Plant Extracts as Components of Edible Antimicrobial Protective Coatings

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Abstract

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The antioxidant and antimicrobial efficiency of edible coating solutions of hydroxypropylmethylcellulose with various plant extracts were formed and determined. Antimicrobial activity of different concentrations (0, 1000, 2000 ppm) of plant extracts such as rosemary, garlic, thyme, clove, and cinnamon against *Bacillus subtilis* and *Pseudomonas fluorescens* was analysed using a plate diffusion test. The antioxidant activity was presented as DPPH radical scavenging activity, Ferric Reducing Antioxidant Power (FRAP), and metal chelating ability. The film containing a rosemary extract reduced the growth of *Bacillus subtilis*. The addition of cinnamon, garlic, and clove extract to the film formulation significantly increased an inhibitory effect on *Pseudomonas fluorescens* growth. Films prepared with clove and rosemary extracts were characterised by the highest antioxidant activity. DPPH values of these extracts were 0.097μM Trolox/ml and 0.129μM Trolox/ml, respectively.

Keywords: antimicrobial activity; antioxidant activity; edible coatings; hydroxypropylmethylcellulose

Edible films and coatings are a modern food protection system. These edible films could form a barrier for physical, chemical and biological changes such as dehydration of fresh and frozen meat or microorganism growth on the food surface. Growth of bacteria on the meat surface is a major cause of food spoilage and discoloration. Considering that ecological and functional demands of consumers are increasing, researchers have focused to a larger extent on biodegradable films including films made from starch or cellulose. These films have good mechanical strength and create a good oxygen barrier (Skurtys *et al.* 2010). It is also possible to incorporate antimicrobial agents into the edible film structure, which could extend the shelf-life of products and provide microbial safety of fresh meat. Many spices and their extracts possess antimicrobial and antioxidant activity which was confirmed by many studies. They may be used as essential tools to improve the food safety and reduce foodborne illnesses. It is possible to use plant extracts instead of chemical preservatives (Arora & Kaur 1999; Czapska *et al.* 2006; Genena *et al.* 2008). Spices and plant extracts are good sources of antioxidant activity, especially rosemary and clove.

The aim of the present study was to form and determine antioxidant and antimicrobial efficiency of edible coating solutions which contain plant extracts.

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MATERIAL AND METHODS

Antibacterial activity. Aqueous plant extracts of rosemary, garlic, thyme, clove and cinnamon were selected for this investigation. Powdery forms of extracts were purchased from RAPS GmbH & Co (Kulmbach, Germany) and were suspended in water. Hydroxypropylmethylcellulose (HPMC) at 1% concentration was used as a carrier, which was dissolved in 0.25% lactic acid solution. Glycerol was added as a plasticiser (25% of dry weight of used polymers). Plant extracts were incorporated into a film solution at various concentrations: 0 ppm (control 1), 1000 ppm and 2000 ppm. Other two reference samples were used in this experiment: control 2 with 0.25% lactic solution and control 3 with distilled water.

Plate diffusion test was used to determine the antibacterial activity of a film solution. *Bacillus subtilis* and *Pseudomonas fluorescens* were investigated in the experiment. The bacterial cultures were grown on a nutrient agar slant and kept at 4°C. *Bacillus subtilis* inoculum was prepared by growing cells in Tryptic Soy Broth (Sigma Aldrich, Poznan, Poland) at 37°C for 24 h and *Pseudomonas fluorescens* in enriched broth (BTL) at 25°C for 24 hours. Optical density of the bacterial culture was measured in a Ray Leigh UV 1800 spectrophotometer at 550 nm. Agar plates were inoculated with 10^6 CFU/ml of bacterial cultures, and samples (50 µl) were applied to a number of holes 9 mm in diameter. The plates were incubated at 37°C for 24 h (*Bacillus subtilis*) and at 25°C for 48 h (*Pseudomonas fluorescens*). During the incubation, bioactive compounds of the film solution diffuse from the hole into the agar and they inhibit the growth of tested bacteria. The diameter of the inhibition zone (mmú was measured using the GIMP 2 program (http://www.gimp.org). The experiment was done in triplicate.

Data were analysed by analysis of variance (ANOVA) using Statistic 9. Differences between means were established by Duncan's Test at 5% significance.

DPPH radical scavenging activity. Free radical scavenging activity of the plant extracts was determined by the method of Chen *et al.* (2007). 0.5 ml of 0.3mM freshly prepared DPPH – ethanol solution was mixed with 1 ml of the extract of varying concentration (1000–2000 ppm) and 1 ml of ethanol. The reaction mixture was shaken well and incubated at ambient temperature for 30 minutes. The reduction of the DPPH free radicals was measured by reading the absorbance at 517 nm. Antioxidant activity of plant extracts was expressed as micromoles of Trolox per millilitre of the film solution (µM Trolox/ml).

