Food Sci. Biotechnol. Vol. 15, No. 3, pp. 462 ~ 465 (2006)

RESEARCH NOTE

Food Science and Biotechnology

Antibacterial Effects of Natural Essential Oils from Ginger and Mustard against *Vibrio* Species Inoculated on Sliced Raw Flatfish

Mi-Ji Yoo, Yong-Suk Kim[1], and Dong-Hwa Shin*

Faculty of Biotechnology (Food Science & Technology Major), Chonbuk National University, Jeonbuk 561-756, Korea
[1]Research Center for Industrial Development of BioFood Materials, Chonbuk National University, Jeonbuk 561-756, Korea

Abstract In order to extend the shelf life of sliced raw flatfish, the antimicrobial effects of natural essential oil from mustard and a mixture of ginger and mustard essential oils were tested at various temperatures. In addition, volatile components of the mixed essential oils were analyzed using gas chromatography and gas chromatography mass spectrometry. The viable cell counts of *Vibrio parahaemolyticus* treated with mixed essential oils from ginger and mustard was 0.7-1.3 log CFU/g lower than those of other treatments during storage at 5°C. During storage at 20°C, the viable cell counts of *V. parahaemolyticus, V. vulnificus* 01, and *V. vulnificus* 02 treated with the essential oils increased slightly from 6.53-6.64 log CFU/g at initial stages to 6.77-7.72 log CFU/g after 24-hr of storage, however they were 1.38-1.97 log CFU/g lower than those of the control group (8.74-9.10 log CFU/g). These results show that the growth of *V. parahaemolyticus* and *V. vulnificus* inoculated on sliced raw flatfish could be inhibited by treatment with natural essential oils from ginger and mustard at 5°C of storage. However, the antibacterial effects of the essential oils on *Vibrio* species observed in this study were not sufficient to merit their use in sliced raw flatfish at temperatures exceeding 20°C.

Keywords: essential oil, ginger, mustard, antibacterial effect, *Vibrio* sp.

Introduction

Ideally, the materials added to prolong shelf-life should not remain in the food, therefore more studies on the use of volatile antibacterial materials for food preservation and the prevention of microorganism growth are required. Due to the increasing consumer trend to avoid foods containing synthetic preservatives, intensive efforts have been made to find natural preservatives from plants and spices (1).

Currently, various antibacterial volatile extracts isolated from spices and herbs have been tested in food. Eugenol (clove extract) and the pimento extract are added to refrigerated cooked beef (2), horseradish essential oil to precooked roast beef (3), allyl isothiocyanate in combination with acetic acid to cooked rice (4), and the extract of *Callicarpa japonica, Polygonum cuspidatum*, and *Styrax japonica* and their volatile constituents to cooked rice (5). In addition, the bactericidal effect of allyl isothiocyanate (6) and the extract of *P. cuspidatum* (7) have also been reported.

About 40 *Vibrio* species are known, and 15 pathogenic strains have been isolated from human feces, including *Vibrio parahaemolyticus* and *V. vulnificus*. *V. parahaemolyticus* lives in brackish saltwater and is present in higher concentrations during the summer. When ingested, it causes watery diarrhea often with abdominal cramping, nausea, vomiting fever, and chills. Severe disease is rare but occurs more commonly in persons with weakened immune systems. *V. vulnificus* can cause disease in those who eat contaminated seafood or have an open wound that is exposed to seawater. In immuno-compromised persons, particularly those with chronic liver disease, *V. vulnificus* can infect the bloodstream, causing a severe and life-threatening illness (8).

Spices such as ginger and mustard are natural foods that add flavor and have antibacterial activities against food-borne microorganisms (9-11).

However, only a few studies involving seafood have been conducted on the antibacterial effects of the essential oils from spices. Therefore, we examined the antibacterial effects of essential oils from ginger and mustard on sliced raw flatfish inoculated with *Vibrio* species.

Materials and Methods

Volatile essential oils Natural essential oils from ginger and mustard for use in antibacterial testing were obtained from Hyangwon-Spice, Inc. (Seongnam, Korea).

Microorganisms The three *Vibrio* species used for antibacterial testing and their culture conditions were as follows: *V. parahaemolyticus* ATCC 17082 was grown at 30°C in brain heart infusion (BHI) broth (Difco, Detroit, MI, USA) and BHI agar medium, *V. vulnificus* 01 and *V. vulnificus* 02 isolated from patients with food poisoning were grown at 37°C in BHI broth and BHI agar medium. The bacteria were grown for 18 hr in sterilized broth medium.

