Evolution of Bactericidal Activity of Selected Food Additives Against Food Borne Microbial Pathogens

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The present study was undertaken in order to examine the bactericidal effects of some selected food additives on food-borne pathogenic bacteria. The antibacterial effect of 9 chemical food additives on our isolates was performed which representing different groups. The study shown that phloxine, nisine, ascorbic acid and sorbic acid had very good antibacterial activity to 4 isolates, this effect was similar to the effect of antibiotics on 4 isolates. Thus, this additives used as preservatives in food. Four isolates were sensitive to small quantities of all tested food additives. Phloxine had best results against gram positive and gram negative isolates, while nisine had more antibacterial activity against gram positive than gram negative. Time kill curves for most potent food additives were performed (phloxine, nisine, sorbic acid, ascorbic acid and sodium benzoate). The results shown that ascorbic acid kill 100% of *E. coli, Salmonella indica* and *Staphylococcus aureus* after 2 hours, while it need 24 hours to kill Bacillus cereus. Phloxine killed 100% of *Bacillus cereus* and *Staphylococcus aureus* after 2, 24 hours respectively. It needs more than 48 hour to kill *E.coli* or *Salmonella indica*. Nisine kill 100% of *E. coli, Salmonella indica* and *Staphylococcus aureus* after 12, 2 and 4 hours respectively. Nisine can't kill *Bacillns cereus* or *Staphylococcus aureus* until 48 hours, while killed *E.coli* and *Salmonella indica* after 2, 8 hours respectively. *E. coli* and *Staphylococcus aureus* killed completely after 2, 24 hours after treated with sodium benzoate, while *Bacillus cereus* and *Salmonella indica* didn't killed completely until 48 hours. The data suggest that these food addatives may have some potential anti-microbial applications.

Key words: Bactericidal, Food additives, Food borne microbial pathogens.

A food additive is any substance not commonly regarded or used as food, which is added to, or used in or on, food at any stage to affect its keeping quality, texture, consistency, taste, colour, alkality or acidity, or to serve any other technological function in relation to food, and includes processing aids in so far as they are added to or used in or on food (The Food Labelling Regulations, 1980 and Food Intolerance and Food Aversion, 1984).

Previous work shows that several halogenated fluoresceins, such as eosin, erytrosin, and Rose Bengal, have shown to have a bactericidal effect on *Staphylococcus aureus* (Rasooly and Weisz, 2002). One halogenated fluorescein, the colorant Phloxine B known as D&C red no. 28

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(20,40,50,70-tetrabromo- 4,5,6,7-tetrachlorofluorecein disodium salt), has bactericidal effect on *S. aureus* and has a potential as a useful antibiotic agent. Phloxine B has a bactericidal effect against gram positive but not against gram-negative bacteria and that the anti-microbial activity is light dependent. (Rasooly, 2005). The anti-*S. aureus* activity of phloxine B, compound 14. FDA-certified batches of phloxine B (D&C red no. 28) are permitted in the United States for use in coloring cosmetics and ingested drugs with an acceptable daily intake for humans set by the FDA at 1.25 mg/kg of body weight (Federal Register, 1982). When dye was added at concentrations of 50 or 100 ug/ml, bacterial growth stopped completely. To assess the effect of phloxine B on MRSA bacteria, seven MRSA strains were incubated with phloxine B. The response of these strains was similar to the response obtained from the control strain, ATCC 13565. On the other hand, the common usage of phloxine B as a color additive in food, drugs, and cosmetics does bring the risk that bacteria might have the opportunity for exposure to small amounts of it over long periods of time, which might result in their developing resistance to its antimicrobial activity. (Rasooly & Weisz, 2002)

