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UTILIZATION OF CARROT JUICE TO INHIBIT *Listeria monocytogenes*

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ABSTRACT

The antibacterial effect of the carrot juice and the distillate of carrot juice, against two strains of *Listeria monocytogenes* (*Listeria monocytogenes* Z_7 and *Listeria monocytogenes* B_4) were investigated (*in vitro*). Fresh minced meat was inoculated with *L.m*. B_4 at level of 3.52 $\log_{10}$ CFU/g of minced meat. The inoculated samples were subjected to treat with 0.6% of carrot juice and stored at 4 and 24°C to study the effect of carrot juice on *Listeria monocytogenes* B_4 (*in vivo*). Psychrotrophic aerobic bacteria and pH of minced meat were determined during storage period at 4 and 24°C. The chemical composition of minced beef meat were also determined. Results revealed that the antimicrobial effect of carrot juice against *Listeria monocytogenes* (*L.m*. Z_7 and *L.m.* B_4) increased with increasing concentration of carrot juice. The carrot juice was bacteriostatic at concentration of 0.4% (v/v) and was bacteriocidal above this concentration. There was a significant difference ($P < 0.05$) between the concentration of 0.5% and 0.6% (v/v), meanwhile there was no significant difference ($P < 0.05$) between the concentration of 0.6 and 0.7% (v/v) for both strains of *L.m*. used in this study. In contrast, the distillate of carrot juice did not show any antimicrobial effects against both strains of *L.m*. at the concentrations tested. Meat pH ranged from 6.11 (Freshness) to 7.41 (Frank spoilage). The increase in pH value during storage was more pronounced at 24°C than at 4°C. The treatment of minced meat with 0.6% carrot juice resulted in a reduction of the number of *L.m*. B_4 on minced meat from $\log_{10}$ CFU/g = 3.52 to 1.55 after 5 days of storage at 4°C. After 1, 2, 3, 4 and 5 days of storage at 4°C, the number of *L.m*. on samples treated with 0.6% of carrot juice was significantly lower as compared with the number on control samples. Likewise the use of 0.6% carrot juice showed antimicrobial effect against *L.m*. on minced meat stored at 24°C. However the effect was more pronounced at 4°C than at 24°C. Minced meat stored at 4 and 24°C, showed shelf life of 4 days and 14 hr respectively. Followed by off odours on day 5 and at 15 hr respectively.

Key words: Carrot juice; Distillate of carrot juice; *Listeria monocytogenes*; minced meat; shelf life.

INTRODUCTION

Listeria monocytogenes is a food borne pathogen of great concern to the food industry; (Frederiksen, 1991; McCarthy, 1997). Recent evidence has linked food containing *Listeria monocytogenes* to documented outbreaks of listeriosis (Ho, *et al.*, 1986; Anon, 1985; Fleming *et al.*, 1985; Schlech *et al.*, 1983). Individuals particularly at risk include pregnant women, newborns or infants, and immunocompromised persons. It is important to note that the mortality rate within these groups is 30% (Golden *et al.*, 1990; Carpenter and Harrison, 1989). *Listeria monocytogenes* is known to occur on meats such as, minced beef (Sheridan *et al.*, 1994), packaged beef (Grou and Vanderlinde, 1990) Pâte (Farber and Daley, 1994), raw chickens (Pini and

Gilbert, 1988), homemade sausage, Cajun meat and rice sausage (Farber and Peterkin, 1991). Growth on such meats during refrigerated storage could increase the risk. However, there is limited information on the ability of *L. monocytogenes* to survive and grow in refrigerated minced meat. Recent studies on experimental rats have shown that infective doses as low as $\log_{10}$ 2 CFU/ml can cause invasive infections of the liver and spleen, the infections observed were found to be dose dependent (Schlech *et al.*, 1993).

Many plants produce organic compounds which possess antimicrobial activities and are widely used in the pharmaceutical industry (Balandrin *et al.*, 1985). Plants possessing antimicrobial principles in human diet include the Allium species: *A. satium* (garlic), *A. cepa* (onion) and *A. porrum* (leek) (Beuchat and Golden, 1989). Many food borne pathogens were reported to be sensitive to extracts from garlic and onion (Johnson and Vaughn, 1969; Saleem and Al-Delaimy, 1982). WU-Yuan *et al.*, (1988) demonstrated that selected plant extracts inhibited growth, adherence and in vitro formation of dental plaque by mutants streptococci and other oral pathogens.

