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INCIDENCE OF *Listeria monocytogenes* AND *Bacillus cereus* IN SOME FOOD OF PLANT ORIGIN AND METHODS OF THEIR CONTROL

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ABSTRACT

This study was carried out to develop a practical and effective method for inhibition or reduction of the growth of *Listeria monocytogenes* and *Bacillus cereus* counts on orange, guava, carrot and green beans at different temperatures: room temperature 25˚C ±1, refrigerator temperature 4˚C ±2 and freezer temperature -18˚C for different storage periods (0, 1, 3, 7, 30, 60, 120 and 180 days). Also screening of the presence of these 2 microorganisms was performed using RT-PCR technique. All samples were spot-inoculated with the pathogenic strains 6 $\log_{10}$ cfu near the stem end and were submerged in two solutions of distilled water containing 1.5% for each of Lactic acid and hydrogen Peroxide (solution A) and 1.5%: 2.0% (solution B) respectively at 40˚C for 15 min. Inoculated samples treated with sterile distilled water at the same temperatures and for the same durations were considered as controls. *Listeria monocytogenes* and *Bacillus cereus* were detected in 6.67% and 13.33% of the examined samples, respectively. The results showed that, bacteria on all treated samples except the green beans samples were completely inhibited by treatment with solution A from the first day of the experiment. Although the injured bacteria were still present till the 1st day for Guava and the 3rd day for carrot, all treatments didn't affect the injured bacteria in case of green beans samples till the end of the experiment (at all treated temperatures). Similar trends were found for solution B treated samples (orange, guava, carrot and green beans).

INTRODUCTION

Food poisoning cases caused by contamination of vegetables and fruits have been reported in the last decade. *Listeria monocytogenes* is considered as one of the most important causes of food poisoning which are characterized by nausea, vomiting, fever, abdominal pain and diarrhea. The complications of the infection with this pathogen are meningitis, faetal malformation, mental retardation and abortion of pregnant women.

Listeria monocytogenes can be isolated from soil, water, animal manure and other environmental sources. Vegetables can become contaminated from the soil or from manure used as fertilizer. The bacterium has been found in a variety of raw foods such as vegetables, fruits, ready-to-eat food, uncooked meat and vegetables, as well as in post contaminated processed foods (Farber and Peterkin, 1991; Lou and Yousef, 1996).

Some countries have established legal limits on the number of *Listeria monocytogenes* organisms that are permissible in foods, especially ready-to-eat products, whereas others have suggested guidelines or criteria that do not have legal standing (Jay, 1996).

The International Commission on Microbiological Specification for Foods has come to the conclusion that if this organism does not exceed 100 organisms/g of food at the point of consumption, the food is considered acceptable for individuals who are not at risk.

Guidelines for some ready to-eat foods establish four quality groups based on the numbers of *Listeria monocytogenes*: not detected in 25 g is satisfactory; $<10^2$/25 g is fairly satisfactory; 10^2 to 10^3 is unsatisfactory; and numbers of $>10^3$ make the food product unacceptable (Gilbert, 1992).

Another important pathogen shares *Listeria monocytogenes* in its food poisoning effect is *Bacillus cereus* which causes the same gastro enteric symptoms with its excreted toxins.

Bacillus cereus is a food-borne pathogen which often contaminates foods of plant origin. Recent investigations have shown that, members of the *Bacillus cereus* group carry genes which have the potential to cause gastrointestinal and somatic diseases mainly because of its produced exotoxins (Hansen *et al.,* 2002).

The epidemiology of *Bacillus cereus* strains responsible for food poisoning is scantly known, mostly because the genotypic and toxigenic properties of the *Bacillus cereus* strains isolated during food-poisoning outbreaks have been never catalogued (Ghelardi *et al.,* 2002).

Improving methods for detection of food poisoning microorganisms in food stuffs plays a very important role in its control. DNA-based methods, such as the PCR, have been increasingly used for rapid, sensitive, and specific detection of *Listeria monocytogenes* (Deener and Boychus, 1991; Olsen *et al.*, 1995; Francis and O'Beirne, 2006) and *Bacillus cereus* (Choo *et al. 2007;* and Abriouel *et al. 2007,* and Masafumi *et al.,* 2006).

The demand of vegetables minimally processed (ready-to-use) has increased partly due to the frequent use of the food services, where the salads are always included in the daily menus (de Curtis *et al.,* 2002). Fresh fruits and vegetables generally do not undergo any processing or cooking to eliminate pathogenic microorganisms before consumption. Therefore, methods to effectively reduce or eliminate pathogenic microorganisms on fruits and vegetables are needed. Currently, there is no processing method that will totally inactivate pathogens on fresh products without compromising sensory quality (Venkitanarayanan *et al.,* 2002).

