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ANTIMICROBIAL ACTIVITY OF SEVERAL HERBAL AND SPICE EXTRACTS AND THEIR ROLE IN THE PRESERVATION OF VACUUM-PACKAGED PORK

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Introduction

Many plant-derived antimicrobial compounds exhibit a wide spectrum of activity against bacteria and fungi, and this has led to the suggestion that they could be used as natural preservatives in food (Farag et al., 1989; Djenanea et al., 2002, 2003). In particular, many kinds of oriental spice plants have long been known to possess antimicrobial effects. Among them are cassia, clove, garlic, sage, oregano, pimento, thyme, rosemary, scutellaria, and forsythia suspensa (Thunb) (Shelef et al., 1980; Yildirim et al., 2000). Many plant oils have also been shown to be effective in inhibiting foodborne pathogens, including *Escherichia coli* O157:H7 and *Salmonella enterica* (Friedman et al., 2004).

Because of its high nutrient density, meat can be an excellent reservoir for food-borne pathogens and other infectious agents, letting alone various spoilage microorganisms. *Escherichia coli*, including the *E. coli* O157:H7 strain, and *Pseudomonas fluorescens*, are potential pathogens that can contaminated animal-derived foods, including ground beef, hamburger patties, poultry, milk, and even ham and cheese sandwiches (Zottola and Smith, 1991; Eriksson et al., 1995). Lactic acid bacteria are the predominant group of microorganisms isolated from vacuum-packaged meat and meat products (Hitchener et al., 1982; Shaw & Harding, 1984; Kato et al., 2000; Sakala et al., 2002). Although the antimicrobial effects of some of herbs and spices have been well documented, few studies have been conducted to investigate the feasibility of using herbal and specie extracts as potential antimicrobial agents for the preservation of chilled meat packaged under vacuum conditions.

Objectives

In the present study, the growth response of three representative bacteria that commonly exist in vacuum-packaged chilled meat and meat products, including *E. coli*, *P. fluorescens* and *L. plantarum*, to the extracts of several herbs and spices was investigated. Specifically, the effect of honeysuckle, scutellaria, forsythia suspensa (Thunb), cinnamon, rosemary and clove water or 70% ethanol extracts, alone and in

combination, on growth of these three bacteria on agar media was examined. The optimum antimicrobial conditions (extract concentrations, types and combinations) established were subsequently used to inhibit microbial growth in vacuum-packaged chilled meat.

Methodology

Preparation of herb and spice extracts

Water extracts of honeysuckle, scutellaria, forsythia suspensa (Thunb), cinnamon, and rosemary were prepared by boiling (100°C) the pulverized dry herbs/spices (50 g each) for 2 h and then filtration with a filter paper. The filtrates were concentrated on a rotary evaporator with a vacuum pump to a final 50 mL volume. The concentration of each of the extracts was assigned a '1 g/mL' unit (based on the herb/spice weight). Ethanol (75%) extracts were prepared by mixing 50 g of pulverized and herbs and spices with 250 mL of 75% ethanol for 48 h with constant agitation. After filtration with a filter paper, the residue was re-extracted with an additional 100 mL of ethanol for another 24 h and then filtered. The combined filtrates were subsequently concentrated on a rotary evaporator with a vacuum pump to 50 mL, and the concentration of the extracts was assigned a '1 g/mL' unit (based on the herb/spice weight).

For both the water and the ethanol extracts, a serial dilution was done to obtain the following concentrations: 1, 0.5 0.25 0.125, and 0.063 g/mL. Olive oil was diluted to 1.0, 0.75, 0.50, and 0.25% with 75% ethanol. Sterilized water and 75% ethanol alone (0 g/mL herb or spice extract) was used as controls. These solutions were used singly or in various combinations to inhibit bacterial growth as described later.

Antimicrobial activity test

The antimicrobial activity of herb/spice extracts was examined using the disk diffusion test (Kim et al., 1995). Briefly, 1 mL of bacterial cultures (Escherichia coli strain ATCC 25922, Pseudomonas fluorescens strain AS1.1802, and Lactobacillus plantarum, 10^{6}-10^{8} CFU/mL) was inoculated into 100 mL of sterile agar (30-37°C) and gently mixed. The mixture was poured into sterile plates and cooled to 20°C to allow solidification. Sterile micro steel cups (0.78d ×1.0h cm) were vertically set on the agar in the plates, and 0.5 mL of the extractives from each of the herbs or spices at each dilution (0.063-1.0 g/mL) or the diluted olive oil (0.25-1.0%) was then pipetted into the steel cups. With the lid on, the plates were incubated at 30°C for 24 h for P. fluorescens and at 37°C for 24 h for E. coli and L. plantarum. The inhibitory effect was assessed by measuring the diameter of the clear zone (circle) around the extract-filled steel cup by means of a vernier caliper.

