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Detection and Quantification of *Listeria monocytogenes* in Ready-to-eat Vegetables, Frozen Vegetables and Sprouts Examined by Culture Methods and Real-time PCR

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Abstract In this study, a total of 175 samples of ready-to-eat vegetables, frozen vegetables and sprouted seeds originating in 10 states of the European Union and from 32 manufacturers were collected during a period of one year and examined for the presence of *Listeria monocytogenes* using standard culture methods and qPCR. In addition to these methods, isolation of *Listeria monocytogenes* was also carried out following a unified sample preparation for combined downstream use in culture and qPCR analysis. Standard culture and culture preceded by unified sample preparation, showed that *L. monocytogenes* was present in 6.9% and 11.4% of analyzed samples, respectively, in low numbers. Application of qPCR revealed only 2.3% of samples to be positive for *L. monocytogenes* in small quantities (less than 10 cells/gram). A statistically significant higher occurrence of *L. monocytogenes* was seen in frozen vegetables compared to ready to eat vegetables ($p<0.01$; Fisher's exact post-hoc tests with Bonferroni's correction) or sprouts ($p<0.05$; Fisher's exact post-hoc tests with Bonferroni's correction). Therefore, temperature abuse in food containing pieces of frozen vegetables without any processing such as cooking, may pose health risks, especially for sensitive individuals such as pregnant women, children, the elderly, and immunocompromised individuals.

Keywords: *Listeria monocytogenes, Foodborne pathogens, Molecular methods, qPCR, Ready-to-eat*

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1. Introduction

Minimally processed vegetables such as cut green leaves or salads and sprouted seeds have become popular foods due to their health benefits and the short time required for their preparation. Since these vegetable foods do not need further treatment such as cooking before consumption, their contamination by foodborne pathogens could pose a high health risk for humans [1]. According to international law, unsafe food should not be placed on the market and to meet this requirement, microbiological testing must be performed on raw materials and finished products, as well as during the manufacturing process.Due to the short shelf life of raw vegetables, fast and automated detection systems for pathogens are required. This has led to a more widespread incorporation of real-time PCR (alternatively denoted as quantitative PCR - qPCR) methods in the food industry [2]. Further advantages of qPCR methods include the possibility of identifying and quantifying more pathogens simultaneously, and the detection of specific genes linked to strain virulence or resistance to antimicrobial agents. The majority of studies that were focused on the screening of *Listeria monocytogenes* used qPCR only for qualitative detection after a pre-enrichment step [3]. Only in a few cases was direct quantification of *L. monocytogenes* using qPCR carried out [4].

Listeriosis is the third leading cause of death by foodborne pathogens [5]. In healthy individuals, the condition usually manifests as self-limiting diarrhea or can be asymptomatic. *L. monocytogenes* in immunosuppressed individuals is the causal agent of invasive listeriosis, which can elicit septicemia, meningitis, encephalitis or abortion [6].

L. monocytogenes is widely distributed in nature (e.g., soil, water and vegetables), and can be found in foods of animal origin (e.g., raw milk, ripened cheeses, meat, poultry and fish products). However, it can also be isolated from the manure of farmed animals or irrigation water [7,8]. *L. monocytogenes* is also able to grow at lower temperatures such as 4°C and can contaminate and persist in plant processing environments; therefore, contamination of vegetables and vegetable products by these pathogens may pose a significant problem for the food industry [7,9].

The goal of the present study was to evaluate the occurrence and quantity of *L. monocytogenes* on ready-to-

eat vegetable produce and sprouts originating in the states of the European Union using a standard culture method and direct qPCR. Due to the necessity of detection and quantification of more pathogens and combination of direct qPCR and culture in the future, another aim of this study was to test how a unified procedure for sample preparation for combined downstream use in culture and direct qPCR analysis of *L. monocytogenes* influences the isolation of this pathogen.

