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Recent sensing technologies for pathogen detection in milk: A review

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ABSTRACT

Quality control utilising Hazard Analysis and Critical Control Points in the dairy industry generates a large volume of samples. The associated costs are significant. The development and application of fast, sensitive and cost effective analytical systems for pathogen detection in milk could aid the industry in the reduction of overheads, find new uses in dairy farming and production precision management and unlock new markets. Recent progress in pathogen sensing technologies for milk analysis, in particular nucleic acid amplification and biosensors, is reviewed here. The importance of representative samples, detection probability and Practical Detection Limit is clarified. Methods for sample pretreatment are discussed in association with the most applicable detection methods. The major findings are summarised and future perspectives are drawn to inspire new ideas in the scientific community.

Contents

1. Introduction

Foodborne pathogen testing has been regulated with the introduction of Hazard Analysis and Critical Control Points (HACCPs)

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(EC Health & Consumer Protection General Directorate, 2005; FDA, 1997). Based on risk analysis along the production and distribution chain, HACCP promotes the identification of critical points for sampling and testing. Its aim is to ensure food safety. As a parallel result, the testing volume has increased and shifted toward pathogen detection on-site at production plants (Alocilja and Radke, 2003). The dairy sector has been flagged with "the highest testing rate per processing plant", but despite quality control, refrigeration, pasteurisation and ultra-high temperature (UHT) treatments, outbreaks of foodborne illnesses due to the consumption of hygenised but contaminated dairy products have been reported (Ackers et al., 2000; De Buyser et al., 2001; Morgan et al., 2009; Schmid et al., 2009; Upton and Coia, 1994).

The dairy industry is in need for a fast, sensitive and cost effective technology for the detection of foodborne pathogens that could successfully address the tasks appointed by legislator bodies. From the FoodMicroSystems (EU FP7/2007-2013 project, grant agreement no. 287634) and its analysis on the dairy industry (FoodMicroSystems, 2013) pathogen detection has emerged as the highest technological priority for the dairy industry. The industry demand for new technologies is mainly motivated by financial considerations in relation to dairy farming, quality control and production precision management, such as efficient use of resources and waste reduction, safety and better use of raw milk that could facilitate commercialisation of new and diversified products. Consumer perspective is mainly driven by cost and safety but also by quality and health. Regarding these factors, the application of innovative and advanced analytical systems for pathogen detection in raw milk is a good example where these technologies could unlock new markets and products. Raw milk and raw milk products are experiencing an increasing worldwide market demand (Food Standards Australia New Zealand, 2009; www.realmilk.com; www.milkmaps.com). Consumers' demand for raw milk access is based on scientific evidence that raw milk has superior nutritional properties (Quigley et al., 2013; Debarry et al., 2007; Ege et al., 2012). In conjunction, farmers are driven by the will to deliver new high quality products and the interest to grow sustainable small and medium enterprises (EC Agriculture and Rural Development, 2005; Farm-to-Consumer Legal Defence Fund, 2013). However, raw milk is a potential vehicle for outbreaks of foodborne illnesses (LeJeune and Rajala-Schultz, 2009) and the medical community warns against the risks posed by unpasteurised raw milk. Local legislations have been developed to address the consumer demand whilst providing standards for the production of raw milk (EC, 2004) or alternatively, to ban the trade of raw milk for human consumption (Farm-to-Consumer Legal Defence Fund, 2013; Food Standards Australia New Zealand, 2012). The reasoning behind the legislation is based on the fact that raw milk cannot be certified safe within its life cycle: raw milk is a highly perishable food whilst pathogen analyses are time consuming. As consequence, the microbial analysis of raw milk is merely retrospective, has statistical value but cannot prevent a disease outbreak. In addition, the available methods are excessively expensive to apply to the certification of every raw milk consignment.

A literature analysis carried out with ISI Web of Knowledge using the keywords "pathogen detection milk" has underlined an increased scientific interest in the topic (Fig. 1). The interest may well be in response to the enforcement of HACCP regulation. When the analyses is refined with the keywords "integrated", "automated", "sensor" and "system" the trend growth is confirmed and the commitment of the scientific community to the development of analytical systems for accurate quality control and effective detection of pathogens in milk is underlined.

This review aims to cover the topic of sensing technology for pathogen detection in milk and the requirements for their application at an industrial level. It provides direction on research and development for advanced and integrated analytical systems that could fulfil the needs of the dairy industry. After an initial discussion of milk sampling and sample preparation, a critical review of the most applicable technologies developed since 2000 is carried out. The main findings are summarised and future perspectives are drawn to inspire new ideas in the scientific community.

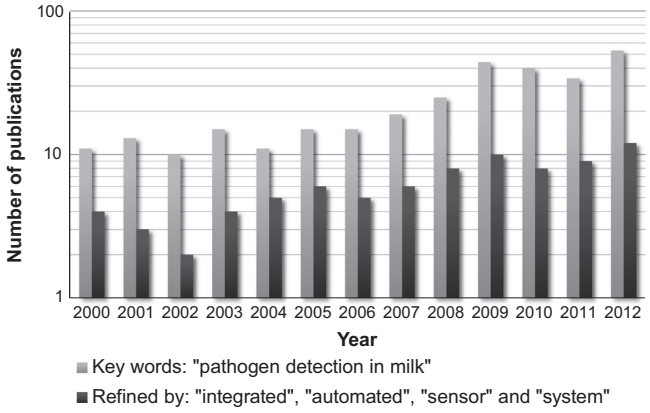

Fig. 1. Number of publications in logarithmic scale since 2000 till 2012. The publications were collected and analysed using ISI Web of Knowledge.

2. Milk sampling and sample preparation

Milk is a complex matrix (Fox, 2011). It is a colloid of butterfat globules and water with dissolved carbohydrates and protein complexes. Bacteria are distributed throughout the emulsion: suspended in solution as well as entrapped and absorbed on proteins micelles and fat globules. Milk is highly nutritious, with neutral pH and high water activity, thus it can support the growth of a rich and complex microbial flora. Produced from a healthy udder, milk is sterile. During the descent and excretion through the teat it is colonised by health promoting and technologically important bacteria, such as *Lactobacillus* spp. Other sources of potential contamination are personnel and milking, transport, storage and processing equipment. Zoonotic pathogens can be introduced by mastitic and unhealthy animals, bedding material, faeces and soil. The most common pathogenic bacteria associated with milk are *Listeria* spp., *Salmonella* spp., *Escherichia coli*, *Campylobacter* spp., *Shigella* spp. and *Brucella* spp. (Quigley et al., 2013). Table 1 reports the acceptable pathogen concentration for microbial analysis of milk as specified in the Commission Regulation (EC) No. 2073/2005 (EC, 2005) along with the respective reference methods, sample volumes and analysis time.

As specified in the guidelines ISO 707:2008 "Milk and milk products: guidance on sampling" (ISO, 2008), a sample of milk has to be representative, thus be equivalent to the whole milk consignment composition. Its weight is of 25 g, corresponding approximately to 25 mL of milk. Sample volume is of particular importance in relation to the probability of sampling pathogenic bacteria, normally defined as Detection Probability and expressed in % value. Pathogens are normally subdominant species and their infection dose is minimal, in the order of 10 and 100 CFU/mL (Dineen et al., 1998). Zero tolerance is applied to particularly virulent microorganisms, such as *Listeria monocytogenes*, *Salmonella*, *E. coli* O157:H7 and *Enterobacteriaceae* (EC, 2005; FDA, 2012). In such cases, pathogen absence has to be certified through microbiological analysis and the analysis sensitivity must be of at least 1 CFU/10 g.