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Plant Organic Acids as Natural Inhibitors of Foodborne Pathogens

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Abstract: Background: Foodborne infections affect approximately 600 million people annually. Simultaneously, many plants contain substances like organic acids, which have antimicrobial activity. The aim of this study was to examine the effects of 21 organic acids, naturally occurring in plants, on four foodborne bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica* Typhimurium) and two fungi (*Geotrichum candidum* and *Penicillium candidum*). Methods: The minimal inhibitory concentrations (MIC) of the organic acids against foodborne bacteria and in silico toxicity prediction of acids were investigated. Results: Benzoic and salicylic acids exhibit the best activity against foodborne bacteria (mean MIC < 1 mg/mL). Acetic, chlorogenic, formic, malic, nicotinic, and rosmarinic acids demonstrate slightly weaker activity (mean MICs 1–2 mg/mL). Other acids have moderate or poor activity. The effectiveness of organic acids against foodborne fungi is weaker than against bacteria. Most acids require high concentrations (from 10 to >100 mg/mL) to inhibit fungal growth effectively. The predicted LD50 of organic acids ranges from 48 to 5000 mg/kg. Those potentially safe as food preservatives (MIC < LD50) include ascorbic, chlorogenic, malic, nicotinic, rosmarinic, salicylic, succinic, tannic, and tartaric acids. The studied organic acids are not carcinogenic but many can cause adverse effects such as skin sensitization, eye irritation, and potential nephrotoxicity, hepatotoxicity, or neurotoxicity. Conclusions: Most of the investigated plant-derived organic acids exhibit good antibacterial activity and moderate or poor antifungal effects. Among 21 acids, only 9 appear to be safe as food preservatives (MIC < LD50). The relationship between MIC and LD50 is crucial in determining the suitability of organic acids as food preservatives, ensuring that they are effective against bacteria or fungi at concentrations that are not harmful to humans.

Keywords: antimicrobials; antiseptics; natural compounds

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1. Introduction

Foodborne infections are among the most common worldwide. According to the World Health Organization (WHO), approximately 600 million people suffer from foodborne illnesses annually and over 400,000 of these cases result in death. Among these infections, diarrheal diseases are the most prevalent, with around 550 million cases each year [1]. Most cases of diarrhea are caused by bacteria, particularly *Campylobacter* spp., *Escherichia coli* (enteropathogenic, enterotoxigenic, and Shiga toxin-producing), non-typhoidal *Salmonella enterica*, *Shigella* spp., and *Vibrio cholerae*. Additionally, foodborne infections can lead to invasive diseases. These are mainly caused by *Brucella* spp., *Listeria monocytogenes*, *Mycobacterium bovis*, *Salmonella* Paratyphi, and *S.* Typhi [1]. Other common foodborne bacteria include *Bacillus cereus*, *Clostridium botulinum*, *C. perfringens*, *Cronobacter sakazakii*, *Staphylococcus aureus*, and *Yersinia enterocolitica* [2].

Foodborne pathogens can be zoonotic, meaning they can be transmitted from animals to humans [3]. Metagenomic studies have shown that the occurrence of bacteria is related to the species of the animal. It was found that *Staphylococcus* and *Clostridium* are present in the feces of all livestock animals, with higher counts in chicken feces compared to cattle and pig feces. Additionally, *Bacillus*, *Listeria*, and *Salmonella* were also found in chicken feces. In cattle feces, *Bacillus*, *Campylobacter*, and *Vibrio* bacteria were detected. Furthermore, in cattle, chicken, and pig feces, other genera potentially pathogenic for humans, such as *Corynebacterium*, *Streptococcus*, *Neisseria*, *Helicobacter*, *Enterobacter*, *Klebsiella*, and *Pseudomonas*, were also identified [3].

Among fungi, there are many foodborne pathogens, including *Paecilomyces* spp., *Xerochrysium* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp., or *Alternaria* spp. [4]. Most fungi cause poisoning through the production of mycotoxins. Infections are less common, such as invasive infections in immunocompromised individuals. These can be caused by, among others, *Absidia corymbifera*, *Aspergillus fumigatus*, *Blastoschizomyces capitatus*, *Candida catenulate*, *Fusarium moniliforme*, *Geotrichum candidum*, *Monascus ruber*, *Mucor circinelloides*, *M. indicus*, *Rhizopus microspores*, *R. oryzae,* and *Saccharomyces cerevisiae,* including *S. boulardii* [5–8].

To reduce the number of pathogens in food, various preventive methods are implemented, including maintaining hygiene and using food preservatives. In many types of food, especially fermented and dairy products, lactic acid bacteria are present [9,10]. These bacteria produce bacteriocins and organic acids that inhibit the growth of other bacteria. Many products also incorporate plant parts, such as mint, sage, thyme, cardamom, and cinnamon. These plants contain essential oils that not only alter the flavor of dishes but also have antimicrobial properties [10]. Upon closer examination, many plants consumed as food contain substances with antimicrobial activity that inhibit pathogen growth. These substances include organic acids, phenols, phenolic acids, quinones, flavonoids, tannins, terpenoids, and alkaloids [11,12]. Some of these compounds have the potential to be used as natural inhibitors of foodborne pathogens. We paid particular attention to organic acids, among other reasons, due to the use of some of them in medicine. Acetic acid, lactic acid, and benzoic acid are used in wound treatment, while citric acid is used in wound treatment and root canal antisepsis [13].

The aim of this study was to examine the effects of 21 organic acids, naturally occurring in plants, on four foodborne bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica* Typhimurium) and two foodborne fungi (*Geotrichum candidum* and *Penicillium candidum*).

2. Materials and Methods

2.1. Chemicals

The following pure organic acids, acetic, aminoacetic, ascorbic, benzoic, caproic, citric, formic, fumaric, glutamic, malic, nicotinic, oleic, oxalic, palmitic, salicylic, succinic, tannic, tartaric, and valeric acid, were obtained from Warchem (Zakręt, Poland). Chlorogenic and rosmarinic acids were obtained from Sigma-Aldrich (Poznan, Poland) and octenidine dihydrochloride was obtained from Schülke and Mayr GmbH (Norderstedt, Germany).

The molecular formula and natural occurrence of the studied acids are presented in Table 1.

Table 1. Molecular formula, pH of prepared solutions, and plant occurrence of studied organic acids [11,12,14–20].

