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ANTIMICROBIAL ACTIVITY OF PRESERVATIVES IN FOOD TECHNOLOGY

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Abstract: *The aim of this study is to determine the antibacterial activity of some preservatives in food industry by minimum inhibitor concentration (MIC) and the paper disc diffusion method for antibiotic susceptibility testing. Some preservatives were chosen namely sodium bisulfite, potassium sorbate and sodium benzoate. The determination of antibacterial activity of these preservatives against gram-negative Escherichia coli (ATCC 25922), Salmonella enteritidis (ATCC 13076), gram-positive: Staphylococcus aureus (ATCC 25923), Bacillus subtilis (ATCC 11774) were investigated. The results show that MIC of sodium bisulfite for E. coli, S. enteritidis were 1.56 mg/mL, 3.125 mg/mL for B. subtilis and 6.25 mg/mL for S. aureus; while MIC of potassium sorbate for E. coli, S. aureus were 400 mg/mL, 800 mg/mL for B. subtilis and no effect on S. enteritidis. MIC of sodium benzoate for E. coli, S. aureus and B. subtilis were 400 mg/mL and no effect on S. enteritidis.*

Key words: *Potassium sorbate, sodium hydrosulfite, sodium benzoate.*

1. Introduction

Strategies to ensure stability, quality and food safety have been and always will be practised [9]. They can use many types of additives for food processing, especially preservatives. Currently, there are some reports that have highlighted that the many food preservatives, including sorbates, sodium bisulfite and sodium benzoate, have the potential to cause health problems, especially if used at higher concentrations in foodstuffs [13]. These compounds have various sources (synthetic or natural origin); although in recent years, food industry and consumers have shown concern about food safety and health however some additives from natural origin might not replace synthic additives because those are high cost, not stable during storage and have a weak antibacterial activity.

Sulfites have had many uses as an antimicrobial effect in drinks where the pH is low, the prevention of enzyme and non-enzyme browning reactions, as bleaching agent, colour stabilizer [6] or antioxidants in food industry and many pharmaceutical formulations [14] while sorbates are used to inhibit primarily molds and yeasts in many foods, such as dairy products (cheese, yogurt, sour

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cream…), bakery product (cakes, pies, doughnuts…), fruit and vegetable products (wine, soft drinks, fruit drinks…), and other food products (dry sausages, margarine, salted fish…) [21]. Besides, sodium benzoate is also used as an antifungal agent to preserve fresh juices, margarine and sweets [22]. These preservatives may be used individually or mixed together. Until now, there have been are many reports on the antibacterial activity of these compounds by various methods but there is not a standard procedure [7]. In this study, using the paper disk diffusion method combined with inhibition zone diameters for determining MIC of these compounds is used.

2. Materials and Methods

2.1. Chemicals and Organisms Collection

All chemicals originated from China and were of analytical reagent grade.

Antibacterial activity and minimum inhibitory concentration (MIC) were determined against two gram-positive bacteria as *Bacillus subtilis* (ATCC 11774) and *Staphylococcus aureus* (ATCC 25923), two gram-negative bacteria as *Escherichia coli* (ATCC 25922) and *Salmonella enteritidis* (ATCC 13076).

2.2. Antimicrobial Assay

The paper disc diffusion method for antibiotic susceptibility testing was used, according to the Kirby-Bauer test [2]. The sterile paper discs of 6 mm diameter were prepared that for using various concentrations of preservatives; gentamicin (10 µg/disc) were used as positive controls to compare the antibacterial activity;5% dimethylsulfoxide (DMSO) was used as negative control. Firstly, 0.1 mL of bacteria suspension (0.5 McFarland standard, approximately 1.5×10^8 cfu/mL) was spread on the surface of the Mueller-Hinton agar media for bacterial strains. Then, sterile paper discs were impregnated with 20 µL of each of solutions. The dishes were incubated for 24 hours at 37^{o}C for bacterial strains. After that, the zones of inhibition were expressed in mm, as the diameters of clear zones around the discs.

2.3. Data Analysis

Experimental results were analyzed by the one-way analysis of variance (ANOVA) method and significant differences among the means from triplicate analyses at ($p<0.05$) were determined by Fisher's least significant difference (LSD) procedure using the Statgraphics software (Centurion XV). The values obtained were expressed in the form of a mean ± standard deviation (SD).

3. Results and Discussion

3.1. Effect of Positive and Negative Control on Zone of Inhibition of Bacteria

DMSO is known as a sulfur organic compound, soluble in water and organic solvents, with molecular formula $(CH_3)_2SO$. Due to its chemical properties, DMSO is very useful for dissolving insoluble-in-water compounds [11]. Based on the results of experiments with DMSO 5%, it does not affect the antimicrobial result, which is confirmed when most of the antibacterial experiments used DMSO as a

negative control. Typically, as in the study by Nitiema et al. [10], DMSO is a negative control for the antibacterial activity (*E. coli* and *Salmonella*) of the extract from phenolic compounds contained in coumarin and quercetin. In addition, DMSO does not show antibacterial activity with *S. aureus* in all extracts of *Polygonum cuspidatum* [23]. As a result, it can be concluded that DMSO dissolves most of the compounds and can be used as a negative control for gram-negative and gram-positive bacteria without compromising the antimicrobial effect.

