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Research Paper

A COMPARATIVE STUDY FOR THE DETERMINATION OF EFFICACY OF COMMONLY USED ANTIMICROBIALS AGAINST SPECIFIC BACTERIAL STRAINS IN TOMATO (*Solanum lycopersicum* L.) JUICE

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Abstract

A large number of bacterial strains are able to spoil a variety of juices like tomato juice and other food products. Currently, approximately 30 different chemicals can legally be used as antimicrobials in food products. The purpose of this research work was to compare the efficacy of commonly used antimicrobial agents like sodium benzoate, potassium sorbate, sodium metabisulphite, benzoic acid and sorbic acid added to tomato juice against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysenteriae, Sarcina lutea, Baciius subtilis, Bacillus cereus, Bacillus megaterium, Shigella sonnei*. To determine the efficacy, different concentrations of antimicrobial solutions of 0.05%, 0.08%, 0.10%, 0.20% and 0.30% in distilled water were prepared. The efficacy of antimicrobials was studied by agar diffusion technique and Minimum Inhibitory Concentration (MIC) method. Based on the maximum number of bacterial population reduction after antimicrobial treatments, these studies suggest that benzoic acid (0.30%) is the most effective antimicrobial agent against *Staphylococcus aureus, Shigella sonnei, Shigella dysenteriae, Sarcina lutea, Salmonella typhi, Baciilus subtilis, Bacillus cereus, Bacillus megaterium* and *Pseudomonas aeruginosa*. Sorbic acid (0.20% & 0.30%) is effective antimicrobials against *Shigella sonnei, Shigella dysenteriae, Sarcina lutea* and *Bacillus cereus*. Potassium sorbate (0.20% & 0.30%) is effective against *Shigella sonnei* and *Sarcina lutea*. Sodium metabisulphite (0.20% & 0.30%) is effective against 10 tested bacterial strains. From this study it is also found that the maximum efficacy of sodium benzoate (0.08%, 0.10%, 0.20% and 0.30%) is against *Baciilus subtilis, Bacillus cereus* and *Bacillus megaterium*.

Key words: *Antimicrobials, Efficacy, Bacterial strains, Tomato juice*.

INTRODUCTION

Food is defined as any chemical substance which when eaten, digested and absorbed by the body, produces energy, promotes growth and repairs the body tissues and regulates these processes [1]. Food is not only important for human consumption but also a good source of microbial growth. These microorganisms can cause unpleasant and sensory changes by different chemical reactions. These result food spoilage and food borne infection and intoxification. The following bacteria such as *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysenteriae, Sarcina lutea, Baciius subtilis, Bacillus cereus, Bacillus megaterium, Shigella sonnei* are normally implicated in food borne diseases. A variety of antimicrobials are used directly to the food products to inactivate or inhibit the growth of such pathogenic and spoilage bacteria. They are added to foods to preserve them from deterioration and extend their shelf life [2]. But due to the improper selection of such antimicrobials, pathogenic bacteria are not inactivated completely by them. This results food spoilage by altering the nutritional value and sensory characteristics like taste, flavor, color appearance of food products. On the other hand excessive use of these agents is hazardous for human health. Different kinds of preservatives are used as antimicrobial agents of food products. In the food industry, sodium benzoate, potassium sorbate, sodium benzoate and sodium nitrite are often used as preservatives. Sodium benzoate is a preservative that is widely used in food industry [3]. Benzoic acid is used in acidic food and beverage like fruit juice, sparkling drinks, soft drinks, pickles or other acidified food. European Commission limits for benzoic acid and sodium benzoate are 0.015-0.5% [4]. Potassium sorbate is used to conserve cheeses, cakes and syrups. The effective concentration is 0.2% [5]. Sorbic acid falls into a group of short-chain organic acids, which, together with their salts, have been shown to exhibit antimicrobial properties. Its salts, such as sodium sorbate, potassium sorbate and calcium sorbate are antimicrobial agents, often used as preservatives in food and drinks to prevent the growth of mold, yeast and fungi. Sorbates are generally used at concentrations of 0.025% to 0.10%.

In our study, we prepared tomato juice and added different concentrations of five antimicrobial agents-sodium benzoate, potassium sorbate, sodium metabisulphite, benzoic acid and sorbic acid. The growth of some selected bacteria named- *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Shigella dysenteriae, Sarcina lutea, Bacillus subtilis, Bacillus cereus, Bacillus megaterium, Shigella sonnei* was observed against the preservative added tomato juices.

The present study is focused to determine the most effective antimicrobials or preservatives and the optimum level of these agents to prevent spoilage and outgrowth of bacterial strains in tomato juices.

