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Original Article

The efficacy of sodium benzoate and potassium sorbate in inhibiting the growth of food fungi and bacteria

Nurul Fatin Amilia Romli[1], Rashidah Sukor[1, 2], Yaya Rukayadi[1], and Aliah Zannierah Mohsin[1*]

[1] *Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor, 43400 Malaysia*

[2] *Institute Tropical Agriculture and Food Security, Universiti Putra Malaysia, Serdang, Selangor, 43400 Malaysia*

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Abstract

Bacterial and fungal resistance to chemical preservatives is a major food safety issue with significant health and economic ramifications. The efficacy of food preservatives in inhibiting the growth of fungi and bacteria is crucial to ensure the safety and quality of food products. This study aimed to determine the efficacy of sodium benzoate and potassium sorbate against food fungi and bacteria. Four spoilage fungi (*Aspergillus* sp., *Trametes* sp., *Penicillium* sp., *Cladosporium* sp.) were isolated from mango, lemon, and orange. Sodium benzoate and potassium sorbate were tested at 100, 200, 300, and 400 mg/L for antimicrobial properties against the isolated fungi and bacteria (*S. enterica*, *E. coli*, *K. pneumonia*, *Proteus sp*., *S. aureus*, *B. cereus*, and *B. subtilis*) using pour plate method. Results showed that sodium benzoate was effective against all microorganisms tested, except for *B. cereus* and *Aspergillus sp*.: the growth of both microorganisms was inhibited at 400 mg/L. Meanwhile, potassium sorbate was found to be effective against all tested microorganisms at 100 mg/L. In conclusion, both preservatives were proven to be effective against bacteria and fungi found in food. This information is useful as guidance for food manufacturers to apply preservatives targeting microorganisms at effective levels.

Keywords: preservatives, sodium benzoate, potassium sorbate, antimicrobial

1. Introduction

Chemical food preservatives in an adequate amount can help extend the shelf life and minimize the prevalence of spoilage and pathogenic microorganisms in food products (Yu, Chin, & Paik, 2021). Sodium benzoate and potassium sorbate are commonly used preservatives in the food industry, particularly in acidic food products such as mayonnaise, juice, and soft drinks. Their antimicrobial activity is dependent on inoculum level, the kind of spoilage organisms, length of shelf life, product storage temperature, pH, and preservative concentration (Wind & Restaino, 1995). The effectiveness of both preservatives is also dependent on the type of food or food system. Although it is difficult to generalize, both preservatives have been shown to inhibit Gram-positive and Gram-negative, catalase-positive, and catalase-negative, aerobes and anaerobes, and thermophilic, mesophilic, and psychotropic bacteria and fungi (Sofos *et al*., 1985). These preservatives destroy the bacterial cell structure by resorting to lipophilic characteristics (Branen & Davidson, 2004; Delamare *et al*., 2007). The gram-positive bacteria are more sensitive than the Gram-negative bacteria, as the peptidoglycan forms the outer membrane of the cell while the cell structure is different, and the peptidoglycan layer lies between the plasma membrane and lipopolysaccharide outer membrane in Gram-negative bacteria; therefore, antimicrobials cannot pass through the outer layer of Gram-negative bacteria easily (Branen & Davidson, 2004). The

*Corresponding author
Email address: aliah_mohsin@upm.edu.my

preservatives also can control the growth of fungi by reducing their metabolism, denaturing fungal cell proteins, or changing the permeability of the cell membrane (Stopforth, Sofos, & Busta, 2005).

Regulatory agencies such as the FDA, the United Nations Food and Agriculture Organization, and the European Food Safety Authority (EFSA) have determined that potassium sorbate and sodium benzoate are "generally regarded as safe," abbreviated as GRAS, if they are applied in certain concentrations. The FDA allows up to a 1 mg/L concentration of sodium benzoate and potassium sorbate by weight in foods and beverages. Excess dietary intake of these preservatives above the allowable daily intake (ADI) levels such as 0-5 mg/kg body weight/day for benzoic acid and benzoate salts and 0-25 mg/kg body weight/day for sorbic acid and sorbate salts (Mischek & Krapfenbauer-Cermak, 2012; World Health Organization [WHO], 2016) may pose health risks to human population. Adverse effects of benzoates have been reported in sensitive patients along with hyperactivity in children (McCann *et al*., 2007; Piper & Piper, 2017). Therefore, to minimize health risks among consumers, the antimicrobial and antifungal properties of these preservatives should be determined so that lower concentrations can be used effectively and specifically in food products. The present work aimed to examine the efficacy of chemical preservatives, i.e., sodium benzoate and potassium sorbate, at different concentrations against selected fungi and bacteria from food sources.

