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Inactivation of Common Species of Food-Poisoning Bacteria by Addition of Some Chemical Preservatives

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ABSTRACT

Inactivation of six common *species of food-poisoning bacteria* (*Listeria monocytogenes. Salmonilla typhimurium, Staphylococcus aureus, Steptococcus faecalis, Escherichia coli and Bacillus cereus*) by potassium sorbate, sodium propionate and sodium bisulfite was studied. Different concentrations of these food additives were individually added to the selective agar medium of each pathogen or to the sterilized milk inoculated with the above pathogens. The minimum inhibitory concentrations of these preservatives greatly differ against tested food-poisoning bacteria. The inhibitory effect of potassium sorbate against *Bacillus cereus* was the greatest, followed by *Escherichia coli*. Sodium propionate had the most inhibitory action against *Staphlococcus aureus*, followed by *Salmonella typhimurium*. Sodium bisulfite was highly active against *E. coli* followed by *B. cereus*. The maximum permissible levels, usually used in foods, of these preservatives were quite enough for complete inactivation of these pathogens in milk and greatly reduced the numbers of other pathogens inoculated into milk.

Key words: Food poisoning, Pathogens, Additives, Preserevatives.

INTRODUCTION

Food borne diseases are having increasing significant adverse effects on the health and productivity of populations in both developing and developed countries. The most common food pathogenic bacteria causing food poisoning are : *Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus,*

Streptococcus faecalis, Escherichia coli and Bacillus cereus (Varnam and Evans, 1991).

The term "food poisoning" caused by the ingestion of microorganism's or their toxins include food – borne illnesses. Whereas, bacterial food infection refers to food-borne illnesses caused by the entrance of bacteria into the body through ingestion of contaminated foods and reaction of the body to their presence or to their metabolites (Frazier and Westhoff, 1988).

The ability of microorganisms to survive and/or proliferate under refrigeration and in reduced oxygen atmospheres, and for some of them, the low number necessary for disease production indicate the seriousness of the potential hazards with which we are faced.

L. monocytogenes is the causative agent of listeriosis, a disease that primarily affects pregnant women, infants and adults (Ralovich, 1984). *Salmonella* transmitts salmonellosis to man has received considerable attention world wide (Sockett, 1991). *Staph. aureus* is an important source of food poisoning throughout the world. This bacterium can contaminate several foods and produce several types of enterotoxins of remarkable stability to heat and radiation causing gastroenteritis (Halpin-Dohnalek and Marth, 1989). *Strep. faecalis* is among bacteria of public health significance but of less acute, while *E. coli* is considered an important agent causing food infections especially in infants (Anonymous, 1993). The role of *B. cereus* as an agent of food poisoning received increasing attention over the past two decades. It has been incriminated in many food poisoning outbreaks. It is associated with meat products, vegetables dishes, different processed cheese, ice-cream, eggs and cooked rice (Ahmed et al.1983).

This proves that food industries follows safety measures properly, among which is the use of preservatives. However, countries experiencing food shortages and low food quality are the countries with inadequate technology of preservation. The use of preservatives in this situation would have an immense impact. Further, although many countries have advanced technology for preservation, methods may either be not applied to their potential or may not be completely effective. Preservatives are thus, depended

upon, either independently or in combination with other forms of preservation, to maintain a food in its original or fabricated state and to prevent excess losses from deterioration. To illustrate, a research was done by Lopez et al.(1996) to study the thermal resistance of *Bacillus stearothermophilus* spores in different heating systems containing some approved chemical preservatives.

Preservatives, in general, may inhibit the growth and activity of microorganisms by interfering with their cell membranes, their enzymes activity, or their genetic mechanisms. Ideally, preservatives should have a wide range of antimicrobial activity; should be nontoxic to human; should be economical; should not have an effect on the flavour, taste, or aroma of the original food; should not be inactivated by the food or any substance in the food; should not encourage the development of resistant strains; should kill rather than inhibit microorganisms (Furia, 1972).