Analysis and identification of volatile constituents Samples were analyzed by gas chromatography (GC, Agilent 6890N; Palo Alto, CA, USA) and GC-Mass spectrometry (Agilent 5973MS) using Supelcowax 10^{TM} fused silica capillary columns (0.25 mm × 60 m, film thickness 0.25 μm). Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The GC oven temperature was 50°C at the initial stage, then increased to 230°C at a rate of 2°C/min, and held for 5 min. The temperatures of the injector

*Corresponding author: Tel: 82-63-270-2570; Fax: 82-63-270-2572
E-mail: dhshin@chonbuk.ac.kr
Received January 9, 2006; accepted March 24, 2006

and the flame ionization detector were 250 and 260°C, respectively. The GC split ratio used was 1:60, and 0.5 μL of essential oil was injected for each run. The mass spectra ranged from 28 to 400 *m/e*, the ionizing voltage was 70 eV, and helium was used as the carrier gas. Molecular components were identified by comparison of the spectra obtained with a mass spectrum library [Wiley 275 L, John Wiley & Sons (Asia) Ptc. Ltd., Singapore].

Testing for antimicrobial effects on sliced raw flatfish Raw flatfish were purchased from local markets in Jeonju, Korea. Raw flatfish were washed two times with 70% ethanol and washed with sterile distilled water. After skinning flatfish with a sterile knife, they were cut into 1×0.5×3 cm sized pieces. Sliced raw flatfish pieces were then soaked in 100 mL of the BHI broth containing *Vibrio* species (4-5 log CFU/mL) for 30 min, the excess liquid drained off, and 20 g portions put into petri-dishes. One thousand ppm of essential oil diluted in 0.1 mL of ethanol was added to a fixed filter paper (Whatman No.2, 3×3 cm), in the center of the petri-dish cover. The plates were then sealed with melted paraffin and incubated for 48 hr at 5, 20, or 37°C (Fig. 1). The sample was homogenized with 80 mL of sterilized 0.85% NaCl saline in a stomacher blender for 1 min. After serial dilution, viable cell counts were determined using the plate count agar method at 24-hr intervals for a total of 48 hr. Ethanol without essential oil was used as a control.

Statistical analysis The Statistic Analysis System (12) software package was used for the analysis of variance. When applicable, Duncan's multiple comparison was carried out to compare the means of test samples. Each sample was tested in triplicate.

Results and Discussion

Volatile constituents of mixed essential oils from ginger and mustard Among the mixed essential oils from ginger, mustard, garlic, and clove tested in a previous study (13), the mixed essential oils from ginger and mustard showed the highest antimicrobial activity. We therefore selected these essential oils for volatile constituent analysis.

Volatile constituents of the mixed essential oils from ginger and mustard (1:1, v/v) are shown in Table 1. Major components of the ginger and mustard essential oil mixture were allyl isothiocyanate (41.67%, peak area), α-zingiberene (5.99%), β-sesquiphellandrene (7.56%), ar-curcumene (5.99%), β-bisabolene (5.43%), campene (3.56 %), α-farnesene (3.41%), and sabinene (3.39%). A previous study reported that natural ginger essential oil contained 45 volatile compounds whereas mustard essential oil contained only 3 volatile compounds. However, when these essential oils were combined in equal proportions, the number of volatile compounds detected decreased to 14 (Table 1). Trace compounds present in both the ginger and the mustard essential oils were not detected in the mixture.

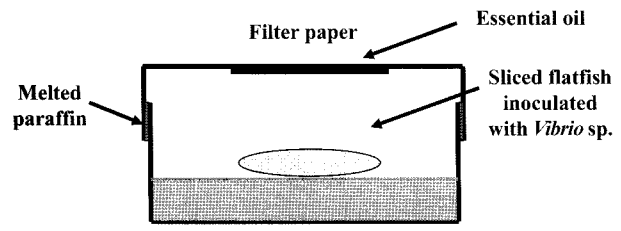


Fig. 1. Scheme for antibacterial tests on sliced raw flatfish.

Application test on sliced raw flatfish In general, the antibacterial effects of preservatives derived from natural sources exhibit less activity in food than in growth medium due to components of the food itself that protect microorganisms (13-16). We, therefore, chose to test the antibacterial activity of the mixed natural essential oils from ginger and mustard on sliced raw flatfish inoculated with *Vibrio* species instead of agar. The antimicrobial effects of essential oils on *Vibrio* species inoculated on raw flatfish and stored at 5, 20, and 37°C are presented in Fig. 2-4.

