Rapid Detection and Identification Systems for the Microbiological Assessment of Processed Soy Foods: A Review

Mitsuru Katase[1] & Kazunobu Tsumura[2]

[1] Fuji Oil Co., Ltd., Osaka, Japan

[2] FUJI OIL HOLDINGS INC., Osaka, Japan

Correspondence: Kazunobu Tsumura, FUJI OIL HOLDINGS INC., 1 Sumiyoshi-cho, Izumisano-shi, Osaka 598-8540, Japan. Tel: 81-72-463-1751. E-mail: tsumura.kazunobu@so.fujioil.co.jp

Abstract

Plant-based diets are gaining interest in promoting physical and environmental health worldwide. The widely growing consumption of processed soy foods results in an increased demand for safe and high quality soy foods. Many of the rapid bacterial detection methods currently available are inhibited by components in the food matrixes. In recent years, high-throughput devices have been developed, which aid in the enumeration and evaluation of microorganisms in processed soy foods (automated fluorescent filter method, high-throughput identification using matrix-assisted laser desorption ionization time-of-flight mass spectrometry, and automated most probable number method). These methods are more rapid and convenient compared to the conventional culture method. This review discusses alternate reliable methods for the microbiological assessment of processed soy foods, which guarantees the safety of the food delivered for consumption.

Keywords: soy foods, microorganisms, risk assessment

1. Introduction

Microorganisms are one of the most important food quality indicators. The consistent manufacture of safe, high-quality foods requires a well-planned hygiene program aimed at controlling and reducing bacterial contamination during and post-processing.

The Japanese food culture has been added to the UNESCO's intangible cultural heritage list (UNESCO, 2013). Soy foods are one of the representatives of the Japanese "Washoku" culture. Therefore, development of analytical methods for the microbiological risk assessment of soy foods is a great importance.

Soybean is one of the most prominent protein sources, and the balance of essential amino acids is similar to that of milk and eggs. Various kinds of soy foods are commercially available. Soy protein isolate is a highly refined powdered protein that has good gelation and emulsifying properties (Hettiarachchy & Kalapathy, 1997; Tsumura, 2009). It is also used as an ingredient in a healthy diet, targeting those with high cholesterol levels. Additionally, soybeans are attracting the most attention among plant proteins, for use as a sustainable protein source (Thrane et al., 2017).

On the other hand, there is little microbial information on processed soy foods (Table 1). Soil-derived *Bacillus*, found in soybean, is the main flora in processed soy foods, and is one of the targets in the sanitation management program (Zhou et al., 2017).

Table 1. Main bacteria isolated from processed soy foods

Items of foods	Detected bacteria	Reference
Tofu	*Streptococcus, Pediococcus, Lactobacillus, Pseudomonas, Pseudomonas, Enterobacter, Enterobacter*	Tuitemwong & Fung, 1991
Tofu	Coliforms, *Pseudomonas, Escherichia, Enterococcus,* Lactic acid bacteria, *Bacillus, Staphylococcus*	Ananchaipattana et al., 2012
Tempeh	*Enterobacteriaceae*, Lactic acid bacteria, *Staphylococcus, Bacillus, Escherichia*	Samson et al., 1987
Kinema	*Bacillus, Enterococcus*	Sarkar et al., 1994
Sufu	Mesophilic aerobic bacteria, Lactic acid bacteria	Han et al., 2001
Doenjang	*Leuconostoc, Tetragenococcus, Enterococcus, Bacillus*	Kim et al., 2009
Meju	*Bacillus, Enterococcus, Pediococcus*	Kim et al., 2011
Cheonggukjang	*Bacillus,* Lactic acid bacteria	Nam et al., 2012
Denjang-meju	*Bacillus*	Jung et al., 2014
Stinky tofu	*Streptococcus, Lactococcus*	Gu et al., 2018

Traditionally, microorganisms in processed soy foods and other retail foods have been evaluated by standard plate count on selective media (International Organization for Standardization, 2003; Official methods of Analysis Online, 2005). However, these methods usually take more than a day to detect the microorganisms (Vasavada & White, 1993). Therefore, a rapid, accurate and simple method is desirable.

