Chapter 4
Advances in Fermentation Technology for Novel Food Products

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Abstract The relevance of fermentation as an important and key aspect of food processing cannot be overemphasized, as it enhances beneficial composition and ensures safety. Fermentation technologies have constantly evolved with advances effectively dealing with the challenges associated with the traditional food fermentation process. Over the years, concerted efforts, intensive scientific research and the advent of modern sophisticated equipment have addressed these challenges and progressed to new approaches for fermentation of foods, subsequently leading to the delivery of novel food products. These advancements are further fueled by competitiveness among industry players based on innovativeness, cost-cutting measures, profit and the understandable desire for process improvement, better yields and quality products. This chapter covers significant advancement and technological applications that can improve food fermentation processes that are applicable for the delivery of better, safer and cost-effective food products.

Keywords Fermentation · Mixed cultures · Carbohydrate · Novel processing techniques · Food metabolomics · Nanotechnology

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Introduction

The onus and imperative for continued development of appropriate technologies for food production have continually risen over the past few years. While conventional food processing techniques still play an important role in the formulation of traditional diets, increasing consumer demand for high-quality, nutritious and safe products propels the industry to search for improved processes. Fermentation remains an age-long food processing technology, practiced, even before the understanding of the underlying processes involved. The techniques and associated knowledge involved in this process are normally handed down from one generation to the other and subsequently passed on within the local communities (Adebo et al. 2017a).

Recently, there has been an increased demand for fermented foods as potential sources of functional foods (Adebo et al. 2017a, b; Adebiyi et al. 2018). The need to meet consumer demand has made it essential to improve conventional fermentation techniques with advanced ones to ensure the delivery of desired fermented foods with consistently better quality, sensory attributes and nutritional benefits. This chapter, thus, provides an overview of the current state and potential developments and advances in fermentation technologies, for the delivery of novel food products. Aspects covered include the use of multi-strain starter cultures for fermentation, novel fermentation processes, carbohydrate for improved processes and other technological applications that can help enhance the development novel fermented foods.

Mixed Starter Cultures for Fermentation

Although most indigenous fermentation processes still largely rely on uncontrolled fermentation techniques (spontaneous fermentation and backslopping), the use of starter cultures (yeasts, bacteria and fungi) is desirable to ensure consistency, maintain hygiene, improve quality and guarantee constant sensory quality and composition. Sequel to the increased consumer demands for products with enhanced beneficial properties, the fermentation industry is constantly exploring ways to select, develop and use these starter cultures to improve the process. The general sequence used for the starter culture selection is depicted in Fig. 4.1. Commercial starter cultures are, however, not necessarily selected in this way but rather done based on rapid acidification and phage resistance (Leroy and De Vuyst 2004).

Starter cultures can be distinguished as single strain (one strain of a species), multi-strain (more than one strain of a single specie) or multi-strain mixed cultures (strains from different species) (Mäyrä-Mäkinen and Bigret 1998; Bader et al. 2010). While the use of single-strain cultures has been the norm and utilized for numerous food products, utilization of multi-strain and mixed cultures has demonstrated different advantages over single-strain use. Challenges of losses in the uniqueness, properties and characteristics of single-strain-fermented foods as

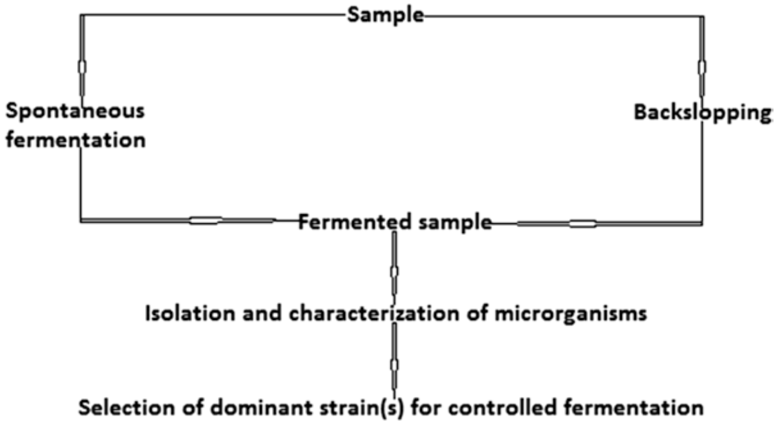

Fig. 4.1 Schematic diagram for strain selection

compared to different microorganisms have been reported (Caplice and Fitzgerald 1999), which could be ascribed to the limited microflora of the food. This could thus inform and suggest the use of multi-cultures, considering that these fermented foods are naturally produced through competitive action of different microorganisms and consequent varying metabolic pathways. Potential for synergistic utilization of different metabolic pathways; multiple biotransformation; increased yield; better organoleptic properties; bulk production of desirable metabolites, enzymes and antimicrobials; and rich biodiversity are added advantages of using mixed cultures (Meyer and Stahl 2003; Brenner et al. 2008; Bader et al. 2010).