MATERIALS AND METHODS

Antimicrobials preparation

The antimicrobial agents used for this study to compare their efficacy were benzoic acid (BDH, England), sodium benzoate (BDH, England), sorbic acid (BDH, England), potassium sorbate (BDH, England) and sodium metabisulfite (BDH, England).These antimicrobials were dissolved at a concentration of 0.05% in distilled water to prepare antimicrobial solution. For all, five different concentrations of the antimicrobials including 0.05%, 0.08%, 0.10%, 0.20% and 0.30% were prepared for the microbiological assays.

Microbial Strains

The efficacy tests of antimicrobials were carried out against ten bacterial strains. Among them five were Gram-positive and the rest were Gram-negative. Gram-positive bacteria are *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium* and Gram-negative bacteria are *Pseudomonas aeruginosa, Escherichia coli, Shigella sonnei, Shigella dysenteriae, Salmonella typhi.* All of the bacterial strains were collected from Food Microbiology Laboratory, Institute of Food Science and Technology, Bangladesh Council of Industrial and Scientific Research, Dhaka, Bangladesh.

Streaking and measurement of bacterial growth

The medium of choice for this study is the Mueller-Hinton agar due to its pH of 7.2 to 7.4. The sterile Mueller Hinton Agar (MHA) was mixed thoroughly with antimicrobials of different tested concentrations (0.05%, 0.08%, 0.10%, 0.20%, and 0.30%).The streaking of a single colony of each bacterial and fungal strain was done carefully in this prepared plate by inoculating loop. The plate was then incubated at 37°c for 24 hours in an incubator and growths were observed. The results were reported as positive (+) if there is inhibition of growth and negative (-) if there is no inhibition of growth.

Tomato juice preparation

Ripened fresh tomatoes were obtained from kallayanpur vegetable market, Dhaka. The collected fresh tomatoes were washed in cold water to remove any external debris and carefully remove the stems with a sterile sharp knife. The washed tomatoes were carefully immersed in boil water for five minutes to keep it microorganisms free. Cut each tomato into quarters, and remove any green, white or mushy spots. The quarters of this sample were blended with a blender which is washed with boil water. After the blending was completed the juice blends were filtered with a sterile muslin cloth to get clear juice. The juice were then stored in a sterile air tight container and preserved under frozen condition for regular use.

Inoculums preparation

A single colony of one bacterial strain grown on Plate Count Agar was inoculated into 20ml of tomato juice in screw capped tube and thoroughly mixed and held it for ten minutes.

Antimicrobials treatment

The amount of antimicrobials such as 0.016gm, 0.02gm, 0.04gm were added with one ml of distilled water in screw capped tubes, shaken vigorously to dissolve antimicrobials and sterilized at the pressure of 15 lbs (121°c) for 15 minutes. The antimicrobial solutions were added to 19ml tomato juice in which bacteria was inoculated in order to obtain the final concentration of 0.08%, 0.10%, and 0.20% in 20ml juice and held it for 10 minutes.

Microbiological examination

A volume of 1ml from 20ml of inoculums was poured into the sterile Petri dishes. A sterile Plate Count Agar medium was added to these plates and incubated at 37°c for 24 hours. The total bacterial populations were recorded and compared with controls to determine the effectiveness of each of the antimicrobials treatment.

Determination of Minimum Inhibitory Concentrations (MIC)

In this study, MIC was determined using "Serial tube dilution technique". In this technique the tubes of broth medium, containing graded doses of antimicrobials are inoculated with the test organisms. After suitable incubation, growth will occur in those tubes where the concentration of antimicrobial is below the inhibitory level and the culture will become turbid (cloudy). Therefore, growth will not occur above the inhibitory level and the tube will remain clear.

RESULTS

Table 1 shows the inhibition of bacterial growth at different concentrations of antimicrobials. Sodium benzoate at the concentration of 0.05%, 0.08%, 0.10%.0.20% and 0.30% did not show any inhibitory effect against all of the tested bacteria. Sodium metabisulphite at the concentration of 0.20% & 0.30% inhibits the growth of all tested bacteria. The growth of *Baciilus subtilis, Bacillus cereus* and *Bacillus megaterium* were inhibited by benzoic acid at the concentration of 0.08%, 0.10%.0.20% and 0.30%. Sorbic acid at the concentration of 0.20% & 0.30% inhibits the growth of *Shigella sonnei, Shigella dysenteriae, Sarcina lutea* and *Bacillus cereus*.