Several rapid detection methods are used in the food industry (Gracias & McKillip, 2004). Polymerase chain reaction (PCR) is one of the most commonly used techniques due to its high sensitivity. In particular, reverse transcription PCR is a valuable method for detecting viable bacteria. However, this method needs to extract nucleic acids from the food samples. In addition, lipids and proteins in food can often interfere with PCR, necessitating the development of other methods (Gadkar & Filion, 2013; Schrader et al., 2012). Fluorescence staining method is widely used for microbial detection without culturing (Miyanaga et al., 2007; Yamaguchi et al., 2007), and flow cytometry is used to count viable bacterial cells (Diaper et al., 1992; Jepras et al., 1995; Khan et al., 2010).

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) has recently been shown to be a rapid and reliable method for bacterial identification (Lay, 2001; Mazzeo et al., 2006). The speed and accuracy of MALDI-TOF MS is widespread as a routine bacterial identification method in food microbiology (Angelakis et al., 2011; Böhme et al., 2011; Hochel et al., 2012; Kuda et al., 2014). Bacterial identification using MALDI-TOF MS eliminates the shortcomings of conventional culture methods because the time required for bacterial identification is very short. However, it is difficult to detect and identify bacteria in high lipid-containing food. Moreover, little information is available on non-culture based bacterial identification in food samples using the MALDI-TOF MS (Barreiro et al., 2012; Ferreira et al., 2010; Furukawa et al., 2013; Katase & Tsumura, 2014).

There are many alternatives methods that have been validated by protocols based on the international certification ISO 16140 standard. Petrifilm method is one of the widely applied methods (Freitas et al., 2009; Silva et al., 2005; de Sousa et al., 2005). This method is convenient and reliable, but is labor intensive. Therefore, a simple and automated method is required for quality assurance in the food industry.

In this review, alternate rapid methods of microbial assessment for the safety of processed soy foods, are discussed.

2. Automated Microbial Cell Counting System with Fluorescent Staining in Processed Soy Foods

Microbiological contamination of processed foods is a key issue in the food industry. Traditional methods serve as a reference for the microbiological quality control of food products, as they are reliable and easy to use for microorganism identification. However, these methods are time consuming and labor intensive. Moreover, they depend on the ability of microorganisms to form visible colonies after an incubation period of typically one to three days. This long time-to-result is an issue for food industries, as process improvement requires the availability of faster methods to control microbiological quality.

Consequently, a lot of technologies have been developed to reduce the time to result. These alternate technologies need to be accurate, sensitive and cost-effective. One of the most representative technologies is the fluorescent filter method (Boulos et al., 1999; Lahtinen et al., 2006; Ootsubo et al., 2003; Pettipher & Rodrigues,

1982).

Bioplorer (Koyo Sangyo Co., Ltd), an optical device designed for measuring microbial count in cosmetics, toiletries and foods, has been reported (Arai et al., 2006; Masakiyo et al., 2010; Nishimura et al., 2006; Nishimura et al., 2008; Shimakita et al., 2006; Shimakita et al., 2007). This device consists of a charge-coupled device camera, an optical unit, a light emitting diode (LED) light source, and a driving stage (Figure 1). Fluorescent dyes such as 4, 6-diamidino-2-phenylindole or propidium iodide are used for microbial staining on a sampling tip, followed by placing the tip on the instrument for automated counting. The advantage of an automated cell counting system is that, it requires much less time compared to microscopy, which consumes more than one hour even if measured by a skilled person (Yamaguchi et al., 2003). Although previous studies have demonstrated that this system could successfully enumerate bacterial cells in various food samples (Shimakita et al., 2006), colloidal and proteinaceous fluid such as soymilk and soy protein isolate solutions are exceptions, owing to filtration problems. To separate microorganisms from a proteinaceous fluid, a conventional isoelectric precipitation method, which is commonly used for the production of soy protein isolates (Petenate & Glatz, 1983; Tsumura et al., 2004).
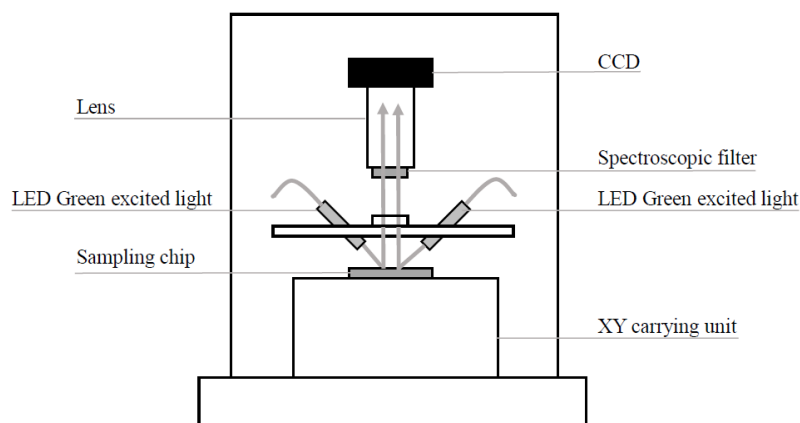

