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INHIBITORY EFFECT OF SOME ANTIBACTERIAL AND HEAT TREATMENTS ON *LISTERIA MONOCYTOGENES* IN VACUUM PACKED CHICKEN LUNCHEON SLICES

(With 3 Figures)

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التأثير المثبط لبعض مضادات البكتريا والمعالجات الحرارية علي ميكروب الليستريا مونوسيتوجين بشرائح لانشون الدواجن المعبأة تحت التفريغ

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لقد تم استخدام طريقتان للتحكم في ميكروب الليستريا مونوسيتوجين في لانشون الدواجن المعباة تحت تفريغ المعدة للبيع المعالجة الاولي أجريت بحقن ميكروب الليستريا مونوستيرجين بتركيز ١٠ ^٧ خلية / جرام في شرائح اللانشون ثم غمرت في ٥% حمض خليك – ٥% خلات صوديوم و٥% خليط من المحلولين. تم تعبئة شرائح اللانشون المحقونة والغير محقونة (ضابطة) تحت تفريغ وتخزينها عند ٤ ° م و١٠ °م وفحصها علي فترات لمدة ٣٠يوم. وأظهرت النتائج ان استخدام خليط من المحلولين كان له تاثير مسبط علي الميكروب بعد ٢٥ و٣٠ يوم من التخزين. المعالجة الثانية أجريت بتسخين الشرائح المعبأة تحت تفريغ في حمام مائي عند ٨٠ ° م لمدة ٠,٥، ١، ٣، ٥ و٧ دقائق وقد لوحظ أن زيادة المدة المحددة للتسخين يؤدي الي زيادة نسبة موت الليستريا مونوستيوجين.

SUMMARY

Two treatments were used to control *L. monocytogenes* on commercial vacuum packaged chicken luncheon. The first treatment was carried out by inoculated *L. monocytogenes* strain (10^7 cfu/g) on luncheon slices, then dipped in acetic acid 5%, sodium diacetate 5% and their combination. The inoculated and non inoculated (control) vacuum packaged slices were stored at 4°C and 10°C and examined intervally up to 30 days. Listericidal effects were observed for the combination of the two antimicrobial tested at days 25 and 30 days of storage. The second treatment was carried out by heating the vacuum packaged slices in a water bath at 80°C for interval periods 0.5, 1, 3, 5 and 7 minutes. Increasing heat treatment time led to increase the leathality for *L. monocytogenes*.

Key words: *L. monocytogenes, chicken luncheon, acetic acid, disodium acetate*

INTRODUCTION

L. monocytogenes is a pathogenic bacterium found in soil, water and on the surface of equipment, floors, and walls. About 99% of reported listeriosis cases were transmitted by foods (U.S. Department of Agriculture, Food Safety and Inspection Service (2003). *L. monocytogenes* is ubiquitous, can be resistant to many food preservation methods (Lou and Yousef., 1991). It has the ability to colonize meat plants (Samelis and Metaxopoulos, 1999). It survives under favorable conditions (Harmayani *et al.* 1993).

Numerous sporadic and outbreak cases of foodborne illness has been linked to consumption of ready to eat (RTE) products contaminated with *L. monocytogenes*. Recontamination may occur after cooking (postcooking contamination) by peeling, slicing and repackaging (U.S. Food Safty and Inspection Service, 2003). Although luncheon meat slices (ready to eat meat products) contain salts such as sodium chloride, nitrite and nitrate, they don't inhibit the growth of *Listeria* during storage at refrigerated temperatures (Mbandi and Shelef, 2002).

Because of irradiation is not approved for use on packaged ready to eat products, interest in the incorporation of generally recognized as safe chemical or biological antimicrobial compounds (El-Shenawy and Marth, 1989; Schmidt, 1995; Wederquist *et al.* 1995; Blom *et al.*, 1997) as safety barriers has been renewed (Kuntz, 1999).