Thus, much work has been conducted on different kinds of preservatives and their ability to inhibit food-poisoning bacteria. Researches concentrated a lot on chemical preservative, where they tried to find best levels and combinations of certain preservatives that would be most effective and least harmful. For instance, the fate of *E. coli* 0157: H7 in unpasteurized apple cider with different levels and combinations of sodium benzoate and potassium sorbate were used (Zhao et al., 1993). Addition of 0.5% sodium acetate, 2% sodium lactate, or 0.26% potassium sorbate significantly decreased growth of *L. monocytogenes* in refrigerated turkey bologna surface inoculated after thermal processing and slicing (Wederquist et al., 1994).

Bacteria of primary significance in foodborne disease include Listeria, *Salmonella, Staphylococcus, Streptococcus, E. coli* and *Bacillus cereus.* Thus, the purpose of this study is to evaluate the inactivation of these food-poisoning bacteria in a media being raw milk using different levels of chemical preservatives such as potassium sorbate, sodium propionate and sodium bisulfite, and natural preservatives such as garlic extract.

Sorbic acid and its potassium salt and propionic acid and its sodium or calcium salts are the preservatives permitted and most

commonly used in many foods as antimicrobial agents especially against moulds and yeasts (Lueck, 1980; Robach and Sofos, 1982; El-Fouly et al., 1986 and Hammad, 1989). Sulfur dioxide, some sulfites and bisulfites are another permitted preservatives used in some foods as anti-microbial agents. They have more inhibitory effect against bacteria than yeasts and moulds (Lueck, 1980 and Armentia-Alvorez et al., 1994). Little attention was given to study the inhibitory action of these preservatives against public health significant bacteria.

The primary purpose of the present study was to evaluate the inhibitory effect of various levels of potassium sorbate, sodium propionate and sodium bisulfite against six common species of food poisoning bacteria, *namely L. monocytogenes, S. typhimurium, Staph. aureus, Strept. faecalis, E. coli and B. cereus* in selective agar media at pH 5.5 and fresh fluid milk during storage at 10°C.

MATERIALS AND METHODS

The food poisoning bacteria (*Listeria monocytogenes, Salmonella typhimurium, Staphylococcus aureus, Streptococcus faecalis, Escherichia coli* and *Bacillus cereus)* were bought from EL-HYATT Drug stores at Sharjah, U.A.E.

Anti microbial food additives:

Potassium sorbate, sodium propionate and sodium bisulfite were individually dissolved in distilled water to give stock solutions of 10%. These solutions were filtered through a 0.45m filter (Millipore Type HA) (Bedford, MA, USA) for sterilization.

Selective media used

Palcam - Listeria – Selective Agar (Oxoid) with palcam-selective-supplement (SR 150 E Oxoid) was used for enumeration of *L.monocytogenes* (wang, 1992).

S. typhimurium was counted on Brilliant agar medium (ISO, 1978), while *Staphylococcus aureus* was counted on Baird – parker agar medium with added Egg-Yolk Tellurite Enrichment (Difco) as described by FDA (1976). Kanamycin aesculin azide agar medium was used for enumeration of *Streptococcus faecalis* and MacConkey agar medium was used far counting of *Escherichia coli* (Oxoid,

1982). Manitol-egg-Yolk-Polymixin (MYP) agar medium was used for enumeration of *Bacillus cereus* (Mossel et al., 1967).

Each specific selective agar medium was sterilized by autoclaving at 121°C for 15 min. (except Brilliant green which was only boiled), then cooled to 46-48°C. Under aseptic conditions, selected concentrations of potassium sorbate or sodium propionate (0.0, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5%) were separately added to each specific selective agar medium. Since soium bisulfite is usually used in foods at concentration lower than that of potassium sorbate or sodium propionate, it was added at concentrations of 0.0125, 0.025, 0.05, 0.01, 0.15 and 0.2%. These preservatives were most effective as anti microbial agents at low pH value and the upper limit of their activity was pH 6.0-6.5 (Lueck, 1980). Therefore, the pH of all the media used was adjusted to 5.5 with sterilized lactic acid (10%).

