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Evaluation of the microbiological quality of some fresh dairy products with Soleris® Automated System

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Abstract. The manufacture of dairy products is an important sector of the food industry. From milking to processing, a number of hygiene rules must be strictly followed. During processing, dairy products can be contaminated with different microorganisms, causing spoilage, infectious diseases, and alterations in the sensory characteristics. There are strict requirements for the quality assurance of milk products. In spite of this, there occur infections linked to milk and dairy product consumption. The

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analysis of the microbiological quality of these products is a health concern, and it also has an economic impact. The increase and development of the global market of processing technologies require rapid monitoring and controlling systems for food products. In our study, we investigated the microbiological quality of some fresh dairy products with the Soleris® test system. For instrument setting, calibration curves were realized with test bacterial strains. With known initial microbial load of the samples, the microbial growth versus time was measured by the above-mentioned system. The occurrence of *Escherichia coli*, *Pseudomonas aeruginosa*, and S*taphylococcus aureus* was evaluated in ten dairy products. Results obtained by the Soleris system showed that the system is efficient for this purpose. Calibration curves with high correlation coefficients permitted the quantitative determination of the aimed bacteria in the dairy product samples.

1 Introduction

Dairy industry is one of the most important sectors of the food industry, characterized by a variety of technologies producing a wide range of food products. In human nutrition, dairy products represent a major energy and nutrient source due to the high-value nutrition components.

The poor hygiene practice in the farm industry or the lack of regulations and supervision results in the contamination of the dairy products with pathogenic microorganisms and/or the occurrence of antibiotic residues. Milk is an ideal environment for bacteria; therefore, when microorganisms from the farm environment contaminate the milk supply, they will multiply rapidly (*Garcia et al.*, 2019). Worldwide several outbreaks were associated with milk-foodborne pathogens (*Landgraf* & *Destro*, 2013; *Desai* & *Trimble*, 2019). Food spoilage is a serious global problem, especially in developing countries, due to inadequate processing and refrigeration equipment. The high nutritional value, high water activity, and near-neutral pH of milk are very favourable for microbial growth. Different foodborne pathogens and food spoilage bacteria were detected in milk products. Diverse species of *Streptococcus* spp. (*Streptococcus agalactiae*, *S. pyogenes*, *S. zooepidemicus*) and *S. aureus* are linked to the health status of the mammary gland and cow herd. The working medium can also be a source of *Listeria monocytogenes*, *Salmonella* spp., *E. coli* O 157:H7, *E. coli* (STEC), *Yersinia enterocolitica*, *Enterobacter sakazakii*, *Campylobacter jejuni*, *Enterococcus faecalis*, *Citrobacter freundii*, and *Bacillus cereus*. Other waterborne microorganisms may appear in milk and milk products as well such as *Leptospira* spp., *Bacillus licheniformis*, *Bacillus subtilis*, *Pseudomonas*

aeruginosa, *Clostridium disporicum*, *Aspergillus* spp., etc. (*Velázquez-Ordoñez et al.*, 2019).

The presence of coliforms in dairy products reflects the poor hygienic conditions during the production process. Coliforms could be eliminated by compliance with personal hygiene requirements. Coliforms are a wide variety of bacteria such as *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Serratia* spp., etc. This group of microbes causes undesirable changes due to various enzyme activities and metabolic by-products (*Laslo & György*, 2018). They are responsible for organoleptic defects in cheese. *Escherichia coli* is a hygienic indicator organism of faecal contamination in dairy products (*Baranceli et al.*, 2014). Based on phenotypic characteristics and virulence factors, different serotypes of *E. coli* are known. These are the enterohaemorrhagic (EHEC), or enteropathogenic (EPEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive (EIEC) *E. coli* strains (*Batt*, 2014).

Staphylococcus aureus represents the third most important foodborne pathogen with enterotoxin-producing capacity and is characterized by multidrug resistance. Several outbreaks are attributed to enterotoxigenic strains. The main sources of contamination of dairy products are humans and poor hygiene. Also, raw milk can be contaminated, which is derived from cow mastitis (*Gillaspy & Iandolo*, 2014).

