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EXPERIMENTAL STUDIES ON THE INHIBITORY EFFECT OF DIFFERENT CONCENTRATIONS OF POTASSIUM SORBATE ON THE VIABILITY OF *YERSINIA ENTEROCOLITICA* IN POULTRY MEAT FILLETS

(With 3 Tables and 3 Figures)

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دراسة معملية عن التأثير المثبط للتركيزات المختلفة لسوربات البوتاسيوم على حيوية ميكروب اليرسينيا إنتيروكوليتيكا فى فيليه لحوم الدواجن

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تم دراسة تأثير التركيزات المختلفة (٣، ٥، ١٠٪) المثبطة لسوربات البوتاسيوم فى صورة حمض السوربيك (٤٠٠، ٦٦٨، ١٣٢٥ جزء فى المليون) على حيوية ميكروب اليرسينيا إنتيروكوليتيكا (عترة نموذجية من نوع ٠,٨) التى تم تلويث عينات فيلية الدواجن بها وتم إجراء التجربة المعملية فى مدة ٢١ يوم عند درجة تبريد قدرها ٤ درجة مئوية وكانت نتائجها كالتالى: التركيز الأعلى للبوتاسيوم سوربات (١٠٪) ١٣٢٥ جزء فى المليون حمض سوربيك عند أقل درجة تركيز هيدروجينى(٥) وكان لها التأثير المبثط الأقوى على ميكروب اليرسينيا إنتروكوليتيكا فى فيلية الدجاج المختبر مما أطال فترة عمر صلاحية للعينات لأكثر من عشرة أيام عن العينات الضابطة وذلك فى درجة حرارة الثلاجة (٤ درجة مئوية). كما أوضحت التجربة أن أعلى كمية من حمض السوربيك (بوتاسيوم سوربات) أستخدمت فى هذه الدراسة المعملية كانت أقل بكثير من الحد المسموح تناولة يوميا من خلال الوجبات الغذائية طبقا للمواصفات الدولية المتعارف عليها. كما تم مناقشة الأهمية الحيوية لإستخدام أملاح السوربات فى الأغذية لإطالة فترة صلاحيتها.

SUMMARY

The effect of different concentrations of potassium sorbate on the survival of *Yersinia enterocolitica* (serotype: O:8) contaminated chicken fillets incubated at 4°C for 21 days were compared. The potassium sorbate concentrations compared included 3, 5, 10% expressed as 400, 668 and 1325 ppm. sorbic acid respectively. The decrease in viable *Yersinia* cells was compared based on the concentration of acid salt dip,

pH determination after 10 days of storage and calculated concentration of sorbic acid throughout and at the end of storage period. Results indicated that 4°C storage temperature and 10% potassium sorbate treatment (1325 ppm sorbic acid) attached to low pH value (5.4) provided the most effective inhibitory system against *Yersinia enterocolitica* in chicken fillets. The shelf life of sorbate treated samples was extended more than 10 days at 4°C over the control samples (without sorbate treatment). Quantities of sorbic acid found in the samples treated with potassium sorbate were below the acceptable daily intake (ADI) established by the Food and Agriculture Organization/World Health Organization.

Key words: *Potassium sorbate, Yersinia enterocolitica, poultry meat*

INTRODUCTION

Yersinia enterocolitica is psychrotrophic bacterium which has only in the last decades become established as food borne human pathogen. During this time a number of food borne disease outbreaks have been attributed to this organism (Abraham *et al.*, 1997and Ackers *et al.*, 2000). There have been numerous studies that have reported on the incidence of *Yersinia enterocolitica* on fresh poultry meat in Egyptian markets ranged from 8.5 to 18% (Abd El-Monem and Saad, 1998). Green Wood and Hooper (1990) found that poultry and meat products were contaminated by *Yersinia enterocolitica* mainly during subsequent processing.

The most common symptoms of *Yersinia* infection is gastroenteritis although other symptoms can include mesenteric lymphadenitis, terminal ileitis and arthritis (Cover and Aber, 1989). Moreover, this organism is unique among common food borne pathogens in being able to mimic appendicitis as a result of invasiveness of the organism via intestinal epithelial tissue in addition to the enterotoxin and enzyme production (Meer *et al.*, 1991). Wei *et al.*, (2001) mentioned that *Yersinia enterocolitica* is a psychrotrophic pathogenic bacterium that has an important public health concern in food industry, this pathogen was well documented as a causative agent of human gastroenteritis and also it could grow under anaerobic condition and at temperature as low at -20°C.