Combination antimicrobial tests in agar media

In addition to testing the antimicrobial activity of the water or ethanol extracts individually (singly), combination antimicrobial tests were also conducted using three herbs/oil (cinnamon, rosemary and clove oil) or four herbs (scutellaria, cinnamon,

honeysuckle, and forsythia suspensa), which was arranged as an orthogonal experiment of three and four factors, respectively, each at three levels (0.125, 0.25, and 0.5 g/mL). Antimicrobial effects in chilled meat

Four ethanol extracts of herbs and three ethanol extracts of spices that exhibited the strongest antimicrobial effects as demonstrated in the agar medium experiment were selected for use as antimicrobial preservatives in chilled meat. Longissimus dorsi muscles were aseptically obtained from pork carcasses 12 h after harvest and divided into 200-250 g chops. Samples were immersed into herb/spice preservatives for 20 s, then vacuum packaged in plastic trays sealed with BOPA/PE films (water and O_2 transmission rates lesser than of 8 and 35$cm^3.m^{-2}.24h.atm^{-1}$, respectively). The products were subsequently stored at 4°C and examined weekly for microbial growth for up to 4 weeks. The microbial analysis was done by means of aerobic plate count (APC) and sensory panel evaluation. The APC counts were recorded by colony forming units per gram of meat sample (CFU/g), and the sensory evaluation was performed with a 6-member train panel that evaluated the meat color using a 5-point scale (5 = bright purplish red, to 1 = brown), and off-odor also using a 5-point scale (5 = intense, to 1 = none).

Statistical analysis

The significance of differences among samples was determined by analysis of variance using the least square difference method of the General Linear Model procedure. Differences were considered significant at the $P < 0.05$ levels.

Results & Discussion

Antimicrobial effects of herbal and spice extracts in agar medium

Both the water and the ethanol extracts of all the herbs/spices examined (honeysuckle, scutellaria, forsythia suspensa, cinnamon, and rosemary) were inhibitory of the growth of the three bacteria (E. coli, P. fluorescens, and L. plantarum) as evidenced by the size enlargement of the diffusion disc. However, the water extract of scutellaria showed the strongest antimicrobial activity especially against E. coli – e.g., the disc diameter (DD) was 17.0 mm at the concentration of 0.5 mg/mL. The same extract also had a strong inhibition to P. fluorescens and L. plantarum, showing a DD of 14.4 mm and 10.3 mm, respectively, at the concentration of 0.5 mg/mL. Water extracts of forsythia and honeysuckle also exhibited considerable antimicrobial activities, especially to E. coli. The water extract of rosemary had a weak inhibition against E. coli., but was suppressive to L. plantarum.

The antimicrobial effects if the ethanol extracts were overall similar to those of water extracts at equal dosages, suggesting that the same type of bioactive components were likely extracted with both solvent systems. The antimicrobial substance of cassia bark has been identified to be trans-cinnamic aldehyde, which showed insecticidal and fumigant activities against Mechoris ursulus (Park et al., 2000). Cinnamic aldehyde has also been isolated from cinnamon shoot (Kim et al., 2004). Zhang et al. (1997) found that the main components of Scutellaria are flavonoids, which can inhibit a variety of bacteria.

Clove oil, which was dissolved in ethanol before application, did produce a remarkable inhibition even at low concentrations against all the three bacteria. However, an inverse relationship was observed between the concentration of the oil and the

microbial growth inhibition, probably due to the poor diffusivity of the oil in the agar, a water-based medium.

