Antimicrobial properties of chosen phenolic compounds against *Escherichia coli* O157:H7

Jakubczak A. 1, Stachelska M.A. 1, Świsłocka R. 1,2,Kalinowska M. 2, Lewandowski W. 1,2.

1 The State College of Computer Science and Business Administration, Food Technology and Nutrition Institute, Łomża, Poland;
2 Białystok Technical University, Department of Chemistry, Białystok, Poland.
Correspondence to: M.A. Stachelska, tel. 513 387 824, e-mail: milenastach@poczta.onet.pl

ABSTRACT
The aim of this research was to examine an antimicrobial activity of salts of phenolic acids against *Escherichia coli* O157:H7 ATCC 8739 and to assess their lowest minimum inhibitory concentrations (MICs). *E. coli* O157:H7 is a pathogen producing verotoxins which provoke hemorrhagic diarrhea at people. There is a demand for the effective natural methods eliminating *E.* coli O157:H7 from food. The following salts were tested: lithium, sodium and potassium salts of syringic, *meta*-anisic and vanillic acids. The 1%, 2%, 3%, 4% and 5% water solutions of each substance were prepared. Agar-well diffusion method was applied. The Petri dishes were incubated at 35°C for 24 h. At the end of the incubation period, inhibition zones which appeared on the medium Petri dishes were calculated in millimeters. It was proved that a lithium salt of syringic acid was the most effective towards *E.* coli O157:H7, while a potassium salt of syringic acid and a potassium salt of vanillic acid were slightly less effective, and a lithium salt of vanillic acid, a sodium salt of vanillic acid and a potassium salt of m-anisic acid indicated comparatively lower antimicrobial activity, and lithium and sodium salts of m-anisic acid were the least effective against the pathogen. The salts of phenolic acids possessing different structural features showed various characteristics against foodborne pathogens. Such research results indicate that phenolic acids and their salts can be a potential bio-alternative for chemical food preservation.

Keywords: *Escherichia coli* O157:H7, antimicrobial activity, phenolic salts

I. INTRODUCTION

Phenolic acids can be easily found in different kinds of plant tissues. As many researches proved their inhibitory activity towards different foodborne pathogens, they may constitute a natural alternative for chemical preservatives and may be applied to eliminate a potential appearance of spoilage microflora and extend the shelf-life of food products. Such substances deserve to be given a careful attention due to the fact that they are effective towards a wide range of unwanted microflora spoiling food (Lopez-Malo et al. 2006, Mann and Markham 1998, Maurya et al. 2007). They enjoy a great popularity because they appear in fruit and vegetables as well as in coffee and tea which means that they may be called natural preservatives (Hocking 1997, Holley and Patel 2005, Jay et al. 1984). New safe methods of guaranteeing the safety of food products and prolongation of their shelf-life should be still searched for and developed to avoid any undesirable side effects for human health provoked by the application of chemical preservatives. Such side effects may include food intolerances and allergic reactions. Apart from having inhibitory properties towards a range of different microflora, they are also safe for human health (Brul and Coote 1999, Juven et al. 1994, Karatzas et al. 2001, Karns et al. 2007, Roller 2003).

E. coli O157:H7 is known to be Gram-negative and enterohemorrhagic pathogen which might be isolated from humans' digestive track. This pathogen was firstly isolated in 1975 from a patient who had bloody diarrhea caused due to the consumption of contaminated meat and dairy products. It can provoke hemolytic uremic syndrome (HUS) which is responsible for provoking hemolytic anemia, thrombocytopenia as well as and acute renal failure. It constitutes a huge danger for people by affecting the large intestine and producing a great number of Shigalike exotoxins (verotoxins) (Denny et al. 2008).

This research estimates the inhibitory properties of salts of syringic, *m*-anisic and vanillic acids against *E. coli* O157:H7 in vitro. Many studies proved that phenolic acids are biologically active compounds which were isolated from different plant species and are applied to eliminate pathogenic microorganisms from food. It is proved that phenolic compounds successfully inhibit phospholipases and membrane functions in cells. These substances also influence lipid metabolism. They naturally appear in green plant cells. They are known to possess anti-oxidative, anti-inflammatory as well as anti-carcinogenic influences. Moreover, they have enjoyed a huge popularity due to their successful influence in medical treatments since ancient times (Park et al. 1999, Penney et al. 2004, Rangel et al. 2005).