Mixed cultures thus offer better complex metabolic activities and provide improved adaption in the food environment. Under such complex conditions, degradation, proteolysis, polymerization and metabolization of the inherent substrate occur through a combined metabolic activity of the inoculated strains. Examples of mixed culture applications for fermentation and delivery of novel food products are summarized in Table 4.1. Accordingly, through improved communication, trading of metabolites, exchange of molecular signals, combining tasks and division of labour among the cultures, better versatility and robustness are experienced under such conditions (Meyer and Stahl 2003; Brenner et al. 2008; Bader et al. 2010). The growth of one strain may however be enhanced or inhibited by the activities of another microorganism, and thus the production of primary and secondary metabolites may be increased or decreased (Keller and Surette 2006; Bader et al. 2010). Nonetheless, these cultures still play potential roles in increasing acidification and acceleration of the fermentation process and improvement of functionality, nutritional quality and health-promoting components.

Equally important are also reduction of cholesterol and biogenic amines and production of ɣ-aminobutyric acid (Ratanaburee et al. 2013; Kantachote et al. 2016) initiated through different interaction modes of mutualism, parasitism, competition, amensalism and commensalism between the strains. Through binding and production of metabolites, yeasts and LAB starter cultures have also been reported to

Table 4.1 Studies demonstrating the use of mixed cultures used for fermentation of food

Product	Raw material	Cocultures	Reference
Bread	Wheat flour, salt, sugar and water	*S. cerevisiae*, *Torulaspora delbrueckii* and *Pichia anómala*	Wahyono et al. (2016)
Cauim	Rice, cassava	*L. plantarum* and *Torulaspora delbrueckii*; *L. acidophilus* and *T. delbrueckii*	Freire et al. (2017)
Fermented milk	Milk	*C. kefyr* and *L. lactis*	Mufandaedza et al. (2006)
Fermented peanut milk	Peanut milk	*L. delbrueckii* ssp. *bulgaricus* *Streptococcus salivarius* ssp. *thermophilus*	Isanga and Zhang (2007)
Fermented sausage	Pork meat	*P. pentosaceus*, *L. sakei*, *S. xylosus*, *S. carnosus* and *Dabaryomyces hansenula*; *P. pentosaceus* and *S. xylosus*; *L. sakei* and *S. xylosus*	Wang et al. (2015)
Feta cheese	Pasteurized whole milk	*Lactococcus lactis*; *L. casei* and *Leuconostoc cremoris*; *L. lactis*, *L. casei* and *Enterococcus durans*; *L. lactis*, *L. casei*, *E. durans* and *Leuc. cremoris*	Litopoulou-Tzanetaki et al. (1993)
Functional beverage	Peanut-soy milk	*Saccharomyces cerevisiae* and *Pediococcus acidilactici*; *S. cerevisiae* and *Lactobacillus acidophilus*; *P. acidilactici* and *L. acidophilus*; *S. cerevisiae*, *P. acidilactici* and *L. acidophilus*	Santos et al. (2014)
Kefir	Milk	*Candida kefyr*, *Lactobacillus* sp., *Kluyveromyces* sp. and *Saccharomyces* sp.	Lopitz-Otsoa et al. (2006)
Moromi	Soy sauce	*Tetragenococcus halophilus* and *Zygosaccharomyces*; *T. halophilus* and *Z. rouxii*; *T. halophilus*, *Z. rouxii* and *Meyerozyma (Pichia) guilliermondii*	Singracha et al. (2017)
Nham	Pork	*P. pentosaceus* and *L. namurensis*	Ratanaburee et al. (2013) and Kantachote et al. (2016)
Probiotic beverage	Cereals	*L. plantarum* and *L. acidophilus*	Rathore et al. (2012)
Salami	Meat	*L. plantarum* and *L. curvatus*	Dicks et al. (2004), Todorov et al. (2007), and Bohme et al. (1996)
		L. sake and *Micrococcus* sp.; *L. curvatus* and *Micrococcus* sp.; *L. sake*, *L. curvatus* and *Micrococcus* sp.	
Suan yu	Fish	*L. plantarum*, *Stap. xylosus* and *S. cerevisiae*; *L. plantarum*, *Stap. xylosus* and *S. cerevisiae*; *P. pentosaceus*; *Stap. xylosus* and *S. cerevisiae*	Zheng et al. (2013)
Sucuk	Meat	*Staphylococcus carnosus* and *P. pentosaceus*; *Stap. carnosus* and *L. sakei*; *Stap. carnosus*, *P. entosaceus* and *L. sakei*	Bingol et al. (2014)