The combination of three or four different ethanol extracts resulted in significantly enhanced ($P < 0.05$) antimicrobial capability essentially for all the herbs and spices against the three bacteria evaluated, with the improvements typically in the 30-50% range (Table 1; Table 2). The best composite herbal antimicrobial system appeared to be 0.125 g/mL scutellaria + 0.25 g/mL cinnamon + 0.5 g/mL honeysuckle + 0.125 g/mL forsythia (Table 1). The R value calculated from the orthogonal experiment results indicated that the relative contributions of the extracts followed that order of cinnamon > honeysuckle > scutellaria > forsythia. The mixed extracts were especially effective against E. coli, surpassing the activity of potassium sorbate at 2-5 mg/mL concentration level. Djenanea et al. (2002) has previously reported that the mixture of rosemary and vitamin C significantly reduced the rates of metmyoglobin formation and lipid oxidation, as well as microbial growth, and it extended the display life of beef steaks from about 10 to about 20 days. Similarly, the combination of three spice ethanol extracts yielded a high antimicrobial activity, with the 0.25 mg/mL cinnamon + 0.125 g/mL rosemary + 0.25% clove oil producing the highest inhibition of microbial growth (Table 2). From the calculated R value, it can be established that the relative importance of the three spices was rosemary > cinnamon > clove oil.

Antimicrobial effect of herbal/spice extracts in refrigerated pork

The aerobic plate counts throughout display of chilled pork are shown in Figure 1. The initial bacterial population was 3.16 $\log_{10}$ CFU/g. From day 5 to day 28, the aerobic plate counts gradually increased to 7.4 $\log_{10}$ CFU/g for control, but for treated meat samples, the values were between 5 and 6.2 $\log_{10}$ CFU/g, which reflected 1.2 to 2.4 log reductions ($P < 0.05$).

The antimicrobial effect of the following combined spice preservative treatment showed the greatest of all: 0.25 g/mL cinnamon + 0.125 g/mL rosemary + 0.25% clove oil, which lowered the $\log_{10}$ CFU/g value by 2.32 when compared with the control after 28 days of storage. The effects of combined all-spice ethanol extracts, 0.5g/mL scutellaria + 0.25 g/mL cinnamon + 0.25 g/mL honeysuckle + 0.25 g/mL forsythia suspense, was also quite significant, lowering the $\log_{10}$ units by 0.45, 1.48, 1.93 and 1.57 CFU/g on days 7, 14, 21 and 28 during storage when compared with control.

The effect of herbal/spice extracts on sensory characteristics of refrigerated pork

The Hunter color L* values of samples treated with herb/spice preservatives were higher than that of control throughout the 28 days of storage ($P < 0.05$). The Hunter color a* value was also higher than that of control, indicating that the herbal/spice extracts contained antioxidant activities. Lee and Shibamoto (2001) showed that extract of clove buds had considerable antioxidant activity.

Djenanea et al. (2003) showed that the use of the antioxidant mixture of rosemary and vitamin C for fresh beef steak packaged in high oxygenation conditions significantly reduced the rate of metmyoglobin formation and lipid oxidation. Mancini et al. (2005) used rosemary and lactate in beef for improving strip lion steak color stability during display in modified atmosphere package, and showed that steaks with rosemary retained more red color during storage. Results of sensory analysis of stored meat showed no difference between samples treated with preservatives and control ($P > 0.05$), although all the meat samples, regardless of herb/spice treatments, showed an increased off-flavor

(from 2 – low to 4 – high) and a decreased red color (from 4.5 – red to 2.5 – brown) during storage. The results supported the notion that the addition of herb/spice preservatives had no adverse effects on sensory characteristics of chilled meat, which was somewhat consistent with the reports of Djenanea et al. (2003). The sensory color evaluation did exactly agree with the Hunter color values, probably due to the variation in the scores assigned by the individual panel members despite the training provided.

Conclusions

The study demonstrated antimicrobial activities of selected herbs and spices in both agar and meat systems, suggesting that they may serve as potential, natural antimicrobial agents for the inhibition of spoilage and pathogenic microorganisms in muscle foods. Although many of the herbs and spices exhibit antibacterial effect, the combination of several herbal/spice extracts would produce stronger microbial inhibition. The lack of side effects of these antimicrobial food ingredients on the color and flavor further indicates the commercial feasibility of this alternative meat quality preservation technique.

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Tables and Figures

Table 1: Factors and levels scheme and results of orthogonal experiment of ethanol extracts from four herbs.