FRAP assay. The ferric reducing antioxidant power assay (FRAP) was performed according to Benzie and Strain (1996). Reagents included 300mM acetate buffer, 10mM TPTZ (2,4,6-tripyridyl-*s*-triazine), 40mM HCl, 20mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing: 25 ml acetate buffer; 2.5 ml TPTZ; 2.5 ml $FeCl_3 \cdot 6H_2O$ solution. Plant extracts (0.2 ml) were added to water (0.8 ml) and mixed with the working solution (3 ml) and were incubated for 10 min in the dark condition. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. Results were expressed in µM $Fe^{(II)}$/ml.

Chelating ability. The chelating of ferrous ions by plant extracts was estimated in accordance with the modified method of Xu *et al.* (2007). Extracts were added to the solution of 2mM $FeCl_2$. After 2 min of incubation at room temperature 1 ml of 5mM ferrozine was added and the mixture was left standing at room temperature for 10 minutes. Absorbance of the solution was measured spectrophotometrically at 562 nm.

RESULTS AND DISCUSSION

Films containing plant extracts such as cinnamon, clove, garlic, thyme and control samples were not effective in the inactivation growth of *Bacillus subtilis* (Table 1). The coating solution produced with rosemary extract showed an inhibitory effect on these bacteria. Increased concentration of rosemary extract caused a significantly larger zone of inhibition. The largest zone of inhibition was observed at 2000 ppm of rosemary extract, and equalled 2.56 mm. Antibacterial activity of rosemary preparations depends on concentration and their types, which is confirmed by our results (Hać-Szymańczuk *et al.* 2009). Also Genena *et al.* (2008) reported that Gram-positive bacteria are more sensitive than Gram-negative ones to the antibacterial compounds from rosemary extract. It was found that control samples 1 and 2 were active against *Pseudomonas fluorescens*, but the differences were not statistically significant (Table 1). The addition of rosemary or thyme extract to the film formulation significantly reduced their antimicrobial activity in comparison with control

Table 1. Antibacterial activity of plant extracts in edible film solution against *Bacillus subtilis* and *Pseudomonas fluorescens*

Extract	*Bacillus subtilis*			*Pseudomonas fluorescens*		
	inhibitory zone (mm)	extract concentration (ppm)	inhibitory zone (mm)	inhibitory zone (mm)	extract concentration (ppm)	inhibitory zone (mm)
Rosemary	2.30[a]	1000	2.03[a]	3.61[b]	1000	3.47[a]
		2000	2.56[b]		2000	3.75[a]
Garlic	0.0[b]	1000	0.0[c]	5.09[a]	1000	5.08[d]
		2000	0.0[c]		2000	5.10[d]
Thyme	0.0[b]	1000	0.0[c]	3.78[b]	1000	3.75[a]
		2000	0.0[c]		2000	3.80[a]
Clove	0.0[b]	1000	0.0[c]	5.10[a]	1000	4.93[c,d]
		2000	0.0[c]		2000	5.27[d]
Cinnamon	0.0[b]	1000	0.0[c]	4.93[a]	1000	4.89[c,d]
		2000	0.0[c]		2000	4.97[c,d]
Control 1	0.0[b]	0	0.0[c]	4.30[c]	0	4.30[b]
Control 2	0.0[b]	0	0.0[c]	4.55[c]	0	4.55[b,c]
Control 3	0.0 [b]	0	0.0[c]	0.00 [d]	0	0.00[e]

[a–c]different superscript letters in the same column are significantly different ($P \leq 0.05$) according to ANOVA

films 1 and 2. Control 3 did not show any inhibitory activity against *Pseudomonas fluorescens*. Coating solutions containing cinnamon, garlic or clove extracts were more effective against tested bacteria than other films. The largest zone of inhibition was observed at 2000 ppm of clove extract, and equalled 5.27 mm. It was also found that *Pseudomonas fluorescens* is sensitive to lactic acid, which was confirmed by Shirazinejad (2010). According to Ankri (1999), garlic also exhibits antibacterial activity against a wide range of Gram-negative and Gram-positive bacteria, antifungal and also antiparasitic activity. Other study confirmed its activity against *Campylobacter, Escherichia, Salmonella, Shigella, Vibrio, Yersinia, Listeria, Klebsiella, Lactobacillus, Enterococcus*, and *Bacteroides* (Ross *et al.* 2001). Minimum inhibitory concentration of powdered garlic extract against *Bacillus subtilis* was obtained at a level of 6.25 mg/ml (Kędzia 2010). According to Hoque *et al.* (2008), essential oils of clove and cinnamon have stronger antimicrobial properties than ethanol and their aqueous extracts.