2. Materials and Methods

2.1 Materials

Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) were purchased from Difco (Sparks, MD, United States). Nutrient Agar (NA), Xylose Lysine Deoxycholate Agar (XLD), Eosin Methylene Blue Agar (EMB), Mannitol Salt Agar (MSA), Buffered Peptone Water (BPW) and Nutrient Broth (NB) were purchased from Merck (Darmstadt, Germany), and Czapek Yeast Agar (CYA) was purchased from Titan Media (New Delhi, India). All media were prepared according to the manufacturers' instructions. Food-grade sodium benzoate and potassium sorbate were purchased from Personal Formula Resources Sdn. Bhd., Puchong, Selangor, Malaysia.

2.2 Isolation of fungi and bacteria from food sources

Bacterial cultures were obtained from the culture collection of the Food Microbiology Laboratory, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, Selangor. The bacterial stocks isolated from various food sources were preserved at -70°C and sub-cultured prior to use. Four Gram-negative bacteria, i.e., *Salmonella* Typhi, *Escherichia coli, Klebsiella pneumoniae,* and *Proteus* sp., and three Gram-positive bacteria, i.e., *Staphylococcus aureus, Bacillus cereus,* and *B. Subtilis,* were cultivated on NA and incubated at 37°C for 18-24 h. Following cultivation, all bacterial cultures were stored at 4°C for seven days until further analysis.

For fungal isolation, three types of fruits, i.e., mango, lemon and orange, were purchased from Pasar Borong Selangor, Seri Kembangan, Selangor, Malaysia, and immediately transported to Food Mycology Laboratory, Faculty of Food Science and Technology, UPM. Fungi were isolated from the fruits using the plate dilution method as described by Bueno, Silva, and Oliver (2004). Briefly, 25 g fruit samples were separately homogenized using a stomacher in 225 mL BPW. Serial dilutions were prepared (10^{-1} to 10^{-5}) and 0.1 mL of each dilution was inoculated onto PDA in triplicates. The plates were incubated upright for seven days at 30°C. After the incubation period, the fungal growth was examined and cultures with different morphology were sub-cultured. Isolated fungi were identified by observation of the morphological characteristics of PDA, MEA, and CYA. Macroscopic characteristics of the isolates, such as colony color, reverse appearance, texture, margin, elevation, pigmentation, and exudate production were examined under a stereomicroscope (Meiji PKL-2, Japan). Meanwhile, microscopic attributes were observed using a light microscope with a camera attached (Nikon Eclipse 80i Binocular, NY). Characteristics such as hyphae, formation of conidial head, conidial shape, and texture were noted. The isolates were identified using the morphological keys of Pitt and Hocking (2009) and De Boer (2002). A seven-day-old fungal culture was harvested and rinsed into a flask containing 20 mL BPW. Then, the flask was agitated at 200 rpm and 30 °C for 2 h to break down the mycelial structure of the fungi. The suspension was then filtered through cotton wool to remove the debris and the concentration of the inoculum was adjusted to yield approximately 10^4 CFU/mL using ten-fold serial dilution prior to antifungal testing.

2.3 Antimicrobial properties of potassium sorbate and sodium benzoate

The bacterial cultures from the stock were sub cultured onto NA by using the four-quadrant streaking method and incubated at 37 °C for 24 h. After the incubation period, a few colonies of bacteria from NA were inoculated into 10 mL NB and incubated further at the same conditions. Next, the concentration of the inoculum was adjusted to yield approximately 10^4 CFU/mL using a spectrophotometer and ten-fold serial dilution prior to antibacterial testing. The preservatives, i.e., potassium sorbate and sodium benzoate were diluted in molten agar to four different concentrations (100, 200, 300, and 400 mg/L). Then, 1.0 mL of the diluted bacterial cultures in NB was pipetted into a sterile plate and the molten agar containing preservatives at each concentration was poured into the plate, gently swirled, and left to solidify at room temperature. Molten agar without the addition of preservatives was used as a control. The number of colonies was counted using a colony counter after a 24 h incubation at 37 °C. The colony forming units per volume (CFU/mL) were calculated using the formula:

$$\text{CFU/mL} = \frac{\text{number of colonies x dilution factor}}{\text{volume added to plate (1.0 mL)}}$$