(continued)

Table 4.1 (continued)

Product	Raw material	Cocultures	Reference
Ting	Sorghum	*L. harbinensis* and *P. acidilactici*; *L. reuteri* and *L. fermentum*; *L. harbinensis* and *L. coryniformis*; *L. plantarum* and *L. parabuchneri*; *L. casei* and *L. plantarum*	Sekwati-Monang and Gänzle (2011)
Wine	Must	*S. cerevisiae* and *Starmerella bacillaris*	Tofalo et al. (2016)
Yakupa	Cassava	*S. cerevisiae* and *L. fermentum*; *T. delbrueckii* and *L. fermentum*; *P. caribbica* and *L. fermentum*	Freire et al. (2015)

detoxify mycotoxins (Adebo et al. 2017c). The use of cocultures/mixed starter cultures during the fermentation process would largely ensure a diversity of microflora that would provide a broad range of beneficial components in fermented foods. An in-depth understanding of the mechanisms of action of these multiple strains in a food system is however needed, necessitating further research in this regard.

Advances in the Use of Carbohydrates for Fermentation

The nature and type of carbohydrate influence inherent microbial and enzymatic actions as well as subsequent modifications to a substrate (Paulová et al. 2013). The complexity in the structural components of plant polysaccharides causes plant-degrading microbes to express numerous carbohydrate-active enzymes (CAZymes) (Lombard et al. 2014), which specifically modify or cleave to a specific type of sugar linkage (Boutard et al. 2014). Studies elucidating these mechanisms and approaches with models describing these interactions have been documented in the literature (Lynd et al. 2002; Boutard et al. 2014; Lombard et al. 2014; Lü et al. 2017).

Particularly, important advances on carbohydrates in fermentation technology are measures applied to improve enzyme accessibility to the active sites, thereby increasing digestibility of substrates during fermentation processes (Taherzadeh and Karimi 2008; Alvira et al. 2010; Lü et al. 2017). These pretreatments could be done using both chemical and physical methods. As for chemical methods, they include water pretreatment making a substrate suitable for enzymatic hydrolysis and subsequent fermentation as well as steam explosion because, high temperature is known to easily remove lignin, which might compromise microbial action (Taherzadeh and Karimi 2008; Thirmal and Dahman 2012). The major physical pretreatment commonly used is milling (Thirmal and Dahman 2012), with the assumption that it would physically increase the surface area of carbohydrates and improve accessibility of substrates to fermenting microbiota (Taherzadeh and Karimi 2008; Thirmal and Dahman 2012; Lü et al. 2017).

Equally important are non-digestible oligosaccharides (NDOs), which are low-molecular-weight carbohydrates, with intermediate properties between sugars and polysaccharides. Dietary fibre, an important member of this class, functions as prebiotics in diets, due to their excellent glycaemic response. Enrichment of fermenting substrates with NDOs gives an avenue for increasing bacterial population, biochemical profile and consequent beneficial physiological effects in the gut (Mussatto and Mancilha 2007). These NDOs have been produced from various carbohydrate sources via direct extraction from natural sources, chemical processes and hydrolyses of polysaccharides or by enzymatic action and chemical synthesis from disaccharides (Mussatto and Mancilha 2007). As such, NDOs are rapidly finding industrial applications both in prebiotic formulations and symbiotic products (containing probiotic organism and prebiotic oligosaccharide) (Mussatto and Mancilha 2007). This could potentially be utilized in different fermented foods for the delivery of desired health benefits.