Nisin is a small cationic lanthionine antibiotic produced by *Lactococcus lactis*. During its antimicrobial action, it targets intermediates in the bacterial cell-wall biosynthesis, lipid II, and undecaprenyl pyrophosphate. Awidely used food preservative, the peptide antibiotic nisin, E 234, is produced by *Lactococcus lactis* and shows high antimicrobial activity against a broad spectrum of Gram-positive bacteria. Nisin is also effective in inhibiting the outgrowth of spores from *Clostridium* and *Bacillus* species. Nisin activity toward Gram-negative organisms also has been reported, although to a lower degree than against Gram-positives and usually in combination with chelating agents (Delves-Broughton, 1990). Nisin is active in the nanomolar range and has no known toxicity to humans, which has placed it in the unique position of worldwide acceptance as a powerful and safe food additive in control of food spoilage (Thomas *et al.,* 2000). The recent discoveries of lipid II as a target for nisin and, in particular, the pivotal role played by the pyrophosphate group, also have brought nisin to the forefront in the battle with resistant human infections as a model case for the design of new antibiotics. Nisin induced leakage of cytoplasmic contents from treated samples, , although it displayed multiple aberrations. Cytokinesis was evident 30 min after addition of nisin, which contradicts the current view of a rapid cell death after antibiotic addition. The principal site of cell-wall inhibition by nisin appears near the division site, where peptidoglycan formation is accelerated. Nisin permeabilizes plasma membranes, accelerates cell division and causes minicell formation. Nisin also deregulates cell envelope formation, which leads to aberrant cell morphogenesis. (Hyde *et al.,* 2006)

Chitin is a natural polymer, found in the exoskeletons of crustaceans and insects, as well as in the cell walls of certain fungi (Rinaudo, 2006). Evidence has been put forward that chitosan possesses various biological activities, viz. antioxidant (Chien *et al.,* 2007), and antibacterial and antifungal properties (Liu *et al.,* 2006; Rhoades and Roller, 2000; Tikhonov *et al.,* 2006 and Uchida *et al.,* 1989), make it an interesting polymer for several applications in food, industries, and also in medicine (Shi *et al.,* 2006). However, its high-molecular weight (MW) has limited its practical applications due to its insolubility at pH values above 6.3 (Okuyama *et al.,* 2000 and Seo *et al.,* 2007). The antimicrobial effect of chitosans is strongly dependent on the type of target microorganism (Gram-negative versus Gram positive) and the MW of chitosan-being higher for lower MW in the case of the Gram-negative bacterium, and the reverse in the case of the Gram-positive one. The stronger antibacterial activity observed, at lower pH, in the juice matrix, coupled with the poor performance of the milk matrix, suggest that the use of chitosans (irrespective of MW) will be limited to food products that possess a low protein content. (Fernandes *et al.,* 2008). Higher MW chitosans (628and100kDa) surrounded both forms of *B. cereus* by forming a polymer layer—which eventually led to the death of the vegetative form by preventing the uptake of nutrients but did not affect the spores since these can survive for extended periods without the access to nutrients. On the other hand, the use of chitooligosaccharides (COS) by itself on *B. cereus* spores was not enough for the destruction of a large number of cells, but it may weaken the spore