The rate of inhibition (%) was calculated using the formula:

$$\% = \frac{(\log \text{CFU/mL at 0 mg/L} - \log \text{CFU/mL at desired concentration}) \times 100}{\log \text{CFU/mL at 0 mg/L}}$$

For antimicrobial tests on fungal isolates, the preservatives were diluted in molten agar into four different concentrations (100, 200, 300, and 400 mg/L). Then, 1.0 mL of diluted fungal culture in BPW was pipetted into a sterile plate and the molten agar containing preservative was poured into the plate, gently swirled, and left to solidify at room temperature. The numbers of colonies were counted after seven days of incubation at 30 °C. The colony forming units per volume (CFU/mL) were calculated using the formula described above.

2.4 Statistical analysis

Data for each group were analyzed and are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed using Minitab17 and the threshold $p<0.05$ was used for 95% statistical significance in Tukey's test.

3. Results and Discussion

3.1 Inhibition by potassium sorbate and sodium benzoate of the fungal isolates

The general colony and morphological characteristics of each of the fungal genera isolated are summarized in Table 1. *Aspergillus* sp., *Trametes* sp., *Penicillium* sp., and *Cladosporium* sp. have been identified as the typical spoilage organisms of food products, leading to off-flavors including strong musty and earthy notes in spoiled foods. In addition to organoleptic property deterioration, spoilage molds such as *Penicillium* and *Aspergillus* spp. can also produce mycotoxins (Garnier, Valence, & Mounier, 2017). The inhibitory effects of potassium sorbate and sodium benzoate on the growth of fungi reduce production of toxins by inhibiting both spore germination and growth of mycelia (Alsudani, 2017; Heydaryinia, Veissi, & Sadadi, 2011). Similarly, Sofos and Busta (1991) found that potassium sorbate and sodium benzoate control the growth of fungi by reducing their metabolism, denaturing fungal cell proteins, or by changing the permeability of the cell membrane. Besides inhibiting microorganisms as a weak-acid preservative, Stratford and Anslow (1998) suggested an inhibitory role for sorbic acid as a membrane-active compound.

Inhibitory properties of potassium sorbate against the growth of isolated fungi are shown in Table 2. There was a reduction in the growth of *Aspergillus* sp., *Trametes* sp., *Penicillium* sp., and *Cladosporium* sp. Each concentration of potassium sorbate tested showed a significant difference ($p<0.05$) when compared to the negative control. The higher the amount of potassium sorbate, the higher the preservative effect that was exerted on the fungal isolates. The antimicrobial properties of potassium sorbate showed no detection in *Aspergillus* sp., *Trametes* sp. and *Cladosporidium* sp. at 300 mg/L, which was not the case for *Penicillium* sp. Even though the minimum inhibitory concentration of sorbate for most molds ranges from 0.001 to 0.1%, it is influenced by many factors including pH, water activity, the presence of sodium chloride, sucrose, and/or organic acids, temperature, species, strain of yeast, and the amount of oxygen present (Alrabadi, 2017). Some microorganisms can produce shock protein once exposed to certain types of preservation effects. This shock protein will make them more resistant to antimicrobial agents; thus, they are able to survive in food products even under extreme conditions (Akinmusire, 2011). In addition, different antimicrobial properties against different fungi might contribute to the efficiency of the sorbic acid to dissociate into their undissociated forms, which may explain why potassium sorbate is more effective than sodium benzoate when equal weights of the two are compared in the acidic product (Wind & Restaino, 1995). Regardless of the findings,

Table 1. General colony and morphological characteristics of fungi isolates according to genera