Major spoilage psychotropic bacteria are the *Pseudomonas* spp. due to their enzymes, such as protease, lipase, and lecithinase, that cause different alterations. *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* are believed to be responsible for the spoilage of raw milk and dairy products (*Arslan et al.*, 2011; *Sharika & Balamurugan*, 2019).

For microbial analysis, different alternative rapid methods with high capacity are developed (*Jasson et al.*, 2010). On the market, different instruments are available that use electrical techniques able to detect different pathogens and food spoilage bacteria besides total viable count in food samples. These methods represent an alternative approach to standard plate counting methods. The systems consist of an incubator and computer with dedicated software. This lets users track impedance curves, create reports, or evaluate calibration curves. Such a system is Soleris® System for Rapid Microbial Detection (*Mozola et al.*, 2013; *Pereault et al.*, 2014). This technique checks the alteration of the chemical characteristics of the growth medium used for microbial detection. The result comes from the changes that appear due to the metabolic activity of microorganisms, which is detected optically. For quantitative determination, a correlation between the microorganism and detection time can be established (*Limberg et al.*, 2016).

In the case of positive *E. coli* detection, the Soleris® instrument tracks the alteration of the medium colour resulted from the pH change. It can be applied for the indication of zero tolerance (*Foti et al.*, 2012).

The advantages of these growth-based automatic measurements in contrast to conventional plate-counting methods are: precision, accuracy, reproducibility, time saving, and cost (*Curda & Sviráková*, 2014; *Blivet*, 2014; Soleris® *Operation Manual*). In the development of an adequate rapid method, several facts are included. *Jasson et al.* (2010) suggested several selection criteria. First at all, the target microbial analysis is the main decision factor. Furthermore, the time and cost are included, referring to them as managerial criteria, and there exist technological and sustainability criteria, too.

Our aim was to calibrate the Soleris® System for the quantitative determination of some pathogenic and food spoilage bacteria as well as to investigate the microbiological quality of some fresh dairy products.

2 Materials and methods

Quantitative detection of Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa with Soleris® System for Rapid Microbial Detection

For the quantitative detection of the targeted bacteria, calibration curves of the tested bacteria were realized. The vials with chromogen-selective mediums (E. coli Medium (EC-104), Staph Medium (SM-118), Pseudomonas medium (VIV-125)) were inoculated with *E. coli, Staphylococcus aureus*, and *Pseudomonas aeruginos*a and incubated at 37 °C for 12 hrs. In every three hours, one ml of each sample was aseptically transferred into 9 ml of physiological solution. From this, serial dilutions to 10^{-1}–10^{-12} were prepared, and a volume of 0.1 ml was spread on the selective agar mediums. After a 24-h incubation at 37 °C, the number of colony-forming units was estimated and used to realize calibration curves with the software of Soleris® Automated System software. Different dairy products were inoculated with *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, and un-inoculated control samples were used for the validity of quantitative detection based on the calibration.

Determination of the occurrence of Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa in some fresh dairy products

During our work, 10 different dairy products (fresh cheese 1, fresh cheese 2, fresh cheese 3, fresh cheese 4, fresh cheese 5, feta-like cheese, salty type

soft cheese, cottage cheese 1, cottage cheese 2, cottage cheese 3) were studied microbiologically with the Soleris Automated System for the detection of the mentioned bacteria. The dairy products sold by weight were obtained from local stores and open-air public market.

11 grams of each sample was aseptically transferred into 99-ml Butterfield's buffer (34 g KH_2PO_4, 175 ml 1 N NaOH, 1,000 ml distilled H_2O, pH=7.2, and diluted 1.25 ml/1,000 ml) and mixed well for 10 minutes. From this homogenate, the adequate volume based on the instruction protocol was added to the Soleris vials (E. coli Medium (EC-104), Staph Medium (SM-118), Pseudomonas medium (VIV-125)) for each bacterial detection. The inoculated Soleris vials were placed into the selected drawer location. After proper adjustments, determinations were initiated. The Soleris software indicated positive test results in less than 24 hrs. Determinations producing no detection within 24 hrs were considered negative. In the case of positive results, the growth curves were evaluated, and the visual validation of medium colour change was also carried out. In the case of positive results, confirmation was done using conventional methods.