Table 1. E numbers of tested food additives

Name	Number	Description	Examples of use
L-Ascorbic Acid (Vitamin C)	E300	Occurs naturally in fruit and vegetables but synthesized biologically. It acts as a preservative, anti-oxidant, meat colour fixative, and flour improver. also avitamin. Ascorbic acid is a sugar acid. Its appearance is white to light-yellow crystals or powder. It is water-soluble.	Found in fruit juices, bread, baked products, powdered mashed potatoes, etc.
Monosodium glutamate (MSG)	E621	Present naturally in seaweed but generally prepared chemically from sugar beet. Flavour enhancer of protein rich foods.	Meat, Chinese foods, packet convenience meals and snacks (eg. Soup), dries products, crisps and potato snacks.
Nisin	E234	Polycyclic peptide amino acid residues. Commercially, it is obtained from *Lactoccus lactis* on natural substrates, such as milk or dextrose, and is not chemically synthesized. It used as preservative.	It is used in processed cheese, meats, beverages, etc. during production to extend shelf life by suppressing Gram-positive spoilage and pathogenic bacteria.
Phloxine B	Not recorded	A red halogenated fluoresceins . Phloxine B has fluorescein skeleton ring	A color additive for food, drugs, and cosmetics.
Riboflavin	E101	Naturally occurring B group vitamin usually obtained from yeast or produced synthetically.	Enrichment and fortification of food. Added to processed cheese as yellow/ orange colour
Sodium Benzoate	E211	Salt of Benzoic Acid	In bottled sauces
Sodium nitrite	E250	Derived from sodium nitrate by chemical or bacterial action. Acts as a preservative and colour fixative. Preservative	Meats, bacon and pork sausages.
Sorbic Acid	E200	Naturally occurring in some fruits but generally manufactured synthetically for use as a food preservatives.	Commonly added to soft drinks, cheese spread, frozen pizza and cakes.
Tartrazine	E102	Widely used yellow / orange colour	Found in soft drinks, cakes, biscuits, puddings,meat products, sauces, tinned and packet convenience foods and confectionery.

(DermNet NZ, 2008).

Table 2. Effect of selected food additives on inhibition of *Bacillus cereus*. Where inhibition zone(mm)- (no effect), +(≤ 9), ++ (< 15), +++ (15-20mm), ++++ (≥20 mm).

Food additives	Concentrations of food additive (mg/ml)														
	0.1	0.5	1	2	4	8	16	32	50	75	100	125	150	175	200
Ascorbic acid•	-	-	-	-	-	-	+	++	++	++	++	++	++	++	++
Nisin•	-	-	-	-	-	-	-	+	+	+	-	+	+	++	++
Ploxine B•	-	-	-	+	++	++	+++	+++	+++	+++	+++	+++	+++	++++	+++++
Riboflavine*	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Sodium benzoate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sodium monoglutamate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium nitrite•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbic acid*	-	-	-	+	+	++	++	++	++	++	+++	+++	+++	+++	+++
Tartrazine•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* = dissolved in Dimethyl formamid. • = dissolved in distelled water.

structure and its ability to contaminate, by inducing exosporiumloss.(Fernandes *et al.,* 2009).

The present study was undertaken in order to examine the bactericidal effects of some selected food additives on some food-borne pathogenic bacteria.

MATERIALAND METHODS

Food additives

A number of food additives include ; ascorbic acid, monosodium glutamate, nicin, phloxine B, riboflavin, sodium benzoate, sodium nitrite,, sorbic acid and tartrazine were chosen. Food additives were purchased from Sigma–Aldrich (Table 1).

Bacterial strains

The food additives were individually tested against the most potent proteolytic bacterial species which selected for this study. Preparation of 24 hours pure culture in saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 hours.

Determination of the antibiotic potency of food additives by Agar diffusion test using paper disc-diffussion method

Briefly, suspension in nutrient broth (NB) of the tested microorganism (200 µl of 10^7-10^8 cells per ml) was spread over the surface of solid Muller Hinton agar plate with a bent glass rod. Paper disc (6mm in diameter) were impregnated with 10µl of each concentrations of each food additive 1, 5, 10, 20, 40, 80, 160, 320, 500, 750, 1000, 1250, 1500, 1750 and 2000 placed on the inoculated plates. These plates were incubated at 37^{o}C for 24h.The diameter of the inhibition zones were measured in millimeters. All the tests were performed in duplicate and repeated twice. (Hayouni *et al.,* 2008)

Minimum bactericidal concentration (MBC)