Efficacy of antimicrobial treatments for the control of bacterial strain in tomato juice

Efficacy of benzoic acid for the control of bacterial strain in tomato juice

Figure-1 shows the level of bacterial colony forming unit (cfu) after the addition of benzoic acid at different concentrations. It is found that at control all the test bacterial strains are at very high level (50 or above cfu/ml). At 0.08%, *Bacillus cereus, Bacillus megaterium* are at moderate level (20 to 39 cfu/ml), *Shigella dysenteriae, Shigella sonnei, Staphylococcus aureus* are at high level (40 to 49 cfu/ml), *Bacillus subtilis, Escherichia coli, Salmonella typhi, Sarcina lutea, Pseudomonas aeruginosa* are at very high level (50 or above cfu/ml). At 0.10%, *Bacillus cereus, Bacillus megaterium, Staphylococcus aureus* are at moderate level (20 to 39 cfu/ml), *Bacillus subtilis, Shigella dysenteriae, Shigella sonnei, Pseudomonas aeruginosa* are at high level (40 to 49 cfu/ml), *Escherichia coli, Salmonella typhi, Sarcina lutea* are at very high level. At 0.20%, *Salmonella typhi, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa* are at very low level (0 to 9 cfu/ml), *Escherichia coli, Bacillus megaterium, Shigella sonnei, Sarcina lutea* are at low level (10 to 19 cfu/ml), *Bacillus subtilis, Shigella dysenteriae* are at moderate level (20 to 39 cfu/ml).

Efficacy of sodium benzoate for the control of bacterial strain in tomato juice

The level of bacterial colony forming unit after the addition of benzoic acid at different concentrations is shown in Figure-2. At control all the test bacterial strains are at very high level (50 or above cfu/ml). At 0.08%, *Bacillus cereus, Bacillus megaterium* are at high level (40 to 49 cfu/ml); *Bacillus subtilis, Escherichia coli, Salmonella typhi, Sarcina lutea, Shigella sonnei, Staphylococcus aureus, Pseudomonas aeruginosa* are at very high level (50 to above cfu/ml). At 0.10% , *Bacillus cereus, Bacillus megaterium, Pseudomonas aeruginosa* were at moderate level (20 to 39 cfu/ml), *Salmonella typhi* is at high level (40 to 49 cfu/ml), *Bacillus subtilis, Escherichia coli, Sarcina lutea, Shigella dysenteriae, Shigella sonnei, Staphylococcus aureus* are at very high level (50 or above cfu/ml). At 0.20%, *Shigella sonnei, Salmonella typhi, Pseudomonas aeruginosa* are at very low level (0 to 9 cfu/ml), *Bacillus cereus, Bacillus megaterium* are at moderate level (20 to 39 cfu/ml), *Bacillus subtilis, Staphylococcus aureus* are at high level (40 to 49 cfu/ml), *Escherichia coli, Sarcina lutea, Shigella dysenteriae* are at very high level (50 or above cfu/ml).

Efficacy of sorbic acid for the control of bacterial strain in tomato juice

Figure 3 is showing the level of bacterial colony forming unit after the addition of sorbic acid at different concentrations. It is observed that at control all the test bacterial strain are at very high level (50 or above cfu/ml). At 0.08%, *Bacillus megaterium, Salmonella typhi, Sarcina lutea, Shigella sonnei* are at moderate level (20 to 39 cfu/ml), *Bacillus cereus, Pseudomonas aeruginosa* are at high level (40 to 49 cfu/ml), *Bacillus subtilis, Escherichia coli, Shigella dysenteriae, Staphylococcus aureus* are at very high level (50 or above cfu/ml). At 0.10%, *Bacillus megaterium* is at low level(10 to 19 cfu/ml), *Salmonella typhi, Sarcina lutea, Shigella sonnei, Bacillus cereus, Pseudomonas aeruginosa* are at moderate level (20 to 39 cfu/ml), *Bacillus subtilis, Escherichia coli, Shigella dysenteriae* are at high level (40 to 49 cfu/ml), *Staphylococcus aureus* is at very high level (50 to above cfu/ml). At 0.20%, *Bacillus megaterium, Salmonella typhi, Sarcina lutea* are at low level (10 to 19 cfu/ml), *Bacillus cereus, Escherichia coli, Shigella sonnei, Pseudomonas aeruginosa* are at moderate level (20 to 39 cfu/ml), *Bacillus subtilis, Shigella dysenteriae, Staphylococcus aureus* are at very high level(50 to above cfu/ml).