Inoculation of raw milk:

Samples (250-ml) of raw milk were placed in 500-ml conical flasks, covered tightly with cotton plugs and double thickness aluminum foil. These flasks were sterilized. Then under aseptic conditions, potassium sorbate or sodium propionate were added to the sterilized milk at concentrations of 0.0, 0.2, 0.3 and 0.4%, while sodium bisulfite was added at concentrations of 0.0, 0.0125, 0.025 and 0.05% (0.0, 125, 250 and 500 p.p.m). A portion of 24-h tryticase soybroth culture of each pathogen was added to each milk sample in order to obtain an initial count of approximately 10^4 cfu/ml.

Storage and microbiological analysis:

The treated and untreated milk samples were stored at 10°C. for 15 days. Milk samples were analyzed immediately after inoculation and at intervals every 5 days. At each interval the tested pathogens were counted, each on its specific selective agar medium, using duplicate surface-plating technique. The plates were incubated at 37°C.

Experimental design:

Two major experiments were conducted. In experiment 1 various concentrations of potassium sorbate (0.0 to 0.5%) and sodium bisulfite (0.0 to 0.2%) were tested for its inhibitory effects on the six common food-poisoning bacteria in selective agar media at

pH 5.5. Applications of these preservatives in a milk model system were carried our at Experiment 2. This experiment consisted of four groups (for each preservative) of sterilized milk inoculated with the pathogens and stored at 10°C. for 15 days. Group A had no preservative added, while in group B, C and D the preservative was added at concentrations of 0.2, 0.3 and 0.4% in case of potassium sorbate or sodium propionate and at concentrations of 0.0125, 0.025 and 0.05% in case of sodium bisulfite.

RESULTS

Effect of preservatives on the growth of pathogens in selective media:-

The results in Table 1 show that potassium sorbate at concentration of 0.2% completely inhibited the growth of *B. cereus*. Complete inactivation of *E. Coli* was occurred when the MacConkey agar medium contained 0.3% potassium sorbate. *L. monocytogenes* and *S. typhimurium* were completely inactivated at potassium sorbate concentration of 0.4%. The maximum concentration of potassium sorbate used, i.e. 0.5%, was not quite sufficient for complete inactivation of *Strep. faecalis* and *Staph. aureus* indicating the relative resistance of these pathogenes to potassium sorbate.

Sodium propionate at concentration of 0.2% greatly reduced the counts of all the tested patogens, while a concentration of 0.3% completely inactivated *S. typhimurium* and *Staph. aureus* (Table 1). A concentration of 0.4% sodium propionate completely inactivated *E. coli* and *L. monocytogenes*, while *B. cereus* and *Strep. faecalis* were not inactivated at the maximum concentration used, i.e. 0.5% indicating its resistance to sodium propionate.

Sodium bisulfite at concentration of only 0.5% (500 ppm) completely inactivated the growth of *E. coli* and *B. cereus* (Table 1). The growth of *Staph. aureus* and *Strep. faecalis* were completely inactivated when their selective agar media contained 0.1% (1000 ppm) of sodium propionate. Complete inactivation of *S. typhimurium* was occurred when its selective agar medium contained 0.15% sodium propionate. *L. monocytogenes* was not inhibited even at the maximum concentration used, i.e. 0.2%.

Effect of the preservatives on the growth of pathogens in a milk model system:

Table (1) shows the effect of potassium sorbate on the growth of *L. monocytogenes* inoculated into sterilized milk during storage at 10°C *L. monocytogenes* counts in control samples (without added potassium sorbate) progressively inbcreased reaching its maximum counts of 6.3 x 10^7 cfu/ml. After 10 days of storage, then these counts decreased at the 15^{th} day. The rate of increase in the counts was much lower when 0.2 or 0.3% potassium sorbate concentration were added to milk.

Addition of 0.4% potassium sorbate decreased the counts of *L. monocytogenes* throughout storage period. The behavior of *S. typhimurium* during storage of milk treated or untreated with potassium sorbate was almost similar to that of *L. monocytogenes* (Table. 2). The numbers of *Staph. aureus* were increased in all milk samples (treated with potassium sorbate or not) up to 10 days of storage, but the rate of increase was lower in samples containing potassium sorbate than that of control samples (Table 2)

The lowest increase in *Staph.aureus* counts was observed when 0.4% potassium sorbate was added to milk samples. The increase in the counts of Strep. faecalis inoculated into milk samples was almost identical to that of *Staph. aureus* (Table 2). Addition of 0.3% potassium sorbate to sterilized milk samples inoculated with the pathogens completely inactivated *E.coli* and *B. cereus* (Table 2) throughout the storage period.

