

ANTIMICROBIAL ACTIVITY ASSESSMENT OF FOOD PRESERVATIVES CONTAINING ORGANIC CARBOXYLIC ACIDS

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Summary

Introduction. Microbial spoilage of food products has been a perennial problem. According to the WHO, the world records between 68.4 to 275 million cases of acute infectious diarrhea annually. The microbiological safety of food products is crucial as one of the criteria for their quality.

Aim. Antimicrobial activity detection of organic carbonic acids against the most common causative agents of food-borne toxic infections.

Materials and methods. The antimicrobial action detection of organic carbonic acids was carried out on bacterial strains *S. aureus*, *S. typhimurium*, *E. coli*, *P. vulgaris*, *C. freundii*, *K. pneumoniae*, *P. aeruginosa* by the dilution method in tryptone-soy broth and in a buffer solution of sodium chloride with peptone (pH 7.0). The minimum inhibitory concentration (MIC) was determined by the highest value from three determinations for each culture taken in the study. The pH of the medium was determined using a portable pH meter AMT16V (Amtast USA Inc).

Results. The growth of all bacterial species, that were used in the study, stopped in tryptone-soy broth with a formic acid content of 0.0625 %, and the concentration of acetic acid for these microorganisms ranged from 0,0625 % to 0,125 %. Propionic and lactic acids inhibited the growth of the vast majority of investigated bacterial species at the same concentration (0,125 %). Citric acid stopped the growth of *Salmonella* spp. and *Klebsiella* spp., at a concentration of 0,5 %. Therefore, *S. aureus*, *P. vulgaris*, and *C. freundii* demonstrated the highest level of sensitivity to the action of organic acids. On the other hand, *Salmonella* spp. were the least sensitive to the acid action, as propionic and lactic acids inactivated them at a concentration of 0,25 %, but citric acid at 0,5 % one.

Conclusions. Organic carboxylic acids demonstrate sufficiently high antimicrobial activity and inhibit the growth of bacteria which are the most common causative agents of food-borne infections.

Keywords: organic carboxylic acids, antimicrobial activity, opportunistic microorganisms

INTRODUCTION

Microbial spoilage of food products has been a perennial problem, the final solution to which is probably impossible, as microorganisms are an integral part of many of them. At the same time, microbial contamination of food products causes not only significant economic losses due to their spoilage but also poses a direct threat to human health. The microbiological safety of food products is a key criterion in assessing their quality since the number of infections, which are transmitted through the consumption of food contaminated with dangerous microorganisms, increases every year. According to the WHO, there are from 68,4 to 275 million cases of acute diarrhea infections registered annually worldwide, more or less related to the consumption

of germ-contaminated products. Almost 2 million people worldwide die each year from food poisoning [8].

Microorganisms can contaminate a food product at various stages of its production: during manufacturing, raw material processing, transportation, storage, and more. World organizations, that are responsible for food safety issues, develop and implement regulatory documents that standardize the production of a particular food product at different stages, general hygiene measures for product safety, and the microbiological quality of raw materials. One of the most important measures aimed at ensuring the microbiological safety of food products provided by international regulations is the use of food additives, namely preservatives [4, 9]. In Ukraine, more than 300

technological additives are allowed for use, and their application and implementation are regulated by the following regulatory and technical documents: the Law of Ukraine «On Sanitary and Epidemiological Well-being of the Population»; «Sanitary Rules and Norms for the Use of Food Additives» (Order of the Ministry of Health of Ukraine No. 222 dated July 23rd, 1996, with amendments); the Law of Ukraine «On the Basic Principles and Requirements for the Safety and Quality of Food Products» [15]. However, after the adoption of the Law of Ukraine «On the Ratification of the Protocol on the Accession of Ukraine to the World Trade Organization» dated April 10th, 2008, No. 250-VI, the use of food additives by EU Regulation No. 1331/2008 of the European Parliament and of the Council of December 16, 2008, is allowed for joint use [13].