Efficacy of potassium sorbate for the control of bacterial strain in tomato juice

The level of bacterial colony forming unit after the addition of benzoic acid at different concentrations is shown in Figure-4. It is observed that at control all the test bacterial strain are at very high level (50 or above cfu/ml). At 0.08%, *Bacillus megaterium* is at moderate level (20 to 39 cfu/ml), *Bacillus cereus* is at high level (40 to 49 cfu/ml), *Bacillus subtilis, Escherichia coli, Salmonella typhi, Sarcina lutea, Shigella dysenteriae, Shigella sonnei, Staphylococcus aureus, Pseudomonas aeruginosa* are at very high level (50 to above cfu/ml). At 0.10%, *Bacillus megaterium, Pseudomonas aeruginosa* are at moderate level (20 to 39 cfu/ml), *Bacillus subtilis, Shigella sonnei, Bacillus cereus* are at high level (40 to 49 cfu/ml), *Escherichia coli, Salmonella typhi, Sarcina lutea, Shigella dysenteriae, Staphylococcus aureus* are at very high level (50 to above cfu/ml). At 0.20%, *Pseudomonas aeruginosa* is at low level (10 to 19 cfu/ml), *Bacillus cereus, Bacillus megaterium, Shigella sonnei* are at moderate level (20 to 39 cfu/ml), *Bacillus subtilis, Staphylococcus aureus* are at high level (40 to 49 cfu/ml), *Escherichia coli, Shigella dysenteriae, Sarcina lutea, Salmonella typhi* are at very high level (50 or above cfu/ml).

Efficacy of sodium metabisulfite for the control of bacterial strain in tomato juice

Figure-5 shows the level of bacterial colony forming unit after the addition of benzoic acid at different concentrations. It is found that at control all the test bacterial strain are at very high level (50 or above cfu/ml). At 0.08%, *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megaterium*, *Shigella dysenteriae*, *Shigella sonnei* are at moderate level (20 to 39 cfu/ml), *Pseudomonas aeruginosa* is at high level (40 to 49 cfu/ml), *Escherichia coli*, *Salmonella typhi*, *Sarcina lutea*, *Staphylococcus aureus* are at very high level (50 to above cfu/ml). At 0.10%, *Bacillus cereus*, *Bacillus megaterium* are at low level (10 to 19 cfu/ml), *Bacillus subtilis*, *Shigella dysenteriae*, *Shigella sonnei* are at moderate level (20 to 39 cfu/ml), *Staphylococcus aureus*, *Pseudomonas* *aeruginosa* are at high level (40 to 49 cfu/ml), *Escherichia coli*, *Salmonella typhi*, *Sarcina lutea* are at very high level (50 to above cfu/ml). At 0.20%, *Shigella sonnei*, *Bacillus megaterium* are at very low level (0 to 9 cfu/ml), *Bacillus subtilis*, *Bacillus cereus* are at low level (10 to 19 cfu/ml), *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* are at moderate level (20 to 39 cfu/ml), *Escherichia coli* is at high level (40 to 49 cfu/ml), *Sarcina lutea* is at very high level (50 or above cfu/ml).

Minimum Inhibitory Concentrations (MIC) of antimicrobials

In Table 2, strong inhibition of antimicrobials against all the tested bacteria were noticed. Antimicrobials were noticed to be vulnerable to all tested bacteria and their MIC values were ranged from 0.0125% to 0.50% (benzoic acid, sorbic acid), 0.125% (sodium metabisulphite), 0.50% (sodium benzoate), 0.125% 10 1.0% (potassium sorbate).

Table (1): Effect of antimicrobials on the growth of tested bacterial strains

	Escherichia coli	*Staphylococcus aureus*	*Shigella sonnei*	*Shigella dysenteriae*	*Sarcina lutea*	*Salmonella typhi*	*Bacillus subtilis*	*Bacillus cereus*	*Bacillus megaterium*	*Pseudomonas aeruginosa*
Benzoic Acid										
0.05%	-	-	-	-	-	-	-	-	-	-
0.08%	-	-	-	-	-	-	+	+	+	-
0.10%	-	-	-	-	-	-	+	+	+	-
0.20%	-	-	+	-	-	-	+	+	+	+
0.30%	-	+	+	+	+	+	+	+	+	+
Sodium Benzoate										
0.05%	-	-	-	-	-	-	-	-	-	-
0.08%	-	-	-	-	-	-	-	-	-	-
0.10%	-	-	-	-	-	-	-	-	-	-
0.20%	-	-	-	-	-	-	-	-	-	-
0.30%	-	-	-	-	-	-	-	-	-	-
Sorbic acid										
0.05%	-	-	-	-	-	-	-	-	-	-
0.08%	-	-	-	-	-	-	-	-	-	-
0.10%	-	-	-	-	-	-	-	-	-	-
0.20%	-	-	+	+	+	-	-	+	-	-
0.30%	-	-	+	+	+	-	-	+	-	-
Potassium sorbate										
0.05%	-	-	-	-	-	-	-	-	-	-
0.08%	-	-	-	-	-	-	-	-	-	-
0.10%	-	-	-	-	-	-	-	-	-	-
0.20%	-	-	+	-	+	-	-	-	-	-
0.30%	-	-	+	-	+	-	-	-	-	-
Sodium metabisulphite										
0.05%	-	-	-	-	-	-	-	-	-	-
0.08%	-	-	-	-	-	-	-	-	-	-
0.10%	-	-	-	-	-	-	-	-	-	-
0.20%	+	+	+	+	+	+	+	+	+	+
0.30%	+	+	+	+	+	+	+	+	+	+