Meanwhile, gentamicin is an amino glycoside antibiotic, produced by the fermentation of *Micromonospora* or *M. echinospora*. It might prevent many types of severe infections caused by gram-negative and gram-positive bacteria. Gentamicin is a complex mixture of several major molecules which have varying degrees of methylation [1]. Gentamycin inhibits the protein synthesis and breaks down the cell membrane of microorganism including many stages. Due to the transport system, Gentamycin diffuses through the outer membrane and is absorbed the cytoplasm. Then, gentamycin moves rapidly to attach the bacterial ribosome, inhibiting protein synthesis. Besides, it also reduces the accuracy of the information RNA (mRNA), resulting in the miscommunication of amino acids in the bacterial polypeptide chain [3], [25].

Gentamicin is commonly used in antimicrobial experiments as the positive control. For instance, Zeraib et al. [26] used the essential oil from *Juniperus thurifera* L. for antimicrobial, in which gram positive is Gentamicin 10 µg/disc and take effect on many bacteria as *S. aureus* (ATCC 25923), *S. aureus* (clinical), *E. coli* (ATCC 25922), etc. In another study, gentamicin is also used to investigate the sensitivity of microorganisms, the extracts were from 46 spices (pepper, cinnamon, coriander, cloves and etc.) and various medicinal herbs [17]. The above result shows that gentamicin inhibits to four bacterial strains. The antibacterial capacity of gentamicin for each type of bacteria is different at the same concentration of 10 µg/disc. Therefore, the order of the bacteria that are sensitive to positive control is *S. enteritidis* < *B. subtilis* < *S. aureus* < *E. coli* (Table 1).

3.2. Effect of Sodium Bisulfite on Zone of Inhibition of Bacteria

The result shows that sodium bisulfite inhibits four bacterial strains at a low concentration (Table 1), indicating its high antibacterial activity. Sodium bisulfite inhibits *E. coli*, *S. enteritidis* at MIC=1.563 mg/mL, *B. subtilis* at MIC=3.125 mg/mL and *S. aureus* at MIC=6.25 mg/mL. It can be deduced that *E. coli* and *S. enteritidis* were more sensitive than *B. subtilis* and *S. aureus* to sodium bisulfite. Besides, inhibition zones of all concentrations of sodium bisulfite were “sensitive” (inhibition zone from 8 to 14 mm), these results were evaluated similarly with the antimicrobial level of some essential oils [15].

Pagano and Zeiger [12] have shown that bisulfites have the potential to directly affect DNA of bacteria. Sodium bisulfite might interact with nucleic acids or their components facilitate the acid reaction causing the gene mutation [5]. Although, the action mechanism of sodium bisulfite on DNA is controversial and there is no specific study. Comparing to the research

result from natural plant, extract from leaves, bark and flowers of *Prosopois juliglora* which inhibit *E. coli*, *B. cereus*, *S. aureus*, *Salmonella* sp. at a concentration of 100 mg/mL (about 16 times higher than this result), or the extract from flowers which does not inhibit *Salmonella* sp. [19]. As a result, the antibacterial activity of sodium hydrosulfite is much stronger than that of natural bioactive compounds. Therefore, sodium bisulfite was used widely in food industry.

Zone of inhibition of sodium bisulfite Table 1

Bacterial strains	Zone of inhibition [mm]						
	DMSO 5 [%]	Gentamycin 10 [µg/disc]	Concentration of sodium bisulfite [mg/mL]				
			6.250	3.125	1.563	0.781	0.390
E. coli	-	19.33 ± 0.58^{Cb}	8.8 ± 0.8^{ABa}	8.0 ± 0.9^{Aa}	7.7 ± 0.6^{Aa}	-	-
S. enteritidis	-	14.33 ± 0.58^{Ac}	9.2 ± 0.3^{Bb}	8.7 ± 0.6^{Ab}	7.5 ± 0.5^{Aa}	-	-
S. aureus	-	15.67 ± 0.5^{Bb}	10.7 ± 0.6^{Ca}	-	-	-	-
B. subtilis	-	15.00 ± 0.0^{ABb}	8.2 ± 0.3^{Aa}	8.5 ± 0.5^{Aa}	-	-	-

-: "not detected".
Various lowercase letters in the same row denote significant difference ($p<0.05$).
Various uppercase letters in the same column denote significant difference ($p<0.05$).

3.3. Effect of Potassium Sorbate on Zone of Inhibition of Bacteria

Table 2 shows that the concentration of potassium sorbate increases with the increase of the antibacterial activity, as it is shown in the zone of inhibition. Potassium sorbate inhibits three strains of microorganisms for instance *E. coli, S. aureus* (MIC = 400 mg/mL), *B. subtilis* (MIC=800 mg/mL) but with *S. enteritidis* there are no signs of inhibition.