Sodium propionate at concentration of 0.3% inhibited the growth of *L. monocytogenes, S. typhimurium* and *Staph. aureus* after only 5 days of storage at 10°C. (Table 3). Addition of 0.4% sodium propionate inactivated the growth of *L. monocytogenes, S. typhimurium, Staph. aureus* and *E. coli* throughout the storage period. Meanwhile, this concentration (0.04%) caused only remarkable decrease in the counts of *Strep. faecalis* and *B. cereus* (Table 3).

The counts of *L. monocytogenes* inoculated into untreated milk samples (without added sodium bisulfite), progressively increased

during storage up to 10 days, then decreased at the 15th day. However, the increase in the counts in treated samples with sodium bisulfite was lower than that in control samples throughout the storage period (Table 3). A concentration of 0.05% (500 p.p.m.) of sodium bisulfite greatly reduced the counts of *S. typhimurium* throughout the storage period (Table 4). Also, this concentration greatly reduced the counts of Staph. aureus and *Strep. faecalis* and completely inhibited the growth of *E. coli* and *B. cereus* (Table 4) throughout the storage period.

DISCUSSION

Throughout the world, particularly in developing countries, the consumption of foods contaminated by food-poisoning bacteria cause a serious public health problem. This problem continue to affect millions of the world populations while no real solution is in sight. Though the methods used to inhibit or inactivate the growth of pathogenic bacteria in the foods are of paramount importance.

The effectiveness of various concentrations of potassium sorbate, sodium propionate and sodium bisulfite on the main food-poisoning bacteria often contaminating foods were studied here in both selective agar media or in a milk model system. The maximum permissible level of potassium sorbate usually used in most foods is 0.3% (Liewen and Marth, 1985). When this level was added to the selective media of the tested Pathogens a great reduction in the numbers of *L. monocytogenes*, *S. typhimurium*, *Staph. aureus* and *Strep. faecalis* were recorded. However, little effect of this level (0.3%) on these pathogens was observed with milk during storage at 10°C. This little effect of potassium sonbate on the above four pathogens inoculated into milk could be due to the fact that the pH of the most fresh milks is nearly neutral. Sofos and Busta (1981) reported that the optimal anti microbial effectiveness of sorbate is at pH 4.75 and the upper limit for sorbate activity is at pH 6.0-6.5. There have been some reports on the inhibitory effect of potassium sorbate against *L. monocytogenes*, *S. typhimurium* and *Staph. aureus*, but none against *Strep. faecalis*. *El-Shenawy* and Marth (1991) found that *L. monocytogones* was inactivated in tryptose broth containing 0.3% potassium sorbate and acidified to pH 5.0 with actic, tartaric, lactic or citric acid, but at pH 5.6 this bacterium grew in the

presence of this concentration. The importance of using sorbate at proper pH values was demonstrated by many investigators, for example, Park and Marth (`1972) showed that *S. typhimurium* could grow in nutrient broth (pH 6.7) or skimmilk (pH 6.4) fortified with 0.3% sorbic acid when the media were not acidified. However, when the pH was reduced to 5.0, growth did not occur. The growth of *Staph. aureus* in the presence of sorbate at concentrations higher than the permissible levels normally used in foods was reported by Lynch and Potter (1982) and Parada et al. (1982).

On the other hand, the maximum permissible level of potassium sorbate was quite sufficient for complete inactivation of *E. coli* and *B. cereus* in both selective agar media and whole fluid milk. The mechanism by which sorbate inhibits microbial growth is not clear and defined. It has implicated inhibition of various enzymes in the carbohydrate metabolism such as enolase and lactic dehydrogenase (York and Vaughn, 1964) and inhibition of respiratory mechanisms. The sensitivity of *E. coli* and *B. cereus* to potassium sorbate was reported by lueck (1980) who mentioned a concentration of only 0.05-0.1% of sorbate at pH 5.2-5.6 has inhibitory effect against them.

