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# Evaluation of Antibacterial Activities of Some Chemical Food Preservatives on Food Associated Bacteria

**Uzoh CV[1]*, Umezuruike KC[2], Braide W[3], Orji C U[4], and Iheukwumere IH[5]**

[1]Department of Microbiology, Federal University Ndufu -Alike Ikwo P.M.B 1010,Abakaliki, Ebonyi State, Nigeria
[2]Department of Biology, Alvan Ikoku Federal College of Education P.M.B 1033 Owerri, Imo State, Nigeria
[3]Department of Microbiology Federal University of Technology P.M.B 1526 Owerri, Imo State, Nigeria
[4]Department of Agricultural Biotechnology, National Biotechnology Development Agency Umaru Musa Yar'Adua Airport Express Way, Lugbe, PMB 5118 Wuse, Abuja Nigeria
[5]Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University P.M.B 02, Uli, Anambra State, Nigeria

## Research Article

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***For Correspondence**

Uzoh C.V, Guizhou Department of Microbiology, Federal University Ndufu -Alike Ikwo P.M.B 1010,Abakaliki, Ebonyi State, Nigeria, Tel: +234-8068582884

**E-mail:** optchuks@yahoo.com

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**ABSTRACT**

There have been several reports on the efficacy of using chemicals to control the growth of food-borne pathogens. This study investigated the antibacterial activity of six chemical food preservatives namely benzoic acid, acetic acid, sodium nitrite, ascorbic acid, citric acid and sodium chloride against food associated bacteria isolated from tinned tomatoes, banana and cooked rice. The bacterial isolates were *Bacillus* sp, *Staphylococcus* sp, *Corynebacterium* sp, *Lactobacillus* sp, *Klebsiella* sp and *Streptococcus* sp. The susceptibility test conducted revealed that benzoic acid had the highest zone of inhibition of 8 mm followed by acetic acid, sodium nitrite, ascorbic acid, citric acid and sodium chloride which had the least zone of inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were 0.5 and 1.0.These findings indicated that benzoic acid could be used to inhibit the growth of food spoilage causing bacteria and food-borne pathogens and can be used to improve the safety of food products by extending their shelf life.

## INTRODUCTION

Food is any substance consumed to provide nutritional support for the body. It also serves as an ideal culture media for microbial growth. The bacteria in food cause offensive and sensory changes, food infections and intoxications in the food as it utilizes it as carbon and energy source. Some of the microorganisms that cause food borne infections and intoxications include *E.coli*, *S. pyogenes*, *Salmonella enterica*, *Yersinia* sp, *Clostridium botulinum*, *Bacillus cereus* [1,2], which are responsible for toxicity in the intestinal tract causing vomiting, diarrhea [3] and also toxic to the liver, kidney and nervous system. The major challenge for the food industry is to eliminate these undesirable changes that occur in foods as a result of the action of these microorganisms and ensuring maximum security in foods. Chemical additives like organic acids which include lactic, acetic and citric acid have been used to combat the actions of these microorganisms [4]. Lactic and citric acid enhance the flavor of fermented foods like pickles and sausage. There are a large number of chemicals that can serve as food preservatives but a small number is allowed in foods due to food and drug administration (FDA) rules which should be strictly adhered to. Hence, some of these compounds that exhibit antimicrobial effect *in vitro* do not show that when added to foods [5]. In order to enhance the shelf life of foods, chemical preservatives have been used. There is paucity of information on the spectrum of activity of these chemical food preservatives on the inhibition of the growth of food borne pathogens found in food products. This research work evaluated the antibacterial activity of some chemical food preservatives against some bacteria associated with some foods.

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## MATERIALS AND METHODS

**Bacterial isolates:** Six food-associated bacteria were isolated from foods like banana, tinned tomatoes and cooked rice. Six chemical food preservatives namely ascorbic acid, benzoic acid, acetic acid, sodium chloride, citric acid and sodium nitrate were evaluated for their antibacterial activity against some selected food-associated bacterial isolates (*Bacillus* sp, *Staphylococcus* sp, *Lactobacillus* sp, *Klebsiella* sp, *Corynebacterium* sp. and *Streptococcus* sp using agar well diffusion method **(Tables 1 and 2).**

**Table 1.** Different bacterial isolates from various food samples.

| Food samples | Isolated organism |
|---|---|
| 1. Tin tomatoes | *Streptococcus* sp<br>*Lactobacillus* sp<br>*Staphylococcus* sp |
| 2. Banana | *Bacillus* spp<br>*Staphylococcus* spp<br>*Klebsiella* spp<br>*Corynebacterium* spp |
| 3. Cooked rice | *Staphylococcus* spp<br>*Bacillus* spp<br>*Lactobacillus* spp |