Organic Acid	Molecular Formula	pH of Prepared Solutions	Exemplary Natural Occurrence
Acetic acid	$C_2H_4O_2$	2.4	Apples, grapes, and blackberries
Aminoacetic acid	$C_2H_5NO_2$	6.2	Common amino acid
Ascorbic acid	$C_6H_8O_6$	2.5	Fruits and vegetables
Benzoic acid	$C_7H_6O_2$	3.8	Cranberries, mushrooms, anise, cherries, raspberries, and food additive (as a preservative)

Table 1. *Cont.*

Organic Acid	Molecular Formula	pH of Prepared Solutions	Exemplary Natural Occurrence
Caproic acid	$C_6H_{12}O_2$	2.7	Vegetable oils
Chlorogenic acid	$C_{16}H_{18}O_9$	4.4	Apples, pears, carrots, tomatoes, sweet potatoes, coffee, and tea
Citric acid	$C_6H_8O_7$	2.9	Fruits
Formic acid	CH_2O_2	2.3	Stinging hairs of nettles
Fumaric acid	$C_4H_4O_4$	4.5	Mosses and mushrooms
Glutamic acid	$C_5H_9NO_4$	2.9	Sunflower seeds, flax seeds, peanut, pistachio, almond, broad bean, Brussels sprout, and lentil
Malic acid	$C_4H_6O_5$	2.0	Fruits
Nicotinic acid	$C_6H_5NO_2$	4.0	Common in plants
Oleic acid	$C_{18}H_{34}O_2$	2.8	Olive oil and grape seed oil
Oxalic acid	$C_2H_2O_4$	1.8	Fruits
Palmitic acid	$C_{16}H_{32}O_2$	4.5	Seeds of beans, sunflowers, and cotton
Rosmarinic acid	$C_{18}H_{16}O_8$	5.8	Rosemary, sage, Spanish sage, basil, oregano, thyme, spearmint, and perilla
Salicylic acid	$C_7H_6O_3$	3.0	Common in plants
Succinic acid	$C_4H_6O_4$	2.4	Fruits and vegetables
Tannic acid	$C_{76}H_{52}O_{46}$	2.6	Bark of oak, beech, American chestnut, spruce, willow, witch hazel, walnut, blackberry, raspberry leaves, blueberries, sloes, rhizome of cinquefoil, hen's weed, and snakeweed
Tartaric acid	$C_4H_6O_6$	1.9	Peaches, apples, grapes, cherries, and strawberries
Valeric acid	$C_5H_{10}O_2$	2.8	Valerian rhizome and angelica root

2.2. Antibacterial and Antifungal Activity

2.2.1. Bacteria and Fungi

The research targeted six foodborne pathogens, including four bacterial strains (Gram-positive *Staphylococcus aureus* and *Listeria monocytogenes* and Gram-negative *Escherichia coli* and *Salmonella enterica* Typhimurium) and two fungal strains (*Geotrichum candidum* and *Penicillium candidum*). All strains were from the collection of the Department of Medical Microbiology at Poznań University of Medical Sciences. Strains were isolated from food or from patients with foodborne infections. Identification was carried out using Mikrolastest biochemical tests (Erba Lachema, Brno, Czech Republic) and Integral System Yeasts Plus (Liofilchem, Roseto degli Abruzzi, Italy).

2.2.2. Minimal Inhibitory Concentrations (MIC)

To determine the minimal inhibitory concentrations (MIC) of the organic acids, the microdilution method was used with 96-well plates (Nest Scientific Biotechnology, Wuxi, China). The MIC methodology was detailed in our published paper [21] and the procedures were based on previous research [22,23]. Bacteria were grown in tryptose-soy broth and fungi in Sabouraud broth (Graso Biotech, Owidz, Poland) and serial dilutions of the organic acids were made to reach final concentrations ranging from 100 mg/mL to 0.02 mg/mL in the wells. The inoculum was adjusted to achieve a final concentration of 10^5 CFU/mL. Plates were incubated at 37 °C for 24–48 h and MIC values were determined visually, using color reactions with 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma, Poznań, Poland) to aid in reading. Each test was performed in triplicate.

2.3. In Silico Bioavailability Toxicity Prediction

Toxicity predictions were conducted using SwissADME [24], Deep-PK [25], and ProTox-3.0 [26] software. Factors considered included predicted LD50, toxicity class, carcinogenicity, hepatotoxicity, neurotoxicity, nephrotoxicity, skin sensitization, and eye irritation. ProTox-3.0 was specifically chosen for predicting LD50 due to its high accuracy in previous evaluations [27].

3. Results

3.1. Antibacterial and Antifungal Activity

Studies have shown that benzoic acid and salicylic acid exhibit the best activity against foodborne bacteria, with an average MIC of less than 1 mg/mL. Acetic acid, chlorogenic acid, formic acid, malic acid, nicotinic acid, and rosmarinic acid demonstrated slightly weaker activity, with average MICs between 1 and 2 mg/mL. Many other acids (ascorbic, caproic, citric, fumaric, oxalic, succinic, tannic, tartaric, and valeric acid) had moderate activity against bacteria. The weakest antibacterial activity was observed in aminoacetic, glutamic, oleic, and palmitic acids, with average MICs ranging from 7.5 to 70.83 mg/mL. Considering that the average MIC for octenidine (positive control) is 0.11 µg/mL, it is evident that the tested organic acids are active at concentrations approximately 10,000 times higher (Table 2).

Table 2. The minimal inhibitory concentrations (MIC) of the organic acids and octenidine against foodborne bacteria. MIC values are presented in mg/mL. The last column shows the mean values and standard deviation (SD) for all readings for a given acid.

Organic Acid	*Staphylococcus aureus*	*Escherichia coli*	*Listeria monocytogenes*	*Salmonella* Typhimurium	Mean MIC ± SD for All Bacteria
Acetic acid	1.25	1.25–2.5	1.25	2.5	1.72 ± 0.65
Aminoacetic acid	100	50	50	50–100	70.83 ± 25.75
Ascorbic acid	1.25	1.25–2.5	2.5	5	2.66 ± 1.56
Benzoic acid	0.63	0.31–0.63	0.31–0.63	0.63–1.25	0.63 ± 0.29
Caproic acid	5	2.5	2.5	5	3.75 ± 1.34
Chlorogenic acid	1.25–2.5	1.25	1.25–2.5	1.25–2.5	1.72 ± 0.65
Citric acid	5	2.5	1.25–2.5	2.5	2.97 ± 1.33
Formic acid	1.25	1.25	1.25	2.5	1.56 ± 0.58
Fumaric acid	1.25–2.5	2.5	2.5–5	2.5	2.66 ± 1.04
Glutamic acid	5	5	10	10	7.50 ± 2.67
Malic acid	2.5	0.63	0.63–1.25	1.25	1.33 ± 0.78
Nicotinic acid	1.25–2.5	0.63–1.25	0.63–1.25	1.25	1.25 ± 0.56
Oleic acid	5	10	5	10	7.50 ± 2.67
Oxalic acid	2.5–5	0.63–1.25	1.25–2.5	2.5	2.27 ± 1.33
Palmitic acid	20–50	20–50	20	20–50	27.5 ± 13.57
Rosmarinic acid	2.5	1.25	1.25–2.5	1.25–2.5	1.88 ± 0.67
Salicylic acid	0.63–1.25	0.31–0.63	0.63	0.63	0.67 ± 0.26
Succinic acid	2.5–5	0.63–1.25	1.25–2.5	2.5	2.27 ± 1.33
Tannic acid	2.5–5	1.25–2.5	0.63–1.25	2.5	2.27 ± 1.33

