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Full Length Research Paper

Antimicrobial activity of some potential active compounds against food spoilage microorganisms

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Antimicrobial activities of six potential active compounds (acetic acid, chitosan, catechin, gallic acid, lysozyme, and nisin) at the concentration of 500 μg/ml against the growth of *Escherichia coli, Staphylococcus aureus, Listeria innocua,* and *Saccharomyces cerevisiae* were determined. Lysozyme showed the highest antimicrobial activity against *L. innocua* and *S. cerevisiae* with an inhibition zone of 19.75 and 17.37 mm, respectively. Catechin was strongly active against *E. coli, L. innocua,* and *S. aureus* with 15.37, 19.38, and 17.00 mm of inhibition zone diameter, respectively. The minimum inhibitory concentration (MIC) value of catechin for *E. coli* and for *S. aureus* was the same at 640 μg/ml, while the minimum bactericidal concentration (MBC) values were 640 and 1,280 μg/ml, respectively. The MIC and MBC values of lysozyme for *L. innocua* were 160 and 640 μg/ml, respectively. *S. cerevisiae* was the most susceptible microorganism to lysozyme among others, since both its MIC and MBC were the lowest (2.5 μg/ml). However, catechin and lysozyme were combined in equal amounts; all tested microorganisms were effectively inhibited as indicated by both qualitative and quantitative antimicrobial activities. This study thus revealed the potential application of some active compounds such as catechin and lysozyme for their usage in food products.

Key words: Antimicrobial activity, catechin, lysozyme, agar disc diffusion, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC).

INTRODUCTION

Food-borne illnesses that result from consuming food contaminated with pathogenic bacteria have been of serious public concern worldwide. According to the Ministry of Public Health of Thailand, 96,383, 109,070, and 85,712 cases of food-borne illnesses were reported between 2009 and 2011, with a total of 11 fatalities. Meanwhile, requirements and standards for safe, stable, and high quality food products have been becoming more rigorous. Microbial activity is a primary reason for the deterioration of many foods and is often responsible for reducing quality and safety. Food-borne illnesses associated with *E. coli 0157:H7, S. aureus, Salmonella enteritidis,* and *L. monocytogenes,* are a major public health concern all over the world (Hall, 1997). *E. coli,* a Gram-negative bacterium, can enter human intestines and can cause urinary tract infection, coleocystitis, or septicaemia (Singh et al., 2000). *S. aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis, and food poisoning (Mylotte et al., 1987). *L. monocytogenes* is responsible for one of the severest food-bome illnesses, listeriosis, which has been recognised as an emerging zoonotic disease over the last two decades (Farber, 2000). A typical strains of *L. innocua* that present phenotypic characteristics similar to those of *L. monocytogenes* were recently isolated from food and from the environment. These isolates tested positive for

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virulence genes specific to *L. monocytogenes* (Moreno et al., 2012). *S. cerevisiae,* the dominant yeast in fermentation, is regarded as a spoilage organism and one of the most resistant strains that spoils most fruits, juices, vegetables, and to a lesser extent, cheese or meat products (Loureiro and Malfeito-Ferreira, 2003).

A number of methods have been employed to control or prevent the growth of these pathogens in food, including the use of physical, biological, or chemical methods, but due to health concerns about the use of chemical agents, there has been increased consumer demand for naturally-derived compounds such as antimicrobial substances. Naturally occurring antimicrobials have been widely investigated, including chitosan, lysozyme, nisin, various plant extracts such as tea, spices and their essential oils, as well as phenolic compounds (Cowan, 1999; Davidson, 2001; Min and Kwon, 2010; Juneja et al., 2012). These compounds have been used to inhibit food-borne bacteria and extend the shelf life of processed food by either reducing the microbial growth rate, or by extending the lag-phase of the target microorganisms. There has been a growing trend among consumers to desire high quality foods that are more natural, minimally processed, and preservative free or naturally derived, while also maintaining food safety. At the same time, much research has been focused on the antimicrobial activity of natural compounds against different bacteria, but data is still lacking for some major food spoilage microorganisms. The purpose of this study is to determine the inhibitory effects of some potential antimicrobial agents against four important species of food spoilage microorganisms.

MATERIALS AND METHODS

Acetic acid glacial was obtained from QRec ASIA Sdn Bhd (Rawang, Selangor). Gallic acid (G7384), catechin hydrolysate (C1251), Nisin (N5764), chitosan (low molecular weight: 448869), and lysozyme (EC 3.2.1.17 from chicken egg white) were procured from Sigma-Aldrich (MO, USA). Mueller-hinton broth (275730) was purchased from Difco (Kansas, USA). Mueller-hinton agar (105437) was purchased from Merck (Darmstadt, Germany). McFarland 0.5 turbidity standard was prepared by adding 0.5 ml of a 1.175% (w/v) barium chloride dihydrate ($BaCl_2$*$2H_20$) solution to 99.5 ml of 1% (v/v) sulfliric acid. 3M Petrifilm™ Aerobic Count Plates, 3M Petrifilm™ *E.* coli/coliform Count Plates, and 3M Petrifilm™ Yeast and Mould Count Plates were all purchased from DKSH (Thailand) Limited, Thailand.