The aims of the present study were to: a) study whether carrot juice and distillate of carrot juice could affect growth of *Listeria monocytogenes* in vitro b) evaluate the potential for the use of natural carrot juice on the growth of *Listeria monocytogenes* cells after inoculation into minced beef and during storage at temperatures of refrigeration (4°C) and a buse (24°C), in the presence of the natural microflora.

MATERIALS AND METHODS

Materials:

Minced beef:

Freshly minced beef (8.5 kg) were purchased locally, immediately placed in ice box and transported to the laboratory of Faculty of Agric., Saba Bacha, within an hour. The compositional analysis of minced meat was determined (fat, protein, moisture and ash contents).

Carrots:

Fresh carrots (*Daucus carota*) (3 kg) were purchased from central market (El-Wkala market) and transported to the laboratory within an hour.

Organisms:

Listeria monocytogenes Z_7 isolated from poultry (Zeitoun and Debevere, 1991) and *Listeria monocytogenes* B_4 isolated from minced beef obtained from Prof. Debevere, J.M. (University of Gent, Belgium) were used in the study.

Preparation of carrot juice and the distillate of juice:

Fresh carrots were washed in sterile water and drained to remove the excess water. Carrot juice was prepared by using household fruit juicer. From 1 kg carrots approximately 420 ml of carrot juice was obtained. Crude juice was centrifuged at 9000 g for 10 min to remove solid materials. Three

hundreds ml of carrot juice were distillated using to study the antimicrobial effect of the distillate of carrot juice.

Preparation of *Listeria monocytogenes* inocula:

Inculding strains: *Listeria monocytogenes* Z_7 and *Listeria monocytogenes* B_4, were maintained individually at 4°C on tryptose phosphate agar (Oxoid CM283) slants and were transferred monthly. Intermediate cultures were prepared by inoculating a loopful from the slant (each) into liquid brain heart infusion (BHI) (Oxoid CM 225) which was then incubated aerobically at 37°C for 24 h (each). One drop of this BHI culture was transferred into a second tube of sterile BHI (each), which was again incubated for 24 h at 37°C. One loop (each) of this culture was streaked on plate count agar (PCA) (Oxoid CM 325) and incubated for 24 h at 37°C (Zeitoun and Debevere, 1991). A colony of the PCA culture (of each strain) was transferred separately into 100 ml of sterile BHI and incubated for 24 h at 37°C. The working cultures, for each strain were prepared by diluting 10 ml of BHI cultures in sterile 90 ml BHI until 10^{-2} and used as inocula. The following two types of experiments were carried out.

(i) Investigation of anti *Listeria* properties of carrot juice and the distillate of juice:

Diluted inocula culture (1ml) (for either *L.m.* Z_7 or *L.m.* B_4) was added to sterile BHI tubes (9 ml) containing 0 (control), 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7% (v/v) of either carrot juice or the distillate of carrot juice (3 tubes were used for each concentration and for each strain).

The carrot juice and the distillate of carrot was added to sterile BHI tubes (after sterilization of BHI tubes). Cold sterilization was used for carrot juice and the distillate of carrot by using microfilter membrane, pore size was 0.2 µm.

The initial contamination level of *L.m.* (CFU/ml) for each strain was determined on plate count agar (PCA, Oxoid CM325) incubated at 37°C for 24 h. All tubes were incubated at 37°C for 24 hr. After incubation period, 1 ml of each tube was aseptically taken and decimal dilutions were made in sterile physiological saline containing 0.1% peptone. *Listeria monocytogenes* colony forming units per ml were determined on plate count agar incubated at 37°C for 24 hr.

(ii) Effect of carrot juice (0.6%) on growth of *Listeria monocytogenes* B_4 and psychrotrophic aerobic bacteria on minced meat:

Eight kg of minced beef meat were inoculated with *L.m.* B_4. The working culture was prepared by diluting 10 ml of BHI culture (as described before) in sterile 90 ml BHI until 10^{-3} and used as inocula. Eighty ml of inocula culture were added to 8 kg minced meat and mixed under aseptical condition. After this artificial contamination, the meat was kept at 4°C for 2 h to drain and to allow the attachment of the *L.m.* B_4 cells on the meat, then devided to two groups (about 4 kg each) (i) first group (control) was stored on foam tray (aproximately 100g/foam) and the foam was covered with polyethylene sheets. Samples were taken to determine the initial contamination level of *L.m.* B_4 CFU/g. (ii) second group was treated with sterile carrot juice (cold

sterilization was used) at concentration of 0.6% (this concentration was chosen as a result of the first experiment). After this treatment, the minced meat was then kept at 4°C for 2 h to drain and then stored on foam tray as above mentioned. Samples were stored at 4 and 24°C.