The Food and Drug Administration proposed that treatments of fruits, vegetables, and commercial fresh juices should be capable of reducing pathogen loads by a minimum of 5.0 log CFU (FDA, 1998).

Washing with cold chlorinated water is the most widely used method, but its efficacy in inactivating pathogenic bacteria on the surfaces of fresh products is minimal (Sapers and Simmons 1998 and Sapers *et al.,* 1999).

Lactic acid and hydrogen peroxide are generally recognized as safe chemicals for specific uses in foods. The antibacterial activity of hydrogen peroxide alone or in combination with lactic acid on fresh products has been reported (Venkitanarayanan *et al.,* 1999; Sapers *et al.,* 2000).

Hence, the treatment of fruits and vegetables with hydrogen peroxide with or without lactic acid may be a practical approach to inactivate

pathogenic bacteria without compromising sensory quality (Chia-Min Lin, 2002).

The aim of this work is to use different technological treatments for controlling the spread of *Listeria monocytogenes* and *Bacillus cereus* through:

1- Screening of the incidence of *Listeria monocytogenes* and *Bacillus cereus* in carrots, green beans, guava and orange using real time PCR technique.
2- Using low temperature treatment at (5 ^{O}C and -18 ^{O}C) for different storage periods and studying their effect on the above mentioned pathogens.
3- Using different concentrations of lactic acid and hydrogen peroxide as surface preservatives and studying their effect on the above mentioned pathogens.

MATERIALSAND METHODS

A- Materials

Samples: Sixty samples of fresh vegetables and fruits (orange, guava, green beans and carrots) were purchased and collected from different Egyptian retail markets in (Giza, Misr Al-Qadima, Nasr city, Imbaba and Maadi) in different seasons to detect *Listeria monocytogenes* and *Bacillus cereus* by Real Time Polymerase Chain Reaction (RT-PCR) as a rapid test. Samples were collected in ice box, were labeled with all required data (Date of Collection, Source…. Etc") and were transported immediately to the laboratory. Also about 15 kg from each of the examined types (orange, guava, green beans and carrots) were collected to study the factors affecting the growth of *Listeria monocytogenes* and *Bacillus cereus* in different storage conditions after treatment with Lactic acid (LA) and Hydrogen peroxides (H_2O_2) at different concentrations and different temperatures.

Bacterial strains: The bacterial strains (*Listeria monocytogenes* and *Bacillus cereus*) were isolated, identified and kindly supplied by Food safety and Biotechnology Lab – Regional Center for Food and Feed – Agriculture Research Center – Egypt.

DNA extraction buffer: DNA extraction was performed using Prepman Ultra sample preparation reagent, Applied Biosystem, USA.

Primers and probe: Sequences of primers and probe used in this study are illustrated in table (1).

Table (1): Primer and Probe sequences of bacteria under investigation:

Listeria monocytogenes Forward primer sequence:	TGC AAG TCC TAA GAC GCCA
Listeria monocytogenes Reverse primer sequence:	CAC TGC ATC TCC GTG GTA TAC TAA
Listeria monocytogenes Probe:	6 – FAM – CGA TTT CAT CCG CGT GTT TCT TTT CG – TAMRA
Bacillus cereus Forward primer sequence:	GTG TTT GAC CAA GGT GGA CAA

Bacillus cereus Reverse primer sequence:	TTA CTC CAT AGA GCA CCC TTG GA
Bacillus cereus Probe:	6 – FAM- CCA AAA CCA GTT GCC AGT GCA TTG G – TAMRA

Master Mix: TaqMan Universal Master Mix (Applied Biosystems, USA) which contained AmpErase uracil-N-glycosylase (UNG), deoxynucleoside triphosphate with dUTPs, 6-carboxyrhodamine as an internal passive fluorogenic reference, and an optimized buffer component.
Mixture (B): Prepared 1.5/1.5 % vol/vol from Lactic acid (LA) and Hydrogen peroxides (H2O2), containing to 1 litre of distilled water.
Mixture ©: Prepared 1.5/2.0 % vol/vol from Lactic acid (LA) and Hydrogen peroxides (H2O2), containing to 1 litre of distilled water.
All concentrations were prepared fresh at the time of work.