Table 2. *Cont.*

Organic Acid	*Staphylococcus aureus*	*Escherichia coli*	*Listeria monocytogenes*	*Salmonella* Typhimurium	Mean MIC ± SD for All Bacteria
Tartaric acid	5	1.25–2.5	2.5	2.5	2.97 ± 1.33
Valeric acid	5	1.25–2.5	2.5	2.5	2.97 ± 1.33
Octenidine dihydrochloride	0.00004–0.00008 (0.04–0.08 μg/mL)	0.00008–0.00016 (0.08–0.16 μg/mL)	0.00008–0.00016 (0.08–0.16 μg/mL)	0.00008–0.00016 (0.08–0.16 μg/mL)	0.00016–0.00005 (0.11 ± 0.05 μg/mL)

In the study, MIC values were determined against two foodborne fungi, *Geotrichum candidum* and *Penicillium candidum*. For all acids tested, the antifungal activity was weaker compared to bacteria. The acetic, benzoic, caproic, chlorogenic, citric, formic, rosmarinic, and valeric acids exhibited the best activity, with mean MICs < 10 mg/mL. Fumaric, oxalic, and tannic acids showed moderate activity, with mean MICs between 10 and 20 mg/mL. Unfortunately, many acids such as ascorbic, malic, oleic, palmitic, succinic, and tartaric had very weak antifungal activity, with MICs > 20 mg/mL, while aminoacetic and glutamic acids had MICs > 100 mg/mL, indicating no activity (Table 3, Figure 1).

Table 3. The minimal inhibitory concentrations (MIC) of the organic acids and octenidine against foodborne fungi. MIC values are presented in mg/mL. The last column shows the mean values and standard deviation (SD) for all readings for a given acid.

Organic Acid	*Geotrichum candidum*	*Penicillium candidum*	Mean MIC ± SD for Both Fungi
Acetic acid	5	10	7.5 ± 2.74
Aminoacetic acid	>100	>100	>100
Ascorbic acid	50–100	100	83.33 ± 25.82
Benzoic acid	2.5–5	10	7.08 ± 3.32
Caproic acid	5	10	7.5 ± 2.74
Chlorogenic acid	1.25–5	5–10	5.63 ± 3.69
Citric acid	5–10	10	8.33 ± 2.58
Formic acid	5	10	7.5 ± 2.74
Fumaric acid	5–10	20	14.17 ± 6.65
Glutamic acid	>100	>100	>100
Malic acid	20–50	50	45 ± 12.25
Nicotinic acid	10	20	15 ± 5.48
Oleic acid	50	50	50 ± 0.0
Oxalic acid	10–20	20	16.67 ± 5.16
Palmitic acid	20–50	20–50	30 ± 15.49
Rosmarinic acid	1.25–5	10	6.46 ± 4.06
Salicylic acid	2.5–5	10	7.08 ± 3.32
Succinic acid	50–100	100	83.33 ± 25.82
Tannic acid	10–20	5–20	14.17 ± 6.65
Tartaric acid	20–50	100	70 ± 34.64
Valeric acid	5	5	5 ± 0.0
Octenidine dihydrochloride	0.00008–0.00016 (0.08–0.16 μg/mL)	0.00008–0.00032 (0.08–0.32 μg/mL)	0.00016 ± 0.00009 (0.16 ± 0.09 μg/mL)

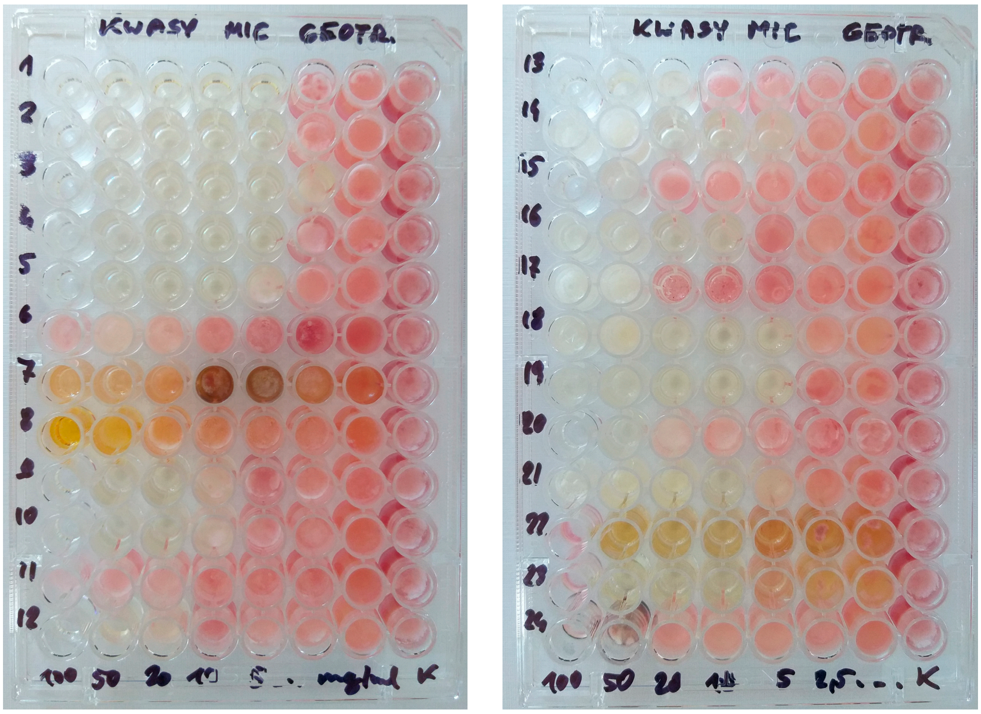

Figure 1. Example images of 96-well plates showing the results of minimal inhibitory concentration (MIC) testing against *Penicillium candidum* (100 to 1.25 mg/mL). Staining was performed using 2,3,5-triphenyltetrazolium chloride (TTC). Legends: 1—acetic acid, 2—valeric acid, 3—formic acid, 4—caproic acid, 5—citric acid, 6—aminoacetic acid, 7—tannic acid, 8—ascorbic acid, 9—oxalic acid, 11—glutamic acid, 12—tartaric acid, 13—malic acid, 14—benzoic acid, 15—succinic acid, 16—nicotinic acid, 17—palmitic acid, 18—salicylic acid, 20—oleic acid, 21—fumaric acid, 22—rosmarinic acid, and 23—chlorogenic acid. Those marked 10, 19, and 24 are acids not presented in this study.