Many scientists have examined their inhibitory properties against *Streptococcus mutans*, *Staphylococcus aureus*, and *Listeria monocytogenes*. However, their antimicrobial properties on *E. coli* O157:H7 strains have not been fully recognized yet. There is a wide variety of naturally occurring extracts which include essential oils, herbs and spices possessing the antimicrobial properties. They are able not only to inhibit but also eliminate the growth of food spoilage and pathogens (Ramachandra et al. 2000, Rojas-Grau et al. 2007, Rupasinghe et al. 2006, Shaughnessy et al. 2006, Shyamala et al. 2007, Sinha et al. 2008, Smith-Palmer et al. 2001). The purpose of this research is to estimate the antimicrobial properties of selected salts of phenolic acids on the growth of *E. coli* O157:H7 in vitro.

II MATERIALS AND METHODS

Sample preparation

The salts were prepared by dissolving suitably weighed amounts of syringic, *m*-anisic and vanillic acids in aqueous solutions of lithium, sodium and potassium hydroxides in a stoichiometric ratio 1:1 The anhydrous compound was obtained after the evaporation the solvent on a water bath, and drying at 120˚C in a dryer.

The 1%, 2%, 3%, 4% and 5% water solutions of each substance were prepared to examine their inhibitory influence on *E. coli* O157:H7.

Microbial strain

The strain *E. coli* O157:H7 ATCC 8739 used for microbiological analysis was obtained from ATCC collections. Culture of *E. coli* O157:H7 was maintained in 5% glycerol in temperature – 20^0C.

Preparation of liquid bacterial culture in tryptic soy broth

A 16-h-old culture inoculated in tryptone soy broth (bioMérieux, Warszawa, Poland) at temperature 35^0C was taken for further experiment. The optical density of this culture after inoculation was determined at 625 nm (Ultraspec III, Pharmacia, Sweden). The incubation was stopped when the optical density achieved a value in the range of 0.8–1.0. The culture suspensions were diluted to an absorbance of 0.1 and used as such for the antimicrobial tests.

Placing the culture dilution on a plate with medium

The medium used for further experiment was Columbia agar with sheep blood on sterilised Petri dish (Ø 10 cm) (bioMérieux, Warszawa, Poland). A 1-ml of a 16-h culture diluted to achieve an absorbance of 0.1 was placed onto the surface of pre-dried Columbia agar with sheep blood Petri dishes (Ø 10 cm), then they were allowed to remain in contact with bacteria for 20 min at room temperature.

Agar-well diffusion method

The antimicrobial activity of the phenolic substances was assessed by the Agar-well diffusion method (Perez et al. 1990). Six equidistant holes were made in each Petri dish using sterile cork borers (Ø 7 mm). A 0,05 ml of each salt at five different concentrations was added to each hole using a pipettor (Eppendorf). Negative controls were tryptone soy broth samples. The inoculated plates were incubated at 35°C for 24 h.

Measuring the inhibition zone diameter

At the end of the incubation period, inhibition zones appearing on the medium Petri dishes were measured in millimeters. All experiments were carried out in triplicate.

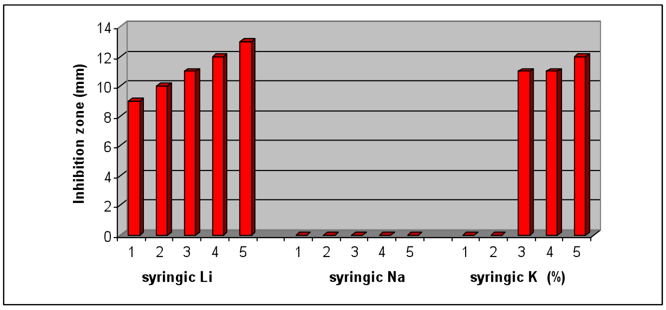
III. RESULTS

The work presents the growth inhibitory properties of nine salts of phenolic acids characterized by various chemical structures against

E. coli O157:H7. It was proved that the examined salts indicate the essentially different antimicrobial activity against the pathogen. The salts used in the experiment showed a huge differentiation in a level of antimicrobial activities. The antimicrobial features of the examined salts and their possibility to be applied as the effective method in fighting with pathogens in food were quantitatively assessed by the measurement of inhibition zone diameter. The results presenting the inhibitory properties of the investigated salts against *E. coli* O157:H7 are shown in Figure 1-3.