					Diameter of the diffusion zone (mm)			
No.	Scutellaria	Cinnamon	Honeysuckle	Forsythia	*Escherichia*	*Pseudomonas*	*Lactobacillus*	
	(g/mL)	(g/mL)	(g/mL)	g/mL)	*coli*	*fluorescens*	*plantarum*	Average
1	0.500	0.500	0.500	0.500	20.58	19.96	17.67	19.40
2	0.300	0.25	0.25	0.25	20.41	11.05	23.97	18.48
3	0.500	0.125	0.125	0.125	18.83	10.40	16.10	15.11
4	0.250	0.500	0.25	0.125	17.40	12.23	20.57	16.73
5	0.250	0.250	0.125	0.500	17.19	12.72	18.22	16.04
6	0.250	0.125	0.500	0.250	16.82	16.14	19.23	16.04
7	0.125	0.250	0.125	0.250	17.12	15.17	22.59	18.29
8	0.125	0.250	0.500	0.125	17.02	19.29	20.42	18.91
9	0.125	0.125	0.25	0.5	14.54	16.68	15.72	15.65
k_1	17.66	18.14	18.12	17.03				
k_2	16.27	17.81	16.95	17.60				
k_3	17.62	15.60	16.48	16.92				
R value	1.39	2.54	1.64	0.68				

Table 2: Factors and levels scheme and results of orthogonal experiment of ethanol extracts of three spices.

				Diameter of the zone of inhibition of bacterial growth (mm)			
	Cinnamon	Rosemary	Clove oil	*Escherichia*	*Pseudomonas*	*Lactobacillus*	
Number	(g/mL)	(g/mL)	(%)	*coli*	*fluorescens*	*plantarum*	Average
1	0.500	0.500	0.500	9.66	1285	13.74	11.26
2	0.500	0.250	0.250	8.58	1169	15.85	12.04
3	0.500	0.125	0.125	8.24	1390	18.90	13.68
4	0.250	0.50	0.125	8.43	1634	11.86	12.21
5	0.250	0.250	0.500	1.363	154	13.03	14.02
6	0.250	0.125	0.250	1/838	1668	12.97	16.01
7	0.125	0.500	0.250	8.56	1356	11.72	11.28
8	0.125	0.250	0.125	9.99	1640	13.01	13.13
9	0.125	0.125	0.500	1.465	1491	12.89	14.15
k_1	13.33	11.60	13.47				
k_2	14.07	13.03	13.30				
k_3	12.83	14.60	12.77				
R value	1.74	3.00	0.70				

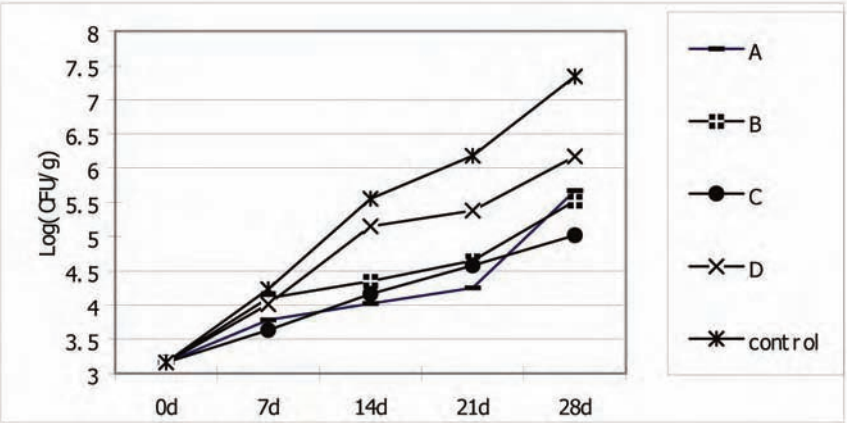

Figure 1: Aerobic plate count numbers in vacuum-packaged pork, treated with combined herbal/spice ethanol extracts, during refrigerated storage (4°C).
A = 0.5 g/mL scutellaria + 0.25 g/mL cinnamon + 0.25 g/mL honeysuckle + 0.25 g/mL forsythia suspensa; B = 0.125 g/mL scutellaria + 0.25 g/mL cinnamon + 0.5 g/mL honeysuckle + 0.125 g/mL forsythia suspense; C = 0.25 g/mL cinnamon + 0.125 g/mL rosemary + 0.25% clove oil; D = 0.125 g/mL cinnamon + 0.125 g/mL rosemary + 0.5% clove oil; Control = 75% ethanol only.