2. Materials and Methods

2.1. Samples

A total of 175 samples originating in 10 states (Czech Republic, Italy, Germany, France, United Kingdom, Austria, Belgium, Netherlands, Poland and Slovakia) of the European Union and from 32 manufacturers were collected during the year 2014 from nine supermarkets in the Czech Republic. Samples included ready to eat freshly cut vegetables such as mixed salads and cut green leaves (e.g., arugula, spinach, parsley or lamb's lettuce), frozen vegetables (e.g., peas, carrot, maize; mixed, packed or not packed) and sprouts. All samples were transported to the laboratory in refrigerated boxes, stored in a refrigerator, frozen vegetables in a freezer and processed within 24 hours after delivery.

2.2. Standard Preparation of Samples and Culture

Standard preparation of samples and culture was carried out according to EN ISO 11290-1 [10]. Twenty-five grams of each sample were homogenized in stomacher (BagMixer® 400 W, Interscience, France) for 2 min with 225 ml of half Fraser broth (BioRad, Marnes-La-Coquette, France). *L. monocytogenes* was enriched in half-concentrated and full-concentrated Fraser selective enrichment broth (BioRad) and isolated on ALOA medium (Agar Listeria according to Ottaviani and Agosti; BioRad). *L. monocytogenes*-positive samples were enumerated according to EN ISO 11290-2 [11].

2.3. Simultaneous Molecular and Culture Analysis

2.3.1. Unified Preparation of Samples for qPCR and Culture

One hundred grams of each sample (fresh cut vegetables, frozen vegetables and sprouts) were respectively placed in a sterile plastic bag with 230 ml of Trizma base-glycine beef extract (TGBE) washing buffer containing 12.1 g Trizma base (Sigma, St. Louis, Missouri), 3.8 g glycine (Sigma, St. Louis, Missouri), 10 g beef extract pH 9.5 (Sigma, St. Louis, Missouri). Samples were then homogenized in a stomacher (BagMixer® 400 W) for 2 min. Subsequently, the liquid phase was transferred to centrifuge tubes and spun at 4 °C for 20 minutes at 8 000 × g. The supernatant was removed and the pellet was re-suspended in 10 ml of buffered peptone water (BPW; Oxoid, Hampshire, United Kingdom). Consequently, 2.0 ml of the 10 ml of buffered peptone water described above, representing 20 g of vegetable samples, were centrifuged again at 5 000 × g for 5 min, supernatant was removed and pellet for DNA isolation was stored at -80 °C and processed within a month. The schematic summary of the unified sample preparation for molecular analysis and culture methods is shown in Figure 1.