From the results one can observe that the effectiveness of potassium sorbate against B. cereus was quite strong, followed by *E. coli, L. monocytogenes* and *S. typhimurium*. No inhibitory effect on *Staph. aureus* and *Strep. faecalis* was shown even when 0.4% sorbate was added to milk or when 0.5% sorbate was added to its selective media at pH 5.5. Propionic acid and its salts are anti-microbial food additive generally recognized as safe (GRAS) with no tolerance level set (Sayer, 1977).

They are highly effective against molds and some yeasts, but have little action against bacteria (Lueck, 1980). Generally, in the present work the counts of the tested pathogens were reduced as the concentration of sodium propionate increased from 0.05 to 0.5% in its selective media and from 0.2 to 0.4% in milk samples. Sodium propionate at concentration of 0.3% completely inhibited the growth of *S.typhimurium* and *Staph. aureus* in its selective agar media. The inhibitory action of propionic acid is due largely to the fact that it accumulates in the cell and blocks the metabolism by inhibiting the

enzymatic system. It also inhibits growth by competing with other substances necessary for the growth of the microbe especially alanin and other amino acids (Lueck, 1980), although the precise mode of fungal static action is not known.

The effectiveness of sodium propionate at concentrations of 0.2 to 0.4% on the tested pathogens which were inoculated into milk was considerable. Countes between 10^6-10^7 cfu/g were reached in the milk without added propionate whitin 5 days of storage at 10°C, while with propoionate these counts never reached even with the minimum concentration used (0.2%) and after 15 days of storage. Sodium propionate was more effective against *S. typhimurium* followed by *Staph. aureus* and *L. monocytogenes. Strept. faecalis* and *B. cereus* could grow in the presence of high concentration of sodium propionate reaching 0.5%. This could be due to the fact that some microorganisms can utilize propionates as a carbon source. Varnam and Evans (1991) reported that *B. cereus* can utilize propionate as a carbon source.

Sulfur dioxide, some sulfites, bisulfites and metabisulfites are permitted in virtually all countries as food preservatives. Practical experience has shown that their action is directed primarily against bacteria (Lueck, 1980). This study showed that only a concentration of 0.0125 (125ppm) sodium bisulfite completely inhibited the growth of *E. coli*, while *B. cereus* was completely inhibited when its selective medium contained 0.025% sodium bisulfite. The inhibitory action of sulfur dioxide, some sulfites and metabisulfites is based essentially on various forms of interference in the enzyme structure of the microbial cell especially enzymes with SH-group. These compounds when dissolved in water form sulfurous acid. Many mechanisms for the action of sulfurous acid on microbial cell have been suggested, including the reduction of disulfide linkages, formation of carbonyl compounds, reaction with ketone groups and inhibition of respiratory mechanisms (Fraizer and westhoff, 1978). The minimum concentration of sodium bisulfite reproted (Lueck, 1980) for complete inactivation of *E. coli, Staph. aureus* and *B. cereus* were lower than that found in the present investigation. The differences in the results could be attributed to many factors which affect the effectiveness of a preservative as an anti microbial additive. Among of these factors are media used, composition of

food used, pH, type of acids used to adjust the pH, water activity, initial contamination and storage conditions as reported by many investigators. (Bell et al, 1959; Sofos and Busta, 1981 and El-Shenawy and Marth, 1992). A concentration of 0.05% (500 p.p.m) of sodium bisulfite completely inactivated the growth of *Staph. aureus*, *E. coli* and *B. cereus* during storage of milk at 10°C and greatly affected the growth of *S. typhimurium* and *Strep. faecalis*. *L. monocytogenes* can grow in milk in the presence of 500 ppm of sodium bisulfite indicating little inhibitory action of this preservative against *L. monocytogenes*.

It could be concluded that the three preservatives used in this work had some anti microbial activity against the tested food poisoning bacteria. However, sodium bisulfite appeared to be more effective in comparison with potassium sorbate and sodium propionate. The effectiveness of each preservative differed from microorganism to another. Owing to the variations in the inhibitory effect of potassium sorbate and sodium propionate against the food poisoning bacteria and on the basis of preferability of use of these two preservatives in a great variety of foods, they can be combined together in practical use in foods, this will improve the antimicrobial spectrum of both.