The MIC of an antibacterial agent for a given organism is the lowest concentration of the agent required to inhibit the growth of an inoculum of the bacterium in a standard test. The MBC is the minimal concentration of antibiotic that kills the inoculum. MICs were determined as the lowest concentrations of food additive at which microorganisms cannot grow in (M–H) broth (Lab M, UK); the strains were inoculated into M–H broth and incubated to the logarithmic growth phase at 37^{o}C. The growth density was adjusted to match a MacFarland 0.5 standard (10^8 CFU/ml) .A 1:100 dilution was prepared in a fresh M–H broth, and used as the inoculum (10^6 CFU/ml). The following concentrations of food additives in mg/ml, 0.1, 0.5, 1, 2, 4, 8, 16, 32, 50, 75, 100, 125, 150, 175 and 200 were prepared. The previous concentrations of food additives were tested, microbial growth was monitored via turbidity by 24 h of incubation at 37^{o}C. MBC was determined by inoculation of 100

Table 3. Effect of selected food additives on inhibition of *E. coli*. Where inhibition zone (mm) - (no effect), +(≤9), ++ (< 15), +++ (15-20mm), ++++ (≥20 mm)

Food additives	Concentrations of food additive (mg/ml)														
	0.1	0.5	1	2	4	8	16	32	50	75	100	125	150	175	200
Ascorbic acid•	-	-	-	-	-	-	-	+	+	+	++	++	++	++	++
Nisin•	-	+	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Ploxine B•	-	-	-	+	++	++	+++	+++	+++	+++	+++	+++	+++	++++	++++
Riboflavine*	-	-	-	-	-	-	-	-	-	-	++	++	++	+++	++++
Sodium benzoate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sodium monoglutamate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium nitrite•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbic acid*	-	-	-	-	+	+	+	+	+	+	+++	+++	+++	+++	+++
Tartrazine•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* = dissolved in Dimethyl formamid.

• = dissolved in distelled water.

Table 4. Effect of selected food additives on inhibition of *Salmonella indica*. Where inhibition zone (mm) - (no effect), +(≤9), ++ (< 15), +++ (15-20mm), ++++ (≥20 mm).

Food additives	Concentrations of food additive (mg/ml)														
	0.1	0.5	1	2	4	8	16	32	50	75	100	125	150	175	200
Ascorbic acid•	-	-	-	-	-	-	-	+	+	+	++	++	++	++	++
Nisin•	-	-	-	-	-	-	+	+	+	+	++	++	++	++	++
Ploxine B•	-	-	+	+	+	+	+++	+++	+++	+++	++++	++++	++++	++++	++++
Riboflavine*	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Sodium benzoate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sodium monoglutamate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium nitrite•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbic acid*	-	-	-	-	-	-	-	-	-	-	++	++	++	++	++
Tartrazine•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* = dissolved in Dimethyl formamid.

• = dissolved in distelled water.

µl-aliquots of negative tubes (absence of turbidity in the MIC determination) on M–H agar, using the spread plate technique (Fernandes *et al.,* 2008).

Time Kill Curve

The method of May *et al.,* (2000) was modified and performed. Ten isolates of each most potent proteolytic activity bacteria were inoculated separately into 10 flasks, one as a growth control and one for each food additive; ascorbic acid, monosodium glutamate, nicin, phloxine B, riboflavin, sodium benzoate, sodium nitrite, sorbic acid and tartrazine. Concentrations of 2MBC of each food additive were added to flasks. All flasks were shaken at 150rpm at 37°C. The modification

Table 5. Effect of selected food additives on inhibition of *Staphylococcus aureus*. Where inhibition zone (mm) - (no effect), +(d” 9), ++ (< 15), +++ (15-20mm), ++++ (≥20 mm).

Food additives	Concentrations of food additive (mg/ml)														
	0.1	0.5	1	2	4	8	16	32	50	75	100	125	150	175	200
Ascorbic acid•	-	-	-	-	-	-	-	+	+	+	++	++	++	++	++
Nisin•	-	-	-	-	-	-	-	++	++	++	++	++	++	++	++
Ploxine B•	-	+	++	++	++	++	+++	+++	+++	++++	++++	++++	++++	++++	++++
Riboflavine*	-	-	-	-	-	-	-	-	-	-	++	+++	+++	+++	++++
Sodium benzoate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sodium monoglutamate•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sodium nitrite•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sorbic acid*	-	-	-	+	+	++	++	++	++	++	+++	+++	+++	+++	+++
Tartrazine•	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* = dissolved in Dimethyl formamid.
• = dissolved in distilled water.

was in time of subculture which was at 0, 2, 4, 8, 12, 24 and 48h of each sample. Then sub cultured isolates incubated at 37 ^{o} C for 24h for all isolates. Time kill curves were determined by counting viable counts to give cfu/ml, and kill curves were plotted with time against the logarithm of the viable count.