Antimicrobial agents have been shown to be valuable in the effort to control *L. monocytogenes* in RTE meat as additives in the formulation of various products, or post processing application. The post processing application of antimicrobial by dipping is more advantageous than their addition in the formulation (Barmpalia *et al* 2004; Geornarsa *et al* 2005).

Post process contamination also may occur in the consumer's refrigerator, even on foods that may not be reheated before consumption. So several studies have evaluated post cook pasteurization to eradicate *Listeria* on fully cooked meat.

Post processing application of antimicrobial and post packaging thermal pasteurization may enhance protection against post processing contamination with *L. monocytogenes* in luncheon meat slices (Farber and Peterkin 1999; Tompkin *et al* 1999).

The objectives of this study were to investigate the antilisterial effect of some chemical agents applied on surface of commercial luncheon vacuum packaged meat slices inoculated after processing and

stored in vacuum packages at 4°C and 10°C. Storage temperature represent potential mild abuse during distribution and retail as well as at the consumer level and also to validate antilisteiral effectiveness of hot water pasteurization post packaging of the same product.

MATERIALS and METHODS

Bacterial culture (inoculum)

The cultures of *Listeria monocytogenes* was maintained on brain heart infusion (BHI) slants. Loopfull from BH1 slants was inoculated into BHI broth and inoculated over night at 35°C. Serial dilutions of the fresh culture was carried out in 0.1% peptone water to obtain a target level of 7 log cfu/cm^2 when 0.1 ml of inoculum was applied to each side of luncheon slice.

Product inoculation

Vacuum packages luncheon slices were obtained from a local supermarket in retail packages, each contain six slices were used in this study: Each retail package of luncheon was aseptically peeled, opened in a laminar flow hood. The slices were placed on aluminum foil. Inoculum of 0.1 ml of *L. monocytogenes* was spread over one side of each slice with sterile bent glass rod, left to stand for 15 minutes to allow for inoculum attachment. The same procedure was repeated for the other side of each slice. Following the inoculation, slices were treated as follows;

1- Treatment 1:

Each inoculated slice was transferred from the aluminum foil with sterile forceps and immersed in different sterile antimicrobial in distilled water for 2 minutes and in water alone. The dipping solutions were (% wt/vol) as follows:

1- Acetic acid (A.A) 5%
2- Sodium diacetate (Na Diacetate) 5%
3- Combination of 1 and 2 in equal volumes
4- Distilled water (control)

After application of the chemical treatments and water slices were drained 1 minute. They (per sample) were stacked on top of each other and were inserted into a vacuum bag, vacuum packaged and stored at 4°C and 10°C for up up 30 days. Duplicate samples were prepared for each treatment; all treatments were microbiologically analyzed immediately after inoculation on day 0 and at 5, 10, 15, 20, 25 and 30 of storage.

2- Treatment 2:

The inoculated luncheon slices were vacuum packaged then treated by submersion in a thermostatically controlled water bath at 80°C for 0.5, 1, 3, 5, 7 and 10 minutes. Samples were removed from the heated water bath, cooled immediately in an ice water bath. All packages were stored at 4°C for up to 3 h until microbiological analysis.

Microbial enumeration of *Listeria monocytogenes*

Each package was wiped with 75% ethanol and aseptically opened, 25 gm of the luncheon slices were transferred into sterile stomacher bag then 225 ml of buffered peptone water (Difco) were added. The contents were mixed for 1 min. Serial dilutions were made in buffered peptone water and plated in duplicate on modified oxford agar. Colonies were enumerated after incubation at 37°C for 24-48 h. To obtain preliminary information on *Listeria monocytogenes* population associated with the samples, random representative samples were used for enumeration of *L. monocytogenes*.

RESULTS

The effects of some antibacterial agents namely acetic acid 5%, sodium diacetate 5% and their combination as well as heat treatment on the growth and survival of *Listeria monocytogenes* in vaccum packaged chicken luncheon are presented in Figures 1-3.

