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Validation of ELISA-based detection of *L. monocytogenes* and *E. coli* O157:H7 in fresh cut vegetables

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Abstract

Innovative diagnostic methods were developed for the detection and quantification of *Listeria monocytogenes* and *Escherichia coli* O157:H7 in minimally processed fresh cut fruits and vegetables. The aim of the present study was to validate the technical efficiency of these methods and evaluate their efficacy and viability for routine analysis. To this purpose, ready-to-eat fresh fruits and vegetables were collected throughout the production chain. A multidisciplinary approach, including a newly developed ELISA method compared to ISO procedures, was applied to detect the pathogenic bacteria after harvesting, processing and shelf-life. Results obtained exhibited the technical efficiency of the developed methods showing similar sensitivity, specificity, negative predictive values and negative likelihood ratios.

Key words: Leafy vegetables, melon, vegetables, ELISA, ISO.

Introduction

Ready to eat fresh-cut vegetables (RTEs) are convenient foods that have increased the volume and value of commercialisation among different European countries. Nevertheless, the economic crisis in the recent years has slightly affected the fresh-cut fruits and vegetables market. This trend can be explained considering the higher quality of the products, which must be convenient and safe [1]. Most of the leafy vegetables used for the fresh-cut preparation are grown in the soil and bacteria contamination can easily occur. Moreover, their short growing cycles require an higher supply of organic matter in order to keep the soil fertility. The organic matter supply is usually performed by manure and as such represents a possible source of contamination of human pathogenic bacteria like *Escherichia coli* O157 L. and *Listeria monocytogenes* L. [2].

Washing treatments are able to reduce the bacteria load and allow to preserve the RTE products for long time [3]. The official standard procedures for the detection of bacterial pathogens require 4-5 days. Unfortunately, the shelf life of leafy vegetable products is limited to 5-7 days that include two days of processing and five days of shelf life. Therefore, faster and reliable methods are required to detect the presence of pathogens without losing the period of commercialisation. In the framework of the EU project QUAFETY - Comprehensive Approach to Enhance Quality and Safety of Ready to Eat Fresh Products different detection methods based on ELISA approach was developed and compared with the standard official ISO procedures.

Materials and Methods

Samples: Rocket and mixed salads were provided by Agronomia s.r.l., (San Paolo d'Argon, BG, Italy) and EuroCatering S.A. (Greece), while Piel De Sapo melons were provided by NoviFruits (Portugal). Samples were collected from raw materials, processed materials (immediately after packaging) and three days shelf-life materials. Thirty-nine samples were analysed corresponding to 2 different batches of melons (Portugal), 7 different batches of rocket (Italy and Greece) and 4 different batches of mixed salad (Italy and Greece).

ELISA-based detection of L. monocytogenes and E. coli O157:H7: Five g of material was homogenised in 1×PBS (1 mM KH_2PO_4, 154 mM NaCl, 3 mM Na_2HPO_4, pH 7.2). Isolation of bacteria, ELISA detection and data analysis were performed according to the protocol described in Cavaiuolo *et al.* [4]. For the detection anti-*E. coli* O157 (ab20976), anti-*L. monocytogenes* (LZA2) (ab11439) and Polyclonal Goat anti-Mouse IgG+IgM H&L (HRP) antibody (ab47827) were purchased from Abcam (Cambridge, UK).

ISO procedures for L. monocytogenes and E. coli O157:H7 detection: Twenty-five g of each sample was used to detect the presence of *L. monocytogenes* and *E. coli* O157:H7 according to ISO 11290-1:1996/Amd 1:2004 and ISO 16654:2001 [5,6], respectively. A sample was characterised as positive for the presence of *L. monocytogenes* when the identity of typical colonies isolated from ALOA agar was verified by series of biochemical tests (Gram stain, catalase test, oxidase reaction, hemolysis, fermentation of rhamnose, xylose, mannitol and methyl α-D-mannopyranoside, and a motility test). In any other case (i.e. identity not verified, absence of typical colonies), the sample was characterised as negative for the presence of *L. monocytogenes*. A sample was characterised as positive for the presence of *E. coli* O157:H7 when

the identity of typical colonies was verified by successive subculturing in BH agar and submittion to *E. coli* O157:H7 Latex Test (Remel, Lenexa, USA). In any other case (i.e. identity not verified, absence of typical colonies) the sample was characterised as negative for the presence of *E. coli* O157:H7.

