



ORIGINAL ARTICLE

Antimicrobial activity of different sodium and potassium salts of carboxylic acid against some common foodborne pathogens and spoilage-associated bacteria



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Abstract Cleaning and disinfection represent the most important activities associated with the elimination of dirt and microorganisms at food processing plants. Improper procedures may lead to cross contamination of food leading to its spoilage or even the transmission of foodborne pathogens. Several strategies have been used in order to achieve a good disinfection of surfaces and products; nevertheless, microbial resistance to common-use-products has developed lately. Due to this fact, the development of new non-toxic-food compatible chemical agents that reduce the impact of foodborne pathogens and spoilage causing microorganisms is desirable for the food industry. The objective of the present study was to evaluate the antimicrobial activity of different sodium and potassium salts of aliphatic and aromatic carboxylic acid on the growth of common food spoilage and pathogenic microorganisms. Growth curves were determined for *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Enterococcus faecalis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella* Enteritidis, and *Listeria monocytogenes* in contact with different concentrations of carboxylic acid salts. The inhibitory effect of both aliphatic and aromatic carboxylic acid salts, in accordance with concentration levels, was 100 > 50 > 25 mg/ml. The inhibitory effect of aliphatic salts was butanoic > hexanoic > octanoic > decanoic and, benzoic > gallic > caffeic acid salts for aromatic salts. In general, sodium salts were more inhibitory than potassium salts ($p \leq 0.05$).

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PALABRAS CLAVE

Actividad
antimicrobiana;
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Patógenos
alimentarios;
Deterioro

Actividad antimicrobiana de diferentes sales de ácidos carboxílicos de sodio y potasio contra algunos microorganismos patógenos y de deterioro asociados a alimentos

Resumen La limpieza y la desinfección representan las actividades más importantes asociadas a la eliminación de suciedad y microorganismos de las plantas procesadoras de alimentos. El uso de procedimientos incorrectos puede llevar a la contaminación cruzada de los alimentos y, por ende, al deterioro de estos o a la transmisión de patógenos de origen alimentario. Se han desarrollado varias estrategias con el fin de obtener una buena desinfección de superficies y productos; no obstante, ha aparecido resistencia microbiana frente a productos de uso común. Debido a esto, el desarrollo de agentes químicos no tóxicos capaces de reducir el impacto de patógenos de origen alimentario y microorganismos causantes de deterioro es deseable para la industria alimentaria. El objetivo de este estudio fue evaluar la actividad antimicrobiana de diferentes sales de sodio y potasio de ácidos carboxílicos alifáticos y aromáticos sobre algunos microorganismos patógenos y asociados a deterioro alimentario, analizando su impacto sobre el crecimiento. Se determinaron las curvas de crecimiento de *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Enterococcus faecalis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* y *Listeria monocytogenes* en presencia de diferentes concentraciones de las sales de ácidos carboxílicos. El efecto inhibitorio de las sales de ácidos carboxílicos alifáticos y aromáticos, según su nivel de concentración, se ordenó del siguiente modo: 100 mg/ml > 50 mg/ml > 25 mg/ml. El efecto de las sales de ácidos alifáticos siguió el orden butanoico > hexanoico > octanoico > decanoico, en tanto que las de ácidos aromáticos se ordenó del siguiente modo: benzoico > gálico > cafeico. En general, las sales de sodio fueron más inhibitorias que las de potasio ($p \leq 0,05$).

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Introduction

Cleaning and disinfection represent the most important activities associated with the elimination of dirt and microorganisms at food processing plants. Improper procedures may lead to cross contamination of food leading to its spoilage or even the transmission of foodborne pathogens⁴. Several strategies have been used in order to achieve a good disinfection of surfaces and products; nevertheless, microbial resistance to common-use-products has developed lately, even though microorganisms vary greatly in their resistance to chemical germicides and sterilization processes⁷. Due to this fact, the development of new non-toxic-food compatible chemical agents that reduce the impact of foodborne pathogens and spoilage-causing microorganisms is desirable for the food industry^{5,17}.