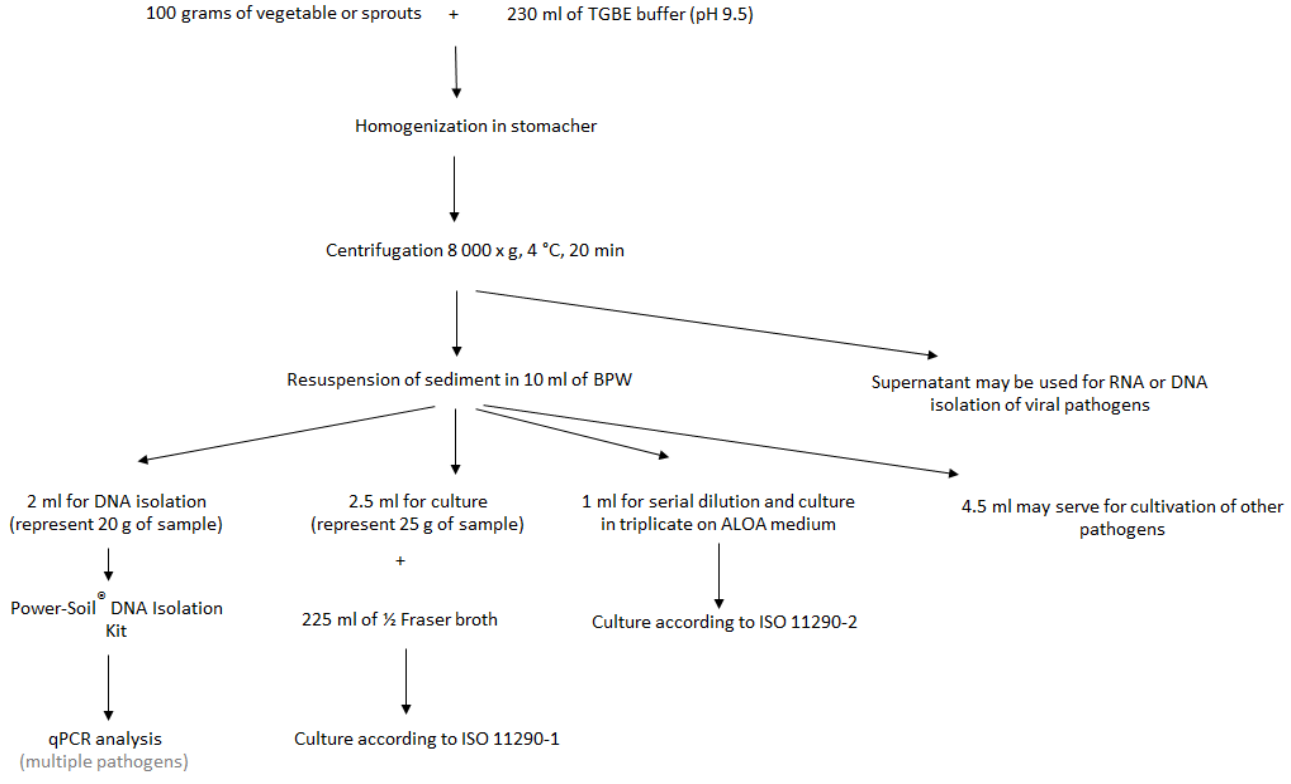

Figure 1. Schematic summary of unified preparation for qPCR and culture

2.3.2. Culture Following the Unified Preparation of Samples

Two and a half milliliters of sample in BPW (corresponding to 25 g of initial vegetable sample) were added to 225 ml of half Fraser Broth (BioRad) for pre-enrichment of *L. monocytogenes*. To determine the quantity of *L. monocytogenes* by culture, 1.0 ml of sample in BPW (corresponding to 10 g of initial vegetable sample) was diluted in BPW and serial dilutions were plated on Petri dishes with ALOA (BioRad) medium in triplicate. The culture and enumeration of *L. monocytogenes* was performed according to EN ISO 11290-1 [10] and EN ISO 11290-2 [11] as in the case of standard preparation and culture (paragraph 2.2.)

2.3.3. DNA Extraction Following the Unified Preparation of Samples

An optimal extraction procedure was selected with regard to results from our previous study [12]. DNA was isolated from prepared pellet using the Power-Soil® DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, California) according to the manufacturer's instructions with minor modifications. To decrease losses of DNA during manipulation, each sample was supplemented with Carrier DNA solution (salmon sperm DNA, 50 ng/µl; Serva, Heidelberg, Germany)