B- Methods
Screening of *Listeria monocytogenes* and *Bacillus cereus* in examined samples:
Preenrichment: All tested samples were enriched in Buffered Peptone Water (BPW) for 24 hours at 37˚C before subjected to DNA extraction.
DNA extraction: DNA extraction was performed using Prepman Ultra sample preparation reagent, Applied Biosystem, USA, according to the user manual attached to the Kit as follows:
One ml from the enriched BPW was transferred to 2 ml microcentrifuge tube and spinned for 3 minutes at 16000 rpm. The resulted supernatant was discarded and the obtained pellet was eluted using 200 *ul* of Prepman Ultra sample preparation reagent. The tube then was incubated in a boiling water bath for 10 minutes with frequent mixing. After reaching room temperature, the tube was centrifuged at 16000 rpm for 3 minutes then 50 *ul* from the supernatant was transferred to a new labeled microcentrifuge tube and stored at -4 ˚C till PCR reaction.
PCR reaction: PCR was performed in a reaction mixture with a total volume of 25 *ul* containing 1 *ul* of extracted DNA, 0.5 mM of the same primers of each pathogen, 0.2 mM of fluorogenic probe (Table 22), and TaqMan Universal Master Mix (Applied Biosystems, USA). The Master Mix contained AmpErase uracil-*N*-glycosylase (UNG), deoxynucleoside triphosphate with dUTPs, 6-carboxyrhodamine as an internal passive fluorogenic reference, and an optimized buffer component. Amplification and detection were carried out in optical-grade 96-well plates in an ABI Prism 7000 sequence detection system (Applied Biosystems) with an initial step of 50˚C for 2 minutes, which is the required optimal AmpErase UNG enzyme activity, and then at 95˚C for 10 minutes, to activate the AmpliTaq Gold DNA polymerase and to deactivate the AmpErase UNG enzyme, followed by 40 cycles of 95˚C for 15 second and 60˚C for 1 minutes. The reaction conditions for amplification and the parameters for fluorescence data collection were programmed into a Dell laptop linked directly to the ABI Prism 7000 sequence detection system by using the SDS 1.6 application software, according to the manufacturer's instructions. After real-time data acquisition, the threshold, which was defined

as being 10-fold higher than the baseline, was determined; and the cycle threshold (*CT*) value was manually set so that it intersected the amplification curves in the linear region of the semilog plot.

Studying the effect of surface treatments, storage temperatures and storage durations on viability of *Listeria monocytogenes* and *Bacillus cereus*:

Samples Preparation:

All collected samples (15 kg from each type) were washed in sterile plastic ice box with soap and rinsing tap water, and wiped with a paper towel, and then immersed in physiological saline, and wiped again with a paper towel, then washed in sterile distilled water and wiped again with a paper towel, After dryness, each type of samples was divided into 3 parts (A, B and C) and each individual unit from each part was subsequently separated into sterile stomacher bag. All bags were placed at 4 ˚C until the time of inoculation.

Preparation of bacterial suspensions:

Listeria monocytogenes and *Bacillus cereus* were inoculated in brain heart infusion broth and incubated for 24 hrs at 30 ˚C and 37 ˚C for *Bacillus cereus* and *Listeria monocytogenes* respectively.

The colony forming units per each milliliter of prepared suspension was estimated by using each *Bacillus cereus* agar and Listeria oxford agar as follows:

Sterile dilutions of the prepared suspensions were done using sterile saline solution, from each dilution 1 ml was inoculated in sterile Petri dish then about 10 ml of the specific media was poured and after good mixing and solidification of the media, the plates were incubated at 30 ˚C and 37 ˚C for *Bacillus cereus* and *Listeria monocytogenes* respectively, Suspension of 10^6 cfu/ml were stored at 4 ˚C till inoculation.

Inoculation of samples under investigation, surface treatment by Hydrogen peroxide, Lactic acid and Water and enumeration of *Listeria monocytogenes* and *Bacillus cereus* in subsamples were performed according to **(**Venkitanarayanan *et al.,* 2002)

RESULTS AND DISCUSSION

Two important criteria that should be considered in developing an antimicrobial treatment for product are that the treatment should be capable of reducing the microbial load on produce to be disinfected by at least 3 log CFU, and preferably by more than 5 log CFU and that it should not adversely affect the sensory characteristics of the treated produce (Venkitanarayana *et al*. 2002).

A spot inoculation method was used to inoculate samples under investigation with the pathogens because this method represents the mode by which they could be contaminated in the field by feces, soil, or hands more realistically than a dip method of inoculation. Furthermore, a spot inoculation method is the most consistent and reproducible method for inoculating a known number of bacterial cells on the surfaces of tested samples (Venkitanarayana *et al*. 2002).