Recently, different researchers have described the inhibitory action of short chain organic acids and/or their salts on the growth of bacteria and molds²⁵, including pathogens^{22,24}. These products are generally recognized as safe (GRAS) compounds frequently used as chemical decontaminants¹⁸.

Acetate, lactate and citrate sodium salts have shown an inhibitory effect on the growth of some food spoilage bacteria and antimicrobial activity against foodborne pathogens, including *Staphylococcus aureus*

and *Yersinia enterocolitica*¹¹, *Listeria monocytogenes*¹⁵, *Escherichia coli*^{11,12}, as well as *Clostridium botulinum*¹. Moreover, a limited antimicrobial capacity has been reported for some organic acid salts against lactic acid bacteria during meat spoilage^{6,14}.

Because of the association of salt consumption with the risk of hypertension, health promoters advise a lower intake of this compound; nevertheless, there has been controversy regarding the effect of salt removal or reduction on the shelf life and safety of food products. Potassium salt intake is actually promoted by the World Health Organization (WHO), and the new guidelines advise adults to consume less than 5 g of salt and at least 3.510 mg of potassium per day in order to reduce the risk of high blood pressure²³.

Potassium salts have also been tested for their antimicrobial effect. Some researchers have described inhibitory effects on pathogenic bacteria, including *L. monocytogenes*, *Vibrio parahaemolyticus* and *Clostridium perfringens*¹; nevertheless, there is still controversy over the subject.

The objective of the present study was to evaluate the inhibitory effect of different sodium and potassium organic salts on the growth of common food spoilage and pathogenic microorganisms including *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Enterococcus faecalis*, *Candida albicans*, *Pseudomonas aeruginosa*, *Salmonella* Enteritidis and *L. monocytogenes*.

Materials and methods

General chemical methods

All glassware and syringes were dried in an oven at 140 °C overnight and flushed with nitrogen immediately prior to use. Tetrahydrofuran (THF) was refluxed and freshly distilled from potassium/benzophenone ketyl under a nitrogen atmosphere.

The reagents used included sodium hydride, potassium hydride, butanoic acid, hexanoic acid, octanoic acid, decanoic acid, benzoic acid, caffeic acid and gallic acid. All the reagents were used without any further purification.

Preparation of sodium carboxylic acid salts

NaH (60% in mineral oil) was poured (0.728 g) into a round bottom flask and kept under a nitrogen atmosphere. Dry THF (20 ml) was added to the flask and stirred; after a few minutes NaH was allowed to settle. The supernatant liquid was removed with a double-tipped cannula under nitrogen and the procedure repeated. The remaining THF was evaporated under vacuum to obtain a dry white solid (0.542 g, 22.6 mmol NaH).

Dry THF (20 ml) was added to the pure NaH obtained as above, and the temperature lowered to 0 °C. Butyric acid (1.99 g, 22.6 mmol), dissolved in THF (20 ml), was added dropwise with stirring in about 15 min. The ice bath was removed and the suspension stirred for 18 h under a nitrogen atmosphere. The solvent was eliminated in a rotary evaporator and the sodium salt was obtained as a white solid.

Potassium salts were prepared using KH instead of NaH, using the above procedure.

Growth curve determination

Type cultures of *L. mesenteroides* (ATCC 8293-2), *L. plantarum* (ATCC 14917), *E. faecalis* (ATCC 29212), *C. albicans* (ATCC 10231), *P. aeruginosa* (ATCC 15442), *Salmonella* Enteritidis (ATCC 13076) and *L. monocytogenes* (ATCC 19116) were kindly supplied by the Bacteriology Lab, Faculty of Microbiology, Universidad de Costa Rica.

Suspension preparation

Each microorganism was inoculated into trypticase soy broth (TSB) + yeast (Oxoid®) and cultured at 37 °C until the desired concentration was attained. The bacterial suspension to be cultured was equivalent to 0.5 McFarland standard, (1.5×10^8 CFU/ml).