Escherichia coli, Staphylococcus aureus, and Saccharomyces cerevisiae were obtained from the Biological Laboratory, Scientific and Technological Instruments Center, at Mae Fah Luang University, Chiang Rai, Thailand. *Listeria innocua* was purchased from the Department of Medical Sciences, Ministry of Public Health, in Bangkok, Thailand. The cultures were streak-plated once a week and a single colony was inoculated into the appropriate medium and incubated overnight in the appropriate atmospheric conditions.

Preparation of inoculums

E. coli, S. aureus, L. innocua, and *S. cerevisiae* were cultured into 5 ml of Mueller-hinton broth and incubated in a shaker incubator at 35 or 40°C for 18 to 24 h. The optical density (OD) of the cultures at 540 nm was adjusted to the standard of McFarland No. 0.5 with 0.85 to 0.9 g sodium chloride/100 ml sterile solution to achieve a concentration of approximately 10^8 colony forming unit (CFU)/ml (Canillac and Mourey, 2001). The final concentration of the approximate cell numbers of 10^6 to 10^7 CFU/ml was obtained by diluting them 100 times with sterile sodium chloride solution. The concentrations of each culture were confirmed by the Petrifilm method.

Antimicrobial susceptibility testing

The antimicrobial agents were tested for their inhibition against the target microorganisms: *E. coli, L. innocua, S. aureus* and *S. cerevisiae.* They were tested by using an agar disc diffusion method modified by EUCAST (2010). All of the test cultures used in the microbiological assay were twice-passaged 16 h cultures grown in Mueller-hinton broth. Cell densities of 10^6 to 10^7 CFU/ml were calculated and prepared.

Whatman no. 1 filter paper was cut into a disc form 5 mm in diameter with a sterilized hole-punch. They were then sterilized with UV light for 30 min (Cooksey, 2000). Each antimicrobial agent (acetic acid, catechin, gallic acid, lysozyme, and nisin) was dissolved in 60% (v/v) ethanol, and chitosan was dissolved in 1% (w/v) acetic acid. They were incorporated into the paper dies at a final concentration of 500 μg per disc. The discs were placed on Mueller-hinton agar plates that had been previously seeded with inoculums containing tested microorganisms in the range of 10^6 to 10^7 CFU/ml. The plates were then incubated at 35°C for 18 h *(E. coli, L. innocua* and *S. aureus)* and 40°C for 24 h *(S. cerevisiae).* The diameters of the inhibitory zone surrounding the paper discs as well as the contact area of the discs with the agar surface were recorded as an indication of inhibition of the microbial species. The evaluation of the inhibitory activity was carried out in quadruplicate by measuring the inhibition zones in millimeters.

Determination of minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC)

The aliquot of 4 ml of Mueller-hinton broth was placed into tubes before the antimicrobial agents were added. The concentrations of antimicrobial agents were adjusted to range from 1280, 640, 320, 160, 80, 40, 20, 10, 5, to 2.5 μg/mL by adding different volumes of the broth. Subsequently, aerobic bacteria were inoculated to the Mueller-hinton broth. The inoculum of 100 μl of the germ suspensions contained 10^6 to 10^7 CFU for aerobic bacteria. The aerobic bacteria were incubated at 35°C for 24 h, and *S. cerevisiae* was incubated at 40°C for 24 h before being visually evaluated for minimal inhibitory concentration (MIC). The MIC was defined as the concentration that resulted in no visible growth or less than 10 CFU, corresponding to an inhibition of 99.9% of the inoculum. For determining the minimal bactericidal concentration (MBC), a variant on the agar dilution method was used. The inoculation spots with no visible growth were cut and top-down streaked on the Mueller-hinton agar. The MBC was determined according to the MIC. The lowest concentration without visible growth corresponded with the MBC.

Statistical analysis

The data was subjected to analysis of variance (ANOVA). A means comparison was carried out by Duncan's multiple range tests. The analysis was performed by using a SPSS package (SPSS 10.0 for window, SPSS Inc, Chicago, IL).

Table 1. Qualitative activity and contact area* of some active compounds against food spoilage microorganisms.