Fig (1): Effect of AA, NaDia and combination of both on *L. monocytogenes* population inoculated on vacuum packaged chicken luncheon slices stored at 4 °C

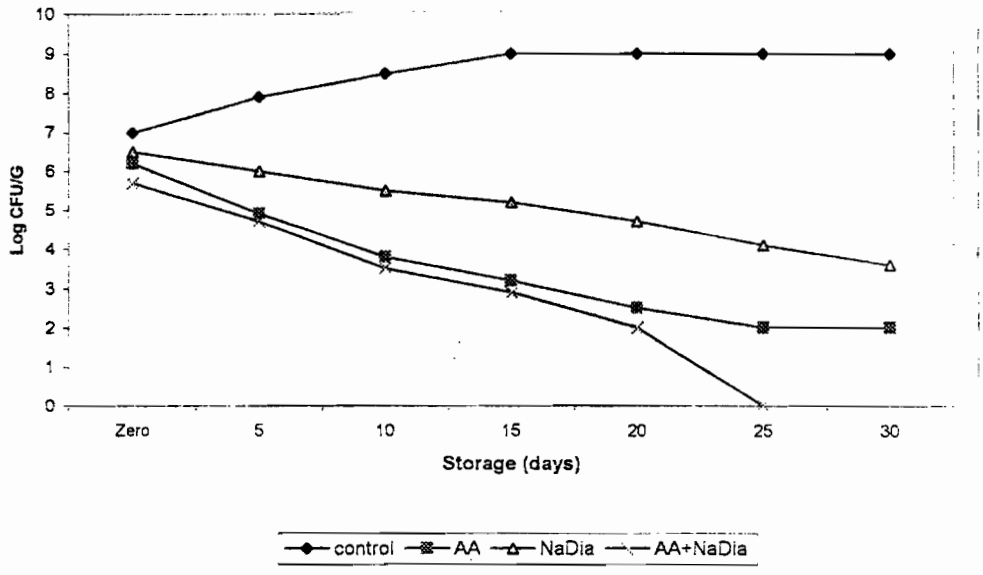


Fig (2): Effect of AA, NaDia and combination of both on L. monocytogenes population inoculated on vacuum packaged chicken luncheon slices stored at 10 oC