Plant extracts used in the experiment have also shown antioxidant activity, which depends on the type and dosage of used extract (Figure 1a). The highest DPPH free radical scavenging activity (0.129μM Trolox/ml) was observed in rosemary extract. A high scavenging effect was also found out

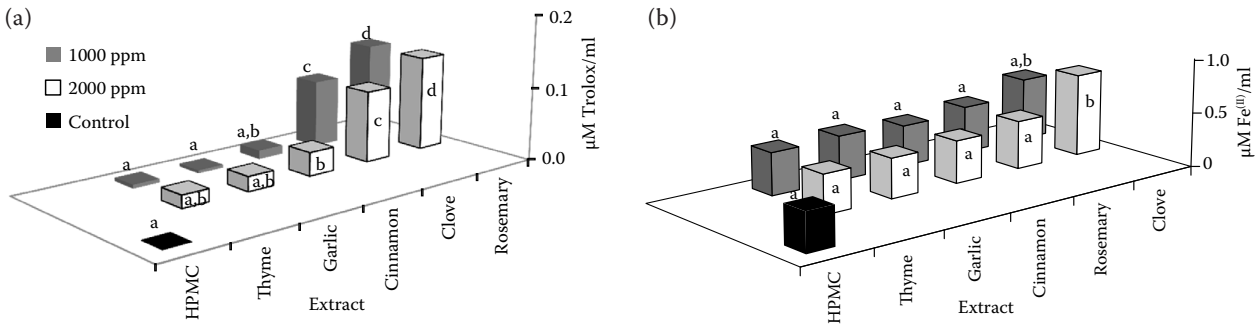

Figure 1. Antioxidant activity of plant extracts determined by the (a) DPPH and (b) FRAP assay

[a–d]different superscript letters in the same column are significantly different ($P \leq 0.05$) according to ANOVA

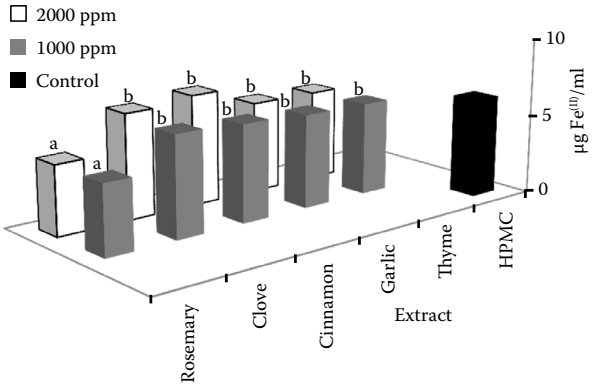

Figure 2. Antioxidant activity of plant extracts determined by the iron chelating activity screening

[a–b]different superscript letters in the same column are significantly different ($P \leq 0.05$) according to ANOVA

in clove extract at 2000 ppm (0.097µM Trolox/ml) and at 1000 ppm (0.092µM Trolox/ml). Statistical analysis proved that other plant extracts such as thyme, garlic, cinnamon had the significantly lower DPPH free radical scavenging activity (Figure 1a).

The FRAP assays showed no differences between control sample (HPMC) and samples containing thyme, garlic, cinnamon, or rosemary extracts. The reducing ability of these extracts was in the range of 0.357–0.439µM $Fe^{(II)}$/ml (Figure 1b). A significant influence of clove extract addition to HPMC film on antioxidant activity was observed. The maximum FRAP value was obtained at 2000 ppm of clove extract (0.748µM $Fe^{(II)}$/ml).

The chelating of ferrous ions by plant extracts was not found with the exception of rosemary extract (Figure 2). Antioxidant activity of plant extracts was confirmed in many studies (De Vries 1997; Gulcin *et al.* 2004; Fernandez-Lopez *et al.* 2005; Wojdyło *et al.* 2007; Woźniak *et al.* 2009). The results pointed out the potential value of HPMC use to produce edible coatings whose biological activity depends on the type of used plant extracts, which was confirmed in the study of Skurtys *et al.* (2010).

CONCLUSIONS

The results have shown that plant extracts exhibit stronger antibacterial properties against *Pseudomonas fluorescens*. They could be incorporated into edible film and coating formulation as antimicrobial agents, which will lead to improved food safety. It creates a possibility to reduce the application of chemical preservatives, which are in common use in the food industry. Extracts of clove, garlic and cinnamon reduce the growth of *Pseudomonas fluorescens*, which is important in chill storage of meat.

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