Selective culture media can inhibit the recovery of stressed cells of bacteria. In this study, Buffered peptone water was used as a nonselective media for the 2 pathogens evaluated, and Oxford agar and *Bacillus cereus* agar were used as the selective media for enumerating *Listeria monocytogenes and Bacillus cereus,* respectively. (Venkitanarayana *et al*. 2002).

Data in Table 2 illustrate the incidence of *Listeria monocytogenes* and *Bacillus cereus* in collected fruits and vegetables samples. It is clear that, Listertia was detected in 6.67% of the examined samples while *Bacillus cereus* was detected in 13.33%.

Table (2): Incidence of *Listeria monocytogenes* and *Bacillus cereus* in examined samples:

	No. of samples	***L. monocytogenes***	***B. cereus***
Carrot	15	2	2
Green Beans	15	1	1
Guava	15	-	4
Orange	15	1	1
Total (number)	60	4	8
Total (%)	-	6.67	13.33

Also the results in Figure (1) showed the amplification blot of the positive samples which is indicated by cutting the threshold line forming a characteristic curve. The curves of the 4 positive *Listeria monocytogenes* samples and the 8 positive *Bacillus cereus* samples are shown in this figure. The negative results are indicated by the noise which appears in the same figure.

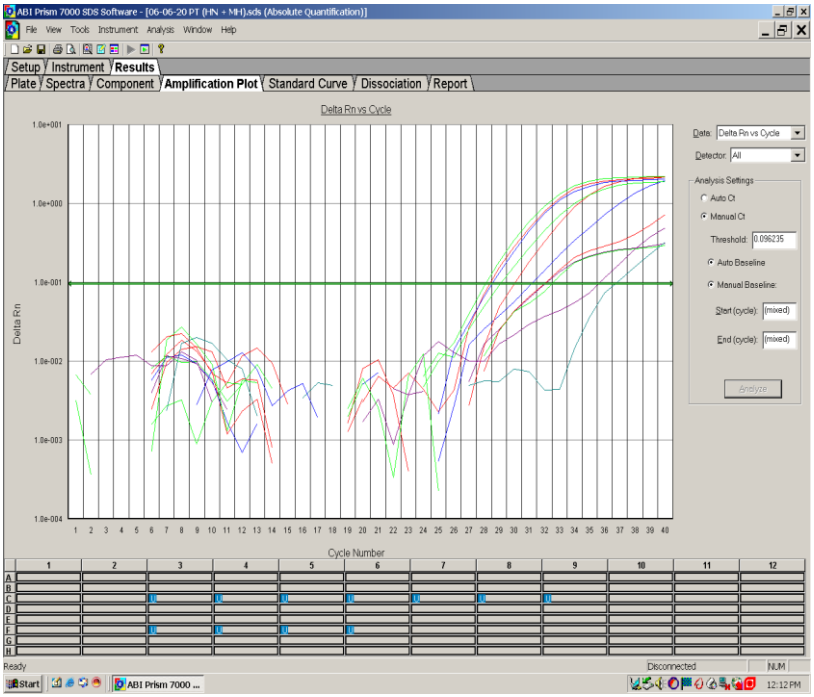

Figure (1): Amplification blots of *Listeria monocytogenes* and *Bacillus cereus* positive samples using RT-PCR.

This data represents greater level than that obtained by other authors like Sagoo *et al.,* 2001, Abadias *et al.,* and Froder *et al.,* who detect *Listeria monocytogenes* in 1.5%, 0.7% and 0.6% of vegetables and fruits respectively. This difference in obtained data may be attributed to the difference in the hygienic practices which may lead to increase in the incidence of this type of bacteria.

It is clear from the obtained data that, at zero time treatment (B group) (Lactic acid : H_2O_2 1.5 : 1.5) was sufficient to injure all *Bacillus cereus and Listeria monocytogenes* which is clear with their negative result in counting and positive result in detection of injured cells; while treatment (C group) (Lactic acid : H_2O_2 1.5 : 2.0) could slightly decrease *Bacillus cereus* count and markedly decrease *Listeria monocytogenes* count. The obtained result agreed with that reported by Venkitanarayanan *et al*. (2002) who observed the marked reduction of *Listeria monocytogenes* count on the surface of some fruits and vegetables after treatment with lactic acid : H_2O_2 1.5 : 1.5.

