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ANTIBACTERIAL EFFECTS OF MUSTARD, GARLIC AND ACETIC ACID AGAINST SALMONELLA TYPHIMURIUM IN GROUND BEEF

(With 2 Tables)

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التأثير المضاد للبكتريا لكل من المستردة والثوم وحمض الخليك على السالمونيلا تيفى ميوريم فى اللحوم المفرومة

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أجريت هذة الدراسة لأستبيان تأثير كل من المستردة والثوم وحمض الخليك كل على حدة او مجمعة على ميكروب السالمونيلا تيفى ميوريم المحقون فى اللحوم المفرومة وقد جهزت هذة المواد بنسب مختلفة وذلك لتكون نسبة اضافة المستردة والثوم ٥% و ١٠ % اما حمض الخليك فقد اضيف بنسبة ٠,٥% و ١%. وقد تم اضافة النسب المذكورة بعد حقنها بميكروب السالمونيلا تيفى ميوريوم وتقسيمها الى مجموعات مختلفة وخفظها عند ٤° م وقد تم متابعة هذا التأثير عند صفر و ٢٤ و٤٨ و٧٢ساعة من الحقن. أظهرت النتائج ان الثوم له تأثير كبير على الميكروب يلية حمض الخليك ثم المستردة التى أظهرت التأثير الأقل والأبطأ اذا ماقورنت بالثوم وحمض الخليك. أما بالنسبة للتأثير المجمع للمواد المذكورة فقد أظهرت النتائج ان ميكروب السالمونيلا لم يكن له وجود بعد ٢٤ ساعة من الحقن فى مجموعة (المستردة ٠,٥ % والثوم ٥ % وحمض الخليك ٠,٥ % وكذلك فى مجموعة المستردة ١٠ % والثوم ١٠ % وحمض الخليك ١ %).

SUMMARY

This study was designed to evaluate the effect of mustard flour, garlic powder and acetic acid either individually or in combined effects on *Salmonella typhimurium*. Samples treatments were prepared to achieve various concentrations by addition of 5% and 10% of mustard flour and garlic powder, while acetic acid was added by 0.5% and 1%. The treatments were added to ground beef samples after inoculation with *Salmonella typhimurium* and stored at 4° C. Samples were followed up after 0, 24, 48 and 72 h. The obtained result indicated that garlic powder showed the greatest antibacterial activity followed by acetic acid then mustard flour

which showed lowest and slowest reduction rate of the tested organism. All combining effect showed remarkable reduction rate where *salmonella typhi* can't be detected after 24 h of inoculation in combining effect of mustard 5 %, garlic 5 % and acetic acid 0.5% also in mustard 10%, garlic 10% and acetic acid 1%.

Key words: *Mustard, Garlic, Acetic acid, S.typhimurium, ground beef.*

INTRODUCTION

Few studies have been addressed the use of spices and some organic acids against pathogens associated with meat. Since *Salmonella typhimurium (S.typhi)* is consider as one of food borne pathogens which are found in a wide variety of foods specially meat and meat products. The organism is the most commonly isolated *Salmonella* serotype, accounting for 23% of Laboratory confirmed Salmonella cases that occur in over 1.4 million each year in the United States (Centers for Disease Control and Prevention, 2001).

In recent years, consumers become more concern about the processed food they eat. Spices and herbs have been added since ancient times not only as flavoring agents, but also as flok medicine and food preservatives (Beuchat, 1994; Nakatani, 1994; Cutler 1995). However there has been increasing interest to replace synthetic preservative,with natural, effective and nontoxic compounds (Smid and Gorris 1999). Allyl isothiocyanate (AITC), a component of mustard oil has potential for use as antimicrobial agent in a variety of food because of its natural origin (Lin *et al.,* 2000a, Park *et al.,* 2000; Weissinger *et al.,* 2001). Garlic is widely used in meat products. It's one of the herbal products consumed world wide because of its beneficial effects on health such as antidiabetic, hypochloesterolemic, antioxidant and cancer preventive (Ali *et al.,* 2007). Garlic extracts have also been to have antibacterial active in different vivo models. The component responsible for the remarkable antibacterial activity of garlic is an oxygenated sulpher compound which termed Allicin from the Latin name (Allium sativuon) of garlic plant (Al Magboul *et al.,* 1988). Acetic acid has also been widely tested as food preservative for killing of food borne pathogenic bacteria (Berry and Cutter 2000; Samelis *et al.,* 2001).