Preservatives are substances of natural or synthetic origin capable of inhibiting microbial growth and reproduction. The main purpose of their use is to limit the reproduction of microorganisms in a food product and prevent its microbial spoilage. In the case of contamination of the product with toxigenic microorganisms, preservatives must prevent their accumulation in dangerous number. There are some general requirements to this group of food additives such as safety for humans, maximum effectiveness in small concentrations, and the absence of a negative impact on the taste qualities of the product. According to the European digital classification of food additives, preservatives belong to the indexes E200–E299. Among them, chemical compounds such as sulfites (E220–229), nitrates (E240–259), phenols, and formates (E230–239) are used. Taking into account possible negative effects on human health, in some countries the national legislation restrict the use of definite substances from this list.

In most countries the most commonly used food preservatives are organic carboxylic acids and their salts, namely: formic, acetic, citric, lactic, and propionic acids. Mostly these compounds are products of normal cellular metabolism, and therefore they are relatively safe for human health. Some of them are used as antibacterials in medical practice [1].

Formic acid has the simplest chemical structure (HCOOH) and is the first member of a group of saturated monocarbon carboxylic acids. It has the highest dissociation coefficient among the organic acids, which are mentioned above. According to the Codex Alimentarius classification system, it is registered as a food additive E236. It is mainly used in the canning industry for strongly acidic products. Its use is limited by a strong odor and the ability to precipitate pectin substances, which blocks drag formation.

There is one more carbon atom in the chemical structure of acetic acid (E260). It also has a strong odor and is widely used in cooking. In the food industry, it is mainly used in the production of canned pickled vegetables and other products. It is also used in mayonnaise, sauces, and at pickling of fish products.

Propionic acid (E280) is also an organic acid with one carboxyl group and one more carbon atom in its hydrocarbon radical compared to acetic acid. A fairly sharp unpleasant odor characterizes it. In the United States and many European countries, it is used as a primary preservative in bakery technologies and the production of confectionery products.

Lactic acid is an alpha-hydroxypropionic oxycarbonic acid with one carboxyl group that differs from propionic acid only by the presence of a hydroxyl group near one of the carbon atoms in the molecule. This feature gives the molecule optical activity, and therefore, lactic acid can exist as L(+) and D(–) isomers as well as the racemate. Unlike propionic acid, lactic acid has no unpleasant odor. Lactic acid is an important intermediate product in the metabolism of living organisms and the end product of lactic acid fermentation, caused by lactic acid bacteria and some other microorganisms. It is naturally present in fermented products and pickled vegetables. It is indexed as L-, D-, and D, L-lactic acid, designated as E270 in the European food additive classification according to the Codex Alimentarius system. It is used as a preservative in confectionery, meat products, and sauces.

In the European food additive classification, citric acid is placed into the group of antioxidants and indexed as E330. Unlike the previous monocarboxylic acids, citric acid is tricarboxylic one and has the most carbon atoms in its chemical structure. Similar to lactic acid, it contains a hydroxyl group near one of the carbon atoms. It is used in citrus beverages, especially in orange, lemon, and lime drinks, as well as in fruit juices, concentrates, and syrups when the natural acid content of fruits is insufficient. Additionally, citric acid is an integral component of many confectionery products, chewing gums, desserts, ice cream, jams, added to mayonnaise, and used in cheese production.

The antimicrobial mode of organic acids action remains poorly understood. Some Ukrainian researchers associate their activity with the ability to influence membrane permeability and the integrity of bacterial cells. Additionally, depending on the degree of their dissociation, carboxylic acids can decrease intracellular pH by releasing hydrogen protons, thereby increasing acidity inside the cell resulting in blockage of metabolic processes. This mechanism of antimicrobial action is typical for organic acids with a high degree of dissociation, such as formic acid. The acids with a low degree of dissociation (such as propionic and acetic acids) can form salts with potassium and sodium ions in cell membranes, which disintegrates bacterial cell membranes and their subsequent destruction. The accumulation of toxic anions of organic acids inside bacterial cells violates DNA replication, thus preventing bacterial multiplication [7].

In any case, the potency of the antimicrobial effect depends on the chemical structure of the organic acid. However, scientific data, that characterizes the

antimicrobial activity of organic acids depending on their chemical structure, is poor.

AIM

Therefore, our study aimed to determine the antimicrobial activity of formic, acetic, citric, lactic, and propionic acids against opportunistic bacteria, which are common causes of food-borne infections.