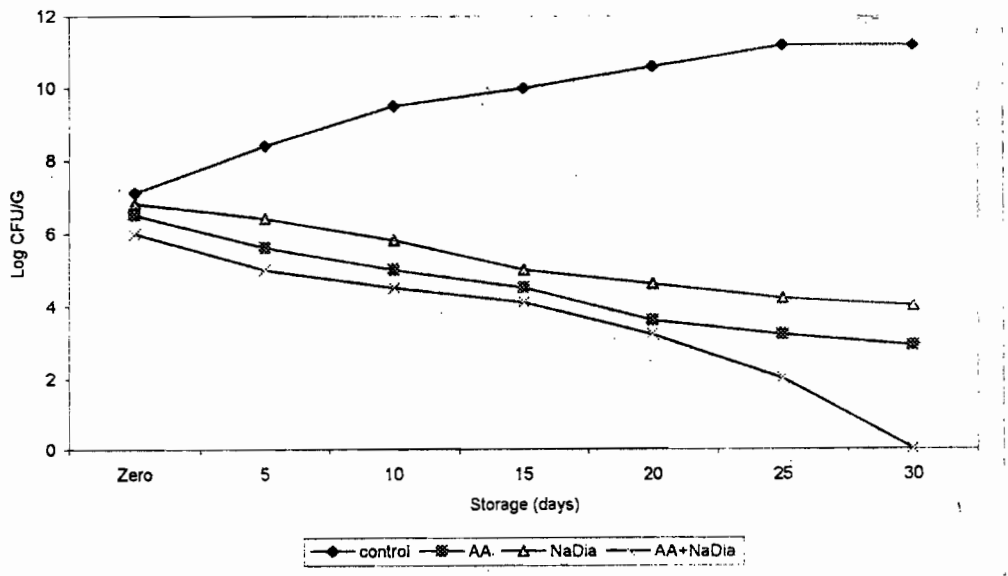
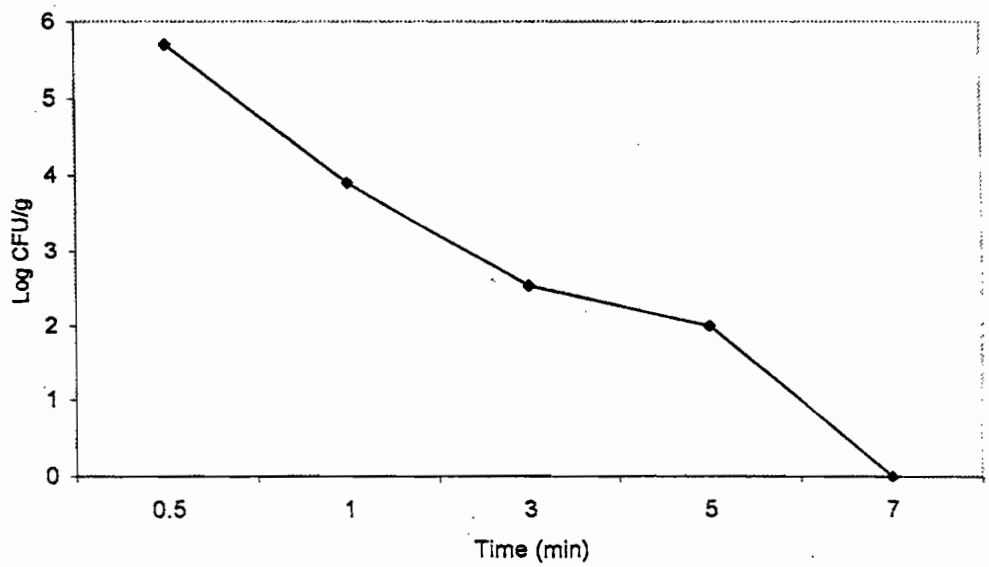


Fig (3): Effect of pasturization on *L. monocytogenes* population inoculated on vacuum packaged chicken luncheon slices



DISCUSSION

The initial sampling of representative luncheon revealed that it contained ≤10 cfu/g of *L. monocytogenes*. The population of *L. monocytogenes* on vacuum packages luncheon samples without treatment (control) increased through out the study as the initial listeriae were 7 log cfu/g on day 0 but increased to 8.5 log cfu in 10 days and eventually reaches 9 log cfu/g with prolonged storage at 10°C (Fig. 1). These results are agreement with previous studies, indicating the ability of *L. monocytogenes* to multiply on cured meat products at refrigeration tempreature (El-Shenawy and Marth, 1989; Buncic *et al.*, 1991; Schmidt, 1995 and Wederquist *et al.*, 1995). Samelis *et al.* (2001) retorted that the pathogen increased from 3 log cfu/cm^2 to above 7 log cfu/cm^2 during 20 days of storage of bologna slices. Likewise, Wederquist *et al.* (1995) reported rapid and extensive growth 9 log cfu /g in 63 days of *L. monocytogenes* on sliced vacuum packaged turkey bologna stored at 4°C, originally inoculated at 2.8 log cfu/g.

Thus, pathogen growth was always more pronounced on processed meat products with high pH and moisture content (Glass and Doyle, 1989; Blom *et al.*, 1997).

At 10°C storage the numbers of the *Listeria* in untreated samples exceeded 9 log cfu/g after 10 days (Fig 2), the rapid growth of the pathogen was expected, because the product didn't contain antimicrobials and stored at abuse temp.

Treatment I:

Treatments of slices of luncheon meat with different chemicals have showed varying effects on growth and behavior of *L. monocytogenes*. The used treatments were acetic acid 5%, sodium diacetate 5% and combination of them. The obtained results showed initial reduction of *L. momocytogenes* at both temperatures compared with the untreated control.