By the end of the 1st week of storage, count of treated samples in (B and C groups) was negative while injured *Bacillus cereus* bacteria were detected in (C group) treated Oranges (Table 3). This data are supported by that obtained by Lin *et al.,* 2002 and Ukuku 2004 who observed the reduction of bacterial count of refrigerated fruits and vegetables treated with Lactic acid : H_2o_2 1.5 : 1.5 by 3 logs. This also agrees with data obtained from tables 6, 9

and 12. The resistance of *Bacillus cereus* bacteria may be due to its being a sporulating pathogen which can resist the unfavorable conditions.

Table (3): Effect of different surface treatments of orange on *Bacillus cereus and Listeria monocytogenes* count (log) at room temperature for different storage periods and incidence of injured bacteria

Orange		Room Temperature					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.60	6.60	-	-	3.95	-
	injured	D	D	D	D	D	D
1 Day	count	3.48	2.60	-	-	3.30	-
	injured	D	D	ND	ND	D	D
3 Day	count	3.38	2.48	-	-	-	-
	injured	D	D	ND	ND	D	ND
7 Day	count	1.77	1.90	-	-	-	-
	injured	D	D	ND	ND	D	ND

(D): detected (ND): not detected (A): Dist. Water…. (B): Lactic: H_2o_2 1.5:1.5.…(C): Lactic: H_2o_2 1.5:2.0…. (B.c): *Bacillus cereus*…. (L.m): *Listeria monocytogenes*.

Storage at +5Cº : +8Cº could keep the *Bacillus cereus and Listeria monocytogenes* count in (A group) in relatively higher count than at room temperature. Also *Bacillus cereus* count in (C group) treated oranges could survive longer than that at room temperature (Table 4). These findings were in accordance with that obtained by Valero (2007) who concluded that, storage of food at 4ºC did not affect the bacterial count. This also can clarify data obtained from table 7, 10 and 13.

Table (4): Effect of different surface treatments of orange on *Bacillus cereus and Listeria monocytogenes* count (log) at refrigerator (+5Cº: +8Cº) for different storage periods and incidence of injured bacteria

Orange		Refrigerator +5Cº: +8Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	**count**	6.60	6.60	-	-	4.36	-
	injured	D	D	D	D	D	D
1 Day	**count**	3.00	2.77	-	-	3.78	-
	injured	D	D	ND	ND	D	D
3 Day	**count**	2.00	3.04	-	-	4.36	-
	injured	D	D	ND	ND	D	ND
7 Day	**count**	1.95	3.59	-	-	-	-
	injured	D	D	ND	ND	D	ND

Listeria monocytogenes in the non treated Oranges (A group) could survive the freezing temperature up to 4 months. These results are supported by that obtained by Iturriaga *et al.,* 2002 who found that *Listeria monocytogenes* and other bacterial strains could survive at freezing temperature for more than 14 months, while *Bacillus cereus* could not be detected at first month. These findings can be clarified by data obtained by Nogueira *et al.,* 2003 who found that, citrus fruits -like orange and lemon- have intrinsic antimicrobial compounds which could inhibit the growth of

certain types of bacteria, so *Bacillus cereus* could not survive this stress factor together with the freezing temperature stress which led to destruction of its living cells, while in case of *Listeria monocytogenes*; its own characteristic which enable it to survive low temperatures was the reason for finding it up to 4 months. The two pathogens in groups (B and C) could not be detected after 7 days of storage (Table 5).

Table (5): Effect of different surface treatments of orange on *Bacillus cereus and Listeria monocytogenes* count (log) at Freezer (-18Cº: -20Cº) for different storage periods and incidence of injured bacteria

Orange		Freezer -18Cº: -20Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.60	6.60	-	-	3.48	-
	injured	D	D	D	D	D	D
7 Day	count	-	1.60	-	-	-	-
	injured	D	D	ND	ND	ND	ND
1 Month	count	-	1.70	-	-	-	-
	injured	ND	D	ND	ND	ND	ND
2 Month	count	-	1.60	-	-	-	-
	injured	ND	D	ND	ND	ND	ND
4 Month	count	-	1.60	-	-	-	-
	injured	ND	D	ND	ND	ND	ND
6 Month	count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND

This finding is supported by that obtained by Lin *et al.,* (2002) Venkitanarayanan *et al.,* (2002), Ukuku *et al.,* (2004) and Alvarado *et al.,* (2007) who raise the strong and reliable antimicrobial effect of lactic acid and hydrogen peroxide on several pathogenic bacteria. These findings explain the findings obtained from tables 8, 11 and 14.