3.2. In Silico Bioavailability and Toxicity Prediction

For the tested organic acids, predicted LD50 ranges from 48 to 5000 mg/kg. The predicted toxicity class for most is between 3 and 5, with an LD50 > 50 mg/kg, indicating that they are toxic or harmful if swallowed, except for acetic acid, which is class 1, and oleic acid, which is class 2. Acetic, benzoic, caproic, formic, oxalic, and valeric acids demonstrate high gastrointestinal tract absorption and are highly bioavailable orally. They generally also exhibit the lowest predicted LD50 values (<1000 mg/kg), indicating higher acute toxicity. High gastrointestinal tract absorption and high oral bioavailability are also demonstrated by aminoacetic, fumaric, glutamic, malic, nicotinic, salicylic, and succinic acids. However, their LD50 levels are above 1000 mg/kg, suggesting they are relatively safer. Chlorogenic and rosmarinic acids appear to be the safest, as they have low gastrointestinal absorption and oral bioavailability, coupled with high predicted LD50 values reaching 5000 mg/kg. None of the tested compounds have carcinogenic properties. Based on the available data, they do not pose a risk of causing cancer.

All the presented acids can lead to skin sensitization and/or eye irritation. Among the 21 acids listed, a total of 11, including benzoic, chlorogenic, formic, fumaric, malic, nicotinic, oxalic, rosmarinic, succinic, tannic, and tartaric acids, exhibit nephrotoxic effects. Nicotinic and salicylic acids may also act neurotoxically and hepatotoxically. Additionally, benzoic acid demonstrates hepatotoxicity. This means that the consumption of organic acids in large quantities can be toxic and lead to various complications. The obtained results for the bioavailability and toxicity prediction of the studied organic acids are presented in Table 4.

Table 4. Bioavailability and toxicity prediction in silico of studied organic acids.

Organic Acid	Gastro-Intestinal Tract Absorption	Human Oral Bioavailability 20%	Predicted LD50 [mg/kg]	Toxicity Class	Carcinogenicity	Hepatotoxicity	Neurotoxicity	Nephrotoxicity	Skin Sensitization	Eye Irritation
Acetic acid	High	Yes	333	1	No	No	No	No	Yes	Yes
Aminoacetic acid	High	Yes	3340	5	No	No	No	No	Yes	Yes
Ascorbic acid	High	No	3367	5	No	No	No	No	Yes	Yes
Benzoic acid	High	Yes	290	3	No	Yes	No	Yes	Yes	Yes
Caproic acid	High	Yes	94	3	No	No	No	No	Yes	Yes
Chlorogenic acid	Low	No	5000	5	No	No	No	Yes	Yes	No
Citric acid	Low	No	80	3	No	No	No	No	No	Yes
Formic acid	High	Yes	162	3	No	No	No	Yes	Yes	Yes
Fumaric acid	High	Yes	1350	4	No	No	No	Yes	Yes	Yes
Glutamic acid	High	Yes	4500	5	No	No	No	No	Yes	Yes
Malic acid	High	Yes	2497	5	No	No	No	Yes	Yes	Yes
Nicotinic acid	High	Yes	3720	5	No	Yes	Yes	Yes	Yes	Yes
Oleic acid	High	No	48	2	No	No	No	No	Yes	Yes
Oxalic acid	High	Yes	660	4	No	No	No	Yes	Yes	Yes
Palmitic acid	High	No	990	4	No	No	No	No	Yes	Yes
Rosmarinic acid	Low	No	5000	5	No	No	No	Yes	Yes	Yes
Salicylic acid	High	Yes	1190	4	No	Yes	Yes	No	Yes	No
Succinic acid	High	Yes	2260	5	No	No	No	Yes	Yes	Yes
Tannic acid	nd	* Low	2260	5	No	No	No	Yes	Yes	Yes
Tartaric acid	Low	Yes	2497	5	No	No	No	Yes	Yes	Yes
Valeric acid	High	Yes	134	3	No	No	No	No	Yes	Yes
Octenidine	Low	No	300	3	No	No	Yes	No	Yes	No

Legends: nd—lack of data, *—due to the large molecule size and the inability to perform calculations, bioavailability data were provided according to DrugBank [28]. Due to toxicity upon ingestion, there are 6 classes: Class 1—lethal (LD50 $\leq$ 5 mg/kg), Class 2—extremely toxic (LD50 5–50 mg/kg), Class 3—toxic (LD50 50–300 mg/kg), Class 4—harmful (LD50 300–2000 mg/kg), Class 5—possibly harmful (LD50 2000–5000 mg/kg) and Class 6—non-toxic (LD50 > 5000 mg/kg) [26].

It is also important to consider the relationship between MIC values and LD50, especially given that they are expressed in different units. Table 5 presents the conversion of MIC values to mg/kg and their comparison with LD50. Chlorogenic acid and rosmarinic acid stand out as both safe and effective food preservatives against bacteria and fungi, with their MIC values being lower (1344–4398 mg/kg for chlorogenic acid and 1213–4168 mg/kg for rosmarinic acid) than their LD50 values (5000 mg/kg). This makes them suitable and safe candidates for food preservation. Some acids, such as ascorbic, malic, nicotinic, salicylic, succinic, tannic, and tartaric acids, show safety for use against bacteria but not fungi. Their MIC values for bacteria are lower than their LD50 values (MIC < LD50), indicating safety for human consumption when used as antibacterial preservatives. Unfortunately, their higher MIC values for fungi than LD50 suggest that it is necessary to choose between limited antifungal effectiveness or potential toxicity. Many of the studied organic acids, including acetic, aminoacetic, benzoic, caproic, citric, formic, fumaric, glutamic, oleic, oxalic, palmitic, and valeric acids, exhibit MIC values that exceed their LD50 values, indicating potential toxicity to humans. These acids are therefore not safe for use as food preservatives, indicating a risk of human toxicity at effective concentrations. The selection of organic acids as food preservatives requires careful evaluation of both their antimicrobial effectiveness and their safety profiles. Balancing these factors is crucial to ensure that the preservatives are effective against microorganisms while being safe for human consumption. Choosing the appropriate acid as a preservative requires consideration of specific preservative properties and the type of microorganisms to be controlled.

Table 5. Relationship between MIC values and LD50 in mg/kg and the safety of studied organic acids as food preservatives.

Organic Acid	Predicted LD50 [mg/kg]	Mean MIC [mg/mL] against Bacteria/Fungi	Density [g/mL]	Mean MIC in 1 kg of Food Product [mg/kg] against Bacteria/Fungi	Safe Use as a Food Preservative (MIC < LD50) against Bacteria/Fungi
Acetic acid	333	1.72/7.5	1.05	1638/7142	No/No
Aminoacetic acid	3340	70.83/>100	1.61	43,994/>62,112	No/No
Ascorbic acid	3367	2.66/83.33	1.65	1612/50,503	**Yes**/No
Benzoic acid	290	0.63/7.08	1.27	496/5574	No/No
Caproic acid	94	3.75/7.5	0.93	4032/8065	No/No
Chlorogenic acid	5000	1.72/5.63	1.28	1344/4398	**Yes**/**Yes**
Citric acid	80	2.97/8.33	1.66	1789/5018	No/No
Formic acid	162	1.56/7.5	1.22	1279/6148	No/No
Fumaric acid	1350	2.66/14.17	1.64	1622/8640	No/No
Glutamic acid	4500	7.50/>100	1.46	5137/>68,493	No/No
Malic acid	2497	1.33/50	1.61	826/31,055	**Yes**/No
Nicotinic acid	3720	1.25/15	1.47	850/10,204	**Yes**/No
Oleic acid	48	7.50/50	0.895	8380/55,866	No/No
Oxalic acid	660	2.27/16.67	1.9	1195/8774	No/No
Palmitic acid	990	27.5/30	0.85	32,706/35,294	No/No
Rosmarinic acid	5000	1.88/6.46	1.55	1213/4168	**Yes**/**Yes**
Salicylic acid	1190	0.67/7.08	1.44	465/4917	**Yes**/No
Succinic acid	2260	2.27/83.33	1.56	1455/53,417	**Yes**/No
Tannic acid	2260	2.27/52.08	2.12	1071/24,566	**Yes**/No
Tartaric acid	2497	2.97/75	1.79	1659/41,899	**Yes**/No
Valeric acid	134	2.97/5.0	0.94	3160/5319	No/No