Genera (Sources)	Colony characteristics	Morphological description
Aspergillus sp. (Isolated from Grape)	Colonies plane, dense, grainy and powdery and rapidly growing. Conidiophores usually black, brown, blue, yellow green, white and brightly coloured. Mycelium sometimes white, pale and colourless.	Septate mycelia. Conidiophore is an unbranched stipe with vesicle arising from foot cell. Phialides that borne directly on vesicle is uniseriate while borne on metullae is biseriate. Conidial colour gives the colour of the colony. Conidia smooth or rough, globose or sub-globose.
Trametes sp. (Isolated from Lemon: Fazly-Ann, Nor-Hafizah, & Rukayadi, 2020).	White mycelia, floccose and cottony.	Simple septate hyphae. Spore cylindrical and ellipsoidal and breaks off from fertile hyphae.
Penicillium sp. (Isolated from Grape: Fazly-Ann, Nor-Hafizah, & Rukayadi, 2020)	Colonies velvety, plane, dull green, yellow, white, greenish grey and bluish green colonies. Some colonies produced bright yellow, light brown, reddish orange and bright red pigments.	Septate mycelia. Phialides grouped together into brush-like structure (penicillus) at the end of conidiophores. Conidiophore can be branched singly on stipe (monoverticillate) or one-stage branched (biverticillate), two-stage branched (terverticillate) or three and more branched (quaterverticillate). Conidia in various shapes and forms, round, ellipsoidal or cylindrical. Smooth or rough walled.
Cladosporium sp. (Isolated from mango)	Colonies olive-ish brown to greyish black and greyish-green. Velvety, wrinkled and hard. Black reverse.	Conidiophore straight and unbranched. Conidia unicellular and sometimes singly septate. Ellipsoidal.

potassium sorbate and sodium benzoate are the most effective agents in controlling the growth of fungi on citrus fruits, especially against *Penicillium* sp. (Valencia-Chamorro, Palou, del Río, & Pérez-Gago, 2008).

Table 3 shows the antifungal properties of sodium benzoate in inhibiting the growth of isolated fungi compared to control, showing significant differences (p<0.05) between the different concentrations of sodium benzoate. As seen in the table, the antifungal effect of sodium benzoate was active in controlling the growth of the four fungal genera tested in the study. Furthermore, an increase in sodium benzoate concentration from 100 to 400 mg/L enhanced the antifungal effects significantly (p<0.05). The acceptable limit of yeast and mold count in fruits like strawberries is <1000 CFU/mL (European Commission, 2012), which means that the number of surviving colonies in Tables 2 and 3 is within the safe limits. This result shows that all four fungal genera can be controlled with 100 mg/L of potassium sorbate or of sodium benzoate. Malaysia Food Regulatory Act 1985 allows up to 350 mg/L and 450 mg/L of sodium benzoate and potassium sorbate in beverages and fruit jams. Other food products have upper regulatory limits for both preservatives. Therefore, below 100 mg/L of potassium sorbate is recommended for use in food, based on its significant activity against all tested fungi. More detailed studies, however, are warranted on other types of spoilage fungi if the amount were to be adopted by the current food industry, since reduced usage of preservatives is favorable as this reduces the production costs.

3.2 Antimicrobial properties of potassium sorbate and sodium benzoate against bacterial isolates

In the investigation of potassium sorbate and sodium benzoate as antibacterial agents, seven different bacteria were used in this study. *S. Typhi*, *E. Coli*, *K. Pneumoniae*, *Proteus* sp., (Gram-negative bacteria), and *S. Aureus*, *B. Cereus*, and *B. subtilis* (Gram-positive bacteria) were considered typical spoilage microorganisms of food products (Gram *et al*., 2002). *Salmonellae* is a group of facultative anaerobic, rod-shaped bacteria with *S. typhimurium* being responsible for more than half of all confirmed salmonellosis cases, mainly in poultry (minced or meat preparations) and turkey meat. Meanwhile, *E. coli*, *Klebsiella pneumoniae*, and *Proteus* are natural inhabitants of the

Table 2. Antifungal properties of potassium sorbate on the growth of isolated fungi (in log CFU/mL) determined using pour plate method