At room temperature, the zero time treatment was insufficient to injure all *Bacillus cereus and Listeria monocytogenes* which is clear with their positive results in counting and detection of injured cells while treatment (C group) (Lactic acid: H_2O_2 1.5: 2.0) could slightly decrease one log count of *Bacillus cereus* on the 1st day and *Listeria monocytogenes* in zero time - and this may be due to the nature of the skin if the green beans which enable the penetration of the pathogen away from the effect of the treatments. By the end of the 1st week of storage, all samples were spoiled while injured bacteria were detected in green beans (Table 6). These findings are supported by that elaborated by Farber *et al.,* (1998) who found that, the storage of fresh vegetables at room temperature caused the increase in number of the bacterial flora causing utilization of all the nutrients of the substrate which led to spoilage of it.

Table (6): Effect of different surface treatments of green beans on *Bacillus cereus and Listeria monocytogenes* count (log) at room temperature for different storage periods and incidence of injured bacteria

Green Beans		Rom. Temperature					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.28	6.90	5.78	6.38	5.48	4.48
	injured	D	D	D	D	D	D
1 Day	count	5.78	6.84	6.26	6.20	5.45	4.48
	injured	D	D	D	D	D	D
3 Day	count	5.48	6.80	6.23	6.30	4.95	5.18
	injured	D	D	D	D	D	D
7 Day	count	Spoilage	Spoilage	Spoilage	Spoilage	Spoilage	Spoilage
	injured	D	D	ND	ND	D	D

Table (7): Effect of different surface treatments of green beans on *Bacillus cereus and Listeria monocytogenes* count (log) at refrigerator (+5Cº: +8Cº) for different storage periods and incidence of injured bacteria.

Green Beans		Refrigerator +5Cº: +8Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.25	6.88	5.59	5.14	5.48	4.48
	injured	D	D	D	D	D	D
1 Day	count	6.17	6.30	5.04	3.60	5.30	3.30
	injured	D	D	D	D	D	D
3 Day	count	6.04	5.43	4.85	3.60	5.08	3.00
	injured	D	D	D	D	D	D
7 Day	count	5.48	5.65	4.53	4.60	5.00	3.00
	injured	D	D	D	D	D	D

Table (8): Effect of different surface treatments of Green Beans on *Bacillus cereus and Listeria monocytogenes* count (log) at Freezer (-18Cº: -20Cº) for different storage periods and incidence of injured bacteria.

Green Beans		Freezer -18Cº: -20Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.23	6.82	5.50	4.60	5.48	4.48
	injured	D	D	D	D	D	D
7 Day	count	4.95	5.08	4.50	3.90	5.00	2.48
	injured	D	D	D	D	D	ND
1 Month	count	4.70	4.70	3.84	1.60	4.50	1.78
	injured	D	D	D	ND	D	ND
2 Month	count	4.65	4.61	3.76	-	4.14	-
	injured	D	D	D	ND	D	ND
4 Month	count	4.30	3.49	3.66	-	3.34	-
	injured	D	D	D	ND	D	ND
6 Month	count	3.65	3.28	2.77	-	2.17	-
	injured	D	D	D	ND	D	ND

Table (9): Effect of different surface treatments of guava on *Bacillus cereus and Listeria monocytogenes* count (log) at room temperature for different storage periods and incidence of injured bacteria.

Cuava		Room Temperature					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.60	6.60	-	-	3.60	1.00
	injured	D	D	D	D	D	D
1 Day	count	3.70	2.30	-	-	-	-
	injured	D	D	D	D	D	D
3 Day	count	2.00	-	-	-	-	-
	injured	D	D	ND	ND	D	D
7 Day	Count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND

Table (10): Effect of different surface treatments of guava on *Bacillus cereus and Listeria monocytogenes* count (log) at refrigerator (+5Cº: +8Cº) for different storage periods and incidence of injured bacteria.

Guava		Refrigerator +5Cº: +8Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.60	6.60	-	-	4.11	-
	injured	D	D	D	D	D	D
1 Day	count	3.60	2.70	-	-	-	1.30
	injured	D	D	D	D	D	D
3 Day	count	2.11	2.89	-	-	-	-
	injured	D	D	ND	ND	ND	ND
7 Day	count	-	2.64	-	-	-	-
	injured	D	D	ND	ND	ND	ND

Table (11): Effect of different surface treatments of guava on *Bacillus cereus and Listeria monocytogenes* count (log) at freezer (-18Cº: -20Cº) for different storage periods and incidence of injured bacteria.