MATERIALS AND METHODS

Clinical isolates of the species *S. aureus*, *S. typhimurium*, *E. coli*, *P. vulgaris*, *C. freundii*, *K. pneumoniae*, and *P. aeruginosa* were used in the research. Bacterial cultivation was carried out in tryptic soy broth and in a buffered solution of sodium chloride with peptone (pH 7,0), manufactured by Graso Biotech (Poland). The determination of bacterial sensitivity to the organic acids was conducted with a dilution test in a liquid nutrient medium [12]. The minimum inhibitory concentration was determined based on the highest value from three measurements for each strain taken in the study.

The pH of the medium was determined with a portable pH meter AMT16V (Amtast USA Inc), which provides an accuracy level of ± 0.02 pH. The device was calibrated in pH units using standard buffer solutions.

RESULTS

It is well known that most causative agents of food-borne infections actively reproduce in a nutrient medium

with pH values around 7,2-7,4, and they maintain viability in a pH range from 4,0 to 9,0. Table 1 presents the determined minimal inhibitory concentrations (MIC) of the investigated carboxylic acids and the corresponding pH values of the nutrient media, illustrating those above. The organic acids inhibited the growth of the studied bacterial species at a pH range between 3,32 and 4,80.

According to a dissociation degree, formic acid is a moderately strong electrolyte, while other carboxylic acids are weak electrolytes. The activity of dissociation decreases with the increase in the length of the hydrocarbon radical. The growth of all bacterial investigated strains was inhibited in tryptone-soy broth containing 0,0625 % formic acid. The addition of such a low concentration of acid decreased the pH of the medium to 3,3 from the initial 7,3.

Acetic acid inhibited the growth of Staphylococci and Proteus isolates at the same concentration as formic acid. However, the pH of the broth was higher (4,48) compared to a similar concentration of formic acid. The multiplication of other causative agents of food infections was blocked at, a concentration of acetic acid twice as high (0,125 %), although the pH of the cultivation medium only decreased to 4,44.

Propionic and lactic acids, having the same number of carbon atoms in the molecule, suppressed the multiplication of the vast majority of the investigated bacterial species at the same concentration (0,125 %), and the pH values of the environment at these concentrations were similar (4,62-4,69).

Table 1

Antimicrobial activity of organic acids for the main causes of food-borne toxic infections under normal conditions

Kind of bacteria	Indicator	Formic acid HCOOH	Acetic acid CH ₃ COOH	Propionic acid CH ₃ CH ₂ COOH	Lactic acid CH ₃ CH(OH)-COOH	Citric acid HOOC-CH ₂ -C(OH) COOH-CH ₂ COOH
S.aureus	MIC*	0,0625	0,0625	0,125	0,125	0,125
	pH	3,32	4,48	4,62	4,69	4,80
E. coli	MIC	0,0625	0,125	0,125	0,125	0,250
	pH	3,32	4,44	4,62	4,69	4,38
P.vulgaris	MIC	0,0625	0,0625	0,0625	0,125	0,125
	pH	3,32	4,48	4,70	4,69	4,80
K.pneumo-niae	MIC	0,0625	0,125	0,125	0,125	0,500
	pH	3,32	4,44	4,62	4,69	4,15
S.enterica	MIC	0,0625	0,125	0,250	0,250	0,500
	pH	3,32	4,44	4,25	4,36	4,15
C. freundii	MIC	0,0625	0,125	0,125	0,125	0,125
	pH	3,32	4,44	4,62	4,69	4,80
P. aeruginosa	MIK	0,0625	0,125	0,125	0,125	0,250
	pH	3,32	4,44	4,62	4,69	4,38

Citric acid exhibited a lower level of antimicrobial activity than other acids, and the pH values of its solutions were the highest. Growth inhibition of Salmonella spp. and Klebsiella spp. was observed only in the presence of a 0,5 % concentration of citric acid at a pH value of the

nutrient medium that was close to the pH in a twice lower concentration of propionic and lactic acids.

Certainly, the final concentration of hydrogen ions is mainly determined by the components of the food product that require preservation. The chemical

composition of the food product may exhibit buffering properties. We determined the MIC of the organic acids for the most resistant to their action species under

cultivation of buffered peptone water. The final pH, after adding preservatives, was higher. The obtained results are presented in table 2.