4. Discussion

In vegetables and fruit juices, many organic acids have been found, including acetic acid, ascorbic acid, aspartic acid, benzoic acid, butyric acid, citric acid, formic acid, gluconic acid, glutamic acid, glycolic acid, isoascorbic acid, lactic acid, malic acid, nicotinic acid, oxalic acid, propionic acid, sorbic acid, succinic acid, and tartaric acid [29]. Some organic acids, such as acetic, ascorbic, citric, lactic, and malic acids, are commonly used as traditional food preservatives [30]. They are also widely used as preservatives in the food industry. According to European legislation, five acids are known as E-additives: E200 sorbic acid, E210 benzoic acid, E260 acetic acid, E270 lactic acid, and E280 propionic acid. Several acids are used as acidifiers: E260 acetic acid, E270 lactic acid, E296 malic acid, E300 ascorbic acid, E330 citric acid, E334 tartaric acid, E355 adipic acid, and E363 succinic acid [19].

The antibacterial activity of organic acids has been confirmed in numerous studies. However, most research has focused on acetic acid, citric acid, formic acid, and malic acid. After searching the PubMed and Scopus databases, it appears that the present work is the first to examine the antibacterial activity of as many as 21 organic acids. We demonstrated that most organic acids exhibit bacteriostatic effects at levels ranging from 0.31 to 5 mg/mL.

The inhibitory concentrations against bacteria reported in the literature vary. Some inhibitory levels are similar to those in the present study. Beier et al. [31] showed that vancomycin-resistant *Enterococcus faecium* is sensitive to acetic acid at a dose of 2 mg/mL, citric acid at doses of 1–4.1 mg/mL, and formic acid at a dose of 1 mg/mL. Similar results were presented by the same research group for *Campylobacter jejuni* obtained from broiler chicken houses. Bacterial growth inhibition occurred at concentrations of acetic acid 0.5–4.1 mg/mL, citric acid 0.26–4.1 mg/mL, and formic acid 0.5–4.1 mg/mL [32]. In another paper, the MIC results demonstrated that acetic acid, citric acid, and tartaric acid inhibited *Salmonella* Typhimurium at concentrations of 0.312% (3.1 mg/mL), 0.625% (6.3 mg/mL), and 0.312% (3.1 mg/mL) for 10^3; CFU/mL [33]. Mine and Boopathy [34] reported that the breakpoints of organic acids against the shrimp pathogen *Vibrio harveyi* were 0.025–0.05% (0.25–0.5 mg/mL) for formic acid and 0.05–0.1% (0.5–1 mg/mL) for acetic acid, which are lower values than those found in this work.

Many data show that higher concentrations than those obtained in this work are required to inhibit foodborne pathogens. In the study by Štempelová et al. [35], the organic acid with the lowest MIC against *Staphylococcus aureus*, *Enterococcus faecium*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* was acetic acid (MIC 0.5–2.0 mg/mL). The remaining acids had higher average MIC levels. The activity of ascorbic acid against these bacteria showed the highest MIC, ranging from 4.0 to 16.0 mg/mL. The MIC values for citric acid ranged from 1.0 to 4.0 mg/mL and for succinic acid from 0.8 to 4.0 mg/mL. Lues and Theron [36] demonstrated that the activity of organic acids depends on the pH. The activity against *Listeria monocytogenes* decreased with increasing pH. For acetic acid, it ranged from 0.5 mM at pH 5.0 to 32 mM at pH 8.0 (30–1920 mg/mL); for citric acid, from 0.5 to 16 mM (96–3072 mg/mL); and for malic acid, from 0.5 to 32 mM (67–4288 mg/mL). Akbas and Cag [37] have shown that citric and malic acids at 1% and 2% (10 and 20 mg/mL, respectively) concentrations inhibit the development of *Bacillus subtilis* biofilm and can destroy mature biofilm. Concentrations of 1% and 2% are several times higher than the MICs obtained in the present work. Similar concentrations were studied in another work [38], where 1%, 2%, and 3% acetic and citric acids reduced the number of *Salmonella* Enteritidis, *Escherichia coli*, and *Listeria monocytogenes* in beef meat. However, only the 3% concentrations led to a significant ($p < 0.05$) reduction in bacteria. In the next paper [39], it was found that acetic acid and citric acid lead to the inactivation of multi-drug-resistant non-typhoidal *Salmonella* and Shiga-toxin-producing *Escherichia coli*. The authors used acids at a concentration of 4.1 mg/mL, which is higher than the average MICs obtained by us. Unfortunately, the authors did not present MIC results, even though they described this study in their methodology.

In studies on sheep and goat meat obtained from freshly slaughtered animals, samples were inoculated with *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella* Typhimurium. The meat samples were then washed with a spray containing 2% lactic acid and a combination of 1.5% acetic acid + 1.5% propionic acid. It was shown that the total number of viable microorganisms in the meat was reduced by approximately 0.52 and 1.16 log units, respectively [40]. Albuquerque et al. [41] demonstrated that 1% (10 mg/mL) citric acid leads to the reduction in *Salmonella* spp., *Staphylococcus* spp., and thermotolerant coliforms in sheep meat. In studies on the effect of organic acids on *Escherichia coli* isolated from fresh pork sausage, the highest level required for antibacterial activity was found to be 1.29 M (247 mg/mL) for citric acid and approximately 4 M for acetic acid (240 mg/mL) [42].

In the literature, there is little on the antifungal activity of organic acids, according to PubMed or Scopus databases. In one publication, similar to our studies, much higher concentrations were required to achieve an antifungal effect. *Penicillium* sp. strains were inhibited by acetic acid at concentrations of 200–800 mM (12,010–48,040 mg/mL) [43]. The activity of tannic acid against *Penicillium digitatum* was better and the MIC value was 1 mg/mL [44]. The above literature data are additionally presented in Table 6.

Table 6. Comparison of obtained MIC values in antibacterial and antifungal activity with the available literature.