Figure 1 presents the inhibitory properties of lithium, sodium and potassium salts of syringic acid against *E. coli* O157:H7.

Fig. 1. Relationship between diameter of inhibition zone (mm) and the active substance concentration (%) of lithium, sodium and potassium salts of syringic acid towards *E. coli* O157:H7.



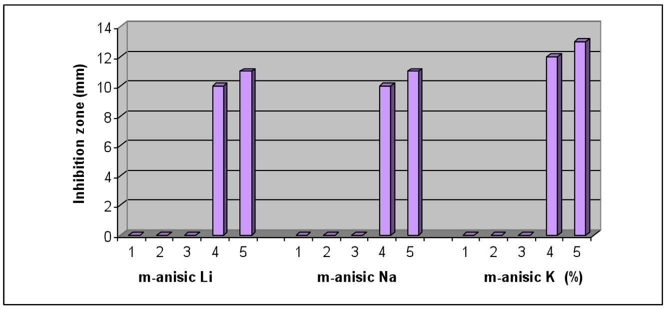
It was proved that lithium and potassium salts of the salts of syringic acid indicate a relatively high inhibitory activity against the examined pathogen. On the other hand, a sodium salt of syringic acid did not show any antibacterial properties against *E. coli* O157:H7. It was proved that a lithium salt of syringic acid was found to be more effective towards the pathogen in comparison to a potassium salt of this acid as its 1 and 2% water solutions caused the growth inhibition. The inhibition zone diameter for a lithium salt of syringic acid amounted to 13 mm at 5% concentration of an active substance, 12 mm at 4% concentration, 11 mm at 3% concentration, 10 mm at 2% concentration and 9 mm at 1% concentration.

It was shown that a potassium salt of syringic acid possessed a slightly lower inhibitory activity against *E. coli* O157:H7 with relation to a lithium salt. The inhibition zone diameter for a potassium salt of syringic acid amounted to 12 mm at 5% concentration of an active substance, 11 mm at 4% concentration, 11 mm at 3% concentration. Its 1 and 2% water solution did not indicate to have any inhibitory influence on the bacteria.

Among examined salts of syringic acid, only a sodium salts was not effective against *E. coli* O157:H7.

Figure 2 illustrates the antimicrobial properties of lithium, sodium and potassium salts of *m*-anisic acid against *E. coli* O157:H7.

Fig. 2. Relationship between diameter of inhibition zone (mm) and the active substance concentration (%) of lithium, sodium and potassium salts of m-anisic acid towards *E. coli* O157:H7.



It can be observed that the salts of *m*-anisic acid are characterized by having similar inhibitory properties against the gram-negative bacteria. It was shown that a potassium salt of this acid had a slightly higher inhibitory activity against *E. coli* O157:H7 compared with lithium and sodium salts which occurred to have the same influence on the pathogen. The activity of a potassium salt was assessed by the measurement of inhibition zone diameter. It amounted to 13 mm at 5% concentration and 12 mm at 4% concentration. Its 1, 2 and 3% water solutions did not prove to have any inhibitory influence on the bacteria.

It was found that lithium and sodium salts of *m*-anisic acid had a similar influence on the growth inhibition of the examined pathogen. Their inhibition zone diameters amounted to 11 mm at 5% concentration of an active substance and 10 mm at 4% concentration. Their 1, 2 and 3% water solution did not possess any inhibitory effect towards the bacteria.

Figure 3 illustrates the growth inhibitory activities of lithium, sodium and potassium salts of vanillic acid against *E. coli* O157:H7.

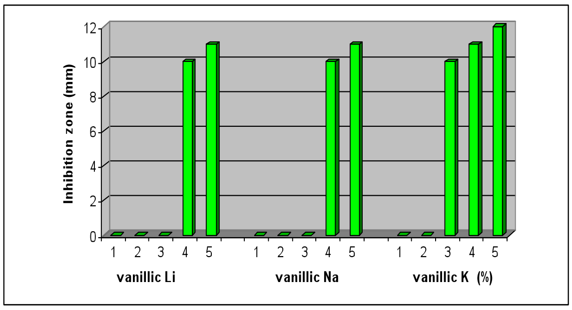


Fig. 3. Relationship between diameter of inhibition zone (mm) and the active substance concentration (%) of lithium, sodium and potassium salts of vanillic acid towards *E. coli* O157:H7.