RESULTS

Antibiotic susceptibility test of food additives

Tables 2, 3, 4 and 5 show the inhibition effect (Antibacterial effect) of studied (selected) food additives on the 4 most potent proteolytic bacterial species (*Bacillus cereus, E.coli, Salmonella indica* and *Staphylococcus aureus*). Four patterns of inhibition effect were observing according to the diameter of inhibition zones in plates provided with the solution of different concentrations of each additive. This 4 patterns were no effect (-) ≥9mm, slow effect (+ &++) (10 - 14mm), intermediate effect (+++) (15-19mm) and high effect (++++) (≥20). Concentration of 16 mg/ml of both ascorbic acid & nisine had no effect on *E.coli* and *Staph aureus*, while this concentration of ascorbic acid was affected on *Bacillus cereus* and not affected on *Salmonella indica*. Vice versa this concentration of nisine had an effect on *Salmonella indica* and did not have an effect on *Bacillus cereus.* The smallest concentrations of ascorbic acid and nisin (8 mg/ml and lower) were not inhibited the growth of all studied bacterial species. The concentrations more than the 32 mg/ml up to 200 mg/ml of ascorbic acid and nisin were affected on *E.coli*, *Salmonella indica* and *Staph aureus*. The concentrations 175 mg/ml and lower of sodium benzoate, sodium monoglutamate, sodium nitrite and tartrazine did not affect on the growth of all 4 studied bacterial species. The concentration of 200 mg/ml of sodium benzoate affected the 4 studied bacteria species.

The concentrations of 75 mg/ml and lower of riboflavine did not affect on all studied bacterial species. The inhibitory effect of riboflavine (10-14mm) was observed at 100 mg/ml and more in

Table 6. The minimum inhibitor concentration (MIC) and minimum bactericidal concentration (MBC) (µg) of food additives against studied bacterial species.

Food additives		Bacterial species			
		Gram positive		Gram negative	
		B. c	*S.a*	*S.i*	*E.c*
Ascorbic acid	MIC	8.0	16	16	16
	MBC	16	32	32	32
Nisin	MIC	16	16	8	16
	MBC	32	32	16	32
Phloxin B	MIC	1.0	0.1	0.5	0.1
	MBC	2.0	0.5	1.0	0.5
Riboflavine	MIC	75	75	75	75
	MBC	100	100	100	100
Sodium benzoate	MIC	175	175	175	175
	MBC	200	200	200	200
Sodium monoglutamate	MIC	0.0	0.0	0.0	0.0
	MBC	0.0	0.0	0.0	0.0
Sodium nitrite	MIC	0.0	0.0	0.0	0.0
	MBC	0.0	0.0	0.0	0.0
Sorbic acid	MIC	1.0	1.0	75	1.0
	MBC	2.0	2.0	100	2.0
Tartrazine	MIC	0.0	0.0	0.0	0.0
	MBC	0.0	0.0	0.0	0.0

Where *B.c.* is *Bacillus cereus, S.a (Staphylococcus aureus), S.i (Salmonella indica), E.c (E.coli).*