3 Results and discussions

Different alternative microbiological methods are used successfully for the detection of different pathogenic and food spoilage microorganisms. According to *Moldenhaue* (2008), alternative methods use bacterial growth that results from the biochemical and physiological changes or the viability of the microorganisms or the detection of the cell components. Also, there are combined methods. *Elegado et al.* (2016) reported several demands for rapid detection methods. These types of methods must be characterized by time saving, selectivity for the desired specific microorganisms, and must be capable to detect more than one microbe simultaneously.

During this study, we calibrated the Soleris® Automated System for the quantitative determination of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. We used this growth-based method for the evaluation of the microbiological quality of some fresh dairy products.

The calibration points were obtained by the plot of mean of the log(10) CFU for each targeted bacteria in function of detection time. In the case of *E. coli*, calibration points are shown in *Fig. 1*. The correlation coefficient was 0.99 with detection time ranging from 0 to 10 hrs.

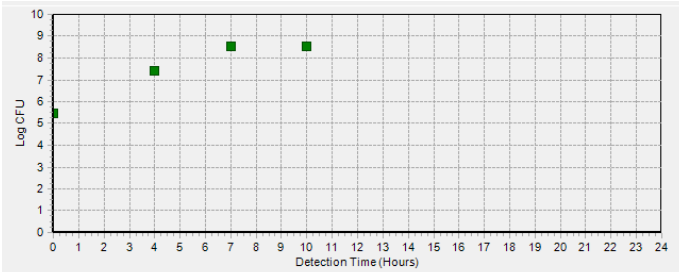


Figure 1: Calibration points: CFU in function of detection time in the case of *E. coli*

Calibration points for *Staphylococcus aureus* are shown in *Fig 2*. The correlation coefficient was 0.99 with detection time ranging from 0 to 10 hrs.

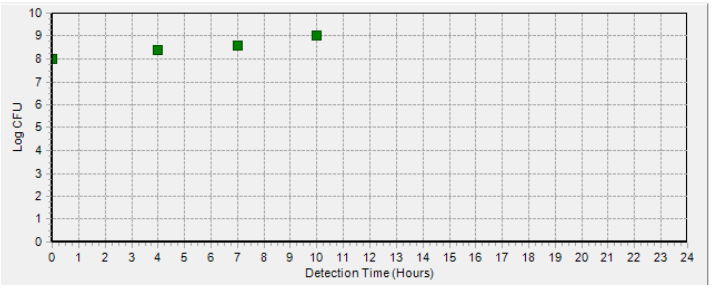


Figure 2: Calibration points in the case of S*taphylococcus aureus*

Calibration points for *P. aeruginosa* are shown in *Fig 3*. The correlation coefficient was 0.97 with detection time ranging from 0 to 10 hrs.

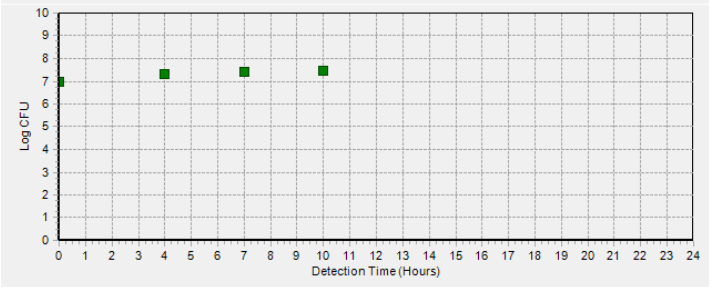


Figure 3: Calibration points in the case of *P. aeruginosa*

The obtained correlation coefficients were higher than the minimum allowed, and the calibration was accepted. The dairy samples have been analysed for the occurrence of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* with the quantified Soleris® System. The results are shown in *Table 1.* The lack of good hygienic practices contributes to the high prevalence of foodborne pathogens.

Escherichia coli is the faecal indicator organism in processed milk products. *E. coli* have been detected in all ten samples with high count (*Table 1*).