Type of fungus		Concentrations (mg/L)				
		0	100	200	300	400
Aspergillus sp.	Surviving number of colonies (log CFU/mL)	3.706 ± 0.072^{a}	3.369 ± 0.084^{b}	3.138 ± 0.161^{b}	nd	nd
	Rate of inhibition (%)		9.17 ± 0.011^{c}	15.326 ± 0.033^{b}	100 ± 0.000^{a}	100 ± 0.000^{a}
Trametes sp.	Surviving number of colonies (log CFU/mL)	3.894 ± 0.010^{a}	3.630 ± 0.012^{b}	3.288 ± 0.016^{c}	nd	nd
	Rate of inhibition (%)		6.779 ± 0.051^{c}	17.103 ± 0.012^{b}	100 ± 0.000^{a}	100 ± 0.000^{a}
Penicillium sp.	Surviving number of colonies (log CFU/mL)	3.756 ± 0.021^{a}	3.625 ± 0.011^{b}	3.583 ± 0.013^{b}	3.043 ± 0.010^{c}	2.916 ± 0.037^{d}
	Rate of inhibition (%)		3.487 ± 0.021^{d}	4.606 ± 0.014^{c}	18.982 ± 0.017^{b}	22.364 ± 0.010^{a}
Cladosporium sp.	Surviving number of colonies (log CFU/mL)	3.883 ± 0.019^{a}	3.630 ± 0.010^{b}	3.284 ± 0.017^{c}	nd	nd
	Rate of inhibition (%)		6.516 ± 0.009^{c}	15.426 ± 0.041^{b}	100 ± 0.000^{a}	100 ± 0.000^{a}

Data are expressed as mean ± SD, (n = 3). Means that do not share the same superscript within the same row are significantly different (p< 0.05); nd= not detected.

Table 3. Antifungal properties of sodium benzoate on the growth of isolated fungi (in log CFU/mL) determined using pour plate method

Type of fungus		Concentrations (mg/L)				
		0	100	200	300	400
Aspergillus sp.	Surviving number of colonies (log CFU/mL)	3.706 ± 0.072^{a}	3.590 ± 0.029^{b}	3.243 ± 0.074^{c}	2.982 ± 0.071^{d}	2.301 ± 0.329^{e}
	Rate of inhibition (%)		3.130 ± 0.032^{d}	12.493 ± 0.091^{c}	19.536 ± 0.063^{b}	37.911 ± 0.221^{a}
Trametes sp.	Surviving number of colonies (log CFU/mL)	3.894 ± 0.010^{a}	3.735 ± 0.009^{b}	3.615 ± 0.007^{c}	3.507 ± 0.015^{d}	3.412 ± 0.012^{e}
	Rate of inhibition (%)		4.083 ± 0.011^{d}	7.165 ± 0.071^{c}	9.938 ± 0.036^{c}	12.378 ± 0.011^{a}
Penicillium sp.	Surviving number of colonies (log CFU/mL)	3.765 ± 0.021^{a}	3.669 ± 0.007^{b}	3.615 ± 0.009^{c}	3.508 ± 0.012^{d}	3.319 ± 0.018^{e}
	Rate of inhibition (%)		2.550 ± 0.011^{d}	3.984 ± 0.081^{c}	6.826 ± 0.041^{b}	11.846 ± 0.020^{a}
Cladosporium sp.	Surviving number of colonies (log CFU/mL)	3.883 ± 0.019^{a}	3.736 ± 0.007^{b}	3.613 ± 0.009^{c}	3.504 ± 0.011^{d}	3.407 ± 0.008^{e}
	Rate of inhibition (%)		3.786 ± 0.051^{d}	6.953 ± 0.011^{c}	9.760 ± 0.101^{b}	12.259 ± 0.005^{a}

Data are expressed as mean ± SD, (n = 3). Means that do not share the same superscript within the same row are significantly different (p<0.05).

gastrointestinal tract microbiome in healthy humans and animals, and are associated with serious infections to ingestion and colonization in the human digestive system (Garnier, Valence, & Mounier, 2017). *S. aureus* is cocci-shaped while *B. cereus* and *B. subtilis* are rod-shaped bacteria found in the environment (soil, vegetation, and food) and in normal human flora. They are the causative agents of nausea, vomiting, diarrhea and multiple human infections. Unlike other the Gram-positive bacteria, the Gram-negative bacteria only possesses a very thin outer peptidoglycan layer that is responsible for the rigidity and strength of the bacterial cell wall, which may allow these antibacterial agents to easily enter the bacterial cell and exhibit their activity (Raftari *et al*., 2009).