Samples treated with 5% acetic acid showed reduction rate 5 and 3.8 log after 25 days storage at 4°C and 10°C respectively (Fig. 1&2). While, lesser reduction rate was observed using 5% Na diacetate than that samples treated with acetic acid. Moreover, combination of 5% acetic acid and Na diacetate reduce the growth of *L. momocytogenes* to undetectable level by the day 25 and 30 of storage at 4°C and 10°C respectively.

In this study the bacterial decreases achieved with the different treatments were found to be in the following order; combinations of 5% acetic acid + 5% Na diacetate 7.7 log units >5% acetic acid 5-5.1 log

units > 5% Na diaconate 2.8-3 log units at 4°C and 10°C after 25 and 30 days of storage respectively. Preliminary experiments showed similar reductions as reported by Samelis *et al.* (2001) where acetic acid combined with citric acid (at 2-5% each) restricted the growth of *L. monocytogenes* on Frankfurters stored at 5°C in vacuum packages. Palumbo and Williams (1994) stated that acetic acid (5%) also showed its effects inhibition on the pathogen.

Mbandi and Shelef (2002) used sodium lactate 2.5% and sodium dilacerate 0.2% and their combination to enhance inhibition of *L. monocytogenes* in beef bologna at 5 and 10°C and found that their combination was listericidal. Buchanan *et al.* (1993); Schlyter *et al.* (1993); Shelef and Addala (1994); Stekelenburg and Kant-Muermans (2001) used sodium salts of short-chain organic acids such as citric, acetic, lactic and their combinations to control *L. monocytogenes* in meat.

Uhart *et al.* (2004) determined the effectiveness of combinations of 3% Sodium diacetate and 6% Sodium lactate and pediocine as aqueous dipping solutions to control *L. monocytogenes* on beef franks stored at 4°C.

Geornaras *et al.* (2005) showed similar reductions when evaluated the effect of chemical dipping solutions on commercial bologna and ham slices in vacuum packages stored at 10°C.

Treatment II:

Post package pasteurization of luncheon samples at 80°C for o.5 minute reduced the pathogen number by 1.3 log and by 5 log for 5 min treatment. So increasing the heating time from 0.5 to 5 minute for the package resulted in greater lethality (Fig. 3). Treating individual luncheon package for 7 minutes often eliminated all inoculated *L. monocytogenes* cells and achieved a 7 log reduction. These data agreed with several related studies on post package pasteurization of RTE products: for example, Roering *et al.* (1998), and Gill *et al.* (2001). They established the feasibility of using pasteurization for control of *L. menocytogenes* in packaged summer sausage and beef salami.

Chen *et al.* (2004) studied post package pasteurization (PPP) of peeled frankfurter using 96°C water reported reduction in *L. monocytogenes* were approximately 2.5 to 3.5 log cfu in 30 to 120 Sec, Murphy *et al.* (2003) reported that a 5 minute exposure of 4 kg of inoculated turkey breast to 96°C water in a cooker with heat transfer coefficient of 800 w/m k resulted in 2 log reduction in *L. monocytogenes* numbers. Also Muriana *et al.* (2004) found that a 2 min exposure of 1.8

to 5 kg of defi turkey product in 189 liters of 93.3°C water always resulted in a 2 log reduction of *L. monocytogenes* count.

From this study it can be concluded that post package (pp) contamination of vacuum packaged luncheon slices with *L. monocytogenes* is an important safety concern and emphasize the hot water pasteurization step after packaging or the antimicrobial solutions to control or reduce the growth of the pathogen, our results show that PPP treatment of the vacuum packaged luncheon cause reduction of the *L. monocytogenes* and was affected by treatment time and the treatment with the exposure or dipping in antimicrobial solutions acetic acid (A.A), sodium diacetate and combination of them before vacuum packaging control the growth of *L. monocytogenes* even at abusive storage temp.

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