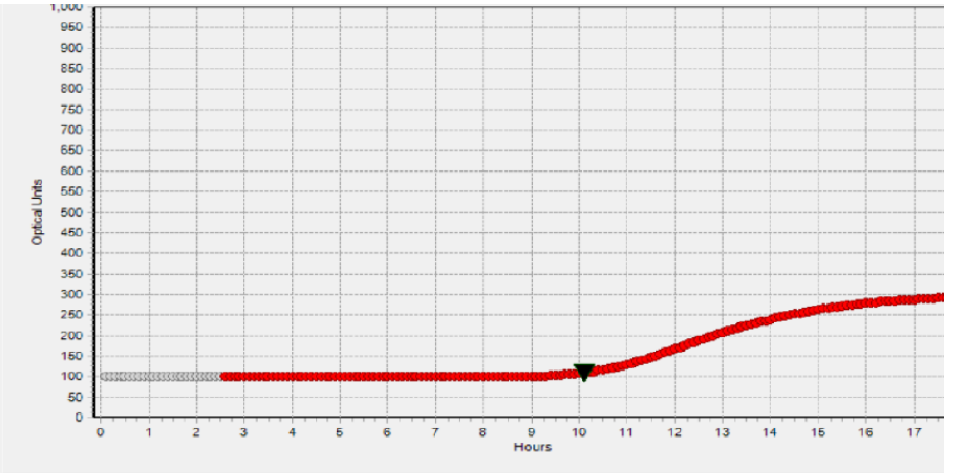
Table 1: *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus* count in the dairy samples

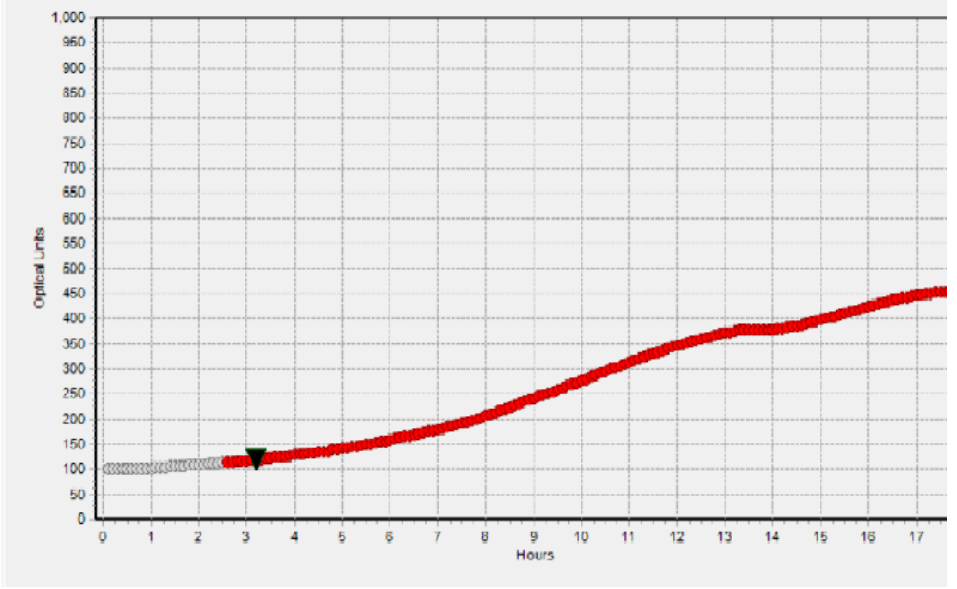
Studied samples	**E. coli CFU/g**	**Detection time (h)**	**P. aeruginosa CFU/g**	**Detection time (h)**	**Staphylococcus aureus CFU/g**	**Detection time (h)**
fresh cheese 1	$1.4{\cdot}10^{9}$	10.1	$2.50{\cdot}10^{7}$	7.6	<10	ND
fresh cheese 2	$5.9{\cdot}10^{8}$	8.9	$2.30{\cdot}10^{7}$	6.7	<10	ND
feta-like cheese	$8.4{\cdot}10^{10}$	16	$3.40{\cdot}10^{7}$	10.4	<10	ND
fresh cheese 3	$1.3{\cdot}10^{8}$	6.7	<10	ND	<10	ND
salty type soft cheese	$2.6{\cdot}10^{8}$	7.7	$1.90{\cdot}10^{7}$	4.8	<10	ND
cottage cheese 1	$1.1{\cdot}10^{7}$	3.2	$1.80{\cdot}10^{7}$	4.5	<10	ND
cottage cheese 2	$1.9{\cdot}10^{11}$	17.2	$2.60{\cdot}10^{7}$	7.9	<10	ND
fresh cheese 4	$5.2{\cdot}10^{8}$	8.7	$4.20{\cdot}10^{7}$	12.3	$1.3{\cdot}10^{8}$	1.2
cottage cheese 3	$6.8{\cdot}10^{8}$	9.1	$2.20{\cdot}10^{7}$	6.3	<10	ND
fresh cheese 5	$6.4{\cdot}10^{8}$	9	$2.60{\cdot}10^{7}$	8	<10	ND