Bacillus cereus and *Salmonella indica*. While the high inhibitory effect 20mm and more were recorded in *E.coli* and *Staph aureus* after the same treatment with the same concentrations of riboflavine. It is important to notice that the concentrations of 16 mg/ml and more of phloxine B (16 up to 200 mg/ml) had a high inhibitory effect against the 4 studied bacterial species. The inhibitory zones were not recorded after the treatment with small concentrations of phloxine B (0.1, 0.5 & 1.0 mg/ml). The intermediate inhibitory effect (++,10-14mm) of sorbic acid was observed in *Salmonella indica* at the concentrations more than 100 mg/ml up to 200 mg/ml, and in *Bacillus cereus* and *Staph aureus* at the concentrations lower than 100 mg/ml up to 8 mg/ml of sorbic acid. While the concentrations lower than 100 mg/ml of sorbic acid did not affect *Salmonella indica.* Also no inhibitory effect was observed at the lower concentrations 0.1, 0.5 and 1 mg/ml in *Bacillus cereus, E.coli* and *Staph aureus*. The high inhibitor effects (+++, 15-19mm) of the concentration more than 100 mg/ml of sorbic acid were recorded in *Bacillus cereus, E.coli* and *Staph. aureus*.

Determination of minimum inhibitory concentration (MIC) & minimum bactericidal concentration (MBC) of food additives.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for food additives which showing the inhibition zones and no inhibition zone. The results in table (6) showed that all studied bacterial species more resistant to sodium nitrite, tartarazine and sodium monoglutamate.This means that these additives had no effect as food preservatives in this study. Nisin and ascorbic acid had a similar intermediate effect on all studied bacterial species. MIC of phloxine-B was 1.0, 0.5, 0.1 mg/ml which effect on *Bacillus cereus*, *Salmonella indica* and both *E.coli* and *Staphylococcus aureus* respectively. phloxine-B was the most effective food additive toward the all studied bacterial species followed by sorbic acid. This means that these two substances were the

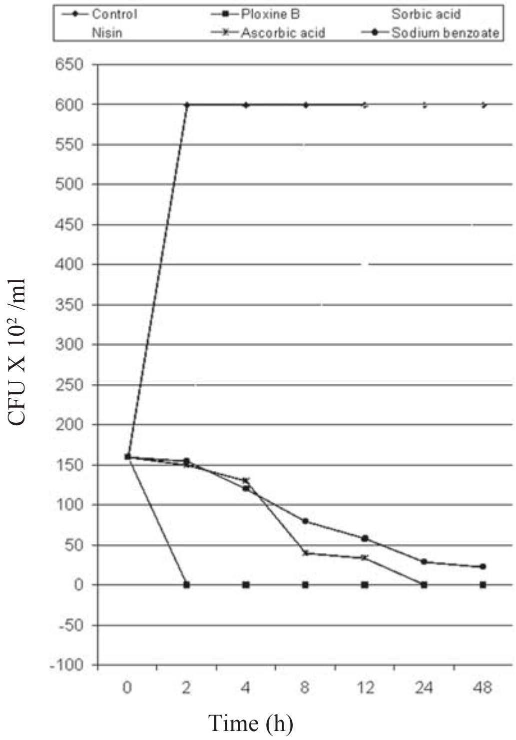

Fig. 1. Time Kill Curves of *Bacillus cereus* by selected food additives.

most effective additives on studied bacterial species in this study. The results in table 6 showed that the MBCs were larger than MICs for all studied bacterial species.

Determination of time kill curves of most effective food additives against the studied bacterial species.