Antimicrobial agents/microbial	*E. coli* Gram (−)		*L. innocua* Gram (+)		*S. aureus* Gram (+)		*S. cerevisiae* (Yeast)	
	Activity[a]	Contact area[b]	Activity	Contact area	Activity	Contact area	Activity	Contact area
Acetic acid	-	-	+	+	-	-	+	+
Chitosan	-	-	-	-	-	-	-	-
Catechin	+++	+	+++	+	+++	+	+	+
Gallic acid	-	-	++	+	+++	+	-	-
Lysozyme	-	-	+++	+	-	-	+++	+
Nisin	-	-	++	+	+	+	+	+
½ Catechin+ ½ Lysozyme	+++	+	+++	+	+++	+	+++	+

[a]Determined by observation on the growth of microorganism on agar surface.+, no growth ; -, microbial growth; +++, totally inhibited (∅>15 mm); ++, partially inhibited (∅12-15 mm); +, slightly inhibited (∅<12 mm); -, no inhibition (∅<0 mm). [b]Contact area is the part of agar on Petri dish directly underneath disc sample; determined by observation on the growth of microorganism under the disc sample on agar surface. +, no microbial growth in contact area; -, indicates microbial growth in contact area.

RESULTS AND DISCUSSION

Qualitative inhibitory activity of antimicrobial agents

The qualitative antimicrobial activity against food spoilage microorganisms of some active compounds is presented in Table 1. The determination of antimicrobial activity was performed in two ways. The first was observation of the growth on the agar surface to determine the inhibition activity of the active compounds. The results obtained were divided into categories of either growth (-) or no growth (+) of the microorganisms. They could then be classified into four levels: (+++) totally inhibited; (++) partially inhibited; (+) slightly inhibited and (-) no inhibition. The results show that catechin presented the highest inhibitory effect against all types of micro-organisms tested (+++ for *E. coli, L. innocua* and *S. aureus),* followed by lysozyme (+++ for *L. innocua* and *S. cerevisiae),* gallic acid (+++ for *S. aureus),* and (+ low efficiency for all, except for *E. coli*). Chitosan used in this experiment had no effect (-) on all tested spoilage microorganisms.

The nicin and acetic acid had low inhibitory effect on Gram-negative microorganisms and yeast when compared to others. The results also show that only catechin could inhibit the growth of Gram-negative microorganism (*E. coli.*). However, the combination of each 50% portion of catechin and lysozyme showed broad antimicrobial activity against all of the tested microorganisms. Lysozyme could only effectively inhibit the growth of *L. innocua* and *S. cerevisiae.* In general, the more sensitive microorganisms were *L. innocua, S. cerevisiae,* and *S. aureus,* while the most resistant was *E. coli.* These results can be attributed to the cell wall lipopolysaccharides of Gram-negative bacteria, which may prevent active components from reaching the cytoplasmic membrane (Ouattara et al., 1997). The difference in resistance of Gram-positive bacteria *(L. innocua* and *S. aureus)* to the active compounds may be due to the variability between the strains of the same species (Gomez-Estaca et al., 2010).

Growth of microorganisms underneath the disc sample on the agar surface was also determined and presented in Table 1 as a contact area. The results of this part were consistent with the qualitative antimicrobial activity. Those compounds possessed antimicrobial activity that provided for the positive inhibitory results presented on the contact area. At the same time, the compounds with no antimicrobial activity were observed to have negative inhibitory effects shown on the contact area. The results also showed that the contact area of some compounds was positive even though the compound had slightly inhibited the target microorganisms (that is, nisin or catechin for *S. cerevisiae*). Chitosan still showed no antimicrobial activity on the contact area. Consistent with previous results, *L. innocua* showed the highest susceptibility to almost all types of active compounds, except to chitosan.

Several categories of antimicrobials have been investi-gated, including organic acids, fungicides, bacteriocins, proteins, enzymes, inorganic gases, silver substitute zeolite, and others. These compounds can control microbial contaminations by either reducing the microbial growth rate or by extending the lag-phase of the target microorganisms. Nisin is an antimicrobial peptide, which is labelled Generally Recognized as Safe (GRAS) (Food and Drug Administration, 21CFR184.1081, 2006), and has shown to effectively inhibit Gram-positive bacteria (Cleveland et al., 2001). Organic acids are natural constituents of many foods and are also widely used as additives for food preservation (Lehrke et al., 2011). Acids and salts are commonly used to control the

Table 2. Quantitative antimicrobial activity of some active compounds against food spoilage microorganisms.