The most contaminated samples were one type of the cottage cheese and feta-like cheese. In the less contaminated sample cottage cheese 1, the *E. coli* count was also high. Detection time varied between 9 and 17 hrs (Fig 4). *Foti et al.* (2012) previously reported that the Soleris® test system could be applied for the assessment of *E. coli* occurrence in mozzarella cheese. The detection time of *E. coli* ranged from 11.4 to 19.1 hrs. The advantages resulting from the use of this alternative system are simplicity and rapidity.

Pseudomonas aeruginosa were found in nine samples. One of the tested dairy products (fresh cheese 3) was free of this bacteria. All positive samples have a high number of *Pseudomonas aeruginosa.* Detection times for *P. aeruginosa* ranged from 4.5 to 12.3 hrs in contrast with *Foti et al.* (2012), where these bacteria were not detected.

In the perspective of public health, *Staphylococcus aureus* represents a serious health threat. In the tested samples, one type of cheese (fresh cheese 4) was detected as positive for this bacteria. There was detected a high count of $1.3 \cdot 10^8$.





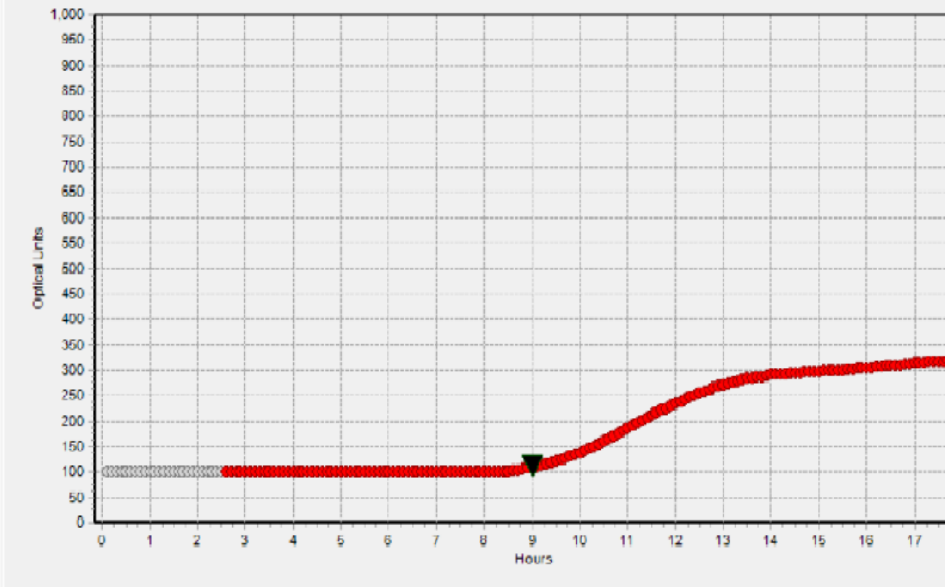
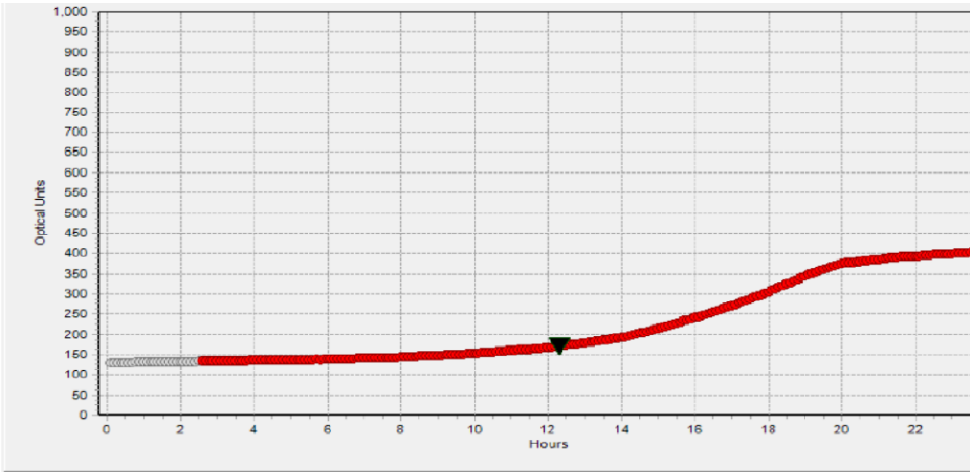