Sampling and enumeration of *L.m.* B_4 and psychrodrophic aerobic bacteria:

At each sampling time, three samples for each storage temperature (at 4 and 24°C) and for each method of treatment were taken (by means of 30 gram of minced meat (each) and homogenized in 270 ml sterile physiological saline supplemented by 0.1% peptone for 2 min using an alcohol sterilized blender (Quickie-Mini Blender National) (Cobb *et al.*, 1976). From this homogenate decimal dilutions were made in physiological saline containing 0.1% peptone. The oxford medium (Oxoid CM856) was used for selective enumeration of *Listeria monocytogenes* B_4 (Curtis *et al.*, 1989; Zeitoun and Debevere, 1991). The plates were incubated aerobically at 37°C for 48 h. Typical *L.m.* colonies were 2-3 mm in diameter, black with a black halo and sunken center (Curtis *et al.*, 1989; Zeitoun and Debevere, 1991). Psychrotrophic aerobic bacteria colony forming units were determined in plate count agar (PCA, Oxoid CM 325), incubated for up to 5 days at 20°C.

Chemical analysis:

At each sampling time, 10 g of minced meat were blended with 90 ml distilled water and the pH values were measured, using a pH meter CG 710 Schoot Gerate (Germany).

Compositional analysis:

Moisture, ash and fat contents were determined according to AOAC (1985).

Protein contents were determined according to Egan *et al.*, (1981).

Statistical analysis:

The data of microbiological examinations and pH values were analyzed using analysis of variances two ways (ANOVA) and Duncan's test at the 5% significance level.

RESULTS AND DISCUSSION

The effects of carrot juice and the distillate of juice on *Listeria monocytogenes* Z_7 and *Listeria monocytogenes* B_4 are shown in Table 1 and 2. The initial contamination level was 22×10^4 and 16×10^4 CFU/ml for *L.m.* Z_7 and *L.m.* B_4, respectively. The antimicrobial activity of carrot juice against *Listeria monocytogenes* (*L.m.* Z_7 and *L.m.* B_4) increased with increasing concentration of carrot juice. The juice of carrot was bacteriostatic at concentration of 0.4% (v/v) above this concentration was bacteriocidal. There was a significant difference ($P < 0.05$) between the concentration of 0.5% and 0.6% (v/v). In contrast there was no significant difference ($P \geq 0.05$) between

the concentration of 0.6 and 0.7% (v/v) for both strains of *L.m.* used in this study. Therefore, the concentration of 0.6% was chosen to apply in minced meat to overcome the action of competitive inhibitors which could be excite in the substrate material. However, no significant differences were observed among the two strains of *L. monocytogenes* in their susceptibility to carrot juice. In contrast, the distillate of carrot juice did not show any antimicrobial effects against both strains (*L.m.* Z_7 and *L.m.* B_4) at the concentrations tested. Indicating that the toxic component in carrot juice against *L.m.* was inactive upon exposure to heat (distillate). Further investigation is warranted to define the characteristics of the carrot juice component (s) responsible for toxic effects on *L. monocytogenes* as well as perhaps other foodborne pathogens.

Table (1). The antimicrobial effect of carrot juice against *Listeria monocytogenes* (*in vitro*).

Concentration of carrot juice % (v/v)	*L.m.* Z_7 CFU/ml	*L.m.* B_4 CFU/ml
0	$42 \times 10^{7\ Aa}$	$35 \times 10^{7\ Aa}$
0.1	$38 \times 10^{7\ Aa}$	$32 \times 10^{7\ Aa}$
0.2	$12 \times 10^{6\ Ab}$	$9 \times 10^{6\ Ab}$
0.3	$15 \times 10^{5\ Ac}$	$11 \times 10^{5\ Ac}$
0.4	$26 \times 10^{4\ Ad}$	$18 \times 10^{4\ Ad}$
0.5	$51 \times 10^{2\ Ae}$	$42 \times 10^{2\ Ae}$
0.6	$27 \times 10^{2\ Af}$	$24 \times 10^{2\ Af}$
0.7	$21 \times 10^{2\ Af}$	$19 \times 10^{2\ Af}$

1. Values with the same superscripts in the same horizontal row (A) or vertical column (a, b, c, d, e and f) are not significantly different ($P \geq 0.05$).
2. The colony forming units (C.F.U./ml) values stated refer to three determi- nations.
3. The initial contamination level for *L.m.* Z_7 was 22×10^4 CFU/ml.
4. The initial contamination level for *L.m.* B_4 was 16×10^4 CFU/ml.