Antimicrobial agents/microbial	*E. coli* Gram (−)	*L. innocua* Gram (+)	*S. aureus* Gram (+)	*S. cerevisiae* (Yeast)
Acetic acid	0 ± 0.00^{aA}	8.75 ± 1.04^{bB}	0 ± 0.00^{aA}	10.10 ± 0.86^{bC}
Chitosan	0 ± 0.00^{aA}	0 ± 0.00^{aA}	0 ± 0.00^{aA}	0 ± 0.00^{aA}
Catechin	15.37 ± 0.48^{bB}	19.38 ± 2.06^{eC}	17.00 ± 1.83^{cBC}	10.75 ± 2.22^{bA}
Gallic acid	0 ± 0.00^{aA}	12.00 ± 1.41^{cB}	16.75 ± 0.87^{cC}	0 ± 0.00^{aA}
Lysozyme	0 ± 0.00^{aA}	19.75 ± 0.50^{eC}	0 ± 0.00^{aA}	17.37 ± 1.11^{cB}
Nisin	0 ± 0.00^{aA}	12.75 ± 0.50^{cD}	7.50 ± 0.58^{bB}	11.38 ± 0.95^{bC}
½ Catechin+ ½ Lysozyme	15.75 ± 1.70^{bA}	15.75 ± 0.96^{dA}	24.00 ± 0.82^{dB}	16.50 ± 0.58^{cA}

Values (n =4) with different superscripts (small letter) in the same column are significantly different ($P < 0.05$). Values (n = 4) with different superscripts (capital letter) in the same row are significantly different ($P < 0.05$). Mean ± standard deviation of inhibition diameter (mm) surrounding film discs.

growth of microorganisms in foods (Phan-Thanh et al., 2000). These include sorbic acid, potassium sorbate, p-amino benzoic acid, acetic acid and sodium diacetate, citric, lactic, malic, tartaric, benzoic, lauric and stearic acid.

Quantitative inhibitory activity of antimicrobial agents

In order to quantify their antimicrobial effect, all of the active compounds were also tested on food spoilage microorganisms: two Gram-positive bacteria *(L. innocua* and *S. aureus),* one Gram-negative bacteria *(E. coli),* and *S. cerevisiae.* The initial screening for antimicrobial activity of the investigated antimicrobial agents was studied against four tested microorganisms by using the agar disc diffusion assay. They were assessed by the presence and absence of inhibition zones. The antimicrobial activity of antimicrobial agents can be classified into four levels: totally inhibited (inhibition zone >15 mm), partially inhibited (12 mm< inhibition zone <15 mm), slightly inhibited (inhibition zone<12 mm), and no inhibition (no inhibition zone present). The results are shown in Table 2. The inhibition zones confirmed what has already been previously described, since the major zones of inhibition corresponded to catechin (10 to 19 mm). Moreover, the results indicate that catechin showed the highest antimicrobial activity against *E. coli* and *S. aureus* with a mean inhibition zone of 15.37 and 17.00 mm, respectively. Lysozyme (19.75 mm) was more effective than catechin (19.38 mm) against *L. innocua* as indicated by the diameter of the inhibition zone. Acetic acid only slightly inhibited *L. innocua* (8.75 mm) and *S. cerevisiae* (10.10 mm), while partially inhibited both Gram-positive bacteria (7.5 to 12.75 mm) and yeast (11.38 mm). Gallic acid was also another effective compound for inhibiting the growth of *S. aureus* (16.75 mm). On the other hand, lysozyme was a suitable antimicrobial agent against both *L. innocua* and *S.cerevisiae* when compared to others. Indeed, lyzozyme exhibits antimicrobial activity by splitting the bonds between N-acetylmuramic acid and N-acetylglucosamine of the peptidoglycan in the bacteria cell wall (Coma, 2008). In this experiment, chitosan was found to have no antimicrobial effect on all types of the microorganisms tested. It is probably because chitosan does not penetrate the lipo-polysaccharide layers of Gram-negative bacteria or the cell walls of other micro-organisms. The high molecular weight of chitosan may affect diffusion into the agar medium during microbial incubation. In general, chitosan antimicrobial activity comes from its positive charges that would interfere with the negatively charged residues of macromolecules on the microbial cell surface, causing the membrane to leak (Joerger, 2007; Dutta et al., 2009; Juneja et al., 2012).

Among the Gram-positive bacteria tested, *L. innocua* was more sensitive than *S. aureus,* yet the most resistant bacteria were *E. coli.* No inhibition was observed between *E. coli* and *S. aureus* when grown with acetic acid, chitosan, and lysozyme. *S. cerevisiae* was resistant to chitosan and gallic acid, while *E. coli* was the most resistant bacteria to all active compounds tested except for catechin. The antimicrobial activities of different active compounds have been studied by different researchers. Green tea polyphenols, especially catechin, have been reported to show inhibitory effects *in vitro* against food spoilage and pathogenic microorganisms including *L. monocytogenes, E. coli. Salmonella typhimurium, S. aureus. Shigella frexneri,* and *V. cholerae* (Hamilton-Miller, 1995; Perumalla and Hettiarachchy, 2011). The antimicrobial activity of tea polyphenols is probably due to the inhibition of DNA and RNA synthesis of bacterial cells. It may also be due to the inhibition of the cytoplasmic membrane function of bacteria and/or the interferance with energy metabolisms of bacteria (Siripatrawan and Naipha, 2012). There are some studies that showed tea extract is capable of inhibiting the growth of a number of spoilage bacteria and food-borne pathogens such as *Bacillus, Clostridium, E. coli, Pseudomonas, Salmonella, S. aureus. Shigella disenteriae, V. cholera, C. jejuni, L. monocytogenes,* etc.