+ = Inhibition of bacteria, - = No inhibition of bacteria

Table (2): Minimum Inhibitory Concentrations (MIC) of antimicrobials

Bacteria	Benzoic acid (%)	Sorbic acid (%)	Sodium metabisulphite (%)	Sodium benzoate (%)	Potassium sorbate (%)
B. cereus	0.125	0.125	0.125	0.50	1.0
B. subtilis	0.125	0.50	0.125	0.50	1.0
B. megaterium	0.125	0.50	0.125	0.50	1.0
E. coli	0.50	0.50	0.125	0.50	0.50
S. aureus	0.25	0.50	0.125	0.50	1.0
S. sonnei	0.125	0.125	0.125	0.50	0.125
S. dysenteriae	0.25	0.125	0.125	0.50	1.0
S. typhi	0.50	0.50	0.125	0.50	0.50
S. lutea	0.25	0.125	0.125	0.50	0.125
P. aeruginosa	0.125	0.50	0.125	0.50	1.0

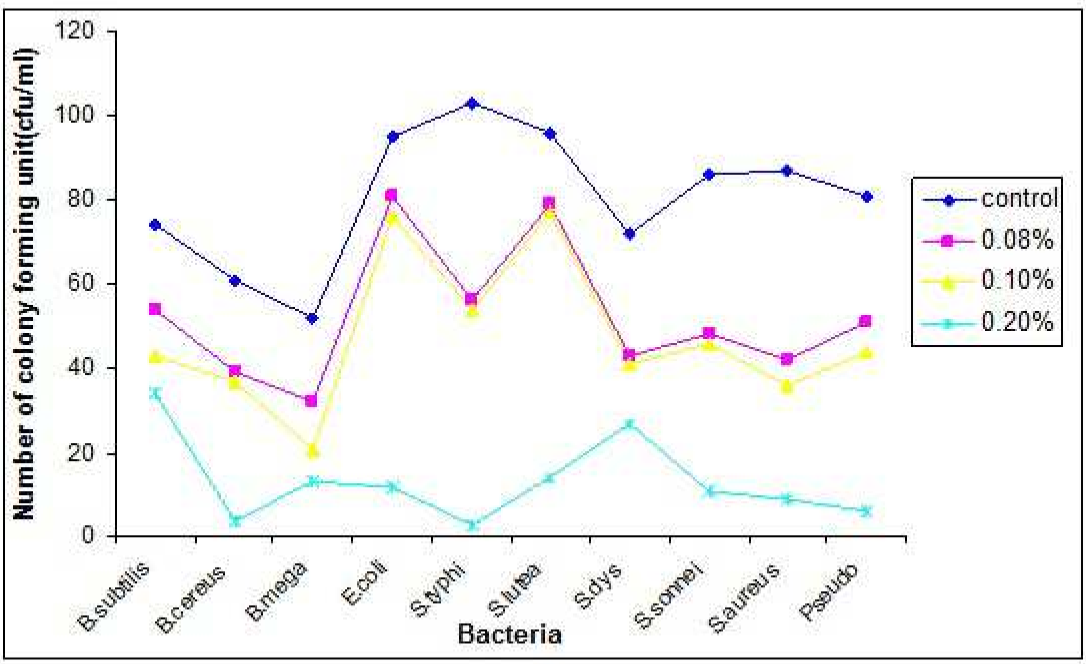

Figure: 1 Reduction of bacterial population after the addition of Benzoic acid

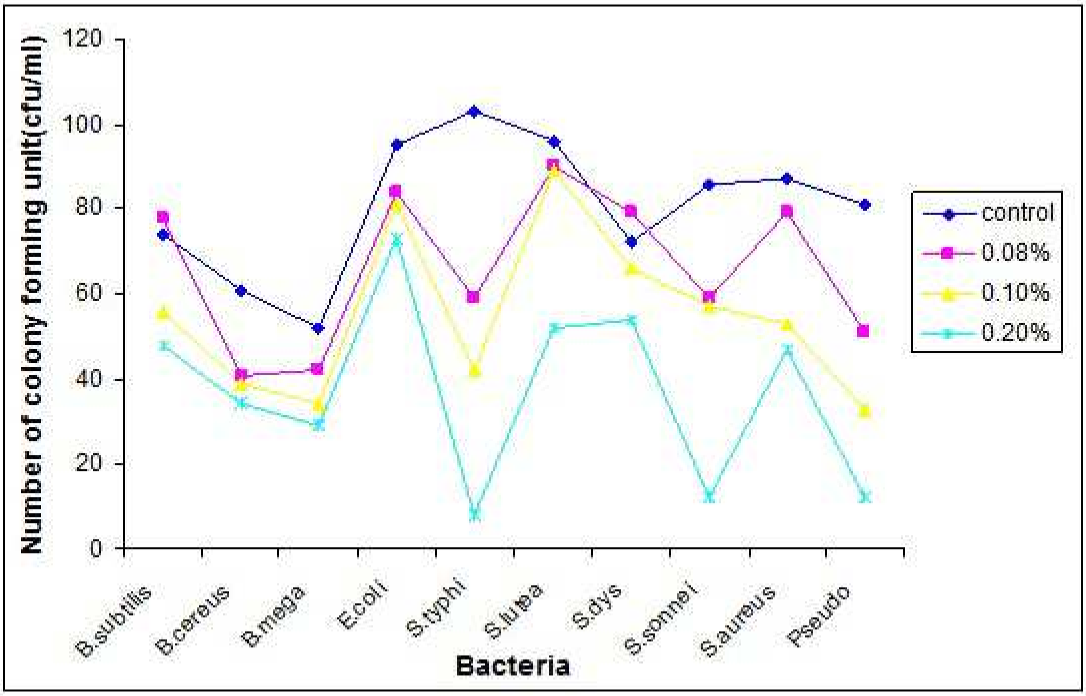

Figure: 2 Reduction of bacterial population after the addition of sodium benzoate