Table (2). The effect of the distillate of carrot juice on *Listeria monocytogenes* (*in vitro*).

Concentration of the distillate of carrot juice % (v/v)	*L.m.* Z_7 CFU/ml	*L.m.* B_4 CFU/ml
0	$42 \times 10^{7\ Aa}$	$35 \times 10^{7\ Aa}$
0.1	$39 \times 10^{7\ Aa}$	$37 \times 10^{7\ Aa}$
0.2	$36 \times 10^{7\ Aa}$	$38 \times 10^{6\ Aa}$
0.3	$37 \times 10^{7\ Aa}$	$39 \times 10^{7\ Aa}$
0.4	$40 \times 10^{7\ Aa}$	$35 \times 10^{7\ Aa}$
0.5	$43 \times 10^{7\ Aa}$	$36 \times 10^{7\ Aa}$
0.6	$45 \times 10^{7\ Aa}$	$40 \times 10^{7\ Aa}$
0.7	$41 \times 10^{7\ Aa}$	$37 \times 10^{7\ Aa}$

1. Values with the same superscripts in the same horizontal row (A) or vertical column (a) are not significantly different ($P \geq 0.05$).
2. The colony forming units (C.F.U./ml) values stated refer to three determi- nations.
3. The initial contamination level for *L.m.* Z_7 was 22×10^4 CFU/ml.
4. The initial contamination level for *L.m.* B_4 was 16×10^4 CFU/ml.

The composition of meat not only influences the microbial spoilage population, but also plays an important role in determining the spoilage pattern. The composition of minced meat (Table 3) used in this study, was 66.15% water, 17.59% protein, 15.62 lipid and 0.91 ash.

Table (3). Composition of minced Beef meat %.

Content	Percentage
Water	66.15 (0.650)
Protein	17.59 (0.382)
Lipid	15.62 (0.240)
Ash	0.91 (0.081)

The values stated refer to three samples, with SD in brackets.

Effect of carrot juice on the growth of psychrotrophic aerobic bacteria on minced meat during storage at 4 and 24°C is illustrated in Table 4. The initial number of psychrotrophic aerobic bacteria was 4.12 log CFU/g on inoculated minced meat. Comparison between numbers in control from one side and inoculated and treated with carrot juice from other side, indicated that carrot juice did not alter the growth rate of the natural microflora or the spoilage pattern of the samples stored at 4 and 24°C. However, the number of psychrotrophic aerobic bacteria on samples treated with carrot juice was slightly lower as compared with the number on control samples. Deterioration occurred more rapidly when minced meats were stored at room temperature (24°C) than at 4°C. Minced meat stored at 4 and 24°C showed shelf life of 4 days and 14 hr respectively. Followed by off odours on the fifth day and at 15 hr, respectively.

Table (4). Effect of carrot juice on psychrotrophic aerobic bacteria on minced meat.

(Stored at 4°C)

Treatment	Log_{10} CFU/g of psychrotrophic aerobic bacteria at n days of storage					
	0	1	2	3	4	5
Control	4.12 ^{Aa}	4.65 ^{Ba}	5.12 ^{Ca}	6.08 ^{Da}	6.69 ^{Ea}	7.48 ^{Ga}
Treated with 0.6% Carrot juice	4.12 ^{Aa}	4.48 ^{Ba}	4.90 ^{Ca}	5.92 ^{Da}	6.58 ^{Ea}	7.22 ^{Ga}

(Stored at 24°C)

Treatment	Log_{10} CFU/g of psychrotrophic aerobic bacteria at n hrs of storage							
	0	4	8	10	12	13	14	15
Control	4.12^{Aa}	4.35^{Aa}	4.87^{Ba}	5.34^{Ca}	6.04^{Da}	6.42^{Ea}	6.88^{Fa}	7.58^{Ga}
Treated with 0.6 Carrot juice	4.12^{Aa}	4.21^{Aa}	4.68^{Ba}	5.13^{Ca}	5.79^{Da}	6.25^{Ea}	6.69^{Fa}	7.39^{Ga}

1. **Values with the same superscripts in the same horizontal row (A, B, C, D, E, F and G) or vertical column (a) are not significantly different ($P \geq 0.05$).**
2. **The log colony forming units (C.F.U.) values stated refer to three samples.**
3. **Control = inoculated with *L.m*. and stored on foam trays.**
4. **Treated = inoculated with *L.m*., treated with 0.6% carrot juice and stored on foam trays.**

Meat pH (Table 5) ranged from 6.11 (Freshness) to 7.41 (Frank spoilage). The increase in pH value during storage, was more pronounced at 24°C than at 4°C.