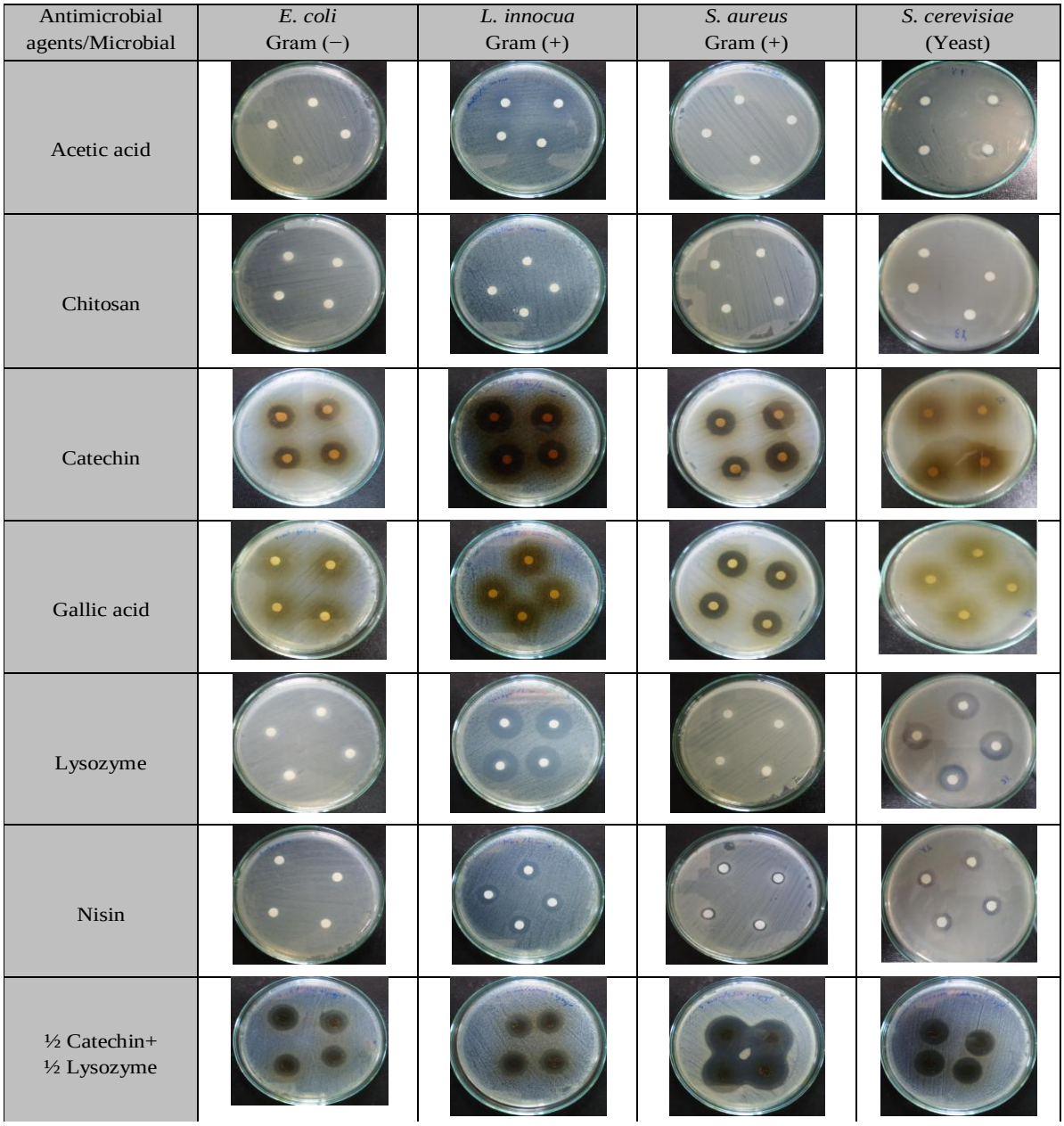

Figure 1. Antimicrobial activity of active compounds against *E.coli, L.innocua, S. aureus, and S. cerevisiae*

(Taguri et al., 2004; Almajano et al., 2008; Xi et al., 2012). Some researchers have found that green tea extract was not effective against *E. coli* (Nazer et al., 2005). Kim et al. (2004) reported that the major food-borne pathogens such as *E. coli, S. typhimurium, L. monocytogenes, S. aureus,* and C. *jejuni* have been reported to be inhibited by tea components, namely by catechins. Concerning phenolic acids studied, it has been reported that the site(s) and number of hydroxyl groups on the benzoic ring could be related to their relative toxicity to microorganism. Increased hydroxylation results in increased toxicity (Cowan, 1999).