In Table 4, each of the concentrations of potassium sorbate tested on the different types of bacteria showed a significant reduction (p<0.05) when compared to the control. The number of bacterial growth (in log CFU/mL) decreased as the amount of antimicrobial agent added was increased, as anticipated. The significant inhibition observed could be due to the effectiveness of the potassium sorbate as a preservative agent in a wider range of pH from 3.0 to 6.5, as compared to other preservative agents which are used at pH below 5.0. At this pH value, the sorbate salt will readily dissolve in water or liquid food products. Once the potassium sorbate is dissolved, it will form undissociated sorbic acid (Fisher & Phillips, 2009). The water molecules will split to form hydrogen ions (H^+) and hydroxide ions (OH^-) and these will be involved in a proton motive force action where the H^+ ions cause the outer part of the membrane of the microorganism present in the food to become acidic and the OH^- causes the pH of the inner part of the microbes to increase in pH towards neutrality (Fisher & Phillips, 2009). At this condition, the undissociated sorbic acid will readily pass through the cellular membrane of the microorganisms. As the intracellular pH of the microorganisms is neutral, the sorbic acid will be ionized into an anion. This anion will eventually increase the pH of the microorganisms intracellularly causing them to become inactive and no more cellular activity can occur (Mehyar, Al-Qadiri, Abu-Blan, & Swanson, 2011). The inactivation of the microorganism will result in its inhibition.

Table 5 shows the antimicrobial properties of sodium benzoate on the growth of selected bacteria (log CFU/mL) using the pour plate method. From the table, each of the concentrations of sodium benzoate tested on different types of bacteria showed a significant difference (p<0.05) as compared to the control, except for against *B. cereus* and *Proteus* sp. For *B. cereus*, the concentration that showed a significant difference when compared to control was 400 mg/L, while for *Proteus* sp. significant difference was shown at a concentration of 200 mg/L. Sodium benzoate was most efficient in inhibiting the growth of *S. aureus* as compared to other tested bacteria, as it showed a significant difference when compared to control at 100 mg/L. This result agrees with the findings of Oladapo, Akinyosoye, and Abiodun, (2014) who found that *S. aureus* could be inhibited at 125 mg/L of sodium benzoate. From the results obtained, it is concluded that all the tested bacteria were sensitive to sodium benzoate except for *B. cereus* and *Proteus* sp. It is also noteworthy that vegetative *B. cereus* bacterial cells may have produced spores as a survival strategy in response to adverse environmental conditions (Yossa *et al*., 2017). *B. cereus* spores are widely recognized as a major concern in the food industry (Setlow, 2014). The antimicrobial properties act on various stages of the life cycle, including spore germination, outgrowth, and vegetative cell division. Gould (1964) reported that sodium sorbate (0.15-0.5 mg/L) allowed some elongation of the cells of bacilli to occur but prevented their division.

Table 4. Anti-bacterial properties of potassium sorbate on the growth of selected bacteria (in log CFU/mL) determined using pour plate method