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Table (1):Effect of potassium sorbate, sodium propionate and sodium bisulfite on the growth of food poisoning bacteria in selective agar media at pH 5.5.

Preserv.	Concent. %	List. Monocyto.	Sal. Typhim.	Staph. aureus	Strept. faecalis	E. coli	B. cereus
Potassium sorbate	0.00	$1.2x10^6$	$4.4x10^7$	$1.9x10^7$	$6.0x10^7$	$2.5x10^7$	$3.9x10^7$
	0.05	$9.0x10^5$	$2.7x10^7$	$1.5x10^7$	$5.5x10^7$	$1.3x10^7$	$6.8x10^6$
	0.10	$4.3x10^5$	$5.0x10^6$	$8.5x10^6$	$2.3x10^7$	$9.7x10^5$	$1.2x10^4$
	0.20	$8.8x10^4$	$1.3x10^5$	$1.6x10^6$	$7.0x10^6$	$5.8x10^3$	$<10^2$
	0.30	$1.9x10^3$	$4.1x10^3$	$2.5x10^5$	$2.4x10^6$	$<10^2$	$<10^2$
	0.40	$1.5x10^2$	$1.8x10^2$	$1.7x10^4$	$3.3x10^5$	$<10^2$	$<10^2$
	0.50	$<10^2$	$<10^2$	$1.2x10^3$	$1.1x10^4$	$<10^2$	$<10^2$
Sodium propionate	0.00	$1.8x10^6$	$4.1x10^7$	$1.2x10^7$	$7.2x10^7$	$2.2x10^7$	$2.7x10^7$
	0.05	$1.2x10^6$	$2.8x10^7$	$9.9x10^6$	$7.5x10^7$	$2.0x10^7$	$2.6x10^7$
	0.10	$8.8x10^5$	$3.0x10^6$	$4.5x10^6$	$6.1x10^7$	$6.2x10^6$	$8.9x10^6$
	0.20	$8.4x10^4$	$1.9x10^4$	$1.0x10^4$	$8.3x10^6$	$4.0x10^6$	$2.5x10^6$
	0.30	$1.0x10^4$	$<10^2$	$<10^2$	$5.5x10^5$	$4.0x10^4$	$1.1x10^5$
	0.40	$<10^2$	$<10^2$	$<10^2$	$7.4x10^3$	$<10^2$	$6.0x10^3$
	0.50	$<10^2$	$<10^2$	$<10^2$	$2.2x10^2$	$<10^2$	$2.0x10^2$
Sodium bisulfite	0.0000	$2.1x10^6$	$4.4x10^7$	$1.7x10^7$	$6.3x10^7$	$3.0x10^7$	$2.4x10^7$
	0.0125	$1.7x10^6$	$2.5x10^7$	$2.3x10^6$	$5.0x10^7$	$8.8x10^4$	$2.8x10^5$
	0.0250	$1.0x10^6$	$2.0x10^7$	$1.5x10^4$	$2.0x10^6$	$<10^2$	$6.4x10^2$
	0.0500	$5.5x10^5$	$5.1x10^6$	$2.3x10^2$	$3.3x10^4$	$<10^2$	$<10^2$
	0.1000	$1.3x10^5$	$1.2x10^4$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	0.1500	$3.3x10^4$	$<10^2$	$<10^2$	$<10^2$	$<10^2$	$<10^2$
	0.2000	$2.4x10^2$	$<10^2$	$<10^2$	$<10^2$	$< 10^2$	$<10^2$

Table (2):Effect of potassium sorbate on the growth of food poisoning bacteria in milk during storage at 10°C.