**Table 2.** Antibacterial activity of the chemical preservative against bacterial isolates.

| | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Bs** | **Ss** | **Ls** | **Sts** | **Ks** | **Cs** | **C** |
| Benzoic acid | 8 | 4 | NA | NA | 2 | 2 | NA |
| Acetic acid | 6 | 2 | 2 | NA | NA | 3 | NA |
| Sodium nitrite | 3 | 2 | 2 | 1 | 1 | NA | NA |
| Ascorbic acid | 2 | NA | NA | 2 | 1 | NA | NA |
| Citric acid | NA | NA | 4 | 2 | 1 | 2 | NA |
| Sodium chloride | NA | 3 | NA | 3 | NA | NA | NA |

Key: Bs-*Bacillus* sp, Ss-*Staphylococcus* sp, Ls-*Lactobacillus* sp, Sts-*Streptococcus* sp, Ks-*Klebsiella* sp, Cs- *Corynebacterium* sp, C: Control, NA: No activity.

### Evaluation of the antibacterial activity of chemical food preservatives

### Preparation of stock solutions

The stock solution of these chemical preservatives were prepared (1% w/v i.e. 0.1 g of the chemical preservative dissolved in enough sterile distilled water to make the final volume of 10 ml) [6].

### Antibacterial activity by agar well diffusion method

Plate Count Agar (PCA) plates were inoculated with 100 µl of the standardized inoculum ($1.5 \times 10^8$ CFU/ml) of bacteria isolated from the food and was uniformly spread with sterile swab stick. Eight millimeter (8 mm) size of wells was made on the agar plates with a sterile cork borer and the lower portion was sealed with a little molten agar. A 100 µl volume of each chemical preservative was poured into the wells of the inoculated plates. A control which was sterilized distilled water poured into a well was used. The plates were left for 15 minutes for diffusion of the chemical preservative and incubated at 370 C for 24 hours. The mean diameter of zone of inhibition was recorded and calculated [7].

### Determination of minimum inhibitory concentration (mic) and minimum bactericidal (mbc) of benzoic acid against food associated bacteria

The minimum inhibitory concentration is the lowest concentration of this chemical food preservative that inhibited the visible growth of microorganisms after 24 hours incubation. Benzoic acid was used for the determination of MIC and MBC by macro dilution agar method **(Table 3).**

**Table 3.** Minimum inhibitory concentration and Minimum bactericidal concentration of benzoic acid against food associated bacteria by macro dilution method.

| Food associated bacterial isolate | Concentration of benzoic acid in percentage (V/V) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **0.031** | **0.062** | **0.125** | **0.25** | **0.5** | **1.0** | **MIC** | **MBC** |
| *Bacillus* sp | + | + | + | + | _ | _ | 0.5 | 0.5 |
| *Staphylococcus* sp | + | + | + | + | _ | _ | 0.5 | 0.5 |
| *Lactobacillus* sp | + | + | + | + | + | _ | 1.0 | 1.0 |
| *Streptococcus* sp | + | + | + | + | + | _ | 1.0 | 1.0 |
| *Klebsiella* sp | + | + | + | + | _ | _ | 0.5 | 0.5 |
| *Corynebacterium* sp | + | + | + | + | + | _ | 1.0 | 1.0 |

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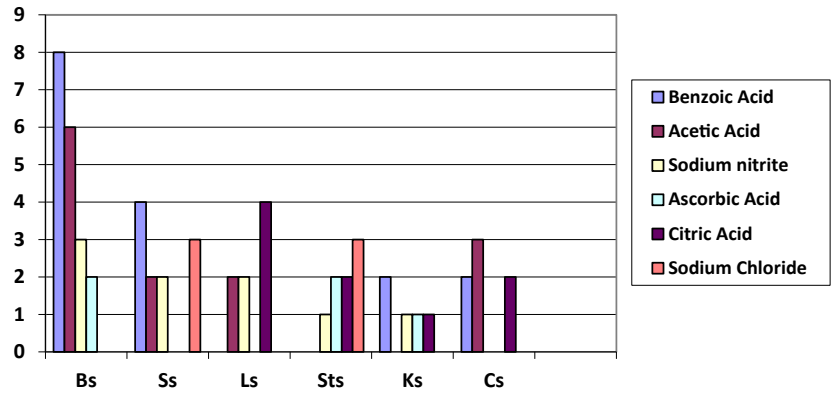
**Macrodilution agar method**

Two fold serial dilution of the benzoic acid was prepared in sterile distilled water to achieve decreasing concentrations from 1.0 to 0.031% v/v in eight sterile tubes labeled 1 to 8. 8 mm diameter sterile cork borer was used to bore well on the pre-solidified Mueller Hinton agar (MHA) plates and 100µl volume of each dilution was aseptically added into the MHA plates containing standardized inoculum (1.5 x $10^8$ CFU/ml) of the food associated bacteria isolated. It was incubated at 37ºC for 24 hours. The lowest concentration that showed a clear zone of inhibition indicated the minimum inhibitory concentration.