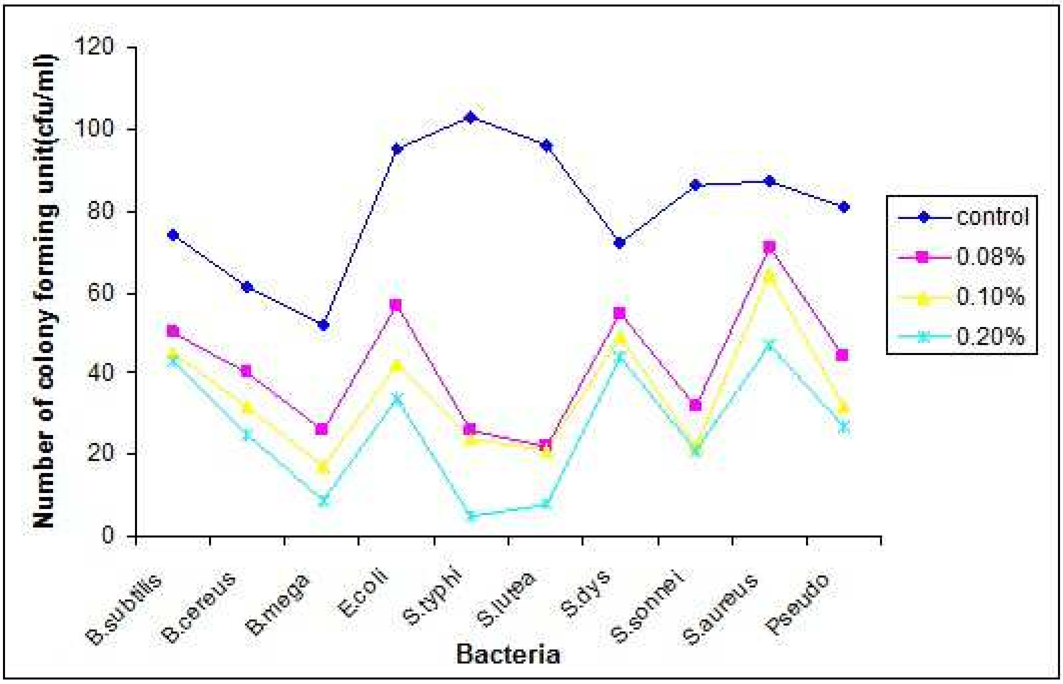

Figure: 3 Reduction of bacterial population after the addition of sorbic acid

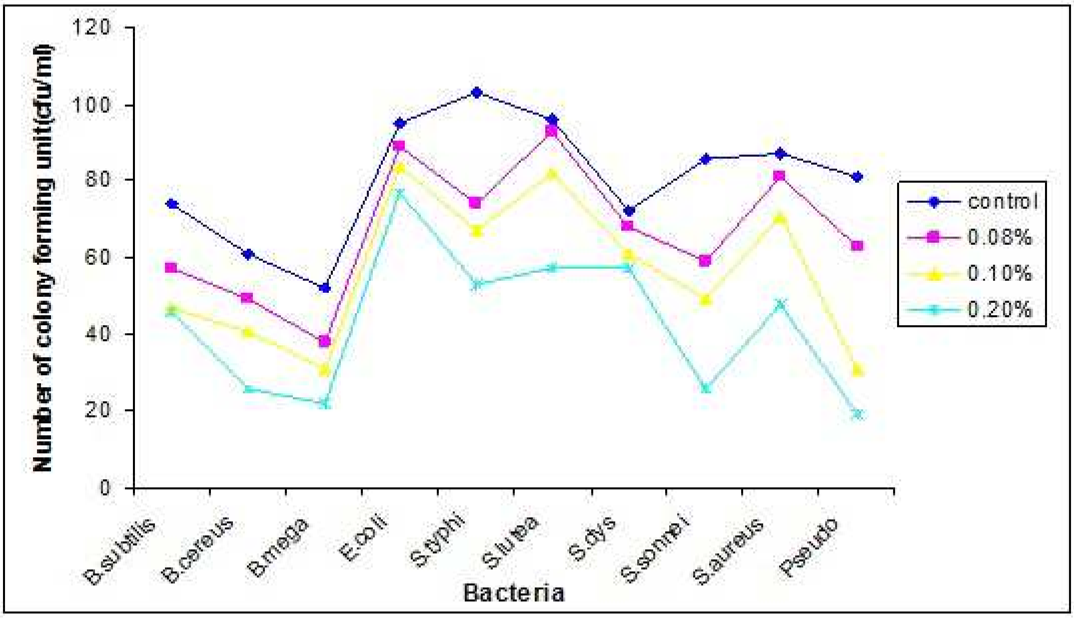

Figure: 4 Reduction of bacterial population after the addition of potassium sorbate

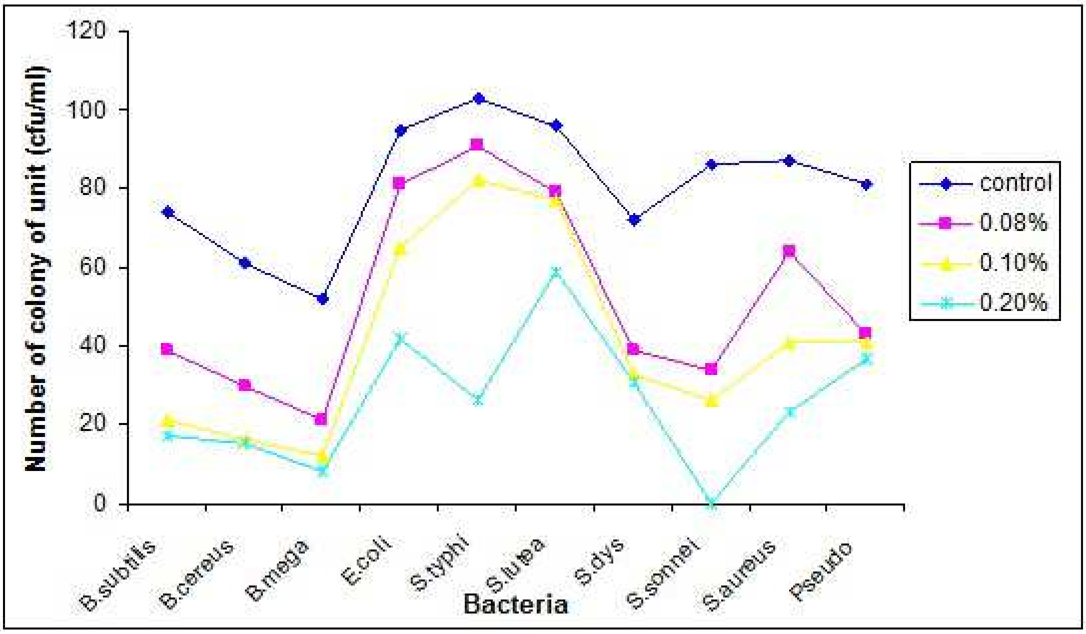

Figure: 5 Reduction of bacterial population after the addition of sodium metabisulphite

DISCUSSION

Antimicrobials are those chemical that prevent the biological deterioration of foods. Currently, approximately 30 different chemicals can legally be used as antimicrobials in food products. But due to the incorrect selection of proper antimicrobials, the sensory characteristics of the food products are adversely affected and become health hazards for consumers. The effectiveness of antimicrobials is not same in all types of food products; it depends on the water activity and pH of the food products, the type and number of microorganisms present in foods. The effectiveness increases as the pH value of the food decreases near the pKa of 4.19 and maximum antimicrobial activity occurs at a pH value 2.5 to 4.0 and significantly lose effectiveness at >pH-4.5. At pH 6.0 antimicrobial activity is only 1% of that at pH 4.0 [6].

In our study, sodium benzoate did not show any inhibitory effect against all tested bacteria. This may be effective with combination with other preservatives. Sodium benzoate at 0.1g/L concentration, in combination which any preservative is found to be effective in preserving the juice at least up to 10 days [7]. But the same preservative at low concentration and in combination with potassium meatbisulphite at high concentration was not effective in decreasing microorganisms. The decrease in the microorganisms may be due to their presence in an unfavourable environment created by sodium benzoate. Sodium benzoate prevents the germination of bacterial spores.