Figure 1. Schematic of the LED-illuminated detection apparatus

Tsumura and Tsuboi (2012) demonstrated that the total bacterial cells in solubilized soymilk could be enumerated using the automated cell counting system. However, for accurate viable cell count, the separation of microorganisms from the complex food matrix is essential (Araki et al., 2010; Benoit & Donahue, 2003; Fukushima et al., 2007; Stevens & Jaykus, 2004). On removal of the food matrix, this system can be implemented and the viable cell count can be obtained by staining with 6-carboxyfluorescein diacetate. Katase et al. (2013) demonstrated the use of this system, combined with a bacterial cell recovery method, in the enumeration of coliforms present in processed soy products. Bacilli are sometimes present as contaminants in processed soy foods (Fang et al., 1999; Pascall et al., 2006). For preferential coliform detection, culture enrichment of coliforms with brilliant green and sodium deoxycholate is performed within 6 h. This method was faster than other conventional methods for detecting trace levels of coliforms in soymilk. An in-house verification test has also been performed, according to the AOAC guidelines (Feldsine et al., 2002), to verify the applicability of this method for the detection of coliforms. This method did not give false positive or false negative results, resulting in higher sensitivity and specificity. A chi square value well below the threshold indicated that the method under investigation, and the reference method, were not statistically different. This alternate technique is more rapid and convenient than the conventional plate count method. Thus, it can be used to quickly detect coliform contamination in processed soy foods such as soymilk. Further improvements are needed for actually detecting specific bacteria by fluorescent *in-situ* hybridization staining (Nishimura et al., 2008; Yamaguchi et al., 2009).

3. MALDI-TOF MS Identification of Bacteria in Processed Soy Foods

In recent years, a rapid method for bacterial identification in processed soy foods using the MALDI-TOF MS system has been generalized (Table 2). However, the fat matrixes in foods interfere with accurate identification of bacteria using the MALDI-TOF MS system. Katase & Tsumura (2014) found that the identification accuracy of MALDI-TOF MS was lower when the fat content of the soymilk sample was ≥ 50%, indicating that the residual fat matrixes might have interfered with the raw mass spectrum and affected the spectrum matching.

Table 2. MALDI-TOF MS bacterial identification of processed soy foods

Items of foods	Identified bacteria	Reference
Korean fermented foods	*Weissella*	Kim et al., 2017
Korean soybean paste (doenjang)	*Bacillus, Paenibacillus, Tetragenococcus, Stapylococcus, Clostridium*	Woo et al., 2015
Soy sauce	*Tetragenococcus*	Kuda et al., 2014
Fried-Tofu, Soy milk	*Enterobacter, Klebsiella, Escherichia, Enterobacter*	Katase & Tsumura, 2014
Soy milk, Tofu, Frozen tofu, Dried tofu	*Leuconostoc, Serratia, Bacillus, Raoultella*	Furukawa et al., 2013
Tofu	*Cronobacter*	Hochel et al., 2012

Other rapid detection methods, such as PCR, are also inhibited by fat matrixes (Gadkar & Filion, 2013; Schrader et al., 2012). According to the International Organization for Standardization (2010), in the bacterial inspection of butter, it is essential to remove the fatty phase.

Katase & Tsumura (2014) demonstrated the separation of coliforms in processed soymilk, using an isoelectric precipitation method, practically applied to produce soy protein isolates (Petenate & Glatz, 1983; Tsumura et al., 2004), in combination with surfactant (sodium dodecyl sulfate) treatment. As a result of this treatment, accurate identification scores were obtained in soymilk samples with high fat content, using the MALDI-TOF MS system.

Contamination of processed foods is not always due to a single bacterial species. However, the coliform concentration in commercially available processed foods is relatively low. The dominant bacterial species in the food product can be identified by selective enrichment methods. The coliforms isolated from commercial soy foods belong to the *Enterobacteriaceae* family (Ananchaipattana et al., 2012; Préstamo et al., 2000). Currently, confirmation test is required following the successful detection of coliforms cultured in brilliant green-bile-lactose broth. On the other hand, since MALDI-TOF MS can directly identify bacterial cells, these confirmation tests are not required. Due to the discriminatory capabilities of MALDI-TOF MS, this direct bacterial identification method can be used in the food industry for quality control, process monitoring, and for confirming the presence of bacteria detected by other tests.