Table 2

Antimicrobial activity of organic acids for the main causes of food-borne toxic infections being cultivated in buffered peptone water

Kind of bacteria	Indicator	Formic acid HCOOH	Acetic acid CH ₃ COOH	Propionic acid CH ₃ CH ₂ COOH	Lactic acid CH ₃ CH(OH)-COOH	Citric acid HOOC-CH ₂ -C(OH) COOH-CH ₂ COOH
K.pneumoniae	MIC	0,0625	0,125	0,312	0,312	0,312
	pH	3,84	4,88	4,90	4,92	4,98
S.enterica	MIC	0,625	0,625	0,625	0,625	0,625
	pH	3,84	4,08	4,32	4,34	4,44
P. aeruginosa	MIC	0,0625	0,312	0,312	0,312	0,312
	pH	3,84	4,62	4,90	4,92	4,98

The MIC of formic acid for investigated isolates remained at the same level in the buffered medium as in the nutrient broth. However, it's worth noting that the pH of the solution was < 4.0. The MIC of acetic acid increased twice for Pseudomonads and fourfold for Salmonella. Nevertheless, the pH of the medium remained quite acidic (<4.8). Propionic, lactic, and citric acids in the buffered solution retained the ability to inhibit the growth of harmful bacteria in concentrations 2–4 times more, even at relatively weakly acidic pH values (4,32–4,98).

DISCUSSION

Our conducted research indicates reasonably high preserving properties of the investigated carboxylic acids. However, there are specific sensitivity features of investigated bacteria to each preservative [10]. For instance, *S. aureus*, *P. vulgaris*, and *C. freundii* demonstrated the highest sensitivity to the action of organic acids. It was detected, that those species were inhibited by 0,125 % concentration of organic acid. On the other hand, Salmonella spp. were the least sensitive to acids, as propionic and lactic acids inhibited their growth at a concentration of 0,25 %, while citric acid did at an amount of 0,5 %. This factor could explain the high incidence of salmonella in the etiological structure of food-borne toxic infections. Annually, in the European Union countries, there are more than 90,000 cases of food-borne toxic infections caused by Salmonella alone [4].

Certainly, there is an undeniable dependence on the antimicrobial action level on the pH of the medium, which changes after the addition of an acid [3]. According to our data, formic and acetic acids, which have higher dissociation constants, can be used as preservatives in lower concentrations than propionic, lactic, and citric acids. It is essential to consider the final concentration of hydrogen ions that arises in the food product after adding the preserving acid. Since high pH values require the addition of acids for preservation purposes in higher concentrations, based on our data, none of the studied causative agents of

food-borne toxic infections demonstrated the ability to grow in the presence of a preservative from the group of carboxylic acids at pH values below 4.98.

There is no single universal international standard for the organic acids concentrations as preservatives in food products. These are determined by the technological regulations of food product manufacturers or, at best, at the national regulatory level. In the United States, lactic acid is permitted for use at a concentration of 2 % in meat products and a concentration of 4,8 % for products with a high risk of *Clostridium botulinum* contamination (canned products). In our country, such clear standards are not established [11].

The lack of a unified approach is due to numerous factors that need to be considered when choosing a preservative, aside from antimicrobial activity. Priority attention is given to the impact on human health. Most chemical preservatives have a toxic effect on the human body, so their concentrations are limited by maximum permissible levels, and these concentrations do not always exert a sufficient preservative effect. For example, according to the recommendations of the Joint FAO/WHO Expert Committee on Food Additives, the acceptable daily intake of formic acid and its salts should not exceed 0,5 mg per 1 kg of body weight [14, 2].

An important factor is the impact of the preservative on the taste and odor of food products. The overall effect of taste sensations depends on both the nature of the substances and their concentrations. Organic food acids can influence both the taste and aroma of a food product. The main taste sensation from the presence of acids is sourness. According to scientific sources, the threshold concentration for perceiving a sour taste is 0,017 % for citric acid and 0,03 % for acetic acid [7]. However, an overly sour taste can spoil the edibility of the product. Reducing the acidic properties of food by adding a lower acid concentration improves the product's taste properties but does not protect it from pathogenic microflora. The sharp odor of formic and acetic acids limits their use as preservatives, and due to the unpleasant odor of propionic acid, the Joint FAO/WHO

Expert Committee on Food Additives does not consider it possible to establish a daily permissible dose for it [2].