Organic Acid	Tested Microorganism	MIC Values from Reference	Our MICs [mg/mL]	Reference
Acetic acid	*Enterococcus faecium*	2 mg/mL	1.25–2.5	[31]
	Campylobacter jejuni	0.5–4.1 mg/mL		[32]
	Salmonella Typhimurium	0.312% (3.1 mg/mL)		[33]
	Vibrio harveyi	0.05–0.1% (0.5–1 mg/mL)		[34]
	Staphylococcus aureus, *E. faecium*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*	0.5–2.0 mg/mL		[35]
	Listeria monocytogenes	0.5–32 mM (30–1920 mg/mL)		[36]
	Salmonella Enteritidis, *E. coli*, *L. monocytogenes*	1–3% (10–30 mg/mL)		[38]
	non-typhoidal *Salmonella*, *E. coli*	4.1 mg/mL		[39]
	S. aureus, *L. monocytogenes*, *E. coli*, *S.* Typhimurium	1.5% (15 mg/mL) acetic + 1.5% propionic acid		[40]
	E. coli	4 M (240 mg/mL)		[42]
	Penicillium sp.	200–800 mM (12,010–48,040 mg/mL)		[43]
Ascorbic acid	*S. aureus*, *E. faecium*, *B. cereus*, *E. coli*, *P. aeruginosa*	4.0–16.0 mg/mL	1.25–5.0	[35]
Citric acid	*E. faecium*	1–4.1 mg/mL	1.25–5.0	[31]
	Campylobacter jejuni	0.26–4.1 mg/mL		[32]
	S. Typhimurium	0.625% (6.3 mg/mL)		[33]
	S. aureus, *E. faecium*, *B. cereus*, *E. coli*, *P. aeruginosa*	1.0–4.0 mg/mL		[35]
	L. monocytogenes	0.5–16 mM (96–3072 mg/mL)		[36]
	B. subtilis	1% (10 mg/mL)		[37]
	S. Enteritidis, *E. coli*, *L. monocytogenes*	1–3% (10–30 mg/mL)		[38]
	non-typhoidal *Salmonella*, *E. coli*	4.1 mg/mL		[39]
	Salmonella spp., *Staphylococcus* spp., and thermotolerant coliforms	1% (10 mg/mL)		[41]
	E. coli	1.29 M (247 mg/mL)		[42]
Formic acid	*E. faecium*	1 mg/mL	1.25–2.5	[31]
	C. jejuni	0.5–4.1 mg/mL		[32]
	V. harveyi	0.025–0.05% (0.25–0.5 mg/mL)		[34]
Malic acid	*L. monocytogenes*	0.5–32 mM (67–4288 mg/mL)	0.63–2.5	[36]
	B. subtilis	2% (20 mg/mL)		[37]
Succinic acid	*S. aureus*, *E. faecium*, *B. cereus*, *E. coli*, *P. aeruginosa*	0.8–4.0 mg/mL	0.63–5.0	[35]
Tannic acid	*Penicillium digitatum*	1 mg/mL		[38]
Tartaric acid	*S.* Typhimurium	0.312% (3.1 mg/mL)	1.25–5.0	[33]

Some organic acids, including acetic, citric, lactic, and malic acids, have been generally recognized as safe (GRAS) [45]. However, in the presented studies, we demonstrated that organic acids vary in terms of toxicity. Unfortunately, for many of them, the required antibacterial concentration is higher than the predicted lethal dose, LD50. The acids that appear to be safe (MIC < LD50) based on our results include ascorbic acid, chlorogenic acid, malic acid, nicotinic acid, rosmarinic acid, salicylic acid, succinic acid, tannic acid, and tartaric acid.

The mechanism of action of organic acids is based on the fact that undissociated molecules are lipophilic and can cross the lipid membrane of microorganisms. After penetrating the bacterial cytoplasmic membrane, they dissociate into anions and protons in the cytoplasm. The protons lower the intracellular pH, leading to the inhibition of bacterial glycolysis, a decrease in ATP, and a reduction in active transport [9,10]. A similar mechanism might occur in mammalian cells. Unfortunately, the literature on the toxicity of organic acids is sparse. In in vivo studies, the LC50 for acetic acid and benzoic acid were reported to be 273 and 277 mg/L for tilapia (*Oreochromis mossambicus*) [46]. These values are similar to the results obtained in this work, namely 333 and 290 mg/kg, respectively. However, the LC50 decreases for other organisms. For cladoceran crustacea (*Moina micrura*), the LC50 values of acetic acid and benzoic acid were 164 and 72 mg/L and for the oligochaete worm (*Branchiura sowerbyi*), they were 15 and 39 mg/L, respectively.

Although the genotoxicity of organic acids was not demonstrated in this study, it has been described for citric acid at a concentration of 20 ppm or 0.02 mg/mL [47]. The high values of the predicted LD50 ranging from several 10s to 5000 mg/kg obtained in this study might explain why the maximum daily intake for many acids has not been determined. The Food and Agriculture Organization (FAO) of the United Nations has not specified a daily intake limit for acetic, citric, lactic, malic, and propionic acids. However, the maximum daily intake for benzoic and sorbic acids is 1 mg/kg, for fumaric acid it is 6 mg/kg, and for tartaric acid it is 30 mg/kg of body weight [19,30].

5. Conclusions

Most of the investigated plant-derived organic acids exhibit antibacterial activity at concentrations ranging from 0.31 to 5 mg/mL. The effectiveness of organic acids against foodborne fungi like *Penicillium candidum* and *Geotrichum candidum* is weaker than against bacteria. Some acids demonstrate moderate antifungal activity with mean MICs of <10 mg/mL, while most acids require higher concentrations (from 10 to >100 mg/mL) to inhibit fungal growth effectively. This highlights the importance of selecting organic acids based on their specific potency against fungal strains in food preservation.

The toxicity profiles of the tested organic acids vary widely, with predicted LD50 values ranging from 48 to 5000 mg/kg. For many of them, the required antibacterial concentration is higher than the predicted lethal dose of LD50. The acids that appear to be safe as food preservatives (MIC < LD50) include ascorbic, chlorogenic, malic, nicotinic, rosmarinic, salicylic, succinic, tannic, and tartaric acids. The relationship between MIC and LD50 is crucial in determining the suitability of organic acids as food preservatives, ensuring that they are effective against bacteria at concentrations that are not harmful to humans. While no presented organic acid is carcinogenic, many can cause adverse effects such as skin sensitization, eye irritation, and potential nephrotoxicity, hepatotoxicity, or neurotoxicity.