4. Automated Enumeration System Based on the Most Probable Number (MPN) Method

Most probable number (MPN) is the primary method for the enumeration of food borne bacteria since it can detect low bacterial levels. However, MPN method is labor intensive and time consuming. TEMPO is an automated enumeration system based on the MPN method for bacterial enumeration in food samples (Kobayashi et al., 2008; Kunicka, 2007; Owen et al., 2010; Paulsen et al., 2006; Paulsen et al., 2008: Torlak et al., 2008;). It is an internationally recognized method validated by ISO and the AOAC, and evaluated in various foods (Crowley et al., 2009; Crowley et al., 2010; Jasson et al., 2010).

Artificially contaminated processed soy products (soy protein isolate, soybean soluble polysaccharides, soymilk and tofu) were evaluated using different TEMPO kits (total aerobic bacteria; total coliform; *Enterobacteriaceae*; yeast and mold; *Staphylococcus aureus*). A high Pearson correlation coefficient was seen between the TEMPO and standard plate method, and no statistically significant difference was observed between the two methods (Katase & Tsumura, 2011).

The standard plate method requires time, materials, and is labor intensive (Vasavada & White, 1993). The confidence that the method has been executed accurately often depends on the skills of the staff. A non-technical staff may not be able to distinguish between a microbial colony and a food particle while performing the bacterial count.

Accurate results were obtained for majority of the processed soy food samples using the TEMPO method. Occasionally, in richly colored soy foods (soy sauce) and fermented soy foods (natto), some results were anomalous. This system provides improved standardization and minimizes economic loss, in terms of labor, by eliminating dilutions, preparation of medium, and plate counting. Therefore, TEMPO is a reliable alternate method for the microbial testing of processed soy foods.

5. Next Generation Sequencing Technique for Microbiological Risk Assessment

In recent years, the next generation sequencer has brought about innovation in microbial analysis (Table 3). In Food and Drug Administration (FDA), and Centers for Disease Control and Prevention (CDC), routine whole genome sequencing (WGS) has successfully identified the sources of food-borne pathogen contamination

(Rantsiou et al., 2018). Without relying on WGS, the analysis of the flora contained in food samples has been successfully examined using the 16S ribosomal RNA sequencing method (Jagadeesan et al., 2019). The 16S metagenomic analysis can be used routinely to evaluate the bacterial quality of food ingredients (Patro et al., 2016). At present, the hurdles are high in terms of cost, for the food companies to use these techniques easily. However, this technology will be used actively in the future for microbiological risk assessment.

Table 3. 16S amplicon sequencing approach for microbial assessment of processed soy foods

Items of foods	Target bacteria	Reference
Fermented soybean products	*Lactobacillus, Enterococcus, Fructobacillus, Staphylococcus, Carnobacterium*	Xie et al., 2019
Soy-daddawa, fermented indigenous food	*Bacillus, Staphylococcus*	Ezeokoli et al., 2018
Da-jiang, fermented soybean condiment	*Staphylococcus, Leuconostoc*	Wu et al., 2018
Fermented soybean foods	*Bacillus, Tetragenococcus, Enterococcus*	Lee et al., 2017
Soybean pastes	predominant phyla (*Firmicutes, Proteobacteria, Actinobacteria*)	Sun et al., 2018
Fermented soy bean paste (doenjang)	*Tetragenococcus, Enterococcus, Leuconostoc, Lactobacillus*	Kim et al., 2016

6. Conclusion

In the clinical field, microbial measurement is being accelerated and automated. However, in the food sector, rapid microbial evaluation technologies are not widespread. This is because the samples to be tested are not simple like water and blood. Foods contain many ingredients such as pigments, proteins, and lipids, which inhibit microbial measurement. Moreover, since the cost of the conventional medium-based method is low, introduction of a new method with high cost is often not preferred. Low cost, simple pre-treatment methods such as protein removal by isoelectric precipitation, and oil removal using surfactants has achieved rapid detection, identification and automation of microbial measurements in processed soy foods. For food companies, the ability to quickly judge the results of microbial tests means enhancing the microbial management system. Compared to the conventional culture method that relies heavily on human labor, automated methods enable each person to obtain similar results, aiding in the advancement of microbial test standardization. Consequently, food manufacturers can focus on making safer products. These findings are considered to contribute greatly in guaranteeing the delivery of safe and high-quality processed soy foods.

Acknowledgments

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