Bactericidal assays

96 well tissue culture microtiter plates (Nalge, Nunc International, Rochester, NY) were used for each experiment. The assay mixture consisted of 50 µl TSB (trypticase soy broth) + yeast (Oxoid®) for all strains evaluated except *L. mesenteroides* and *L. plantarum* where MRS (Man, Rogosa and Sharpe) broth (Oxoid®) was used. Fifty (50) microliters of each bacteria suspension was used as well as 50 µl of

the chemical agent at the different concentrations to be tested (25, 50 and 100 mg/ml). Seven wells were used as positive growth control, one well was used as negative control (no bacteria added). For each bacteria tested, 4 wells of the microtiter plate were used. The first well was used to evaluate the 25 mg/ml final concentration of each chemical agent, the second well for the 50 mg/ml concentration and the third one for the 100 mg/ml concentration. The fourth well was used as positive growth control of the microorganism.

Growth curves were determined using the Biotek Synergy HT multi detection reader (Vermont, US). Protocol followed included 600 nm readings every 30 min for 24 h at 37 °C incubation temperature.

Data analysis

All experiments were performed in triplicate. Data was analyzed using the SPSS-PC program (Statistical Package for the Social Sciences) with a 95% ($p \leq 0.05$) confidence level.

Results

The antimicrobial activity of different sodium and potassium salts of aliphatic carboxylic acid was evaluated in this study (Table 1). Differences ($p \leq 0.05$) between the antimicrobial activity of sodium and potassium salts were observed for some of the microorganisms tested.

The inhibitory effect of the aliphatic acid salts according to concentration levels was $100 > 50 > 25$ mg/ml, and according to chemical nature was butanoic > hexanoic > octanoic > decanoic acid salts. The comparison of ions shows that sodium salts have a higher antimicrobial effect than potassium salts.

L. mesenteroides was inactivated by sodium and potassium butanoate and sodium and potassium hexanoate at a minimal inhibitory concentration (MIC) of 25 mg/ml. However, significant differences between sodium (MIC of 25 mg/ml) and potassium (MIC of 50 mg/ml) salts were observed in the case of the octanoic salt (Table 1). A MIC of 100 mg/ml was observed for both decanoic acid salts. Similar results were observed for *L. plantarum*.

Most of the antimicrobials were effective against *L. monocytogenes* (MIC of 25 mg/ml) with the exception of the octanoic and decanoic acid salts, where higher concentrations (50 and 100 mg/ml) were necessary to observe complete inhibition. *Salmonella* Enteritidis, *P. aeruginosa*, *E. faecalis* and *C. albicans* presented all higher MICs for sodium and potassium octanoate and decanoate salts (100 mg/ml) as well. Furthermore, *P. aeruginosa* presented higher MICs for potassium salts (50 mg/ml) than for sodium salts (25 mg/ml) when butanoate and hexanoate salts were evaluated.

The results of the antimicrobial activity of different aromatic sodium and potassium salts are presented in Table 2. There were statistical differences ($p \leq 0.05$) between the antimicrobial activity of sodium salts compared to potassium salts.

The antimicrobial effect of all aromatic salts tested according to concentration levels was $100 > 50 > 25$ mg/ml, and the antimicrobial effect according to chemical nature

Table 1 MICs of the different aliphatic salts tested against seven different microorganisms

Organic acid salt	Minimal inhibitory concentration (mg/ml)						
	<i>Leuconostoc mesenteroides</i>	<i>Lactobacillus plantarum</i>	<i>Listeria monocytogenes</i>	<i>Salmonella Enteritidis</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Candida albicans</i>
Sodium butanoate	≤25	≤25	≤25	≤25	≤25	≤25	≤25
Potassium butanoate	≤25	≤25	≤25	≤25	50*	≤25	50
Sodium hexanoate	≤25	≤25	≤25	≤25	≤25	≤25	≤25
Potassium hexanoate	≤25	50*	≤25	≤25	50*	≤25	≤25
Sodium octanoate	≤25	≤25	≤25	100	100	100	100
Potassium octanoate	50*	50*	50*	100	100	100	100
Sodium decanoate	100	100	100	100	100	100	100
Potassium decanoate	100	100	100	100	100	100	100