Figure 4: Detection of *E. coli* in fresh cheese 1, cottage cheese 1, fresh cheese 4, and cottage cheese 2

Globally, the occurrence of this bacteria was detected in various types of cheese. The high nutritional value (proteins, vitamins, and minerals) favours the growth and activity of *Staphylococcus aureus*. According to *Baran et al.* (2017), various causes are responsible for the staphylococcal intoxications transmitted by cheese. One of them is the raw milk contamination of *Staphylococcus aureus*, which can occur during cheese processing or after production.

Results revealed that the Soleris® Test System as a rapid, alternative method can be used for microbial quality determination. It is remarkable that the sensitivity of this method ranges to 10^8 CFU/ml (*Jasson et al.*, 2010). In three cases, the bacterial count was higher than the aforementioned value, while these samples were also examined with the traditional plate count method in order to confirm the results – the results turned out to be identical.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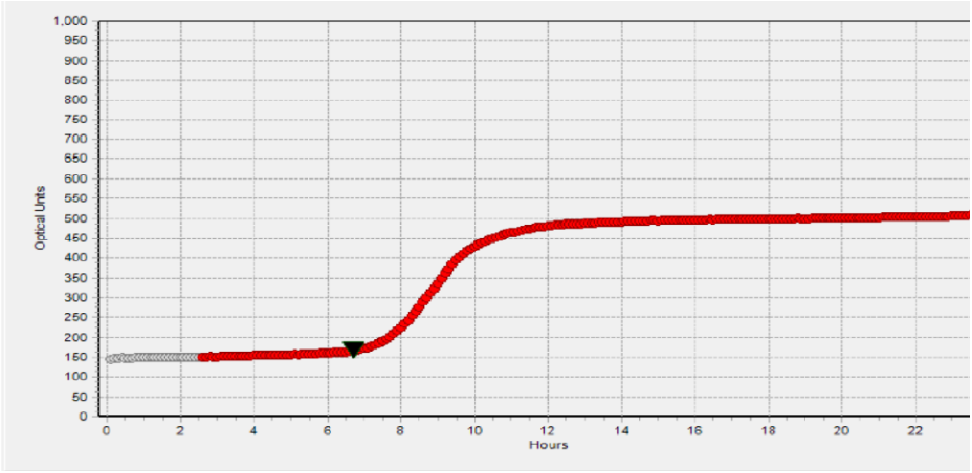
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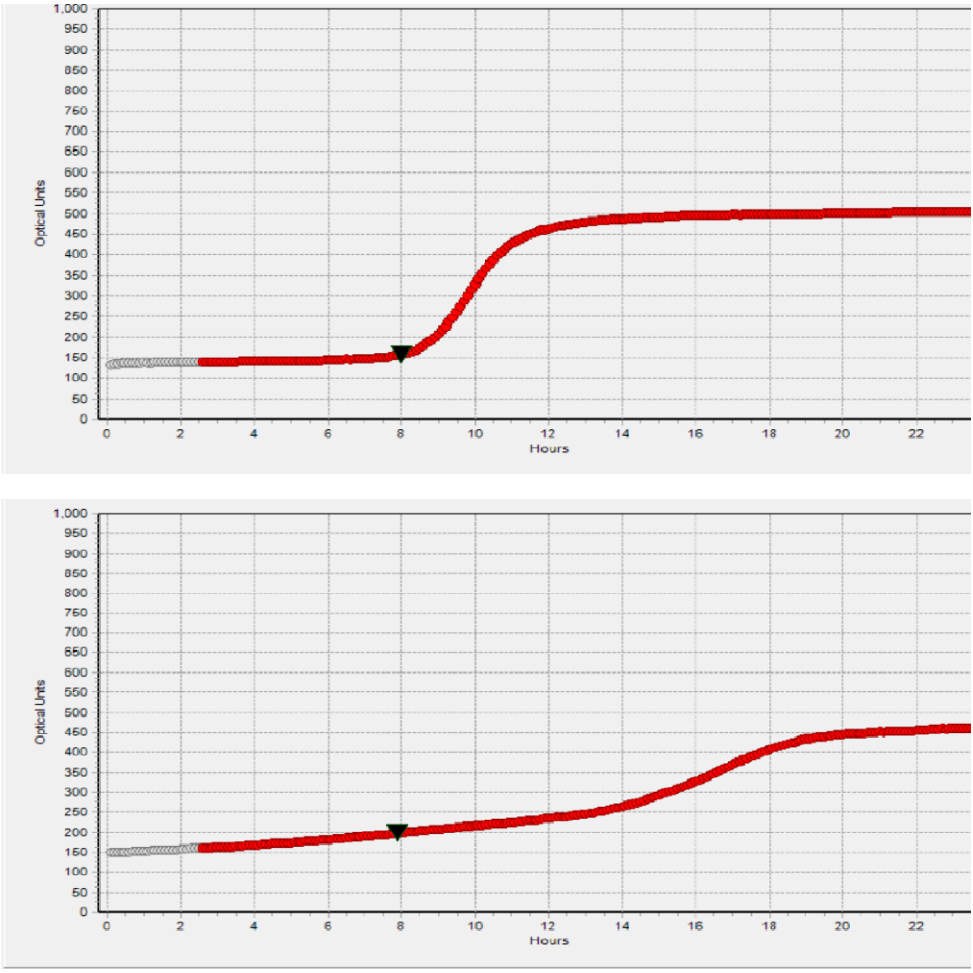