Time kill curves of most effective food additives toward most potent proteolytic bacterial species (*Bacillus cereus, E.coli, Salmonella indica* and *Staphylococcus aureus*) were determined in Figures 1, 2, 3 and 4. Fig. 1 showed that the time-kill curve of most effective food additive against *Bacillus cereus*. Phloxine-B, had acompletely killing effect (100% death rate) to ward *Bacillus cereus* after 2 hour and still constant at 4, 8, 12, 24 & 48 hours. The starting killing time of ascorbic acid and sodium benzoate (6.25% death rate) was 2 hour toward *Bacillus cereus*. Time of completely kill (100 % death rate) of ascorbic acid was 24 hours. It is important to notice the increasing of death rates of *Bacillus cereus* were 21.8%, 50%, 59.3%, 83.1% and 84.1% after 4, 8, 12, 24 and 48 hours respectively. Nisin and sorbic acid had no killing effect against *Bacillus cereus*. The growth rate was 275% and 193.7% respectively, these results were recorded at 12 hours and 48 hours respectively. Fig 2. showsed that the time kill curves of most effective food additives. It is important to notice that two observation were observed. The first; time kill curve (completely killing 100% death rate) of sorbic acid, nisin,ascorbic acid and sodium benzoate) was 2 hours toward E. coli. The second observation was nokilling for phloxin-B. After 2 hours a good growth was observed. The growth rate was 375% after 2 hours. The similar growth rate 375% was observed in control culture. Fig. 3 showed the time-kill curves of selected food additives against *Salmonella indica*. The time-kill curves (100% death rate) of ascorbic acid, sorbic acid and nisin toward *Salmonella indica* was 2, 8, and 12 hours respectively. The death rate of *Salmonella indica* was 6.25%, 9.0%, 43.75%, 50%, 68.7% and 81% after 4, 8, 12, 24 and 48 hours. Phloxin-B has no killing effect toward *Salmonella indica*. Fig. 4

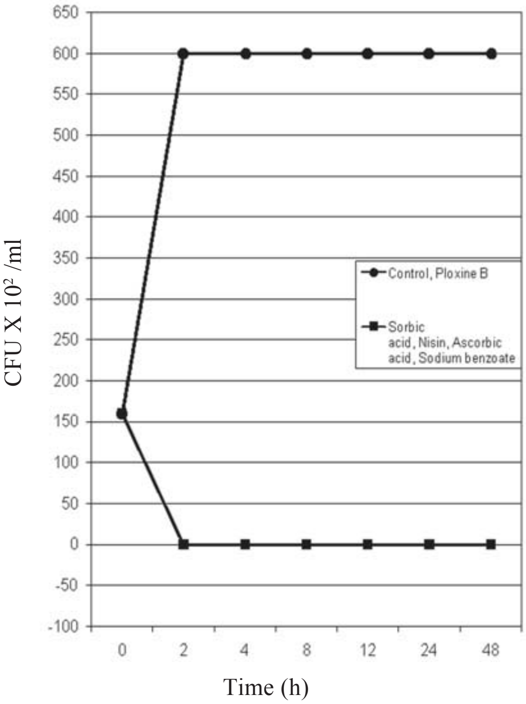

Fig. 2. Time Kill Curves of *Escherichia coli*

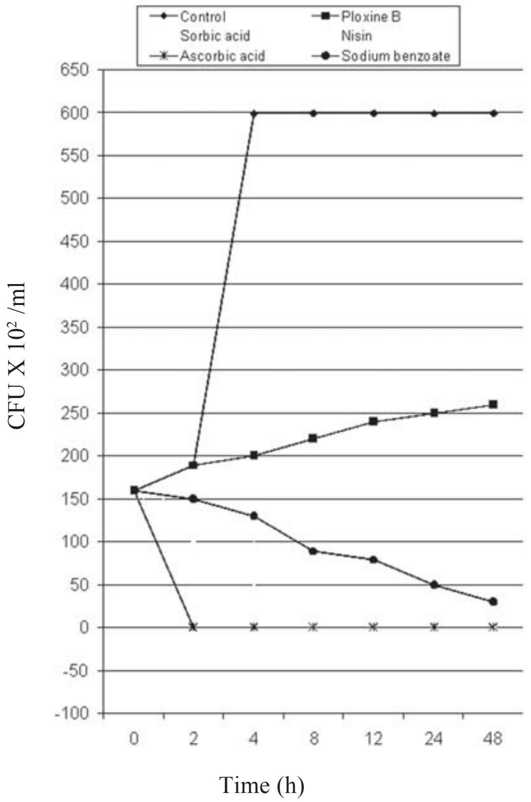

Fig. 3. Time Kill Curves of *Salmonella indica* by selected food additives.