The ability of food borne pathogens to survive in acidic environments is important because acidity is often used in foods to

control these bacteria, *Yersinia enterocolitica* is one pathogen that has shown this ability.

Stern and Pierson (1979) reported that four separate strains of *Yersinia enterocolitica* grew at pH 4.6, Kendall and Gilbert (1980) demonstrated that this organism grew at pH 4.4 and above and survived for at least 72 hours at pH 4.2 and Robert, (1987) also found viable *Yersinia enterocolitica* cells after 21 days at pH 4.0 at 5°C. Helms *et al.*, (2003) stated that short and long term mortality was associated with infection with *Yersinia enterocolitica* and added that in Denmark from 1991-1999 the number of patients suffering from *Yersinia* was 4045 and the number of death was 32 persons (0.08%).

Extensive researchers on the use of sorbic acid and its salts as preservative in foods was reviewed (Sofa and Busta, 1981; Sofa and Busta, 1983 and Elliott *et al.*, 1985).

Sorbic acid and potassium sorbate were originally used to inhibit the growth of molds and yeasts, but it has been found that they also act upon *Staphylococuus aureus*, *Colstridium botulinum*, *salmonellae*, *Pseudomonas* and *Yersinia*. Antimicrobial effect in meat products has been studied for inhibiting bacterial growth in sausage (Tompkin *et al.*, 1974), for prolonging shelf life of poultry (Robach and Sofa, 1982). Myers *et al.*, (1983) studied the effect of sorbate to diminish the growth of *Yersinia* species and mentioned that the results were highly satisfactory. Greer (1982) investigated the influence of this preservative on the growth of psychrtrophic bacteria which deteriorate beef.

The antimicrobial properties of sorbic acid salts especially potassium sorbate are very important and more preferable application due to high solubility in water. The low water solubility is a disadvantage of sorbic acid. At 25°C, the solubility of acid in water is 0.16% and of potassium sorbate in water is over 50%. An increase in fat content of tissue will also lower the amount of sorbic acid in the aqueous phase where it is needed for microbial control. Sorbic acid is a lipophilic acid preservative with a short chain length and this kind of substance inhibits both Gram positive and Gram negative bacteria (Sofa and Busta, 1981).

The purpose of the present study was to evaluate the effectiveness of potassium sorbate (different concentrations) expressed as sorbic acid (ppm), storage temperature (4°C) and pH (5.3-5.9) on the quality of refrigerated chicken fillets artificially contaminated with *Yersinia enterocolitica* (serotype O:8).

MATERIALS and METHODS

Cultures:

Culture used for this study was *Yersinia enterocolitica* (serotype O:8 strain) provided by Department of Food Control, Faculty of Veterinary Medicine, Zagazig University. Before used in experiments culture was checked for purity by streaking onto Cefsulodin Irgasan-Novobiocin (CIN) agar; Oxoid), and incubated at 32°C for 18-24 hours, up to 5 typical forms dark red bulls eye like colonies with a transparent border were picked up and maintained on Tryptic Soy Agar salnts (TSA; Difco) incubated for 24 hours at 25°C and held at 5°C until use.

Preparation of samples:

Chicken fillets from freshly slaughtered chicken were received from the processing plant in a chilled condition (muscles were cut in slices, transversely to the fibers, 10 cm in diameter and one cm thick, 120 gm weight). Samples (16 slices) were divided into 4 groups, 3 groups as examined samples and one control. All samples were exposed to ultraviolet rays (lamp, Clean Air Techniek bv, Netherlands) for surface sterilization and the treatment began within 3 hours upon receipt.

Appropriate *Yersinia* culture suspension was diluted into one liter of phosphate buffer (pH 7.2) to give a cell suspension of 10^4 cfu/ml. Experimentally examined samples and control ones (4 groups) were inoculated by dipping in *Yersinia* suspension for 1 minute and drained dry. Each group of experimentally examined samples were then dipped for one minute in 3% aqueous solution of potassium sorbate for the first group, 5% for the second group and 10% for the third one. Control group was dipped in sterile distilled water, all samples were individually bagged and stored at 4°C for 21 days.