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References

1. Havelaar, A.H.; Kirk, M.D.; Torgerson, P.R.; Gibb, H.J.; Hald, T.; Lake, R.J.; Praet, N.; Bellinger, D.C.; de Silva, N.R.; Gargouri, N.; et al. World Health Organization Global Estimates and Regional Comparisons of the Burden of Foodborne Disease in 2010. *PLoS Med.* **2015**, *12*, e1001923. [CrossRef] [PubMed]
2. Bintsis, T. Foodborne Pathogens. *AIMS Microbiol.* **2017**, *3*, 529–563. [CrossRef] [PubMed]
3. Kim, H.; Cho, J.H.; Song, M.; Cho, J.H.; Kim, S.; Kim, E.S.; Keum, G.B.; Kim, H.B.; Lee, J.-H. Evaluating the Prevalence of Foodborne Pathogens in Livestock Using Metagenomics Approach. *J. Microbiol. Biotechnol.* **2021**, *31*, 1701–1708. [CrossRef] [PubMed]
4. Houbraken, J.; Samson, R.A. Current Taxonomy and Identification of Foodborne Fungi. *Curr. Opin. Food Sci.* **2017**, *17*, 84–88. [CrossRef]
5. Benedict, K.; Chiller, T.M.; Mody, R.K. Invasive Fungal Infections Acquired from Contaminated Food or Nutritional Supplements: A Review of the Literature. *Foodborne Pathog. Dis.* **2016**, *13*, 343–349. [CrossRef] [PubMed]
6. Skora, M.; Witalis, J.; Krzysciak, P.; Macura, A.B. Fungal genus *Geotrichum*: An opportunistic pathogen of humans. *Postępy Mikrobiol.* **2009**, *48*, 125–132.
7. Rannikko, J.; Holmberg, V.; Karppelin, M.; Arvola, P.; Huttunen, R.; Mattila, E.; Kerttula, N.; Puhto, T.; Tamm, Ü.; Koivula, I.; et al. Fungemia and Other Fungal Infections Associated with Use of *Saccharomyces boulardii* Probiotic Supplements. *Emerg. Infect. Dis.* **2021**, *27*, 2090–2096. [CrossRef] [PubMed]
8. Enache-Angoulvant, A.; Hennequin, C. Invasive Saccharomyces Infection: A Comprehensive Review. *Clin. Infect. Dis.* **2005**, *41*, 1559–1568. [CrossRef] [PubMed]
9. Karpiński, T.M.; Szkaradkiewicz, A.K. Characteristic of Bacteriocines and Their Application. *Pol. J. Microbiol.* **2013**, *62*, 223–235. [CrossRef]
10. Farid, N.; Waheed, A.; Motwani, S. Synthetic and Natural Antimicrobials as a Control against Food Borne Pathogens: A Review. *Heliyon* **2023**, *9*, e17021. [CrossRef]
11. Alibi, S.; Crespo, D.; Navas, J. Plant-Derivatives Small Molecules with Antibacterial Activity. *Antibiotics* **2021**, *10*, 231. [CrossRef] [PubMed]
12. Adamczak, A.; Ożarowski, M.; Karpiński, T.M. Antibacterial Activity of Some Flavonoids and Organic Acids Widely Distributed in Plants. *J. Clin. Med.* **2019**, *9*, 109. [CrossRef] [PubMed]
13. Mira, N.P.; Marshall, R.; Pinheiro, M.J.F.; Dieckmann, R.; Dahouk, S.A.; Skroza, N.; Rudnicka, K.; Lund, P.A.; De Biase, D.; Working Group 3 of the COST Action EuroMicropH. On the Potential Role of Naturally Occurring Carboxylic Organic Acids as Anti-Infective Agents: Opportunities and Challenges. *Int. J. Infect. Dis.* **2024**, *140*, 119–123. [CrossRef] [PubMed]
14. Pereira, C.; Barros, L.; Carvalho, A.M.; Ferreira, I.C.F.R. Use of UFLC-PDA for the Analysis of Organic Acids in Thirty-Five Species of Food and Medicinal Plants. *Food Anal. Methods* **2013**, *6*, 1337–1344. [CrossRef]
15. Raj, K.; Węglarz, Z.; Przybył, J.L.; Kosakowska, O.; Pawełczak, A.; Gontar, Ł.; Puchta-Jasińska, M.; Bączek, K. Chemical Diversity of Wild-Growing and Cultivated Common Valerian (*Valeriana officinalis* L. s.l.) Originating from Poland. *Molecules* **2023**, *29*, 112. [CrossRef] [PubMed]
16. Ricke, S.C.; Dittoe, D.K.; Richardson, K.E. Formic Acid as an Antimicrobial for Poultry Production: A Review. *Front. Vet. Sci.* **2020**, *7*, 563. [CrossRef] [PubMed]
17. Ma, B.; Yuan, Y.; Gao, M.; Li, C.; Ogutu, C.; Li, M.; Ma, F. Determination of Predominant Organic Acid Components in *Malus* Species: Correlation with Apple Domestication. *Metabolites* **2018**, *8*, 74. [CrossRef] [PubMed]
18. Li, M.; Su, J.; Yang, H.; Feng, L.; Wang, M.; Xu, G.; Shao, J.; Ma, C. Grape Tartaric Acid: Chemistry, Function, Metabolism, and Regulation. *Horticulturae* **2023**, *9*, 1173. [CrossRef]
19. Ben Braïek, O.; Smaoui, S. Chemistry, Safety, and Challenges of the Use of Organic Acids and Their Derivative Salts in Meat Preservation. *J. Food Qual.* **2021**, *2021*, 6653190. [CrossRef]
20. Coban, H.B. Organic Acids as Antimicrobial Food Agents: Applications and Microbial Productions. *Bioprocess Biosyst. Eng.* **2020**, *43*, 569–591. [CrossRef]
21. Korbecka-Paczkowska, M.; Karpiński, T.M. In Vitro Assessment of Antifungal and Antibiofilm Efficacy of Commercial Mouthwashes against *Candida albicans*. *Antibiotics* **2024**, *13*, 117. [CrossRef] [PubMed]
22. Karpiński, T.M.; Ożarowski, M.; Seremak-Mrozikiewicz, A.; Wolski, H. Anti-Candida and Antibiofilm Activity of Selected Lamiaceae Essential Oils. *Front. Biosci. (Landmark Ed.)* **2023**, *28*, 28. [CrossRef] [PubMed]
23. Paczkowska-Walendowska, M.; Rosiak, N.; Plech, T.; Karpiński, T.M.; Miklaszewski, A.; Witkowska, K.; Jaskólski, M.; Erdem, C.; Cielecka-Piontek, J. Electrospun Nanofibers Loaded with Marigold Extract Based on PVP/HPβCD and PCL/PVP Scaffolds for Wound Healing Applications. *Materials* **2024**, *17*, 1736. [CrossRef] [PubMed]