2.3.4. Detection and Quantification of *L. monocytogenes* Using qPCR

L. monocytogenes was detected and enumerated using triplex qPCR assays. The generic internal amplification control nep/F57 was added to reactions in an amount of 10^3 copies to eliminate false negative samples [12]. *L. monocytogenes* was detected using primers targeting the species-specific *hly* gene (encoding virulence factor listeriolysin O) and genus-specific region of the *23S rRNA* gene [13]. The quantification of *L. monocytogenes* was performed according to a standard curve derived from a 10-fold serial dilution of plasmid gradient constructed for the *hly* gene in a range of 5×10^5 and 5×10^0 copies per 5 µl of qPCR reaction. qPCR reactions were performed in a total volume of 20 µl containing 2x Light Cycler® 480 Probes Master (Roche Applied Science, Manheim, Germany), with addition of 0.2 U of Uracil-DNA Glycosylase (Roche) to prevent DNA carry-over; primer concentration was 500 nM and probe concentration was 50 nM. The qPCR reactions were performed on a Light Cycler 480 II (Roche) with the following protocol: pre-incubation at 95 °C for 7 min, followed by 45 amplification cycles consisting of 95 °C for 10 s, 60 °C for 30 s (fluorescence acquisition), and 72 °C for 1 s. The "Fit point analysis" option of the LightCycler 480 software (version 1.5.0.39) was used to determine the absolute numbers of bacterial cells. The quantity of bacterial cells was subsequently recalculated according to the parameters of DNA isolation to grams of initial amount of sample [12].

2.4. Statistical Analysis

To compare the frequencies of positive findings between the standard culture method and culture following the unified preparation of the sample, and between the standard culture method and detection of *L. monocytogenes* by qPCR, Fisher's exact test was used. The association of positive findings in the qPCR and in the standard culture with the produce type was assessed by the χ^2-test for independence, followed by Fisher's exact post-hoc tests with Bonferroni's correction of p-values. All tests were performed using GraphPad Prism 5.04 software (GraphPad, Inc., San Diego, CA, USA). Differences were considered statistically significant if $p<0.05$.

3. Results and Discussion

Many reports of foodborne outbreaks or sporadic cases have demonstrated the importance of monitoring the presence and the exact level of pathogens in food [5,7,14]. Listeriosis is a sporadic disease, but regardless of the number of hospitalizations, deaths and annual costs, *L. monocytogenes* is considered as one of the most important foodborne pathogens [5]. In this study, results obtained by standard culture showed a relatively low occurrence as well as low numbers of *L. monocytogenes* in ready-to-eat vegetables and sprouts originating from the states of the European Union (Table 1). Application of standard culture methods revealed the presence of *L. monocytogenes* in 6.9% of analyzed samples, in counts of under 100 CFU/g.

The occurrence of *L. monocytogenes* was 2.1% for cut green leaves or mixed salads and 2.9% for sprouts (Table 1). A low occurrence of this pathogen in minimally processed vegetables has been reported by other authors [15,16,17].

A statistically significant higher occurrence of *L. monocytogenes* was found in frozen vegetables ($p<0.05$ at least; Fisher's exact post-hoc tests with Bonferroni's correction) originating from various manufacturers. Using standard culture, *L. monocytogenes* was detected in almost 20.9% of these samples. Similar results were observed previously [18]where *L. monocytogenes* in very low counts was isolated from 25.4% of the samples of frozen vegetable salads. This observation shows that frozen vegetable products may be prone to *L. monocytogenes* contamination that may occur from the soil or irrigation water on fields, or during processing in a plant. The long-term persistence of *L. monocytogenes* in food processing plants is an important factor for the transmission of this pathogen to food. Strains of *L. monocytogenes* were found to persist and could be recovered from a produce-processing plant over a period of more than two years even when regular daily disinfection was carried out [9].

The application of real-time PCR is one of the most promising advances in food safety and the technique represents a suitable supplementary method for the fast and automatized screening of foodborne pathogens in food matrices. The main advantages of qPCR include the ability of detecting and quantifying more pathogens (also viruses and protozoa) isolated from a single sample, with the possibility of the application of a multiplex format within a single qPCR reaction [2]. Many qPCR systems for the detection of foodborne pathogens are currently commercially available [2]. However, studies comparing direct qPCR and conventional culture methods for different food matrices are still few in number. In this study, we compared the results of *L. monocytogenes* detection, using both methods, from different foods of

plant origin. Using qPCR, only 2.3% of samples (one fresh mixed vegetable salad and three samples of frozen vegetables) were positive for *L. monocytogenes* in small quantities (less than 10 cells/gram; Table 1). Due to the small number of samples examined in this study this difference between qPCR and the standard culture method was not statistically significant ($p>0.05$; Fisher's exact test). The low numbers of qPCR-positive samples are probably due to the absence of any enrichment procedure before qPCR.