The experimental data we obtained indicate that acetic, propionic, lactic, and citric acids can provide a sufficient preservative effect with a content in the product at a concentration of 1 %, while formic acid, even at a concentration of 0,1 %. However, when choosing a preservative, it is necessary to consider the chemical composition of the preserved product in terms of its possible influence on the final pH of a product.

CONCLUSIONS

Therefore, organic carboxylic acids exhibit sufficiently high antimicrobial activity and inhibit the growth of bacteria, which are the most common causative agents of food toxic infections, at concentrations less than 1 % in the medium for cultivation. According to the degree of antimicrobial activity, the investigated carboxylic acids are ranked in decreasing order as follows: formic > acetic > propionic > lactic > citric.

Prospects for further research. In order to develop recommendations for the technological regulations of producers of technological products, it is necessary to investigate the influence of organic acids on a wider range of microorganisms, covering not only the causative agents of foodborne infections but also other bacteria and fungi capable of causing food spoilage. In addition, precise determination of the threshold concentrations of each acid that do not alter the taste characteristics of the food product is necessary.

COMPLIANCE WITH ETHICAL REQUIREMENTS

The authors complied with all relevant ethical standards in conducting this study. The study did not use personal data of patients, animals or humans.

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The authors declare no conflict of interest. The authors received no financial support for their study.

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Резюме**ОЦІНКА ПРОТИМІКРОБНОЇ АКТИВНОСТІ ХАРЧОВИХ КОНСЕРВАНТІВ З ЧИСЛА ОРГАНІЧНИХ КАРБОНОВИХ КИСЛОТ****Надія С. Фоміна, Валентин П. Ковальчук, Ірина М. Вовк, Олександр О. Фомін, Ірина М. Коваленко**

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Вступ. Проблема псування мікробних продуктів є споконвічною. За даними ВООЗ, у світі щорічно реєструють від 68,4 до 275 млн. випадків гострих діарей інфекційної природи. Важливою є мікробіологічна безпека харчових продуктів як один із критеріїв їх якості.

Мета. Визначення рівня протимікробної активності органічних карбонових кислот щодо найбільш частих збудників харчових токсикоінфекцій.

Матеріали та методи. Визначення протимікробного ефекту органічних карбонових кислот щодо штамів бактерій *S. aureus*, *S. typhimurium*, *E. coli*, *P. vulgaris*, *C. freundii*, *K. pneumoniae*, *P. aeruginosa* проводили методом послідовних серійних розведень у триптон-соевому бульйоні та у буферному розчині хлориду натрію з пептоном (рН 7,0). Мінімальну інгібуючу концентрацію (МІК) визначали за найбільшим показником з трьох визначень для кожної взятої у досліді культури. Визначення рН середовища проводили з використанням портативного рН-метра АМТ16В (Amtast USA Inc).

Результати. Розмноження усіх використаних у дослідженні видів бактерій припинялось у триптон-соевому бульйоні із вмістом мурашиної кислоти 0,0625 %, концентрація оцтової кислоти для цих же мікроорганізмів становила від 0,0625 % до 0,125 %. Пропіонова і молочна кислоти пригнічували розмноження переважної більшості досліджених видів бактерій у однаковій концентрації (0,125 %). Лимонна кислота зупиняла ріст сальмонел і клебсіел у 0,5 % концентрації.

Отже, найвищий рівень чутливості до дії органічних кислот виявляють *S. aureus*, *P. vulgaris* та *C. freundii*. Найменш чутливими до дії кислот є сальмонели, адже пропіонова і молочна кислоти зупиняють їх розмноження у концентрації 0,25 %, а лимонної кислоти необхідно 0,5 %.

Висновки. Органічні карбонові кислоти виявляють достатньо високу протимікробну активність і зупиняють вегетацію бактерій, що є найбільш частими збудниками харчових токсикоінфекцій.

Ключові слова: органічні карбонові кислоти, протимікробна активність, умовно-патогенні мікроорганізми