However many questions still raised about formulation, the best preservation and microbiological safety of various food to modified what we have been doing to minimize hazards that might exist in various food.

The present study was designed to evaluate the effect of mustard, garlic and acetic acid on reduction the numbers of *S. typhi* in ground beef.

MATERIALS and METHODS

Bacterial strain:

The strain of *Salmonella typhimurium* was obtained from the Bacteriological laboratory Department, Animal Health Research Institute, Ministry of Agriculture. The stock culture was maintained on Typticase soya agar slants (Difico) at 4° C

Cell suspension and Inoculation:

The strain of *S.typhi* was cultured on Trypticase soya broth (Difico), for 24 h. at 37 °C before use in experiments. The cell suspension was serially diluted with 0.1% sterile peptone water to an approximate concentration calculated to yield 10^6- 10^7 CFU/ml of sample according to Min_Suk *et al.* (2003). This working solution was used to inoculate all groups in this experiment

Sample treatment:

Mustard flour, garlic powder and commercial vingar (containing 5% acetic acid) were obtained from local supermarket. Samples treatments were prepared to achieve various concentrations of 5 % and 10 %of mustard flour and garlic powder. (In aqueous solution). The concentration of acetic acid was added to achieve 0.5% and 1% (vol/wt) by adding 10 and 20 ml of the commercial vingar.

Ground beef samples:

About 2 Kg of ground beef were purchased from local supermarket and transferred to the Laboratory in an ice box with minimal of delay, then prove to be free from the tested pathogenic organism according to the method carried out by ISO 6579 (2002).

Experimental design:

Two main studies were carried out on 12 groups of ground beef samples (each of 100 gm) All groups were inoculated with 1 ml of the bacterial inoculm (working solution).

1- Study of antimicrobial effect of individual treatment on *S. typhi* inoculated in ground beef samples:

Seven groups of ground meat sample were used: First and second groups were inoculated with 5 % and 10 % of mustard flour respectively. Third and fourth groups were inoculated with 5% and 10% of garlic powder respectively. Fifth and sixth groups were inoculated with 0.5% and 1% of acetic acid respectively. Seventh group was kept as control without treatment.

2- Study of Antimicrobial effect of combining treatment on *s. typhi* inoculated in ground beef samples:

Five group of ground meat samples were used:

First group was inoculated with mustard 5% and acetic acid 1%. Second group was inoculated with mustard 10% and acetic acid 0.5%. Third group was inoculated with mustard 5%, garlic 5% and acetic acid 0.5%. Fourth group was inoculated with mustard 10%, garlic 10% and acetic acid 1%. The last group was kept as control without any addition.

Both bacterial inoculum and treatments were distributed in the ground meat by hand Kneading under complete aseptic condition with application of all hygienic measures required for 5 min. All samples and control were stored at 4 °C. Samples were followed up on xylose lysine desoxycholate agar (oxoid) plate at 0 h, 24 h, 48h and 72 hr. of storage for enumeration of *S. typhi* as recommended by Nottingham *et al.* (1975). The average of duplicate plates counts was converted to log10

RESULTS

Table 1: Antimicrobial effect of mustard flour, garlic and acetic acid on *Salmonella typhi* at (4 °C)

Times	Log CFU/ml						
	Mustard		Garlic		Acetic acid		control
	5%	10%	5%	10%	0.5%	1%	
0 h	6.22	6. 01	6.00	6.02	6.00	6.00	6.26
24h	5.92	5.53	5.11	4.48	5.22	5.08	6.10
48 h	5.00	5.08	4.48	Non detectable	4.90	Non detectable	5.92
72 h	4.60	4.30	Non detectable	Non detectable	Non detectable	Non detectable	5.83

Table 2: Antimicrobial effect of combining treatment on *Salmonella typhi* at (4 °C)

Treatment		Time			
Group		0 h	24 h	48 h	72 h
1	Mustard (5%) Acetic acid (1%)	6.00	4.84	Non detectable	Non detectable
2	Mustard (10%) Acetic acid (0.5%)	5.90	4.69	Non detectable	Non detectable
3	Mustard (5%) Garlic (5%) Acetic acid (0.5%)	5.92	Non detectable	Non detectable	Non detectable
4	Mustard (10%) Garlic (10%) Acetic acid (1%)	5.58	Non detectable	Non detectable	Non detectable
5	Control	6.24	6.22	5.92	5.83

DISCUSSION

The survivals of *S. typhi* in various treatments at 4 °C either in individual or in combining treatments are presented in Table (1) and Table (2). *S. typhi* was reduced in all treatments except for control.