It was indicated that a potassium salt of vanillic acid is characterized by the highest inhibitory activity while lithium and sodium salts proved to be slightly lower effective and had the same inhibitory activities towards *E. coli* O157:H7. The inhibition zone diameter for a potassium salts of vanillic acid amounted to 12 mm at 5% concentration, 11 mm at 4% concentration and 10 mm at 3% concentration. Its 1 and 2% water solutions did not indicate to possess any inhibitory effect towards the examined bacteria. The inhibition zone diameters for lithium and sodium salts of vanillic acid were the same and amounted to 11 mm at 5% concentration of an active substance and 10 mm at 4% concentration. Their 1, 2 and 3% water solutions were insufficient to cause any growth inhibition.

The antimicrobial properties of salts of phenolic acids stay in a close correlation with their chemical structure and concentration (Aziz et al. 1998, Moon et al. 2006, Mourtzinos et al. 2009, Ngarmsak et al. 2006). The salts used in the experiment were proved to possess the antimicrobial influence (Burt 2004, Fitzgerald et al. 2004a, 2004b). It is widely known that the aldehyde group present in phenolic compounds has got a huge influence on the elimination of pathogens from food. What is more, the type and position of some of the lateral groups in the benzene ring play also a huge role in the inactivation of unwanted microorganisms (Fitzgerald et al. 2005, Gastélum et al. 2010).

Such phenolic compounds possess the hydrophobic nature (Cava et al. 2007, Fitzgerald et al. 2003). They are able to damage the plasmatic membrane of the microbial cells due to the interaction with lipids and proteins causing a loss of ionic gradient and inhibition of the respiratory activity (Chobpattana et al. 2002, Chao et al. 2000). The results of many investigations showed that phenolic compounds are usually less effective towards Gram-negative rather than Gram-positive bacteria because of their more complex double membrane (Ahrabi et al. 1998, Helander et al. 1998). The relatively high sensitivity of *E. coli* O157:H7 on the activity of the chosen phenolic compounds might be explained by the fact that there is the presence of some important proteins in the external membrane, which are responsible for making channels enabling the penetration of low molecular weight compounds (Evrendilek 2007, Ferrante et al. 2007). There are also some papers which report the existence of some links between phenolic compounds and proteins which interact together and are responsible for microorganism inactivation (Lambert et al. 2000, Li et al. 2000, Lirdprapamongkol et al. 2005).

The results of many investigations indicated that fat present in food products decreased the antimicrobial properties of phenolic compounds (Char et al. 2009, Char et al. 2010). It has been proved that fat might be responsible for creating a kind of protective film around the bacterial cells and diminishes the antimicrobial performance (Mejlholm and Dalgaard 2002). On the other side, there is a possibility of the migration of the antimicrobial substances to the food fat phase, which means that the water phase remains to be free of antimicrobial compounds and the inhibitory activity of these compounds is significantly diminished (Davidson et al. 1989, Tassou et al. 1995).

To sum up, it was found that a lithium salt of syringic acid was the most effective towards *E.* coli O157:H7, while a potassium salt of syringic acid and a potassium salt of vanillic acid were slightly less effective, and a lithium salt of vanillic acid, a sodium salt of vanillic acid and a potassium salt of m-anisic acid indicated comparatively lower antimicrobial activity, and lithium and sodium salts of m-anisic acid were the least effective against the pathogen. The salts of phenolic acids which possess various chemical structures are characterized by different inhibitory properties against foodborne pathogens.

IV CONCLUSIONS

The results of carried out experiment showed that the salts of phenolic acids are known to possess the inhibitory activity against the examined foodborne pathogen. Such activity is differentiated with relation to various chemical structures of analyzed salts. As the salts occurred to be effective towards the pathogen, many further researches should be performed to develop the most effective and suitable methods of fighting with pathogens in food products. The phenolic compounds may be applied instead of chemical preservatives for the purpose of guaranteeing the health safety and prolongation of shelf-life of food products.

Acknowledgements

This work was supported by grant no. N N312 427639.

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