Sorbate analysis:

Sorbate assay in chicken meat fillets was performed by triplicate on samples 10 cm diameter and 1 cm thick using ultraviolet spectrophotometer with ethyl ether extraction in acid medium according to AOAC (1990). A Double-Beam UV-Visible digital spectrophotometer model UV-150 (Shimad ZU Corp., Tokyo) was used, and the absorbance was determined at 250 nm. Measurments were performed throughout and at the end of the storage period. Total concentration of sorbate was expressed as ppm. sorbic acid based on the weight of the sample (Zamora and Zaritzky, 1987).

PH determination:

PH determination was performed after 10 days of storage period by using pH meter model 3310 (Jenway LTd. UK).

Microbiological analysis:

After each storage interval (0, 3, 7, 10, 14, 17 and 21 days), The packages were opened aseptically at random using sterile forceps. A ribbon ($10cm_2$ in surface and approximately 0.3 cm thick) was removed along the lateral area of the muscle by means of a sterile forceps and scalpel. Each sample (20 g) was then placed in 500 ml flask containing 180 ml of sterile 0.1% peptone broth and shaken for 15 minutes at 250 rpm and 30°C (shaker Model G-52). Appropriate dilutions were made with sterile 0.1% peptone broth and duplicate plates of CIP agar were used, enumeration of typical *Yersinia* colonies was performed at 32°C for 24 hours.

RESULTS

Table 1: Determination of potassium sorbate (as sorbic acid ppm) in the examined chilled poultry fillets.

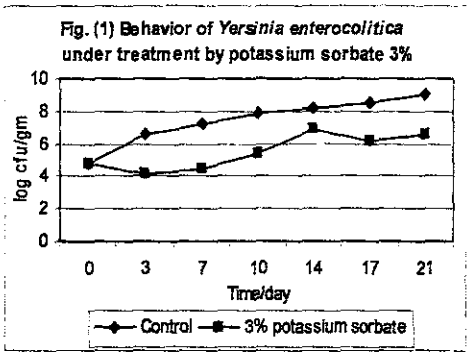
pH of inoculated samples	% of potassium sorbate dip	Sorbic acid (ppm)	No. of samples
	0	0	4 (control)
5.8	3.0	400	4 (samples)
6.0	5.0	668	4 (samples)
6.2	10.0	1325	4 (samples)

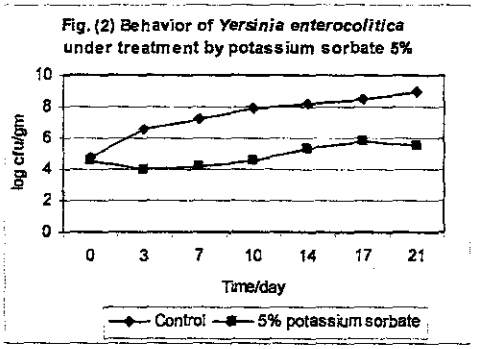
Table 2: Effect of storage period on residual sorbic acid in the examined samples.

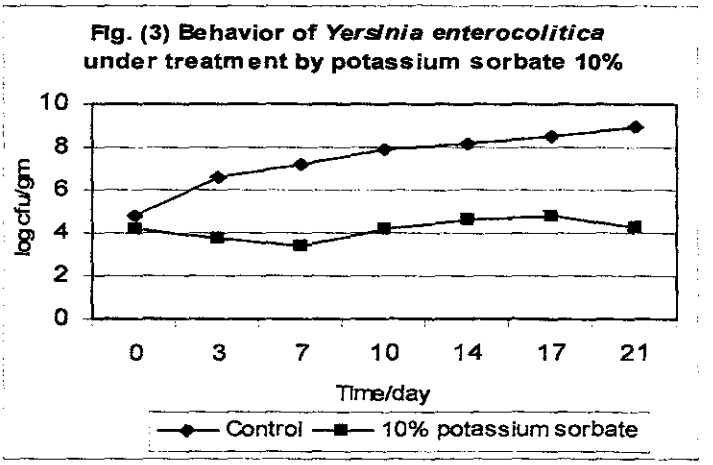
Sorbic acid in chicken meat (ppm)	Storage period (days)						
	0	3	7	10	14	17	21
400	397	399	400	402	400	395	392
668	663	665	665	670	668	665	670
1325	1321	1327	1329	1330	1328	1327	1327

Table 3: Behavior of *Yersinia enterocolitica* under treatment by different potassium sorbate concentrations.