Table 1. Detection of *Listeria monocytogenes* in produce using culture methods and direct qPCR

Produce type	No. of samples	Standard culture[a]		Unified culture[d]		qPCR	
		Positive[b]	Quantity[c]	Positive[b]	Quantity[c]	Positive[e]	Quantity[f]
Cut green leaves or salads	97	2 (2.1%)	< 50	4 (4.1%)	< 50	1 (1.0%)	10^0
Frozen vegetables	43	9 (20.9%)	< 50	15 (34.9%)	< 50	3 (7.0%)	10^0
Sprouts	35	1 (2.9%)	< 50	1 (2.9%)	< 50	0 (0.0%)	-
Total	175	12 (6.9%)	< 50	20 (11.4%)	< 50	4 (2.3%)	-

[a] Standardized cultivation - cultivation according to ISO 11290-1 and ISO 11290-2 preceded by standard sample preparation
[b] Number (percentage) of positive samples for *L. monocytogenes* after the enrichment of the sample
[c] Number of colony forming units in gram of sample (CFU/g)
[d] Unified cultivation - cultivation according to ISO 11290-1 and ISO 11290-2 preceded by the unified sample preparation described in this paper (sample preparation is common to both qPCR and cultivation)
[e] Number (percentage) of qPCR samples positive for the *hly* gene (*L. monocytogenes)*
[f] Number of cells in gram of sample did not exceed the level 10^0

Table 2. *Listeria monocytogenes*-positive samples detected using culture with standard and modified methods and direct qPCR

No. order	Sample	Producer[a]	Country of origin[b]	Store[c]	Culture		qPCR	
					Standard[d,e]	Unified[f,e]	*hly* gene[g]	Quantity (cells/gram)
1	Sprouts	a	CR	A	-	+	-	
2	Sprouts	b	CR	B	+	-	-	
3	fresh spinach	c	PL	C	-	+	-	
4	fresh rocket	d	IT	B	+	-	-	
5	fresh mixed salad	e	CR	B	-	-	+	$3.48x10^0$
6	fresh mixed salad	f	CR	C	-	+	-	
7	fresh spinach	g	EU	D	-	+	-	
8	fresh spinach	f	CR	A	+	+	-	
9	frozen spinach	h	na	B	+	+	-	
10	frozen mixed vegetables	h	na	B	+	-	-	
11	frozen green beans	h	na	B	+	-	-	
12	frozen pepper	h	na	B	+	-	-	
13	frozen mixed vegetables	i	PL	B	-	+	-	
14	frozen mixed vegetables	i	PL	B	+	+	-	
15	frozen maize	j	CR	E	+	+	-	
16	frozen mixed vegetables	k	SR	B	-	+	-	
17	frozen peas	k	SR	B	-	+	-	
18	frozen peas	k	SR	B	-	+	-	
19	frozen peas	k	CR	B	-	+	-	
20	frozen mixed vegetables	l	CR	B	-	+	+	$1.20x10^0$
21	frozen mixed vegetables	l	CR	B	-	+	-	
22	frozen mixed vegetables	m	CR	B	-	+	+	$2.50x10^0$
23	frozen peas	n	A	B	-	+	+	$5.60x10^0$
24	frozen mixed vegetables	n	A	B	+	+	-	
25	frozen peas	o	NL	A	+	+	-	
26	frozen peas	p	CR	B	+	+	-	
	Total	16	6+1	5	12	20	4	