We found Sodium metabisulphite both at 0.20% and 0.30% was effective against all tested bacteria. Sodium metabisulfite reacts mostly with sulfide linkages to interfere with enzymes of the bacteria or may cause DNA breakdown [8,9]. *Bacillus subtilis, Bacillus megaterium* and *Shigella sonnei* was unable to growth in tomato juice that contained metabisulfite but was able to grow in juice that did not contain metabisulfites [10]. However, the authors of the latter study did not state what concentration of sodium metabisulfite was present in the juice.

From this study it was found that benzoic acid (0.20%) is most effective antimicrobial against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, and Shigella dysenteriae* while sorbic acid (0.20%) is against *Sarcina lutea* and sodium metabisulfite (0.20%) is against *Bacillus subtilis, Bacillus megaterium* and *Shigella sonnei* because reduction of maximum number of population of test organisms was caused by antimicrobials mentioned above. The results of this study are similar to that of Angela D Hartman [11], although the lower level of reduction of test bacterial populations was caused by sodium benzoate and potassium sorbate.

Food poisoning and spore-forming bacteria are generally inhibited by 0.01%-0.02% undissociated benzoic acid, but many spoilage bacteria are much more resistant. Sorbic acid has been commonly used in food such as dairy products, bakery products, fruit and vegetable products, fat emulsion products, certain meat and fish products, and confectionary items, with levels present at approximately 0.02 to 0.3% sorbic acid. In beverages, wines, carbonated and non-carbonated beverages, and fruit drinks, sorbate is generally used at levels of 0.02% to 0.1%, which imparts some antimicrobial inhibition while not imparting strong off-flavors in juices. However, sorbates above 0.10% may result in undesirable off flavors in fruit products [11].

The Minimum Inhibitory Concentration (MIC) of antimicrobials was also studied using Serial tube dilution technique. From this study, the minimum inhibitory concentrations of benzoic acid against *Escherichia coli, Salmonella typhi* were found to be 0.50%, 0.25% against *Sarcina lutea, Staphylococcus aureus, Shigella dysenteriae* and 0.125% against *Pseudomonas aeruginosa, Shigella sonnei, Bacillus subtilis, Bacillus megaterium, Bacillus cereus*; the MIC value of sorbic acid were found to be 0.125% against *Sarcina lutea, Shigella sonnei, Shigella dysenteriae, Bacillus cereus* and 0.50% against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Bacillus megaterium*; the MIC value of potassium sorbate were found to be 1% against *Bacillus subtilis, Bacillus megaterium, Bacillus cereus, Shigella dysenteriae, Staphylococcus aureus, Pseudomonas aeruginosa*), 0.50% against *Escherichia coli, Salmonella typhi* and 0.125% against *Shigella sonnei, Sarcina lutea*; the MIC value of sodium benzoate and sodium metabisulfite against all test organisms were 0.50% and 0.125% respectively.

Both benzoic acid and sodium benzoate are moderately toxic by ingestive, intramuscular and intraperitoneal routes [12]. Based upon the results of extensive human feeding investigations conducted in this century, Chittenden *et al.*, 1909 [13] and Dakin, 1909 [14] concluded that sodium benzoate was not deleterious to human health. **Sodium benzoates** can trigger allergies and may cause brain damage. Sax and Lewis, 1989 [12] listed benzoic acid as a human skin and eye irritant.

Since the permissible level of test antimicrobials is 0.05% to 0.10%, food producer have to be careful during the application of these antimicrobials in foods to restrict within this level. The efficacy of antimicrobials can be further investigated against other food spoilage bacteria to find out more evidence that benzoic acid, sorbic acid and sodium metabisulfite are more effective antimicrobials than others. This study suggested that benzoic acid, sorbic acid and sodium metabisulfite can be used more effectively to prevent deterioration of food, thus increasing their shelf life.

CONCLUSION

Among the antimicrobials analyzed, benzoic acid is the most effective antimicrobial against *Escherichia coli, Salmonella typhi, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus, Shigella dysenteriae* while sorbic acid is against *Sarcina lutea* and sodium metabisulfite is against *Bacillus subtilis, Bacillus megaterium, and Shigella sonnei.* This study will help the food producer or food processor to select the proper antimicrobials against specific bacterial strain and thus reducing the spoilage of food products which contribute to the reduction of prevalence of food borne illness commonly found in developing countries like Bangladesh, India, Nepal, and Pakistan. At the same time it will help the consumers to keep safe by consuming recommended level, since excessive intake of antimicrobials may become health hazard.

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