Novel Food Processing Technologies for Improved Fermentation Processes

Novel and emerging food processing technologies for fermentation have increasingly gained interest over the past years. They are broadly categorized as a nonthermal and thermal process. The available novel nonthermal processes are high pressure processing (HPP), ultrasound (US) irradiation [gamma irradiation (ɣ-irradiation), microwave irradiation (MI)] and pulsed electric field (PEF); meanwhile thermal processes include ohmic heating (OH), radio frequency (RF) and microwave heating (MH). While the former could be aimed at accelerating the rate of chemical reactions (oxidation, polymerization, condensation and esterification) and fermentation, used for monitoring fermentation and for pasteurization, the latter may be adopted to improve shelf life, inactivate pathogenic and deleterious microorganisms, improve metabolic activities and production of enzymes as well as shorten the fermentation process. These techniques have recently been extensively described and adequately documented (Garde-Cerdán et al. 2016; George and Rastogi 2016; Koubaa et al. 2016a, b; Ojha et al. 2016, 2017). Available studies reporting the use of these technologies are summarized in Table 4.2.

HPP is conventionally applied to food products as a final mitigation step for products already packaged, which cannot be heat treated (Bajovic et al. 2012). They have received considerable attention as a technique for eliminating pathogens in fermented foods, although with mixed results. Some studies have indicated that HPP may not be desirable (Marcos et al. 2013; Omer et al. 2015), while others have encouraged the potential use of HPP in fermented foods (Table 4.2). Significant reduction and elimination in microbial loads of fermented foods have been reported (Omer et al. 2010; Gill and Ramaswamy 2008; Avila et al. 2016), with other studies indicating that HPP shortens wine ageing duration and enhances composition (Oey et al. 2008; Tchabo et al. 2017).

Table 4.2 Summary of studies reporting the use of novel processing techniques for fermented foods

Fermented product	Processing technique	Observation	Reference
Beer	US	Enhanced ethanol production	Choi et al. (2015)
Changran Jeotkal	ɣ-irradiation	Reduction of microbial levels, better chemical stability and improved overall acceptance	Jo et al. (2004)
Coffee	RF	Identification and characterization of behaviours during fermentation	Correa et al. (2014)
Dry-aged loins	US	Faster and better proteolytic changes in dry-aged meat cuts	Stadnik et al. (2014)
Fermented juice	OH	Retention of nutrients, inactivation of microorganisms	Profir and Vizireanu (2013)
Fermented milk	HPP	Reduced viable counts of *Candida* spoilage yeasts	Daryaei et al. (2010)
Fermented minced pepper	HPP	Lower levels of biogenic amines, lower microbial level, better sensory quality	Li et al. (2016)
Fermented sausage	ɣ-irradiation	Controlled the occurrence of undesirable and pathogenic microorganisms, reduction of *E. coli* O157:H7 load	Johnson et al. (2000), Chouliara et al. (2006), and Lim et al. (2008)
Fermented soybean paste	ɣ-irradiation	Reduction of biogenic amines	Kim et al. (2003)
Full-fat yoghurt	US	Higher water holding capacity, viscosity, lower syneresis and a reduction in fermentation	Hongyu et al. (2000)
Gochunjang	OH	Better pasteurization with no reduction in quality	Cho et al. (2016)
Kimchi	ɣ-irradiation	Controlled ageing and improved the shelf life of *kimchi*, sterilization of the product, softening of texture and better sensory quality	Song et al. (2004) and Park et al. (2008)
Kombucha analogues	PEF	Inactivation of acetic acid bacteria from *kombucha* consortium	Vazquez-Cabral et al. (2016)
Morr, *salami*	HPP	Reduction in *E. coli* O103:H25 and *E. coli* O157 counts	Gill and Ramaswamy (2008) and Omer et al. (2010)
Must	HPP	Reduction/elimination of wild microorganisms, especially yeasts	Bañuelos et al. (2016)
Must	MI	Reduction in fermentation time up to 40%, better alcohol yield	Kapcsándi et al. (2013)
Phellinus igniarius mycelial fermentation	US	Improved polysaccharides production, accelerated transfer of nutrients and metabolites	Zhang et al. (2014)

(continued)

Table 4.2 (continued)