Table (5). Effect of carrot juice on pH of minced meat artificially contaminated with *L. monocytogenes*.

(Stored at 4°C)

Treatment	PH of the minced meat at n days of storage					
	0	1	2	3	4	5
Control	6.11 ^{Aa}	6.18 ^{Aa}	6.36 ^{Ba}	6.62 ^{Ca}	6.98 ^{Da}	7.23 ^{Ea}
Treated with 0.6% Carrot juice	6.11 ^{Aa}	6.34 ^{Bb}	6.47 ^{Ca}	6.75 ^{Da}	7.07 ^{Ea}	7.25 ^{Fa}

(Stored at 24°C)

Treatment	pH of the minced meat at n hrs of storage							
	0	4	8	10	12	13	14	15
Control	6.11 ^{Aa}	6.15 ^{Aa}	6.41 ^{Ba}	6.52 ^{Ba}	6.61 ^{Ca}	6.80 ^{Da}	6.95 ^{Ea}	7.38 ^{Fa}
Treated with 0.6% Carrot juice	6.11 ^{Aa}	6.23 ^{Aa}	6.55 ^{Ca}	6.64 ^{CDa}	6.73 ^{Da}	6.93 ^{Eb}	7.12 ^{Fb}	7.41 ^{Ga}

1. **Values with the same superscripts in the same horizontal row (A, B, C, D, E, F and G) or vertical column (a and b) are not significantly different (P ≥ 0.05).**
2. **The pH values stated refer to three samples.**
3. **Control = inoculated with *L.m*. and stored on foam trays.**
4. **Treated = inoculated with *L.m*., treated with 0.6% carrot juice and stored on foam trays.**

Driven largely by consumer demand for “all natural ingredient” food products. Some ingredients which fall into this broad category are antimicrobial agents. Wild plants can provide a variety of safe and inexpensive food ingredient, which can be added to various foods to enhance safety and increasing thus their quality and their nutritional value (Saleem and Al-Delaimy, 1982, Beuchat and Golden, 1989). The antimicrobial effect of carrot juice against *L.m.* on minced meat stored at 4 and 24°C is presented in Table 6. The initial contamination level of *L.m.* B_4 was 3.52 $\log_{10}$ CFU/g. After 1, 2, 3, 4 and 5 days of storage at 4°C, the number of *L.m.* on samples treated with a 0.6% of carrot juice was significantly lower as compared with the number on control samples. The use of 0.6% of carrot juice resulted in a reduction of the *L.m.* CFU/g from $\log_{10}$ = 3.52 to 1.55 after 5 days of storage at 4°C. No change in the number of *L.m.* on control samples was detected during storage at 4°C for 5 days. Johnson *et al.*, (1988) reported survival, but no growth, of two strains of *L.m.* in ground beef held at 4°C. These results are similar to observations in the present study. The number of *L.m.* on control samples stored at 24°C, increased rapidly from 3.52 to 5.36 $\log_{10}$ CFU/g after 15 hr. In contrast the number of L.m. on samples treated with 0.6% carrot juice still similar to the initial number after 15 h of storage at 24°C (spoilage). This was due to the antimicrobial effect of carrot juice against *L. m*. The carrot juice (0.6%) showed antimicrobial effect against *L. m.* at 4 and

Table (6). Effect of carrot juice on *Listeria monocytogenes* on minced meat.