Pathogens	Storage Period (Days)	Concentrations %			
		0.0	0.2	0.3	0.4
List. Monocytogenes	0	$4.3x10^4$	$4.3x10^4$	$4.3x10^4$	$4.3x10^4$
	5	$6.4x10^6$	$4.5x10^4$	$4.5x10^4$	$4.1x10^4$
	10	$7.7x10^7$	$5.3x10^5$	$5.0x10^4$	$3.9x10^3$
	15	$7.2x10^7$	$4.9x10^4$	$4.7x10^4$	$2.2x10^2$
Sal. Typhimurium	0	$4.2x10^4$	$4.2x10^4$	$4.2x10^4$	$4.2x10^4$
	5	$6.1x10^6$	$4.5x10^4$	$4.4x10^4$	$3.9x10^3$
	10	$7.0x10^7$	$4.9x10^4$	$4.5x10^4$	$3.0x10^3$
	15	$6.7x10^6$	$4.7x10^4$	$4.3x10^4$	$2.5x10^2$
Staph. aureus	0	$5.3x10^5$	$5.3x10^5$	$5.3x10^5$	$5.3x10^5$
	5	$7.5x10^7$	$6.2x10^6$	$5.5x10^5$	$5.5x10^5$
	10	$8.1x10^8$	$6.7x10^6$	$6.0x10^6$	$5.6x10^5$
	15	$7.8x10^7$	$6.2x10^6$	$5.7x10^5$	$5.2x10^5$
Strept. faecalis	0	$6.0x10^6$	$6.0x10^6$	$6.0x10^6$	$6.0x10^6$
	5	$7.9x10^7$	$6.5x10^6$	$6.3x10^6$	$6.1x10^6$
	10	$8.1x10^8$	$7.1x10^7$	$6.5x10^6$	$6.3x10^6$
	15	$7.0x10^7$	$6.8x10^6$	$6.4x10^6$	$5.8x10^5$
E. coli	0	$3.5x10^5$	$3.5x10^5$	$3.5x10^5$	$3.5x10^5$
	5	$1.6x10^7$	$2.9x10^5$	$<10^2$	$<10^2$
	10	$2.6x10^8$	$3.8x10^5$	$<10^2$	$<10^2$
	15	$5.0x10^7$	$1.1x10^5$	$<10^2$	$<10^2$
B. cereus	0	$2.0x10^4$	$2.0x10^4$	$2.0x10^4$	$2.0x10^4$
	5	$8.7x10^5$	$2.0x10^3$	$<10^2$	$<10^2$
	10	$6.3x10^6$	$<10^2$	$<10^2$	$<10^2$
	15	$1.0x10^6$	$<10^2$	$<10^2$	$<10^2$

Table (3):Effect of Sodium propionate on the growth of Food-poisoning bacteria in milk during storage at 10°C.

Pathogens	Storage period (Days)	Concentrations %			
		0.0	0.2	0.3	0.4
List. Monocytogenes	0	1.2×10^{4}	1.2×10^{4}	1.2×10^{4}	1.2×10^{4}
	5	5.0×10^{6}	9.0×10^{4}	3.8×10^{3}	$< 10^{2}$
	10	6.9×10^{7}	3.8×10^{5}	$< 10^{2}$	$< 10^{2}$
	15	3.2×10^{7}	1.2×10^{5}	$< 10^{2}$	$< 10^{2}$
Sal. typhimurium	0	3.3×10^{4}	3.3×10^{4}	3.3×10^{4}	3.3×10^{4}
	5	4.1×10^{6}	5.3×10^{4}	5.6×10^{3}	$< 10^{2}$
	10	7.4×10^{7}	3.2×10^{4}	$< 10^{2}$	$< 10^{2}$
	15	1.2×10^{7}	8.5×10^{3}	$< 10^{2}$	$< 10^{2}$
Staph. aureus	0	7.5×10^{4}	7.5×10^{4}	7.5×10^{4}	7.5×10^{4}
	5	9.8×10^{6}	2.5×10^{4}	2.9×10^{3}	$< 10^{2}$
	10	1.1×10^{8}	3.0×10^{3}	$< 10^{2}$	$< 10^{2}$
	15	2.4×10^{7}	2.1×10^{2}	$< 10^{2}$	$< 10^{2}$
Strept. faecolis	0	5.2×10^{5}	5.2×10^{5}	5.2×10^{5}	5.2×10^{5}
	5	7.8×10^{7}	6.5×10^{6}	5.3×10^{5}	5.0×10^{5}
	10	8.0×10^{8}	6.7×10^{6}	5.5×10^{5}	4.9×10^{4}
	15	7.8×10^{7}	5.7×10^{5}	5.0×10^{5}	4.5×10^{4}
E. coli	0	5.3×10^{5}	5.3×10^{5}	5.3×10^{5}	5.3×10^{5}
	5	8.2×10^{7}	4.0×10^{6}	2.3×10^{5}	$< 10^{2}$
	10	7.5×10^{8}	2.8×10^{7}	1.1×10^{5}	$< 10^{2}$
	15	6.6×10^{7}	5.0×10^{6}	6.8×10^{4}	$< 10^{2}$
B. cereus	0	4.5×10^{4}	4.5×10^{4}	4.5×10^{4}	4.5×10^{4}
	5	5.7×10^{5}	5.5×10^{5}	4.3×10^{4}	3.9×10^{3}
	10	6.2×10^{6}	5.7×10^{5}	4.1×10^{4}	3.4×10^{3}
	15	5.8×10^{5}	4.7×10^{4}	3.8×10^{3}	2.4×10^{2}