* MIC values from the same column are statistically different ($p \leq 0.05$).

was benzoic > gallic > caffeic salts. The comparison of ions shows that sodium salts have the same activity as potassium ions for benzoic salts; however, a higher antimicrobial activity was observed for the sodium salts ($p \leq 0.05$) in the case of gallic and caffeic acids. All microorganisms tested presented a MIC of 25 mg/ml for sodium and potassium benzoates. In the case of the sodium gallic salt, MICs of 25–50 mg/ml were observed for all the microorganisms and higher concentrations of caffeic acid sodium salt (100 mg/ml) were necessary to accomplish complete inhibition of all the microorganisms with the exception of *L. mesenteroides* (25 mg/ml). MIC for potassium gallate was of 100 mg/ml for all the microorganisms

evaluated and the potassium salt of caffeic acid did not inhibit any of the microorganism tested (Table 2).

Discussion

Food product contamination can occur through different environmental routes, including air, water, people and even surfaces. The latter route is the most important one to control for any sanitation program²⁰.

The increase in antimicrobial resistance has been attributed to the inadequate use of antimicrobial compounds and the transference of resistance genes within and

Table 2 MICs of the different aromatic salts tested against seven different microorganisms

Organic acid salt	Minimal inhibitory concentration (mg/ml)						
	<i>Leuconostoc mesenteroides</i>	<i>Lactobacillus plantarum</i>	<i>Listeria monocytogenes</i>	<i>Salmonella Enteritidis</i>	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Candida albicans</i>
Sodium benzoate	25	25	25	25	25	25	25
Potassium benzoate	25	25	25	25	25	25	25
Sodium caffeinate	25	100	100	100	100	100	100
Potassium caffeinate	>100*	>100	>100	>100	>100	>100	>100
Sodium gallate	25	25	50	50	25	25	25
Potassium gallate	100*	100*	100*	100*	100*	100*	100*

* MIC values from the same column are statistically different ($p \leq 0.05$).

between strains³. The development of novel, non-toxic-food compatible chemical products could help to reduce the presence of pathogens and spoilage microorganisms from environments, including surfaces, equipment and even workers' hands.

The results obtained in this work for sodium and potassium salts of aliphatic carboxylic acid show that the antimicrobial activity of these compounds is related to the number of carbon atoms. High antimicrobial activity was observed for compounds with shorter carbon chains such as butanoate and hexanoate. Raftari et al.¹⁶ reported that the lethal effect of organic acids increases with chain length until a cut off phenomenon occurs. Kim et al.⁹, and Kubo et al.¹⁰ reported similar results for phenolic acids and for alkenals, respectively. However, other reports in the literature established that short and medium chain fatty acid salts were more germicidal at lower pH values than long chain molecules²¹. In our study, the average pH value of the experimental matrix (TSA or MRS broth) was 5.9. Lower antimicrobial activity of long chain fatty acid salts at acidic conditions could be correlated with a decrease in solubility²¹. These results indicate that short chain fatty acid salts could be efficient antimicrobials in food products with an acidic pH.

Other studies have also reported the antimicrobial activity of organic acid salts. Nakai et al. describe a MIC for *L. plantarum* of 11.4 mg/ml using caproic acid, coinciding with the value reported in this study for sodium and potassium hexanoate¹³. The inhibition of these bacteria with different organic acid salt treatments, including sodium acetate, sodium lactate and sodium citrate has been reported in sliced salmon⁸.