24. Daina, A.; Michielin, O.; Zoete, V. SwissADME: A Free Web Tool to Evaluate Pharmacokinetics, Drug-Likeness and Medicinal Chemistry Friendliness of Small Molecules. *Sci. Rep.* **2017**, *7*, 42717. [CrossRef] [PubMed]
25. Myung, Y.; de Sá, A.G.C.; Ascher, D.B. Deep-PK: Deep Learning for Small Molecule Pharmacokinetic and Toxicity Prediction. *Nucleic Acids Res* **2024**, *52*, W469–W475. [CrossRef] [PubMed]
26. Banerjee, P.; Kemmler, E.; Dunkel, M.; Preissner, R. ProTox 3.0: A Webserver for the Prediction of Toxicity of Chemicals. *Nucleic Acids Res.* **2024**, *52*, W513–W520. [CrossRef] [PubMed]
27. Noga, M.; Michalska, A.; Jurowski, K. The Prediction of Acute Toxicity (LD50) for Organophosphorus-Based Chemical Warfare Agents (V-Series) Using Toxicology in Silico Methods. *Arch. Toxicol.* **2024**, *98*, 267–275. [CrossRef]
28. Tannic Acid. Available online: https://go.drugbank.com/drugs/DB09372 (accessed on 12 July 2024).
29. Klampfl, C.W.; Buchberger, W.; Haddad, P.R. Determination of Organic Acids in Food Samples by Capillary Zone Electrophoresis. *J. Chromatogr. A* **2000**, *881*, 357–364. [CrossRef]
30. Gurtler, J.B.; Mai, T.L. Preservatives | Traditional Preservatives—Organic Acids. In *Encyclopedia of Food Microbiology*, 2nd ed.; Batt, C.A., Tortorello, M.L., Eds.; Academic Press: Oxford, UK, 2014; pp. 119–130, ISBN 978-0-12-384733-1.
31. Beier, R.C.; Harvey, R.B.; Poole, T.L.; Hume, M.E.; Crippen, T.L.; Highfield, L.D.; Alali, W.Q.; Andrews, K.; Anderson, R.C.; Nisbet, D.J. Interactions of Organic Acids with Vancomycin-Resistant *Enterococcus faecium* Isolated from Community Wastewater in Texas. *J. Appl. Microbiol.* **2019**, *126*, 480–488. [CrossRef]
32. Beier, R.C.; Byrd, J.A.; Caldwell, D.; Andrews, K.; Crippen, T.L.; Anderson, R.C.; Nisbet, D.J. Inhibition and Interactions of *Campylobacter jejuni* from Broiler Chicken Houses with Organic Acids. *Microorganisms* **2019**, *7*, 223. [CrossRef]
33. El Baaboua, A.; El Maadoudi, M.; Bouyahya, A.; Belmehdi, O.; Kounnoun, A.; Zahli, R.; Abrini, J. Evaluation of Antimicrobial Activity of Four Organic Acids Used in Chicks Feed to Control *Salmonella typhimurium*: Suggestion of Amendment in the Search Standard. *Int. J. Microbiol.* **2018**, *2018*, 7352593. [CrossRef] [PubMed]
34. Mine, S.; Boopathy, R. Effect of Organic Acids on Shrimp Pathogen, *Vibrio harveyi*. *Curr. Microbiol.* **2011**, *63*, 1–7. [CrossRef] [PubMed]
35. Štempelová, L.; Kubašová, I.; Bujňáková, D.; Karahutová, L.; Gálová, J.; Kužma, E.; Strompfová, V. Antimicrobial Activity of Organic Acids against Canine Skin Bacteria. *Vet. Res. Commun.* **2023**, *47*, 999–1005. [CrossRef] [PubMed]
36. Lues, J.F.R.; Theron, M.M. Comparing Organic Acids and Salt Derivatives as Antimicrobials against Selected Poultry-Borne *Listeria monocytogenes* Strains in Vitro. *Foodborne Pathog. Dis.* **2012**, *9*, 1126–1129. [CrossRef] [PubMed]
37. Akbas, M.Y.; Cag, S. Use of Organic Acids for Prevention and Removal of *Bacillus subtilis* Biofilms on Food Contact Surfaces. *Food Sci. Technol. Int.* **2016**, *22*, 587–597. [CrossRef]
38. Dan, S.D.; Mihaiu, M.; Reget, O.; Oltean, D.; Tabaran, A. Pathogens Contamination Level Reduction on Beef Using Organic Acids Decontamination Methods. *Bull. UASVM Vet. Med.* **2017**, *74*, 212–217. [CrossRef]
39. Castro, V.S.; da Silva Mutz, Y.; Rosario, D.K.A.; Cunha-Neto, A.; de Souza Figueiredo, E.E.; Conte-Junior, C.A. Inactivation of Multi-Drug Resistant Non-Typhoidal *Salmonella* and Wild-Type *Escherichia coli* STEC Using Organic Acids: A Potential Alternative to the Food Industry. *Pathogens* **2020**, *9*, 849. [CrossRef] [PubMed]
40. Dubal, Z.B.; Paturkar, A.M.; Waskar, V.S.; Zende, R.J.; Latha, C.; Rawool, D.B.; Kadam, M.M. Effect of Food Grade Organic Acids on Inoculated *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* in Sheep/Goat Meat Stored at Refrigeration Temperature. *Meat Sci.* **2004**, *66*, 817–821. [CrossRef]
41. Albuquerque, G.N.; Costa, R.G.; Barba, F.J.; Gómez, B.; Ribeiro, N.L.; Beltrão Filho, E.M.; Sousa, S.; Santos, J.G.; Lorenzo, J.M. Effect of Organic Acids on the Quality of Sheep "Buchada": From Food Safety to Physicochemical, Nutritional, and Sensorial Evaluation. *J. Food Process. Preserv.* **2019**, *43*, e13877. [CrossRef]
42. Dias, F.S.; da Silva Ávila, C.L.; Schwan, R.F. In Situ Inhibition of *Escherichia coli* Isolated from Fresh Pork Sausage by Organic Acids. *J. Food Sci.* **2011**, *76*, M605–M610. [CrossRef]
43. Moro, C.B.; Lemos, J.G.; Gasperini, A.M.; Stefanello, A.; Garcia, M.V.; Copetti, M.V. Efficacy of Weak Acid Preservatives on Spoilage Fungi of Bakery Products. *Int. J. Food Microbiol.* **2022**, *374*, 109723. [CrossRef] [PubMed]
44. Zhu, C.; Lei, M.; Andargie, M.; Zeng, J.; Li, J. Antifungal Activity and Mechanism of Action of Tannic Acid against *Penicillium digitatum*. *Physiol. Mol. Plant Pathol.* **2019**, *107*, 46–50. [CrossRef]
45. Maimaitiyiming, R.; Yang, Y.; Mulati, A.; Aihaiti, A.; Wang, J. The Use of Ultraviolet Irradiation to Improve the Efficacy of Acids That Are Generally Recognized as Safe for Disinfecting Fresh Produce in the Ready-to-Eat Stage. *Foods* **2024**, *13*, 1723. [CrossRef] [PubMed]
46. Saha, N.C.; Bhunia, F.; Kaviraj, A. Comparative Toxicity of Three Organic Acids to Freshwater Organisms and Their Impact on Aquatic Ecosystems. *Hum. Ecol. Risk Assess.* **2006**, *12*, 192–202. [CrossRef]
47. Türkoğlu, Ş. Genotoxicity of Five Food Preservatives Tested on Root Tips of *Allium cepa* L. *Mutat. Res./Genet. Toxicol. Environ. Mutagen.* **2007**, *626*, 4–14. [CrossRef]