Antimicrobial activity

The antimicrobial activities of all active compounds against *E. coli, L. innocua S. aureus,* and *S. cerevisiae,* are shown in Figure 1. The inhibition zones were formed depending on the strain, the kind of active compounds, and the concentration of active compounds used. After comparing the six active antimicrobial compounds tested, it became clear that catechin was the most active antimicrobial activity because it showed the largest inhibition zone against all other tested microorganisms. In addition, when catechin and lysozyme were combined,

Table 3. Minimum inhibitory concentration and Minimum bactericidal concentration of some active compounds against food spoilage microorganisms.

Compound	Microorganism	MIC* (µg/mL)	MBC** (µg/mL)
Catechin	*E. coli*	640	640
	S. aureus	640	1,280
Lysozyme	*L. innocua*	160	640
	S. cerevisiae	2.5	2.5

*MIC, Minimum inhibitory concentration; **MBC, minimum bactericidal concentration.

higher inhibitory activity for some microorganism was observed. These results confirmed the results presented in Table 1 and Table 2. Lysozyme showed higher growth inhibition against *L. innocua* than *S. cerevisiae,* while catechin showed the most effective inhibition for *S. aureus.* However, the compounds differ significantly in their activity against tested microorganisms. The most active compound was catechin, which showed broad-spectrum antimicrobial activity against *E. coli, L. innocua, S. aureus,* and *S. cerevisiae,* 15.37, 19.38, 17.00, and 10.75 mm, respectively.

MIC and MBC of antimicrobial agents

MIC is an accepted and well-used criterion for measuring the susceptibility of microorganisms to inhibitors. Many factors affect the obtained MIC value, including temperature, inoculum size, and type of microorganisms (Lambert, 2000). The MIC of tested compounds against selected microorganisms is presented in Table 3. The MIC of the catechin was determined against *E. coli.* and *S. aureus,* while lysozyme was tested for *L. innocua* and *S. cerevisiae.* The MIC values of the compounds ranging from 2.5 to 640 µg/mL are also shown in Table 3. Lysozyme showed very strong activity against *S. cerevisiae* with the lowest MIC (2.5 µg/mL). The lowest MIC for *E. coli* and *S. aureus* was obtained with catechin at 640 µg/mL, whereas the highest MIC was by lysozyme at 160 µg/mL for *L. innocua.* Both catechin and lysozyme exhibited concentration-dependent inhibition of growth. It is possible that these antimicrobial agents could limit bacterial growth by interfering with the bacterial protein biosynthesis, DNA replication, or other aspects of bacterial cellular metabolism. In this experiment, the MIC of Gram-positive bacteria *(S. aureus)* was the same as Gram-negative bacteria *(E. coli).*

The different concentrations of catechin and lysozyme showed various degrees of growth inhibition against *E. coli, S. aureus, L. innocua,* and *S. cerevisiae,* respectively, by using the broth dilution method. The growth of *E. coli* and *S. aureus* was inhibited by catechin at different concentrations, which delayed the lag phase and lowered the growth rate and final cell concentration of the microorganisms. When the concentration of catechin reached 640 and 1,280 µg/mL, the complete inhibition of *E. coli* and *S. aureus* growth was observed (Figure 2). In addition, the growth of *S. cerevisiae* and *L. innocua* was completely inhibited by lysozyme at the concentration of 2.5 and 640 µg/mL (Figure 2). The antibacterial properties of these active compounds may be associated with their lipophilic character. This leads to changes in membrane potential and increases the permeability of the cytoplasm membrane for protons and potassium ions, including depletion of the intracellular ATP pool (Juneja et al., 2012). Lysozyme is an antimicrobial peptide that is effective against Gram-positive (and sometimes gram-negative) bacteria. It is naturally present in egg white, plants, and animal secretions. Its antimicrobial properties are associated with the hydrolysis of peptidoglycan layers in the bacterial cell wall and also with membrane perturbation (Masschalck et al., 2002; Perez-Espitia et al., 2012). According to the MIC and MBC, the Gram-negative bacteria were more resistant than the Gram-positive bacteria. This may be due to the different natures of the Gram-negative cell envelope (made up of lipopolysaccharide), which restricts access to the membrane more than in Gram-negative bacteria. In the present study, the highest MBC was shown by *S. aureus*

Conclusion

Catechin and lysozyme showed promise for retaining antibacterial activity and inhibiting bacterial contaminants. Each active compound showed specific growth inhibition of food spoilage microorganisms in different ways. The results suggest that the combination of catechin and lysozyme may provide a unique functional barrier that could increase the shelf life of food products. Further studies will be carried out for incorporating these potential active compound combinations (catechin-lysozyme) into gelatin film models.