Regarding to Table (1) the most effective treatments on the population of *S. typhi* was toward acetic acid 1% and garlic powder 10%, thus *S.typhi* was undetectable at 48h of inoculation in both of them. On the other hand mustard flour treatment showed the slowest and least effect on growth of *S.typhi* in comparative to the effect of garlic powder and acetic acid .There is a reduction by 1.62 log CFU/ g and 1.71 log CFU/ g in mustard flour at 5% and 10% respectively from 0 time to 72h . In this aspect Lin *et al.* (2000b) demonstrated that *S. typhi* was less resistant to mustard than other type of gram negative bacteria.

In concerning to the antibacterial effect of garlic powder as shown in Table (1) *S. typhi* can't be detected at 48 h of treatment with garlic 10% and at 72 h of 5% treatment of garlic. There is a reduction by 1.52 log CFU/g at 5% treatment between 0 h and 48 h and by 1.54 log CFU/g at garlic 10% between 0 time and 24h of inoculation. Our result of garlic treatment was supported by Uhart *et al.* (2006) and Marija and Nevena (2009). However the anti bacterial effect of garlic is due to the principal antimicrobial component of garlic which is allicin, garlic bulbs do not contain allicin in its active form, but contain its precursor, upon crushing garlic bulbs,alliin is enzymatically hyrolsed to allicin which is an inhibitor of respiratory SH-group enzymes (Beuchat and Golden 1989) and

acetyl_COA synthetase, Focke *et al.* (1990). On the other hand Ali *et al.* (2000) recorded in their study that the chopped garlic has a slow down effect on microbial growth of *Salmonella typhi* in ground meat on the garlic concentration, but they explained that this effect was not at an expected level even at the highest concentration because potential antimicrobial agent in chopped garlic were probably insufficiently extracted.

Regarding to the antimicrobial effect of acetic acid as shown in Table (1), *S. typhi* was undetectable at 48 h of treatment with 1 % and at 72 h of treatment with 0.5%. The level of *S. typhi* dropped by 1.1 log CFU/g at 5% treatment between 0 time and 48 h and by 0.92 log CFU/g at 1% treatment between 0 time and 24 h of inoculation .Such results are nearly inagreement with those recorded by Min_Suk *et al.* (2003) and Cosansu and Ayhan (2010). Acetic acid antimicrobial activity is based primarily on its pH_loweing effect, and it exhibits this antimicrobial activity only in an undissociated form (Sofos *et al.*, 1998)

The results tabulated in Table (2) show strongly reduction of *S.typhi* in all combining treatments except for control, this result was nearly inagreement with those recorded by Min_Suk *et al.* (2003) and Uhart *et al.* (2006) *S. typhi* can 't be detected after 24 h of inoculation in the combining effect of (mustard 5 %, garlic 5 % and acetic acid 0.5%) also in (mustard 10%, garlic 10% and acetic acid 1%). Combined treatment of mustard 5% and acetic acid 1% resulted in reduction of 1.16 log CFU/g between 0 time and 24 h, while combined treatment of mustard 10% and acetic acid 0.5% resulted in reduction by 1.21 log CFU/g between 0 time and 24 h of inoculation. Uhart *et al.* (2006) reported that combination of other interventions with spices may help inhibit *S. typhi* in food systems such as ground beef.

The combining effect of Allyl isothiocyante component of mustard which is used as an antimicrobial agent (Ward *et al.*, 1998) acetic acid which is widly used as food preservative for killing food borne pathogenic bacteria (Strat Ford and Anslow 1996) and garlic which is responsible for remarkable antibacterial activity (Almagboul *et al.*, 1988; Kumar and Berwal 1998), all of them make strong reduction against *S. typhi* better than each component individual in different ratio used .

Comparatively both control samples showed slightly and slowest decrease through out the storage period and this may be due to the effect of low storage temp at 4 °C and possible presence of competitive psychrotrophic microorganism

However different antibacterial studies have revealed varying magnitudes of its effects depending on the organism and treatment used.

From the achieved results, it could be concluded that *S. typhi*,is more sensitive to garlic powder and acetic acid than mustard flour. Therefore research should be focused on addition of spices and acetic acid in certain concentrations to obtain effective antimicrobial activity without adversely influence flavors and odours.

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