Fermented product	Processing technique	Observation	Reference
Salami	HPP	Inactivation of *L. monocytogenes*, *E. coli* O157:H7, *Salmonella* spp. and/or *T. spiralis* larvae	Proto-Fett et al. (2010)
Salted and fermented squid	ɣ-irradiation	Adequate squid fermentation, prevented putrefaction and prolonged shelf stability	Byun et al. (2000)
Seeds of *Plantago asiatica* L.	MH	Enhanced production of value-added polysaccharides	Hu et al. (2013)
Semihard cheese	HPP	Inactivation of *Clostridium tyrobutyricum* vegetative cells and prevention of late blowing defect	Avila et al. (2016)
Sugar cane must	ɣ-irradiation	Decrease in contaminating bacterial counts, decreasing acidity, improved ethanol yield	Alcarde et al. (2003)
Sweet whey	US	Reduced fermentation time, with higher viable counts	Barukcic et al. (2015)
Wine	ɣ-irradiation	Shortening of ageing time, improving rice wine defects, production of a higher taste quality	Chang (2003) and Chang (2004)
Wine	HPP	Increase in esters, aldehydes, ketones, terpenes, lactones and furans contents, reduction of fermentation time	Buzrul (2012) and Tchabo et al. (2017)
Wine	US	Shortened ageing time	Chang and Chen (2002), Chang (2004), and Liu et al. (2016)
Wine	PEF	Increased colour intensity, anthocyanins and total phenols, better extraction of bioactive compounds, higher flavonols and phenolics, reduction in the fermentation process time, alternative technique to stop fermentation (instead of using SO_2)	Lopez et al. (2008), Donsi et al. (2010), Puértolas et al. (2010), El Darra et al. (2013), Abca and Evrendilek (2015), Delsart et al. (2015), Mattar et al. (2015), and El Darra et al. (2016)
Wine	HVEF	Shortened wine maturation process	Zeng et al. (2008)
Wine	RF	Monitoring and quality control of traditional wine manufacturing	Song et al. (2015)
Yeast fermentation	US	Process signature which may be related to product and process quality was captured	Hoche et al. (2016)
Yoghurt	MH	Improved shelf life	Turgut (2016)
Yoghurt	US	Quality control and monitoring the fermentation stages of yoghurt	Alouache et al. (2015)

ɣ-irradiation gamma irradiation, *HPP* high pressure processing, *HVEF* high-voltage electric field, *MH* microwave heating, *OH* ohmic heating, *PEF* pulsed electric field, *RF* radio frequency, *US* ultrasound

Irradiating meat (with doses up to 3 kGy) prior to production of dry fermented pepperoni was reported to reduce microbial load of *E. coli* O157:H7, with resultant products possessing intact quality parameters (Johnson et al. 2000; Chouliara et al. 2006). Likewise is the use of microwave irradiation and heating, which have been applied in sterilization, material treatment and reduction in processing time, thus attracting a great deal of attention (Rasmussen et al. 2001; Hoai et al. 2011; Kapcsandi et al. 2013). The use of US also improves microorganism and/or enzyme activity, ensures high-quality product and safety (Alouache et al. 2015) and promotes esterification, oxidation and condensation reactions leading to the production of more esters, acids and esters in ageing processed wine (Tchabo et al. 2017) and milk (Nguyen et al. 2009, 2012).

The possibilities of future application and vast current use of electric current for fermentation and production of value-added products are promising (Cho et al. 2016). OH have been successfully applied in electroporation of microorganisms (Sastry 2005; Loghavi et al. 2008, 2009). In comparison with conventional heating, a decrease in lag fermentation phase with OH was demonstrated by Cho et al. (1996), suggesting it as a better technique for pasteurization and sterilization of viscous foods (Cho et al. 2016). Several applications of PEF in fermentation-related processes have been reported (Table 4.2), demonstrating improvement in the secretion of phenolic substances and anthocyanins (Puértolas et al. 2010), reduction of fermentation time, lesser browning and an improvement in yeast metabolism (Delsart et al. 2015; Mattar et al. 2015).

Limited studies have been presented on the use of RF in fermentation-related processes, with one of such observing increased homogeneity, retention of important microbes and no detrimental effect on storage stability of the yoghurt (Siefarth et al. 2014). Limitations of these novel technologies could relate to high investment costs, other variables during the process, standardization and optimization of the process to meet required regulations. Most of these applications reported are also under laboratory conditions, and simulating such under industrial conditions is needed to fully understand them and facilitate their subsequent implementation.