Species		Concentrations (mg/L)				
		0	100	200	300	400
Bacillus cereus	Surviving number of colonies (log CFU/mL)	3.670 ± 0.030^{a}	2.829 ± 0.036^{b}	2.748 ± 0.028^{bc}	2.656 ± 0.034^{c}	2.424 ± 0.098^{d}
	Rate of inhibition (%)		22.916 ± 0.051^{d}	25.122 ± 0.231^{c}	27.629 ± 0.011^{b}	33.951 ± 0.050^{a}
Bacillus subtilis	Surviving number of colonies (log CFU/mL)	3.121 ± 0.072^{a}	2.167 ± 0.112^{b}	1.916 ± 0.078^{c}	1.752 ± 0.046^{c}	1.418 ± 0.102^{d}
	Rate of inhibition (%)		30.567 ± 0.221^{d}	38.609 ± 0.091^{c}	43.864 ± 0.019^{b}	54.566 ± 0.011^{a}
Staphylococcus aureus	Surviving number of colonies (log CFU/mL)	3.526 ± 0.054^{a}	2.760 ± 0.042^{b}	2.653 ± 0.025^{bc}	2.553 ± 0.067^{c}	2.403 ± 0.036^{d}
	Rate of inhibition (%)		21.724 ± 0.061^{d}	24.759 ± 0.044^{c}	27.595 ± 0.081^{b}	31.849 ± 0.072^{a}
Klebsiella pneumoniae	Surviving number of colonies (log CFU/mL)	3.103 ± 0.082^{a}	2.557 ± 0.066^{b}	2.390 ± 0.053^{c}	2.174 ± 0.049^{d}	2.054 ± 0.022^{d}
	Rate of inhibition (%)		17.596 ± 0.073^{d}	22.978 ± 0.081^{c}	29.939 ± 0.062^{b}	33.806 ± 0.033^{a}
Proteus sp.	Surviving number of colonies (log CFU/mL)	3.736 ± 0.032^{a}	3.596 ± 0.007^{b}	3.357 ± 0.016^{c}	3.098 ± 0.035^{d}	2.971 ± 0.026^{e}
	Rate of inhibition (%)		3.747 ± 0.021^{a}	10.145 ± 0.029^{c}	17.077 ± 0.055^{b}	20.476 ± 0.033^{a}
Salmonella typhi	Surviving number of colonies (log CFU/mL)	3.385 ± 0.077^{a}	2.879 ± 0.024^{b}	2.778 ± 0.026^{b}	2.517 ± 0.040^{c}	2.111 ± 0.067^{d}
	Rate of inhibition (%)		14.948 ± 0.041^{d}	17.932 ± 0.052^{c}	25.643 ± 0.072^{b}	37.647 ± 0.091^{a}
Escherichia coli	Surviving number of colonies (log CFU/mL)	3.419 ± 0.058^{a}	3.001 ± 0.011^{b}	2.860 ± 0.019^{c}	2.680 ± 0.032^{d}	2.387 ± 0.078^{e}
	Rate of inhibition (%)		12.226 ± 0.035^{d}	16.350 ± 0.033^{c}	21.615 ± 0.065^{b}	30.184 ± 0.088^{a}

Data are expressed as mean ± SD, (n = 3). Means that do not share the same superscript within the same row are significantly different (p < 0.05).

Table 5. Antibacterial properties of sodium benzoate on the growth of selected bacteria (in log CFU/mL)

Species		Concentrations (mg/L)				
		0	100	200	300	400
Bacillus cereus	Surviving number of colonies (log CFU/mL)	3.670 ± 0.030^{a}	3.497 ± 0.008^{a}	3.421 ± 0.015^{ab}	3.332 ± 0.011^{ab}	2.714 ± 0.597^{b}
	Rate of inhibition (%)		4.713 ± 0.011^{d}	6.785 ± 0.033^{c}	9.210 ± 0.034^{b}	26.049 ± 0.094^{a}
Bacillus subtilis	Surviving number of colonies (log CFU/mL)	3.121 ± 0.072^{a}	2.896 ± 0.044^{b}	2.753 ± 0.029^{c}	2.656 ± 0.030^{c}	2.491 ± 0.014^{d}
	Rate of inhibition (%)		7.209 ± 0.052^{d}	11.791 ± 0.079^{c}	14.899 ± 0.104^{b}	20.186 ± 0.094^{a}
Staphylococcus aureus	Surviving number of colonies (log CFU/mL)	3.526 ± 0.054^{a}	3.235 ± 0.016^{b}	3.093 ± 0.023^{c}	2.876 ± 0.035^{d}	2.701 ± 0.039^{e}
	Rate of inhibition (%)		8.253 ± 0.054^{d}	12.280 ± 0.088^{c}	18.434 ± 0.074^{b}	23.398 ± 0.059^{a}
Klebsiella pneumoniae	Surviving number of colonies (log CFU/mL)	3.103 ± 0.082^{a}	2.826 ± 0.026^{b}	2.719 ± 0.021^{b}	2.551 ± 0.043^{c}	2.270 ± 0.036^{d}
	Rate of inhibition (%)		8.927 ± 0.045^{d}	12.375 ± 0.064^{c}	17.789 ± 0.114^{b}	26.845 ± 0.124^{a}
Proteus sp.	Surviving number of colonies (log CFU/mL)	3.736 ± 0.032^{a}	3.668 ± 0.012^{a}	3.542 ± 0.042^{b}	3.310 ± 0.024^{c}	3.050 ± 0.030^{d}
	Rate of inhibition (%)		1.820 ± 0.044^{d}	5.192 ± 0.106^{c}	11.403 ± 0.124^{b}	18.362 ± 0.111^{a}
Salmonella typhi	Surviving number of colonies (log CFU/mL)	3.385 ± 0.077^{a}	2.994 ± 0.040^{b}	2.715 ± 0.034^{c}	2.467 ± 0.017^{d}	2.317 ± 0.084^{d}
	Rate of inhibition (%)		11.551 ± 0.094^{a}	19.793 ± 0.081^{c}	27.120 ± 0.074^{b}	31.551 ± 0.154^{a}
Escherichia coli	Surviving number of colonies (log CFU/mL)	3.419 ± 0.058^{a}	3.064 ± 0.021^{b}	2.934 ± 0.018^{c}	2.910 ± 0.022^{c}	2.664 ± 0.046^{d}
	Rate of inhibition (%)		10.383 ± 0.081^{d}	14.185 ± 0.024^{c}	14.887 ± 0.094^{b}	22.082 ± 0.094^{a}