(Stored at 4°C)

Treatment	Log_{10} CFU/g of *L.m.* at n days of storage					
	0	1	2	3	4	5
Control	3.52 ^{Aa}	3.58 ^{Aa}	3.62 ^{Aa}	3.42 ^{Aa}	3.55 ^{Aa}	3.48 ^{Aa}
Treated with 0.6% Carrot juice	3.52 ^{Aa}	2.14 ^{Bb}	1.88 ^{CDEb}	1.65 ^{DEFb}	1.72 ^{EFb}	1.55 ^{Fb}

(Stored at 24°C)

Treatment	Log_{10} CFU/g of *L.m.* at n hrs of storage							
	0	4	8	10	12	13	14	15
Control	3.52 ^{Aa}	3.59 ^{Aa}	3.92 ^{Ba}	4.25 ^{Ca}	4.38 ^{Ca}	4.84 ^{Da}	5.12 ^{Ea}	5.36 ^{Ea}
Treated with 0.6% Carrot juice	3.52 ^{Aa}	2.46 ^{BCDEFGHB}	2.18 ^{CDEb}	2.35^{DEGb}	2.42^{EFGHb}	2.65^{FGHb}	2.48 ^{GHb}	2.62 ^{Hb}

1.Values with the same superscripts in the same horizontal row (A, B, C, D, E, F, G and H) or vertical column (a and b) are not significantly different (P ≥ 0.05).
2.The log colony forming units (C.F.U.) values stated refer to three samples.
3.Control = inoculated with *L.m*. and stored on foam trays.
4.Treated = inoculated with *L.m*., treated with 0.6% carrot juice and stored on foam trays.

24°C (temperature of storage). However, the effect was more pronounced at 4°C than at 24°C.

Since the control growth of *L. m*. as a food associated pathogens has been the major concern in this study, the carrot juice is suggest as incorporation of generally recognized, safe, natural compounds as additives to minced meat and to other common food systems which could be highly beneficial to human health.

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في هذا البحث تم اختبار التأثير المضاد في عصير الجزر وأيضا المتقطر من عصير الجزر ضد سلالتين من بكتريا الليستريا هما *Listeria monocytogenes* B_4 و *Listeria monocytogenes* Z_7. أيضا تم عمل تلقيح اللحم المفروم الطازج ببكتريا *L.m* B_4 بمعدل (3.52 log CFU) لكل جرام من اللحم المفروم وتم معاملة العينات الملقحة بـ ٠,٦% عصير جزر ثم خزنت علي ٤ ، ٢٤ °م لدراسة تأثير عصير الجزر المضاف علي بكتريا *L.m* way$_4$. تم تقدير البكتريا الهوائية المحبة للبرودة ورقم الـ pH أثناء التخزين علي ٤ ، ٢٤ °م وأيضا تم تقدير التركيب الكيماوي للحم المفروم . أوضحت النتائج أن التأثير المضاد في عصير الجزر ضد بكتريا الليستريا *L.m* B_4 ، *L.m* Z_7 ازداد بزيادة تركيز عصير الجزر ، وكان عصير الجزر له تأثير Bacteriostatic وتأثير Bacteriocidal عند تركيز أعلي من ذلك وأيضا أظهرت النتائج أن هناك معنوية في التأثير بين تركيز ٠,٥% ، ٠,٦% بينما لم يكن هناك فرق معنوي في التأثير بين تركيز ٠,٦% ، ٠,٧% علي كل من السلالتين . بينما لم يعطي متقطر عصير الجزر أي تأثير مضاد ضد البكتريا عند التركيزات المختبرة . رقم الـ pH للحم المفروم تراوح من ٦,١١ للحم الطازج إلي ٧,٤١ للحم عند بداية الفساد وكان الزيادة في رقم الـ pH أكثر وضوحا في حالة التخزين علي ٢٤ °م عن ٤°م . وقد أدي استخدام معاملة اللحم المفروم بعصير الجزر انخفاض عدد البكتريا الـ *L.m* B_4 من ٣,٥٢ إلي ١,٥٥ (Log_{10} CFU) بعدة أيام من التخزين علي درجة ٤°م . وكان عدد الـ *L.m* B_4 في العينات المعاملة بـ ٠,٦% عصير جزر أقل معنويا إذا ما قورن بالعدد في العينة الغير معاملة . وبالمثل كان التأثير المضاد لاستخدام ٠,٦% عصير الجزر علي *L.m* B_4 في اللحم المفروم عندما خزن علي ٢٤°م . ولكن التأثير كان أكثر وضوحا عندما تم التخزين عند ٤°م عما في حالة التخزين عند ٢٤°م . اللحم المفروم المخزن علي ٤°م و ٢٤°م أوضح فترة صلاحية ٤ أيام و ١٤ ساعة علي التوالي وتبع ذلك الرائحة غير المرغوبة عند ٥ أيام و ١٥ ساعة علي التوالي أيضا .