Log cfu/gm / Time/day	Control	Potassium sorbate concentration		
		3%	5%	10%
0	4.8	4.8	4.6	4.2
3	6.6	4.1	4.0	3.7
7	7.2	4.5	4.2	3.4
10	7.9	5.4	4.6	4.2
14	8.2	6.9	5.3	4.6
17	8.5	6.2	5.8	4.8
21	9.0	6.6	5.5	4.3







DISSCUSION

Results of this investigation revealed that there were apparent differences in the antimicrobial activity of various sorbic acid concentrations on *Yersinia enterocolitica*. In this way these results support those found in similar studies with other microorganisms (Minor and Marth, 1972).

The initial sorbate concentration layer at the surface of samples diffuses into the meat bulk due to the concentration gradient (Torres *et al.*, 1985). During prolonged storage time sorbate profile tends to reach a uniform distribution, and it is convenient to express this concentration based on sample weight. Solutions of potassium sorbate in a range 3-10% left residues between 400-1325 ppm of sorbic acid in poultry meat (Table 2).

Observed difference between residual sorbate levels in refrigerated meat samples during storage period (Table 1) were not statistically significant ($p>0.05$). These results agreed with data reported by Robach *et al.* (1980). Zamora and Zairtzky (1987) reported that, average of residual potassium sorbate (as sorbic acid-669 ppm) in beef slightly increased during the storage period (38 days at 4°C) up to 679 ppm). Data obtained from this study indicated that a one minute dip of chicken fillets in 5% (w/v) potassium sorbate significantly improved their storage life at ^{4o}C between 0 and 21 days of storage.

Sorbate treated slices by 3% had about 2 log cycle reductions in *Yersinia* count per cm_2 compared to control ones, but reached to 4 log cycle reduction at 10% potassium sorbate concentration comparable with control samples (Table 3 and Figure 1).

A marked difference in *Yersinia enterocolitica* counts between control and sorbate treated samples was observed after 10 days of storage period. Figure (1) showed that counts reduction of sorbate treated slices were 3, 3.5 and 4 log cycle per cm^2 at 3%, 5% and 10% potassium sorbate concentration and pH values 5.6, 5.6, 5.4 respectively compared by control samples. These results agree with that obtained by to and Robach (1979); Elliott *et al.* (1985) and Robert (1987).

Spoilage of control samples, as judged by slime and odour formation started after 7 days while sorbate treated samples were in good condition up to 17 days of storage at 3°C. Zamora and Zaritzky (1987) studied the effectiveness of potassium sorbate in poultry, finding that a dipping of 30 seconds of 8% solution (residue of 1200-1300 ppm of

sorbic acid) prolonged the shelf life of the product at 3°C from 10 days (control) to 17 days.

The birds in this study were processed in a commercial plant; hence results should have commercial application.

Growth of *Yersinia enterocolitica* was controlled by sorbate treatment so, sorbates are considered as Generally Regarded As Safe (GRAS) food additives, no resistant bacterial strains were developed by its use in poultry and treated poultry is highly acceptable organoleptically (To and Robach, 1979).

Zamora and Zaritzky (1987) determined that application of 3200 ppm sorbic acid in fresh poultry (10% potassium sorbate used as a dip for 60 seconds) did not affect sensory characteristics of cooked poultry.

Sorbic acid is considered to be a non-toxic compound for human beings, as it is metabolized like fatty acids, i.e. to carbon and water.

An Admitted Daily Intake (ADI) of up to 1500 mg of sorbic acid for a 60 kg body weight was established by FAO/WHO (1974). Assuming an average daily consumption of beef (150 gm) and considering the maximum residue in the slices determined in the present study (1325 ppm sorbic acid). The mean daily intake resulted in 198.75 mg of sorbic acid, this value is well below the ADI within the limits established by the results, and the risk for the use of potassium sorbate in beef was negligible, because the amount ingested was quite low.

The benefits included the accomplishment of an optimal quality product (chicken meat fillets) with a longer shelf life, which made marketing for longer periods of time possible.

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