Data are expressed as mean ± SD, (n = 3). Means that do not share the same superscript within the same row are significantly different ($p < 0.05$).

Possible sites of action by sorbate include: (1) the cell wall, cytoplasmic membrane and mechanisms associated with transport systems required to maintain a viable and active cell; (2) any number of enzymes necessary in metabolic pathways; and (3) genetic material such as DNA, RNA, and ribosomes.

The number of colonies of all bacteria and fungi found in this study remained at an acceptable level of microbial number counts in foods, based on Food Standards Australia New Zealand (2016) and the European Commission (2012). For *S. aureus*, *Bacillus*, and other pathogens, the satisfactory level is $<10^3$ CFU/g. For Salmonella, the satisfactory level is not detected in 25 g of food sample. For *E. coli*, the satisfactory level is $<10^2$ CFU/g. Therefore, potassium sorbate and sodium benzoate were effective against *S. Typhi*, *E. coli*, *K. pneumoniae*, (Gram-negative bacteria), *S. aureus*, and *B. subtilis* at 100 mg/L , and against *B. cereus* and *Proteus* sp. at 300 mg/L, since these concentrations reduce the numbers of bacteria and fungal colonies significantly from the control. As mentioned before, Malaysia Food Regulatory Act 1985 allows up to 350 mg/L and 450 mg/L of sodium benzoate and potassium sorbate in beverages and fruit jams. However, a lower concentration of both preservatives can be recommended for use in food, based on these significant reductions in all tested bacteria.

The antimicrobial mechanism of sodium benzoate was similar to that of potassium sorbate. However, the pH range for its effectiveness is much narrower as compared to potassium sorbate. It is only functional as a preservative agent at a pH of approximately 3.0 to 4.5, making it less efficient than potassium sorbate (Stopforth, Sofos, & Busta, 2005). A limitation of this study is that the pH of sodium benzoate and potassium sorbate at each diluted concentration applied during the antimicrobial test was not determined, which might have contributed to more conclusive results. In addition, the antimicrobial properties of potassium sorbate and sodium benzoate were determined using the molten agar dilution method. This technique is suitable for both antibacterial and antifungal susceptibility testing (Balouiri, Sadiki, & Ibnsouda, 2016). The temperature of the molten agar is hypothesized to have a synergistic effect that broadens the spectrum of antimicrobial action. In this study, the preservative was added to the molten agar at a temperature above room temperature, just before the agar solidified. However, we did not record the exact temperature at this stage, which is considered another limitation of our study.

4. Conclusions

It can be concluded that potassium sorbate and sodium benzoate exhibited good antimicrobial properties against Gram-negative and Gram-positive bacteria as well as against the fungi tested in the study. Potassium sorbate and sodium benzoate were effective against all tested fungi at concentrations as low as 100 mg/L since each concentration reduced the numbers of bacteria and fungal colonies significantly. These results would assist food industry to use antimicrobial agents, such as potassium sorbate and sodium benzoate at allowable levels in products that are easily contaminated with bacteria and fungi. Furthermore, it will benefit the industries to decide on suitable preservatives to be used in their food products to inhibit different microorganisms. However, the efficacy of these preservatives was not tested at lower pH levels where they might exhibit different efficiency, and this could be explored in further studies. The effect of these preservatives on antimicrobial properties in the food matrix is also necessary for application in food products. Spore germination and mycelium inhibition tests should be included to get a more conclusive result on the

antifungal properties of those preservatives. In short, the minimum level of the use of both preservatives in food products can be further refined in the legislation.

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