Guava		Freezer -18Cº: -20Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.60	6.60	-	-	3.07	-
	injured	D	D	D	D	D	D
7 Day	count	-	-	-	-	-	-
	injured	D	D	D	ND	D	ND
1 Month	count	-	-	-	-	-	-
	injured	D	ND	ND	ND	ND	ND
2 Month	Count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND
4 Month	count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND
6 Month	count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND

On the 3ed day (C group) (lactic acid: H_2O_2 1.5: 2.0) was sufficient to injure all *Bacillus cereus and Listeria monocytogenes* which are clear with their negative results in counting and detection of injured cells; while

treatment (B group) (Lactic acid: H_2o_2 1.5: 1.5) could slightly decrease *Bacillus cereus* count and markedly decrease *Listeria monocytogenes* count. By the end of the 1st week of storage, count of treated samples in (B and C groups) was negative while injured bacteria were detected in (A group) treated Carrots (Table 12). These results are supported by that obtained by Farber *et al.,* (1998) who found that by the end of one week storage, the carrot's bacterial load count declined more than 2 logs.

Table (12): Effect of different surface treatments of carrots on *Bacillus cereus and Listeria monocytogenes* count (log) at room temperature for different storage periods and incidence of injured bacteria.

Carrot		Rom. Temperature					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.60	6.60	1.00	1.60	1.30	-
	injured	D	D	D	D	D	D
1 Day	count	1.90	4.30	-	-	-	-
	injured	D	D	D	D	D	D
3 Day	count	1.70	3.48	-	-	-	-
	injured	D	D	D	ND	ND	ND
7 Day	count	1.00	2.30	-	-	-	-
	injured	D	D	ND	ND	ND	ND

Storage at +5Cº : +8Cº could keep the *Bacillus cereus and Listeria monocytogenes* count in (A group) in relatively higher count than at room temperature. Also *Bacillus cereus* count in (C group) treated Carrots could survive longer than that at room temperature. The two pathogens were not detected in the 3ed day in (B group) (Table 13).

Table (13): Effect of different surface treatments of carrots on *Bacillus cereus and Listeria monocytogenes* count (log) at Refrigerator (+5Cº: +8Cº) for different storage periods and incidence of injured bacteria.

Carrot		Refrigerator +5Cº: +8Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	Count	6.60	6.60	1.30	1.48	1.30	-
	Injured	D	D	D	D	D	D
1 Day	Count	3.45	4.43	-	-	-	-
	Injured	D	D	D	D	D	ND
3 Day	Count	2.84	4.08	-	-	-	-
	Injured	D	D	ND	ND	D	ND
7 Day	Count	2.00	3.08	-	-	-	-
	Injured	D	D	ND	ND	ND	ND

Bacillus cereus and Listeria monocytogenes in the non treated Carrots (A group) could survive the freezing temperature up to the 7th day. The two pathogens in groups (B and C) could not be detected after zero time of storage (Table 14).

Table (14): Effect of different surface treatments of carrots on *Bacillus cereus and Listeria monocytogenes* count (log) at freezer (-18Cº: -20Cº) for different storage periods and incidence of injured bacteria.

Carrot		Freezer -18Cº: -20Cº					
		A		B		C	
		B.c	L.m	B.c	L.m	B.c	L.m
0 Day	count	6.60	6.60	1.00	1.30	1.00	-
	injured	D	D	D	D	D	D
7 Day	count	2.00	3.11	-	-	-	-
	injured	D	D	ND	ND	ND	ND
1 Month	count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND
2 Month	count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND
4 Month	count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND
6 Month	count	-	-	-	-	-	-
	injured	ND	ND	ND	ND	ND	ND

Obtained results indicate that the treatment of apple, guava, carrots and green bean with 1.5% lactic acid plus 1.5% hydrogen peroxide at 40ºC for 15 min can effectively reduce populations of *Listeria monocytogenes* and *Bacillus cereus* by about 5.0 log CFU per fruit.

There were no marked changes in the sensory evaluation of the examined samples (odor, color, …….etc) till the end of the experiment when compared with the control samples, assuring the results obtained by McWatters *et al.,* (2002) who conducted an extensive sensory study to determine consumer acceptance of treated fruits with the chemical wash solution. The results of this study revealed that, the sensory and qualitative characteristics of fruits were not adversely affected by the treatment, and panelists could not perceive any significant differences between the treated and the control samples.