## RESULTS AND DISCUSSION

Among the six chemical food preservatives tested for their antibacterial activity, benzoic acid was found to be the best antibacterial agent on *Bacillus* sp. This differs from the result of Rosenquist and Hansen [8], where acetic acid was the most effective against *B. subtilis*. The second most active chemical food preservative was acetic acid. This corroborates the work of Bell et al., [9] who reported that dipping beef into 1.2% acetic acid for just 10 seconds and refrigerating for 20 hours at 50°C could reduce *Salmonella typhimurium* by 73.3%. Doores, [10] equally reported that acetic acid inhibited the growth of *Bacillus* sp, *Clostridium* sp, *P. aeruginosa*, *E.coli* and *Staphylococcus aureus*. Acetic acid commonly called vinegar was able to exhibit these antimicrobial capabilities due to its ability to lower the pH and cause instability of bacteria cell membranes [11]. Sodium nitrate equally showed antibacterial activity. This could be the reason why sodium nitrate and related salts like sodium acetate are widely used, economical and generally "recognized-as-safe" and could be used to prevent rope forming bacteria (*Bacillus subtilis*) in baked foods [12]. Citric acid has a strong antibacterial activity on *Lactobacillus* sp, *Streptococcus* sp, *Klebsiella* sp and *Corynebacterium* sp. This agrees with the work of Xiong et al. [13] who reported the inhibitory activity of citric acid against *S. aureus*. The antibacterial activity of citric acid is dependent on pH, concentration and anion effects [14]. The *in vitro* minimum inhibitory concentration (MIC) of benzoic acid, which had been found to be the best chemical food preservative was evaluated against the six food associated bacteria using macro dilution agar plate method. The MIC values of acetic acid ranged between 0.5 and 1.0% (V/V) against the food associated bacteria isolated. *Bacillus* sp, *Staphylococcus* sp and *Klebsiella* sp were found to be the most sensitive which survived only up to 0.5% concentration of benzoic acid. Thus benzoic acid was found to be the most effective with the lowest MIC of 0.5 against six bacterial isolates. The minimum bactericidal concentration (MBC), which was the observed bacterial growth on inoculated plates incubated at 37°C for 24 hr taken from the various concentrations of benzoic acid (0.5) and 1.0% (v/v) as prepared for the MIC. The minimum bactericidal concentration (MBC) equaled the MIC of benzoic acid, which finally was able to inhibit food-associated bacterial growth. In conclusion, the benzoic acid has the highest antimicrobial property than other organic acids tested and hence can be used to improve the safety of food products, preserve and prolong their shelf-life **(Figures 1 and 2).**




Bs-*Bacillus* sp, Ss-*Staphylococcus* sp, Ls-*Lactobacillus* sp, Sts-*Streptococcus* sp, Ks-*Klebsiella* sp, Cs- *Corynebacterium* sp.

**Figure 1.** Antibacterial activity of six chemical preservatives against food associated bacterial isolates.

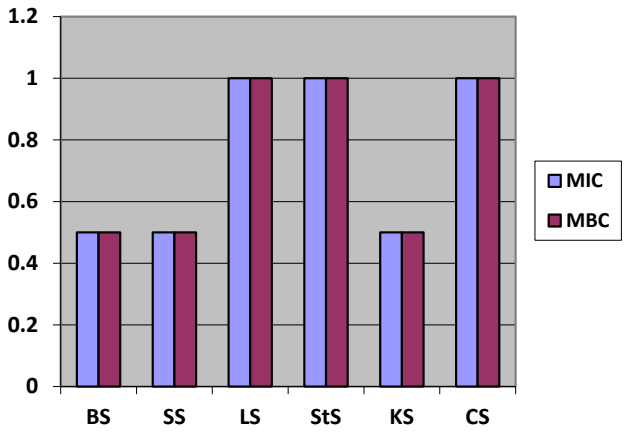



**Figure 2.** Minimum inhibitory concentration and minimum bactericidal concentration of benzoic acid on food associated bacteria.

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