Potassium sorbate plays a major role in antifungal activity, however, the growth of bacteria is also inhibited by various mechanisms. Sorbate is a potent inhibitor of bacterial spore germination. However, the actual mechanism of spore germination and associated reactions are not well determined [20]. It inhibits the initial stages of germination, the interference with transport of cell. Sorbate alters spore membrane permeability and causes inhibition of cation transport functions in the later stages of germination [4]. Besides, it may inhibit some certain enzymes in the cell [18], bear influence on cell walls and membranes, which may alter their integrity and permeability. In addition, sorbate may affect other processes such as electron transport or proton-motive force [20], etc.

Mohammad [8] reports that the number of bacterial cells (CFU/mL) of *E. coli* and *S. aureus* decrease with the increase of concentration of potassium sorbate. *S. aureus* does not develop at concentrations of 0.2% for 24 hours and 0.1% for 48 hours while *E. coli* does not develop at 0.2% for 48 hours. However, the received result has the concentration of potassium sorbate which is used higher in this study due to different bacteria strains or different methods.

3.4. Effect of Sodium Benzoate on Zone of Inhibition of Bacteria

The result of sodium benzoat is similar to that of potassium sorbate, which inhibited *E. coli* strains, *B. subtilis*, *S. aureus* (MIC=400 mg/mL), while there are no signs of the inhibition of *S. enteritidis* (Table 3).

Sodium benzoate may strongly inhibit strongly yeast, mold and bacteria. It acts as a preservative at a lower pH because the ratio of undissociated benzoic acid to ionized benzoic acid increases leads to the increase of the pH value. It is generally accepted that the undissociated benzoic acid is the active antimicrobial agent. Although no definite theory has been yet proposed to explain this antimicrobial effect, it is believed to be related to the high lipoid solubility of the undissociated benzoic acid which allows it to accumulate on the cell membranes or on various structures and surfaces of the bacterial cell, effectively inhibiting its cellular activity [24]. In addition, there have been some studies on the antimicrobial activity of sodium benzoate for some microbial species characteristics, but the results obtained from those studies are not really convincing and do not have enough reliability to used as the basis for the action of sodium benzoate in antimicrobial activity.

Zone of inhibition of potassium sorbate Table 2

Bacterial strains	Zone of inhibition [mm]						
	DMSO 5 [%]	Gentamycin 10 [µg/disc]	Concentration of sodium bisulfite [mg/mL]				
			800	400	200	100	50
E. coli	-	19.33 ± 0.58^{Cc}	15.7 ± 1.5^{Bb}	10.3 ± 0.6^{Aa}	-	-	-
S. enteritidis	-	14.33 ± 0.58^{A}	-	-	-	-	-
S. aureus	-	15.67 ± 0.5^{Bb}	10.7 ± 2.6^{Aa}	9.0 ± 1.7^{Aa}	-	-	-
B. subtilis	-	15.00 ± 0.0^{ABb}	9.0 ± 0.0^{Aa}	-	-	-	-

-: "not detected".
Various lowercase letters in the same row denote significant difference ($p<0.05$).
Various uppercase letters in the same column denote significant difference ($p<0.05$).

Zone of inhibition of sodium benzoate Table 3

Bacterial strains	Zone of inhibition [mm]						
	DMSO 5 [%]	Gentamycin 10 [µg/disc]	Concentration of sodium bisulfite [mg/mL]				
			400	200	100	50	25
E. coli	-	19.33 ± 0.58^{Cb}	7.7 ± 0.6^{Aa}	-	-	-	-
S. enteritidis	-	14.33 ± 0.58^{A}	-	-	-	-	-
S. aureus	-	15.67 ± 0.5^{Bb}	9.0 ± 1.0^{Ba}	-	-	-	-
B. subtilis	-	15.00 ± 0.0^{ABb}	9.0 ± 0.0^{Ba}	-	-	-	-

-: "not detected".
Various lowercase letters in the same row denote significant difference ($p<0.05$).
Various uppercase letters in the same column denote significant difference ($p<0.05$).

The received result was lower than that of polyphenol extract from plants, for instance the polyphenol extract from blackthorn inhibited *E. coli* (MIC=250 µg/mL), *B. subtilis* (MIC=250 µg/mL), *S. enteritidis* (MIC=250 µg/mL) and *S. aureus* (MIC=15.6 µg/mL) [16]. It shows that the bioactive compounds were better than the synthesis preservatives in some cases. Besides, potassium sorbate and sodium benzoate are two main additives used to prevent mold, so their antibacterial capacity is not effective.

4. Conclusions

The results of this study demonstrate the effective antimicrobial action of preservatives when used individually and sodium sulfite has the best effect of antibacterial capacity. The combination of MIC and the paper disk diffusion method can contribute to a new outlook on the antibacterial method.

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