Table (4):Effect of Sodium bisulfite on the growth of Food-poisoning bacteria in milk during storage at 10°C.

Pathogens	Storage period (Days)	Concentrations %			
		0.0000	0.0125	0.0250	0.0500
List. Monocytogenes	0	4.5×10^{4}	4.5×10^{4}	4.5×10^{4}	4.5×10^{4}
	5	6.8×10^{6}	6.3×10^{6}	4.9×10^{4}	4.5×10^{4}
	10	8.0×10^{8}	6.7×10^{6}	6.5×10^{6}	4.9×10^{4}
	15	6.7×10^{6}	6.5×10^{6}	5.5×10^{5}	4.3×10^{4}
Sal. Typhimurium	0	4.1×10^{4}	4.1×10^{4}	4.1×10^{4}	4.1×10^{4}
	5	6.8×10^{6}	5.5×10^{5}	4.2×10^{4}	3.8×10^{3}
	10	7.8×10^{7}	6.7×10^{6}	4.0×10^{4}	3.6×10^{3}
	15	7.3×10^{7}	6.2×10^{6}	3.7×10^{3}	2.2×10^{2}
Staph. aureus	0	3.6×10^{4}	3.6×10^{4}	3.6×10^{4}	3.6×10^{4}
	5	8.1×10^{6}	4.0×10^{5}	5.3×10^{3}	1.4×10^{2}
	10	8.7×10^{7}	3.0×10^{6}	4.2×10^{2}	$< 10^{2}$
	15	1.2×10^{7}	6.4×10^{5}	$< 10^{2}$	$< 10^{2}$
Strept. faecolis	0	5.1×10^{5}	5.1×10^{5}	5.1×10^{5}	5.1×10^{5}
	5	9.6×10^{6}	2.7×10^{6}	3.2×10^{5}	3.9×10^{4}
	10	7.0×10^{7}	1.0×10^{7}	7.6×10^{4}	2.7×10^{3}
	15	1.6×10^{7}	9.2×10^{5}	1.8×10^{4}	$< 10^{2}$
E. coli	0	4.4×10^{5}	4.4×10^{5}	4.4×10^{5}	4.4×10^{5}
	5	5.8×10^{6}	1.4×10^{6}	$< 10^{2}$	$< 10^{2}$
	10	2.9×10^{7}	8.8×10^{6}	$< 10^{2}$	$< 10^{2}$
	15	6.8×10^{6}	3.8×10^{5}	$< 10^{2}$	$< 10^{2}$
B. cereus	0	5.6×10^{5}	5.6×10^{4}	1.2×10^{4}	5.6×10^{4}
	5	6.0×10^{6}	4.5×10^{5}	$< 10^{2}$	$< 10^{2}$
	10	1.2×10^{7}	2.5×10^{6}	$< 10^{2}$	$< 10^{2}$
	15	2.2×10^{6}	1.9×10^{5}	$< 10^{2}$	$< 10^{2}$