[a] Producers were labeled with the letters "a" to "p"
[b] CR – Czech Republic, PL – Poland, IT – Italy, EU – The European Union, SR – Slovak Republic, A – Austria, NL - Netherlands
[c] Stores were labeled with the letters "A" to "E"
[d] Cultivation according to ISO 11290-1 preceded by standard sample preparation
[e] +, -: presence, absence of culturable *L. monocytogenes*, all isolates were obtained after multiplication in enrichment medium
[f] Cultivation according to ISO 11290-1 preceded by the unified sample preparation method described in this paper (sample preparation is common to both qPCR and culture)
[g] +, -: presence, absence of the *hly* gene (specific gene to *L. monocytogenes*)
na – not available

To simplify simultaneous detection of foodborne pathogens by qPCR and culture, we have tested a procedure for sample preparation that is convenient for qPCR and also for culture. The advantage of this approach is that it could be used in a broad range of applications including simultaneous culture of various types of bacteria.

Similar to standard culture, culture following the unified preparation of samples included detection as well as enumeration of *L. monocytogenes*. Using standard culture, *L. monocytogenes* was isolated from 6.9% of studied samples compared to the 11.4% of positive samples observed using culture preceded by unified preparation. In total, using both culture methods *L. monocytogenes* was isolated from 14.3% of samples. A greater difference in *L. monocytogenes* detection was demonstrated in frozen vegetables where 20.9% of samples were positive using standard culture and 34.9% using culture following unified preparation. However, no statistically significant difference between the methods was observed in either case ($p>0.05$; Fisher's exact test), due to the small number of tested samples. Despite this, it can be concluded that the modifications of the sample preparation method tested in this study positively influenced the probability of *L. monocytogenes* detection in terms of the number of samples testing positive. Comparison of the results of standard culture and culture with unified preparation showed considerable differences in *L. monocytogenes* presence in the studied samples (Table 2). The discrepancy between the results of the two methods (ISO and unified protocol) is likely caused by the Poisson distribution of the low numbers of bacteria in the analyzed samples. The same explanation might also account for the higher detection rates and probability of detection in samples analyzed using the unified protocol as the larger volume of sample (100 g) provides better homogeneity after the sample processing compared to the standard ISO procedure (25 g of sample).

4. Conclusion

In conclusion, the results of standard culture showed that *L. monocytogenes* was usually present in low numbers and at levels below the limit of detection of the qPCR, which generally ranges between 10^2 to 10^3 cells/gram for these types of matrices [12]. Samples of frozen vegetables tested positive more often than freshly cut vegetables or sprouts. Therefore, temperature abuse in food containing pieces of frozen vegetables without any processing such as cooking may represent a risk to human health. The results of culture following the unified procedure of sample preparation showed that it is possible to use this modification for the detection of *L. monocytogenes*. Further, comparison of the two culture analyses revealed a large variability in *L. monocytogenes* detection rates in these samples, which could be caused by a cumulative effect of the heterogenic distribution of pathogen on the surface of the sample.

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References

[1] Callejon, R.M., Rodriguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C. and Troncoso, A.M, "Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes" *Foodborne Pathogens and Disease*, 12 (1). 32-38. January 2015.

[2] Chapela, M.J., Garrido-Maestu, A. and Cabado, A.G, "Detection of foodborne pathogens by qPCR: A practical approach for food industry applications." *Cogent Food and Agriculture*, *1* (1). 1013771. March 2015.

[3] Losio, M.N., Pavoni, E., Bilei, S., Bertasi, B., Bove, D., Capuano, F., Farneti, S., Blasi, G., Comin, D., Cardamone, C., Decastelli, L., Delibato, E., De Santis, P., Di Pasquale, S., Gattuso, A., Goffredo, E., Fadda, A., Pisanu, M. and De Medici, D, "Microbiological survey of raw and ready-to-eat leafy green vegetables marketed in Italy" *International Journal of Food Microbiology*, 210. 88-91. October 2015.

[4] Berrada, H., Soriano, J.M., Pico, Y. and Manes, J, "Quantification of *Listeria monocytogenes* in salads by real time quantitative PCR" *International Journal of Food Microbiology*, 107 (2). 202-206. March 2006.