Results and Discussion

While all melon samples were negative for both bacteria (average OD_{450nm} 0.09, negative control (NC) OD_{450nm} 0.06), in fresh cut salads the ELISA method identified few suspected samples. Based on the NC absorbance values (average OD_{450nm} 0.101), on the coefficient of variation (CV) among samples and assays and on the OD_{450nm} values of different concentrations of *E. coli* O157:H7 and *L. monocytogenes* reported in the work of Cavaiuolo *et al.* [4], the thresholds were set for classifying the samples as sure negative, suspect and sure positive. Samples with and $OD_{450nm} \leq 0.3$ were considered sure negative and samples with $OD_{450nm} \geq 0.5$ were considered sure positive for *E. coli* O157:H7. Only three samples were considered as suspect: Rocket raw (OD_{450nm} 0.36), mix raw (OD_{450nm} 0.321) and mix three days-shelf (OD_{450nm} 0.322). Samples with and $OD_{450nm} \leq 0.5$ were considered sure negative and samples with $OD_{450nm} \geq 0.6$ were considered sure positive for *L. monocytogenes* (Table 1). Based on these thresholds, two suspected samples were isolated: Mix salad raw (OD_{450nm} 0.52) and mix three days shelf (OD_{450nm} of 0.566).

Table 1. Detection of *E. coli* O157:H7 and *L. monocytogenes* in rocket and mix salad samples using the ELISA method.

	E.coli 0157:H7			*L. monocytogenes*		
Sample	OD_{450nm} mean	Stdev		OD_{450nm} mean	Stdev	
Rocket raw	0.360	0.204	Suspect	0.449	0.181	Negative
Rocket processed	0.152	0.08	Negative	0.203	0.138	Negative
Rocket shelf-life	0.202	0.105	Negative	0.285	0.171	Negative
Mix raw	0.321	0.08	Suspect	0.519	0.148	Suspect
Mix processed	0.242	0.05	Negative	0.419	0.202	Negative
Mix shelf-life	0.322	0.06	Suspect	0.566	0.228	Suspect
Negative	0.101	0.017		0.095	0.030	
Melon raw	0.091	0.002	Negative	0.076	0.025	Negative
Melon processed	0.087	0.001	Negative	0.088	0.002	Negative
Melon shelf-life	0.094	0.001	Negative	0.092	0.001	Negative
Negative	0.061	0.003		0.063	0.001	

The use of classical microbiological approaches verified the presence of *E. coli* O157:H7 in mixed salad raw and rocket three days shelf-life and absence in the rest of the samples. Absence of *L. monocytogenes* was verified in all samples.

If the samples classified as 'suspects' are considered positives, then a 2×2 contingency table summarising the results obtained by the ELISA can be formed and the samples can be differentiated into true positives, true negatives, false positives and false negatives (Table 2). Furthermore, the performance indices of the newly developed methods used to detect *L. monocytogenes* and *E. coli* O157:H7 can be calculated (Table 3).

Table 2. The 2 × 2 contingency table obtained from the application of the newly ELISA method used to detect *L. monocytogenes* and *E. coli* O157:H7.

	TP	TN	FP	FN
L. monocytogenes	2	37	2	0
E. coli O157:H7	3	35	2	1

TP: True positive. TN: True negative. FP: False positive. FN: False negative.

Table 3. Performance indices of the newly ELISA method used to detect *L. monocytogenes* and *E. coli* O157:H7.

	L. monocytogenes	*E. coli* O157:H7
Sensitivity	1	0.75
Specificity	0.94	0.94
Positive predictive value	0.5	0.06
Negative predictive value	1	0.97
Positive likelihood ratio	19.5	13.87
Negative likelihood ratio	0	0.26

Conclusions

Results obtained exhibited the technical efficiency of the developed methods. More accurately, all methods compared had similar sensitivity, specificity, negative predictive values and negative likelihood ratios. False positive results obtained by the ELISA method resulted in the reduction of positive predictive values. Regarding their efficacy and viability for routine analysis it is mostly dependent upon available equipment and technical expertise.

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