both productions of aberrant recombinants and cloning-related artifacts. In the near future, next-generation DNA sequencing will enable to add a molecular dimension to understanding flavor, taste, and texture of fermented foods during the fermentation process.

As known, fermented foods are sources probiotic-rich food which well suited to promoting the positive health image of consumers. In the near future, whole genome sequencing (WGS) and real-time PCR will become the best choice and more important as rapid molecular tools for identifying, screening and analyzing ferment microbiota compositions including probiotic species if it is not possible to establish any other identification tool or techniques to analyze the microbial content in a much faster way. In addition, the availability of both methods supported by decreasing costs will make them more applicable.

Authors Contributions Statement

Dr. Aly Farag El Sheikha designed and wrote the manuscript. Dr. Dian-Ming Hu revised the manuscript.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Abbreviations

AFLP	Amplified Fragment Length Polymorphism
ARISA	Automated Ribosomal Intergenic Spacer Analysis
CZE	Capillary Zone Electrophoresis
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic Acid
FAO	Food and Agriculture Organization of the United Nations
FISH	Fluorescence *In Situ* Hybridization
ITS	Internal Transcribed Spacer
LAB	Lactic Acid Bacteria
LH-PCR	Length Heterogeneity- Polymerase Chain Reaction
MALDI-TOF MS	Matrix-Assisted Laser Desorption Ionizing-Time of Flight Mass Spectrometry
NGS	Next-Generation Sequencing
PCR	Polymerase Chain Reaction
PFGE	Pulsed-Field Gel Electrophoresis
qPCR	Quantitative-Polymerase Chain Reaction
RAPD-PCR	Random Amplified Polymorphic DNA-Polymerase Chain Reaction
rDNA	Ribosomal Deoxyribonucleic Acid
REA	Restriction Enzyme Analysis
Rep-PCR	Repetitive Element-Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
rRNA	Ribosomal Ribonucleic Acid
SSCP	Single-Strand Conformation Polymorphism
TGGE	Temperature Gradient Gel Electrophoresis
T-RFLP	Terminal Restriction Fragment Length Polymorphism
WGS	Whole Genome Sequencing

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