In conclusion, the RT-PCR technique is proved to be one of the most advanced, specific and sensitive tools for estimation of the incidence of food poisoning bacteria in different kinds of food specially food of plant origin. Also, treatment with the fresh product with hydrogen peroxide and/or lactic acid in various concentrations could reduce and sometimes can completely eliminate *Listeria monocytogenes* and *Bacillus cereus* bacteria from the surface of the tested fruits and vegetables without producing any negative effects on its sensory parameters.

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دراسة مدى تواجد ميكروبي الليستيريا مونوسيتوجينس و الباسيلس سيريس في بعض الأغذية ذات الأصل النباتي و طرق السيطرة عليها

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تهدف هذه الدراسة إلى إختيار طريقة عمليّة وتقييم فاعليتها فى الحد من أو تثبيط التلوث الميكروبى الناشئ من العدوى بميكروبي الليستيريا مونوسيتوجينس و الباسيلس سيريس لثمار كل من: البرتقال و الجوافة و الجزر والفاصوليا الخضراء و التخزين على درجات الحرارة المختلفة: درجة حرارة الغرفة ٢٥ ± ٢م° و درجة حرارة الثلاجة ٤ ± ٢م° و درجة حرارة الفريزر -١٨ : -٢٠م° لمدد تخزينية مختلفة (٠، ١، ٣، ٧، ٣٠، ٦٠، ١٢٠، ١٨٠ يوم) ، و كذلك عمل مسح لعدد ٦٠ عينة للكشف عن الميكروبات المذكورة بإستخدام RT-PCR .

تم حقن كلّ العينات بالبكتيريا الممرضة بمنطقة العنق ثم قسمت العينات إلى ثلاث مجموعات :

(١) مجموعة تم غمرها فى محلول (أ) يحتوى على ١,٥ % حمض اللاكتيك : ١,٥ % فوق أكسيد الهيدروجين تم تحضيره باستخدام الماء المقطر المعقم.

(٢) مجموعة تم غمرها فى محلول (ب) يحتوى على ١,٥ % حمض اللاكتيك : ٢,٠ % فوق أكسيد الهيدروجين تم تحضيره أيضاً باستخدام الماء المقطر المعقم.

(٣) مجموعة الكنترول و التى تم استبدال محاليل اللاكتيك و فوق أكسيد الهيدروجين بالماء المقطر المعقم بنفس حجم المحاليل.

تم ترك العينات على درجة حرارة ٤٠ م° لمدة ربع ساعة.

أوضحت النتائج المتحصل عليها عزل ميكروب الليستيريا مونوسيتوجينس بنسبة ٦,٦٧% و ميكروب الباسيلس سيريس بنسبة ١٣,٣٣% بإستخدام تقنية ال RT-PCR ..

كما أوضحت النتائج أيضاً أن جميع عينات ثمار الفاكهة و الخضر التى تم معاملتها بالمحلول الأول (أ) ١,٥: ١,٥ % (حمض اللاكتيك : فوق أكسيد الهيدروجين) قد تم تثبيط جميع الميكروبات المرضية بها (لكل من الليستيريا مونوسيتوجينس و الباسيلس سيريس) و ذلك بعد مرور يوم واحد من التجربة فيما عدا عينات ثمار الفاصوليا الخضراء.

تواجدت البكتيريا المضعفة حتى اليوم الأول لعينات ثمار الجوافة و اليوم الثالث لعينات الجزر، بينما لم نتمكن من إكتشاف تلك البكتيريا فى حالة ثمار البرتقال، و قد أظهرت النتائج عدم فاعليةالمعاملة بالمحلول الأول (أ) لثمار الفاصوليا الخضراء حيث استمر تواجد البكتيريا المجروحة حتى نهاية التجربة على درجات حرارة التخزين المختلفة (درجة حرارة الغرفة و الثلاجة و الفريزر).

و من النتائج إتضح أيضاً أن المعاملة بالمحلول (ب) أعطت نفس الإتجاه السائد بنتائج المعاملة بالمحلول الأول (أ) مع وجود إختلافات بسيطة و غير مؤثرة (أو معنوية).

ومن هذه النتائج يتضح فائدة إستخدام المعاملات المشار إليها في الحد من التلوث الميكروبي للخضروات و الفاكهة وكذلك زيادة فترة الحفظ دون التأثير على الجودة ، وأيضاً تتضح أهمية التقنية المستخدمة (RT-PCR) في الكشف على الميكروبات المذكورة حيث أثبتت حساسيتها و دقتها في إجراء التحليل.