The highest MICs were found in *Pseudomonas*. The MIC obtained with octanoate and decanoate sodium and potassium salts was of 100 mg/ml or higher; nevertheless smaller chain salts presented MICs varying from 25 to 50 mg/ml. *L. plantarum* and *P. aeruginosa* showed a decreased susceptibility to potassium salts even with the short chain fatty acids. These data indicate that the antimicrobial effect of fatty acid salts is dependent on the type of microorganism.

In the case of foodborne pathogens, *Salmonella* Enteritidis and *L. monocytogenes* growth was prevented or inhibited by all the salts tested. Raftari et al.¹⁶ have also described a significant decrease in populations of *Salmonella typhimurium* and *L. monocytogenes* after spraying meat tissues with formic, lactic, acetic and propionic acid. Same behavior is described by Silva et al. when they tested sodium lactate and sodium propionate, combined with sodium acetate in soft cheeses¹⁹. Sodium and potassium salts of fatty acids could be a practical option to control both spoilage and pathogenic microorganisms in different food products.

Phenolic products have a basic skeleton; nevertheless the number and position of the hydroxyl groups on the aromatic ring, as well as the type of substituents cause significant changes in their properties²⁵. The site and number of hydroxyl groups in the phenol groups are thought to be related to their toxicity to microorganisms with higher antimicrobial capacity observed with higher hydroxylation¹⁶. Moreover, some authors have reported that more highly oxidized phenols have higher antimicrobial properties¹⁶.

Antimicrobial activity of phenolic products may be due to the destabilization and permeabilization of the cytoplasmic membrane and enzyme inhibition. Phenols can also inhibit the synthesis of nucleic acids of both gram negative and gram positive bacteria⁴. In this study, sodium gallate showed a MIC of less than 25 mg/ml for all microorganisms tested with the exception of *L. monocytogenes* and *Salmonella* spp (50 mg/ml). These results agree with a previous report by Borges et al., where gallic acid presented a MIC of 0.5 mg/ml for *P. aeruginosa* and 20 mg/ml for *L. monocytogenes*². The potassium salts of gallic acid showed MICs of 100 mg/ml and higher for all the microorganisms evaluated. In the case of sodium salts of caffeic acid, a low MIC (25 mg/ml) was observed for *L. mesenteroides*; however, a low antimicrobial activity was reported for the other microorganisms evaluated (MIC = 100 mg/ml). A similar situation was observed for potassium caffeinate. These results contrast with the low MICs obtained for sodium gallic salts. This difference may be due to the fact that gallic acid is a more highly hydroxylated phenol than caffeic acid¹⁶.

Sanchez Maldonado et al.¹⁸ reported MIC values similar to our study for different compounds including benzoic, caffeic and gallic acids. These authors found that gram positive bacteria are generally more resistant than gram negative microorganisms and that the increase in the number of hydroxyl groups increased the inhibitory activity of hydroxybenzoic and hydroxycinnamic acids. They also stated that the substitution of hydroxyl groups with methoxy groups increased the activity of hydroxybenzoic acids but not of hydroxycinnamic acid.

Interestingly, sodium gallic and caffeic salts presented lower MICs when compared with potassium salts, a fact that contrasts with Borges et al. results², which establish that potassium chloride has an equivalent antimicrobial effect over some pathogens such as sodium chloride if it is calculated on a molar basis. Moreover, Wang et al.²² have emphasized that the chemical structure and composition of the phenolic products is a determinant of the biological function of the product.

The chemical compounds analyzed in this project show promising results; however, it is important to take into account that MIC results may vary in diverse studies reported in the literature. This discrepancy is due to the different methods used for the determination of antimicrobial activity combined with the use of different microorganisms and strains. Furthermore, it is well known that no strain can represent the behavior of a complete species⁶.

The main objective of this study is to devise chemical products that can reduce pathogens and spoilage microorganisms in foods. The compounds analyzed in this study showed promising results; nevertheless, further studies have to be done to study their safety, solubility, sensory properties (including color and flavor) as well as their stability on different food matrices.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that they have followed the protocols of their work centre on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Conflict of interest

The authors declare that they have no conflicts of interest.

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