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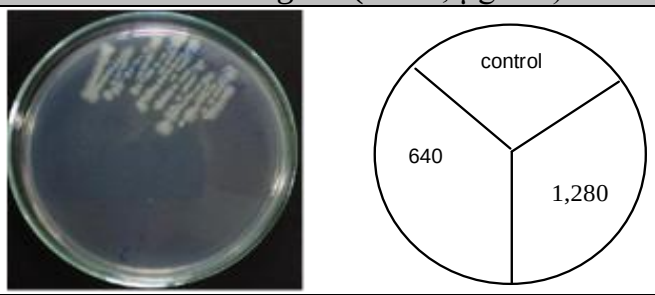
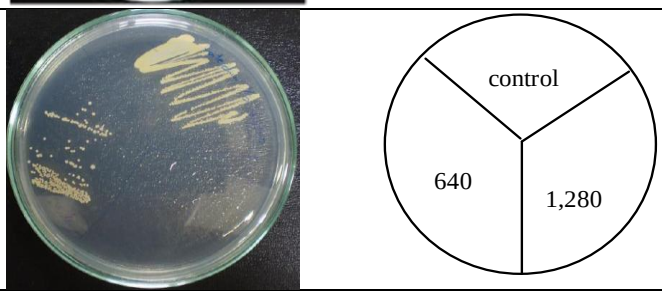
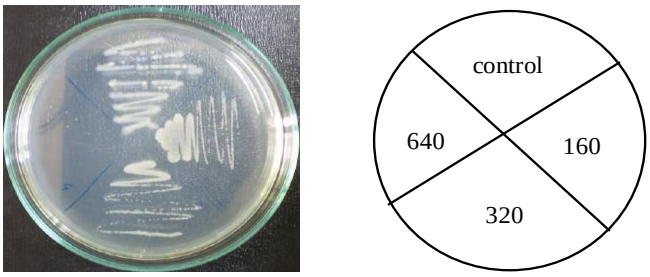
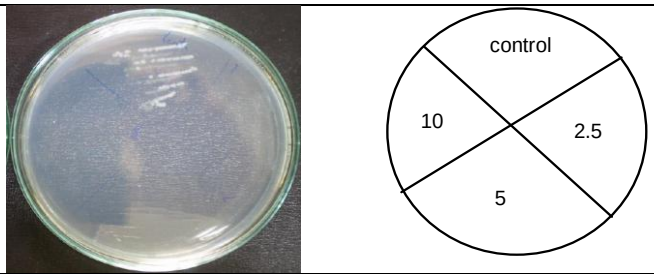
Compounds	Microorganisms	Photo and Legend (MBC; μg/mL)
Catechin	*E. coli*	
	S. aureus	
Lysozyme	*L. innocua*	
	S. cerevisiae	

Figure 2. Minimum bactericidal concentration (MBC) of catechin and lysozyme against *S. cerevisiae, L. innocua, S. aureus* and *E. coli.*

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REFERENCES

Almajano MP, Carbo R, Jimenez JAL, Gordon MH (2008). Antioxidant and antimicrobial activities of tea infusions. Food Chem. 108:55-63.

Canillac N, Mourey A (2001). Antibacterial activity of the oils of Picea excelsa on *Listeria monocytogenes Staphylococcus aureus* and coliform bacteria. Food Microbiol. 18:261-268.

Cleveland J, Montville TJ, Nes IF, Chikindas ML (2001). Bacteriocins: safe natural antimicrobials for food preservation. Int. J. Food Microbiol. 71:1-20.

Coma V (2008). Bioactive packaging technologies for extended shelf life of meat-based products. Meat Sci. 78:90-103.

Cooksey K (2000). Utilisation of antimicrobial packaging films for inhibition of selected microorganisms. In: Risch SJ (editor). Food packaging: testing methods and applications. Washington, DC: American Chemical Society pp.17-25.

Cowan M (1999). Plant products as antimicrobial agents,' Clin. Microbiol. Rev. 12:564-582.

Davidson PM (2001). Chemical preservatives and natural antimicrobial compounds, pp. 593-627. In: Food Microbiology Fundamentals and Frontiers. Doyle MP, Beuchat LR, Montville TJ (eds). ASM Press, Washington, DC, USA.

Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009). Perspectives for chitosan based antimicrobial films in food applications. Food Chem.

114:173-1182.
European Committee on Antimicrobial Susceptibility Testing: EUCAST. (2010). Breakpoint tables for interpretation of MICs and zone diameters. Version 1.1. 2010. (Accessed 26 November 2010).
Farber JM (2000). Present situation in Canada regarding Listeria monocytogenes and ready-to-eat seafood products. Int. J. Food Microbiol. 62:247-251.
Gomez-Estaca J, Lopez de Lacey A, Lopez-Caballero ME, Gomez-Guillen MC, Montero P (2010). Biodegradable gelatin-chitosan films incorporated with essential oils as antimicrobial agents for fish preservation. Food Microbiol. 27:889-896.
Hall RL (1997). Food-bome illness: implications for the fiiture. Emerg. Infect. Dis. 3:555-559.
Hamilton-Miller JM (1995). Antimicrobial properties of tea (*Camellia sinensis L*.). Antimicrob. Agents Chemother. 39:2315-2311.
Joerger RD (2007). Antimicrobial films for food applications: a quantitative analysis of their effectiveness. Packag. Technol. Sci. 20:231-273.
Juneja VK, Dwivedi HP, Yan X (2012). Novel natural food antimicrobials. Annu. Rev. Food Sci. Technol. 3:381-403.
Kim S, Ruengwilysup C, Fung DYC (2004). Antibacterial effect of water-soluble tea extracts on foodborne pathogens in laboratory medium and in a food model. J. Food Prot. 67:2608-2612.
Lambert RJW (2000). Susceptibility testing: inoculum size dependency of inhibition using the Colworth MIC technique. J. Appl. Microbiol. 89:275-279.
Lehrke G, Hemaez L, Mugliaroli LS, Staszewski MV, Jagus RJ (2011). Sensitization of *Listeria innocua* to inorganic and organic acids by natural antimicrobials. LWT- Food Sci.Technol. 44:984-991.
Loureiro V, Malfeito-Ferreira M (2003). Spoilage yeasts in the wine industry. Int. J. Food Microbiol. 86:23-50.
Masschalck B, Deckers D, Michiels CW (2002). Lytic and nonlytic mechanism of inactivation of Gram-positive bacteria by lysozyme under atmospheric and high hydrostatic pressure. J. Food Prot. 65:1916-1923.
Min KJ, Kwon KY (2010). Effect of various antimicrobials on the growth kinetics of food-borne pathogens in ready-to-eat, *pyeonyuk* (cooked and pressed pork). Food Sci. Biotechnol. 19:99-106.
Moreno LZ, Paixao R, Gobbi DD, Raimundo DC, Ferreira TP, Hofer E, Matte MH, Moreno AM (2012). Characterization of atypical Listeria innocua isolated from swine slaughterhouses and meat markets. Res. Micro. 163:268-271.
Mylotte JM, McDermott C, Spooner JA (1987). Prospective study of 114 consecutive episodes of *Staphylococcus aureus* bacteremia. Rev. Infect. Dis. 9:891-907.
Nazer AI, Kobilinsky A, Tholozan JL, Dubois-Brissonnet F (2005). Combinations of food antimicrobials at low levels to inhibit the growth of Salmonella sv. Typhimurium: A synergistic effect? Food Microbiol. 22:391-398.
Ouattara B, Simard RE, Holley RA, Piette GJP, Begin A (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. Int. J. Food Microbiol. 37:155-162.
Perez-Espitia PJ, Ferreira-Soares NF, Coimbra JSR, Andrade NJ, Cruz RS, Alves-Medeiros EA (2012). Bioactive peptides: synthesis, properties, and applications in the packaging and preservation of food. Comp. Rev. Food Sci. Technol. 11:187-204.
Perumalla AVS, Hettiarachchy NS (2011). Green tea and grape seed extracts e potential applications in food safety and quality. Food Res. Int. 44:827-839.
Phan-Thanh L, Mahouin F, Alige S (2000). Acid responses of *Listeria monocytogenes.* Int. J. Food Microbiol. 55:121-126.
Singh R, Chandra R, Bose M, Luthra PM (2000). Antibacterial activity of curcuma longa rhizome extract on pathogenic bacteria. Curr. Sci. 83:737-740.
Siripatrawan U, Noipha S (2012). Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. Food Hydrocolloid. 27:102-108.
Taguri TT, Tanaka T, Kouno I (2004). Antimicrobial activity of 10 different plant polyphenols against bacteria causing food-bone disease. Biol. Pharm. Bull. 27:1965-1969.
Xi D, Liu C, Su YC (2012). Effects of green tea extract on reducing *Vibrio parahaemolyticus* and increasing shelf life of oyster meats. Food Control. 25:368-373.