Figure 5: Detection of *Pseudomonas aeruginosa* in fresh cheese 1, fresh cheese 3, fresh cheese 5, and cottage cheese 3

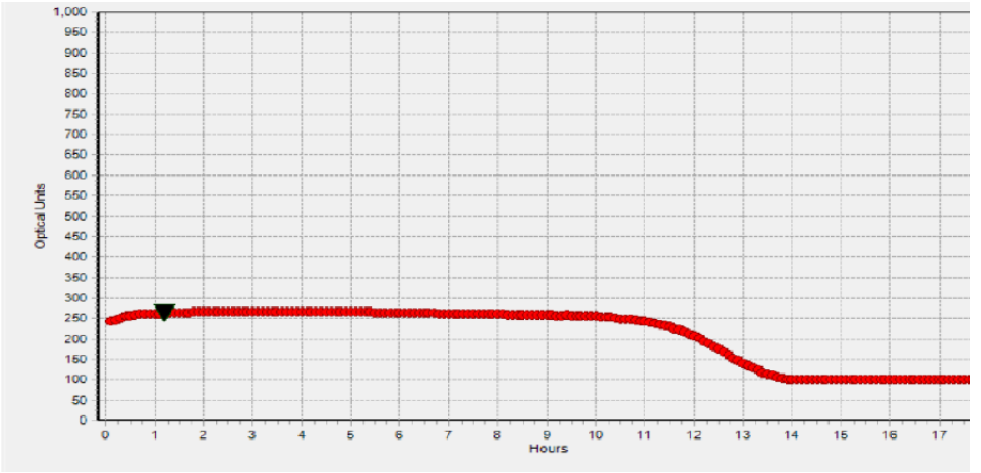

Figure 6: Detection of *Staphylococcus aureus* in fresh cheese 4

4 Conclusions

The Soleris® Automated System was successfully calibrated. Our results indicate that the Soleris® Automated System can be utilized as an alternative method to the standard plate count methods for the quantitative detection of *E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* in different dairy products. Our findings also revealed that dairy products sold by weight obtained from local stores and open-air public markets were highly contaminated. Besides poor hygienic practices during production, storage and distribution can also act as sources of contamination.

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