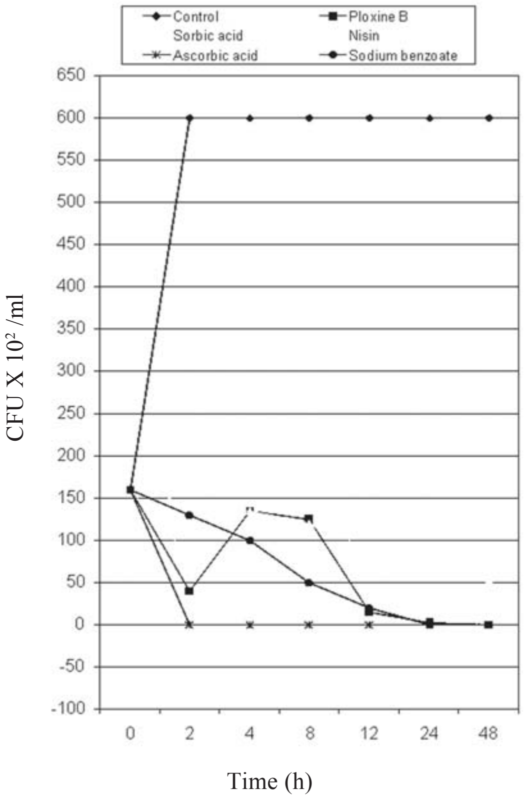

Fig. 4. Time Kill Curves of *Staphylococcus aureus* by selected food additives

shows the time-kill curves of selected food additives against *Staphylococcus aureus*. The time-kill curves (100% death rate) of ascorbic acid, nisin, phloxin-B and sodium benzoate at 2, 4 and 24 hours respectively. It is important to notice that after 2 hours the death rates of *Staphylococcus aureus* were 12.5%, 18.7%, 53%, 75% and 100% after treatment by sorbic acid, sodium benzoate, nisin, phloxin-B and ascorbic acid.

DISCUSSION

The susceptibility of antibiotic on the studied bacterial species consider as a database for comparison between the known effect of antibiotic and the unknown effect of food additive. Food additives may play role in limiting or preventing microbial proliferation. Obviously, the number of chemical compounds permitted to be used as food and pharmaceutical preservatives is limited (Branen 1983 and Hugo and Russel, 1991). The growth in the use of food additives has increased enormously in the past 30 years, totally now over 200,000 tons per year.

Therefore it has been estimated that today about 75% of the western diet is made up of various processed foods, each person is now consuming an average 8-10 lbs of additives per year with same possibily eating considerably more (Miller 1985 and ACARD, 1982). Chemical additives have generally be used to combats specific microorganisms and also can enhance or contribute to the flavour of acidified or fermented food. The susceptibility of antibiotic against the studied bacterial species was clear as follow; *Bacillus cereus* was resistant to ampicillin and azeteronan while *Bacillus cereus, Staph aureus* and *Salmonella indica* were resistant to azternan only. Both *Salmonella indica* and *E.coli* were resistant to vancomycin. The same susceptibility effect was recorded in case phloxine B, nisine, ascorbic acid and sorbic acid, therefore this 4 food additives were used as food preservatives. All studied bacterial species were susceptible to small concentrations of the all tested food additives.

Phloxin B was the most effective additive toward the all studied bacterial species; this means that this substance was available using as food preservative. This result was agreement with Rasooly, 2005 and Federal Reigister, (1982) they showed that phloxin B has antibacterial effect against gram positive and gram negative bacteria. The bacterial growth was stopped completely after the addition of 50-100ug/ml from phloxin B to the growth media (Rasooly and Weisz, 2002). Nisin shows high antibacterial activity against on growth of gram positive followed by gram negative bacteria. Nisin can help control of spoilage bacteria in dairy products, fish, juice and vegetables; this result was agreement with results. Organic acid can help control bacteria, molds and yeast in bakery products, meat, juices and other food. In general organic acids are abroad spectrum in using in food production, processing and industry.

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