[5] Scallan, E., Hoekstra, R.M., Angulo, F.J., Tauxe, R.V., Widdowson, M.A., Roy, S.L., Jones, J.L. and Griffin, P.M, "Foodborne illness acquired in the United States-major pathogens" *Emerging Infectious Diseases*, 17 (1). 7-15. January 2011.

[6] de Noordhout, C.M., Devleesschauwer, B., Angulo, F.J., Verbeke, G., Haagsma, J., Kirk, M., Havelaar, A. and Speybroeck, N, "The global burden of listeriosis: a systematic review and meta-analysis" *Lancet Infectious Diseases*, 14 (11). 1073-1082. November 2014.

[7] European Food Safety Authority (EFSA). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2015. *EFSA Journal* 14 (12):4634. 2016.

[8] Hutchison, M.L., Walters, L.D., Avery, S.M., Synge, B.A. and Moore, A, "Levels of zoonotic agents in British livestock manures" *Letters in Applied Microbiology*, 39 (2). 207-214. July 2004.

[9] Pappelbaum, K., Grif, K., Heller, I., Wuirzner, R., Hein, I., Ellerbroek, L. and Wagner, M, "Monitoring hygiene on- and at-line is critical for controlling *Listeria monocytogenes* during produce processing" *Journal of Food Protection*, 71 (4). 735-741. April 2008.

[10] International Organization for Standardization. Microbiology of food and animal feeding stuffs — horizontal method for detection and enumeration of *Listeria monocytogenes*, Part 1 – Detection Method (EN ISO 11290-1:1996). International Organization for Standardization, Geneva, Switzerland. 1996.

[11] International Organization for Standardization. Microbiology of food and animal feeding stuffs — horizontal method for detection and enumeration of *Listeria monocytogenes*, Part 2 – Detection Method (EN ISO 11290-2:1998). International Organization for Standardization, Geneva, Switzerland. 1998.

[12] Vojkovska, H., Kubikova, I. and Kralik, P, "Evaluation of DNA extraction methods for PCR-based detection of *Listeria monocytogenes* from vegetables" *Letters in Applied Microbiology,* 60 (3). 265-272. March 2015.

[13] Rodriguez-Lazaro, D., Hernandez, M. and Pla, M, "Simultaneous quantitative detection of *Listeria* spp. and *Listeria monocytogenes* using a duplex real-time PCR-based assay" *FEMS Microbiology Letters*, 233 (2). 257-267. April 2004.

[14] Malorny, B., Lofstrom, C., Wagner, M., Kramer, N. and Hoorfar, J, "Enumeration of salmonella bacteria in food and feed samples by real-time PCR for quantitative microbial risk assessment" *Applied and Environmental Microbiology*, 74 (5). 1299-1304. March 2008.

[15] Caponigro, V., Ventura, M., Chiancone, I., Amato, L., Parente, E. and Piro, F, "Variation of microbial load and visual quality of ready-to-eat salads by vegetable type, season, processor and retailer" *Food Microbiology*, 27 (8). 1071-1077. December 2010.

[16] De Giusti, M., Aurigemma, C., Marinelli, L., Tufi, D., De Medici, D., Di Pasquale, S., De Vito, C. and Boccia, A, "The evaluation of the microbial safety of fresh ready-to-eat vegetables produced by different technologies in Italy" *Journal of Applied Microbiology*, 109 (3). 996-1006. March 2010.

[17] Froder, H., Martins, C.G., De Souza, K.L., Landgraf, M., Franco, B.D. and Destro, M.T, "Minimally processed vegetable salads: microbial quality evaluation" *Journal of Food Protection*, 70 (5). 1277-1280. May 2007.

[18] Cordano, A.M. and Jacquet, C, "*Listeria monocytogenes* isolated from vegetable salads sold at supermarkets in Santiago, Chile: prevalence and strain characterization" *International Journal of Food Microbiology*, 132 (2-3). 176-179. June 2009.