Other Techniques for Advancing and Improving Fermentation Processes

While other major technologies for the advancement of the fermentation process have been discussed, other potential technologies such as encapsulation, metabolomics and the use of extremophiles for the delivery of novel fermented food products are also highlighted in this section of the chapter.

Encapsulation for the Delivery of Novel Fermented Products

Encapsulation is a technique used to entrap active agents embedded in a carrier material to improve delivery of desired components into foods. It equally ensures protection of inherent materials (such as sensitive bioactive materials) against environmental extremes, stabilizes ingredients, immobilizes cells and enzymes during fermentation and can potentially mask unpleasant sensory qualities. Encapsulated starter cultures have demonstrated excellent applications in foods when compared to their nonencapsulated counterparts. They ensured stability and slow release of cultures during fermentation and production of heat-processed *sucuks* (Bilenler et al. 2017), higher viability during storage (Peredo et al. 2016) and an increase in fermentation efficiency and better microbial survival (De Prisco and Mauriello 2016; Simo et al. 2017).

Accordingly, encapsulation has been effectively used for the delivery of bioactive compounds and development of functional fermented foods. Increased folate-enriched functional foods was achieved using alginate and mannitol encapsulated LABs (Divya and Nampoothiri 2015), while a functional yoghurt was successfully produced by co-encapsulating bioactive compounds (Comunian et al. 2017). Bioactive compounds may also be nanoencapsulated such that their potential for use as antioxidants and antimicrobials is improved to ensure safety against opportunistic pathogenic microorganisms in fermented foods (Cushen et al. 2012). Nanoencapsulation has been applied to improve stability, protect nutraceuticals against degradation, enhance bioavailability and ensure the delivery of functional ingredients to potential consumers (Dasgupta et al. 2015).

Extremophiles for Fermentation

Extremophiles are microorganisms known to thrive in extreme conditions of pressure, pH, radiation, salinity and temperature, high levels of chemicals and osmotic barriers. Due to their ability to thrive under such conditions, they possess adaptive capabilities and contain enzymes with potential applications in diverse fields of biotechnology (Gomes and Steiner 2004; Adebo et al. 2017e). Extremozymes (enzymes from extremophiles) can effectively be applied to produce novel fermented foods mainly because they have naturally developed resistance to drastic changes and reactions during food processing. Examples of such extremozymes with potential applications include amylases, cellulases, proteases, catalases, xylanases, keratinases, pectinases, esterases, lipases, phytases and peroxidases (Gomes and Steiner 2004). Cold-active β-galactosidase has been utilized in the production of lactose free milk and cheese (Khan and Sathya 2017) and serine proteases applied for the hydrolysis of proteins to peptides (Mayr et al. 1996; de Carvalho 2011). Extremophilic lipases and esterasescan hydrolyze glycerols and fatty acids, with possibility of producing health promoting poly-unsaturated fatty acids in fermented foods (Schreck and Grunden 2014). Likewise are piezophilic extremozymes, which are also valuable to fermented food products requiring high-pressure processes (Zhang et al. 2015).

Food Metabolomics for the Delivery of Novel Food Products

Food metabolomics (foodomics) has facilitated the characterization and simultaneous determination of the comprehensive profile of foods (Adebo et al. 2017d). Qualitative and quantitative determinations of a complex food metabolome such as that of fermented foods, which had seemed technically challenging, can now be done sequel to the availability of sophisticated analytical equipment and chemometric tools. This profiling technique offers enormous potentials to generate in-depth information on the composition of fermented foods, metabolic interactions that can be associated with the functionalities and nutraceutical potentials embedded in fermented foods. Through the application of this technique, a thorough understanding of the effect of fermentation on the development of functional and novel fermented foods is feasible. Further to this is a better understanding of fermentation and how it influences product quality, functionality and desired properties.

Future Prospects and Conclusion

There is no doubt that fermentation is an integral and important processing technology employed in developing novel food products. Significant advances have been made over these past years on effective technologies needed for improving the fermentation processes. Different advanced technologies have emerged, and successful developments of novel food processing techniques and food products have equally been developed. The need, however, for improvement is inevitable with evolving food habits, consumer demand for better quality as well as stringent regulations. The use of the techniques highlighted in this chapter seems promising for modern industrial processes; nevertheless more detailed studies and optimization may still be required before they can be fully implemented on a large scale.

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