After 24-hr of storage at 5°C (Fig. 2), the viable cell counts of *V. parahaemolytocus* treated with the mixed essential oils from ginger and mustard were 0.7-1.3 log CFU/g lower than those of other treatments. However, the viable cell counts of *V. vulnificus* 01 and 02 were not affected by the treatment with essential oils. In addition, the viable cell counts of inoculated *Vibrio* species with all treatments decreased slightly during storage at 5°C.

During storage at 20°C (Fig. 3), the viable cell counts of *V. parahaemolyticus, V. vulnificus* 01, and *V. vulnificus* 02 treated with the ginger/mustard mixture or mustard essential oil alone increased from 6.53-6.64 log CFU/g at initial stages to 6.77-7.72 log CFU/g at 24-hr of storage, while those of the control group (without added essential

Table 1. Volatile constituents of mixed natural essential oils from ginger and mustard

Peak No.	RT (min)[1)]	Compounds	Peak area (%)[2)]
1	13.340	α-Pinene	1.10
2	15.539	Camphene	3.56
3	23.361	1,8-Cineole	0.76
4	23.993	Sabinene	3.39
5	34.435	Allyl isothiocyanate	41.67
6	54.907	Germacrene D	0.69
7	55.318	γ-Cadinene	0.87
8	55.921	α-Zingiberene	20.89
9	56.224	β-Bisabolene	5.43
10	57.397	*e,e*-α-Farnesene	3.41
11	58.649	β-Sesquiphellandrene	7.56
12	58.910	ar-Curcumene	5.99
13	95.509	1,2-Benzendicarboxylic acid	0
14	96.357	Campherenone	0
		Total	95.32

[1)]Retention time (min) based on the peak of the GC chromatogram.
[2)]Peak area on the GC chromatogram.

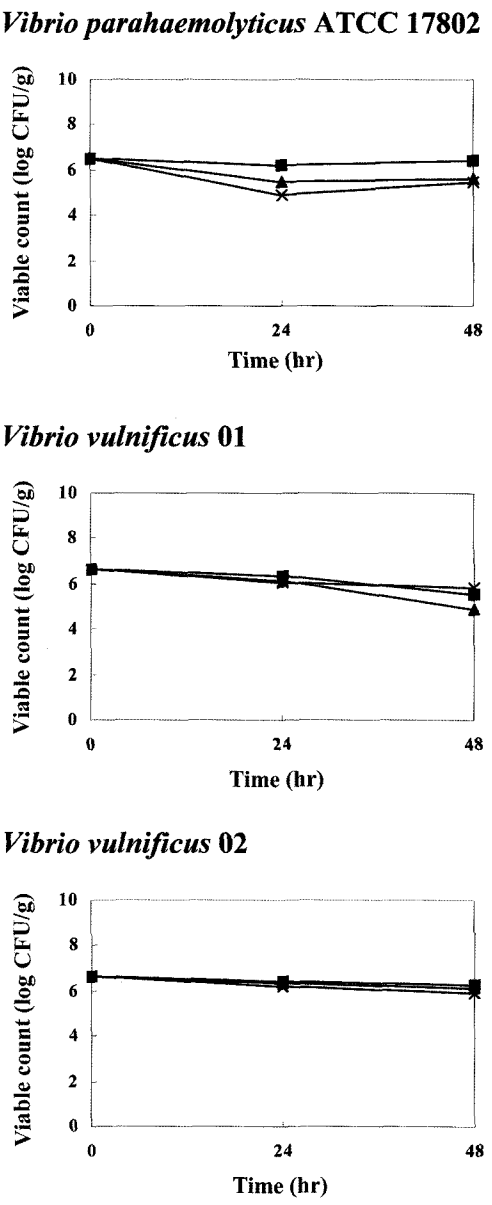


Fig. 2. Changes of viable cell counts of *Vibrio* species inoculated on sliced raw flatfish during storage at 5°C. -■-, sliced raw flatfish inoculated with *Vibrio* sp. (control); -▲-, natural mustard essential oil + inoculated with *Vibrio*; -×-, natural ginger essential oil + natural mustard essential oil (1:1) + inoculated with *Vibrio* sp.

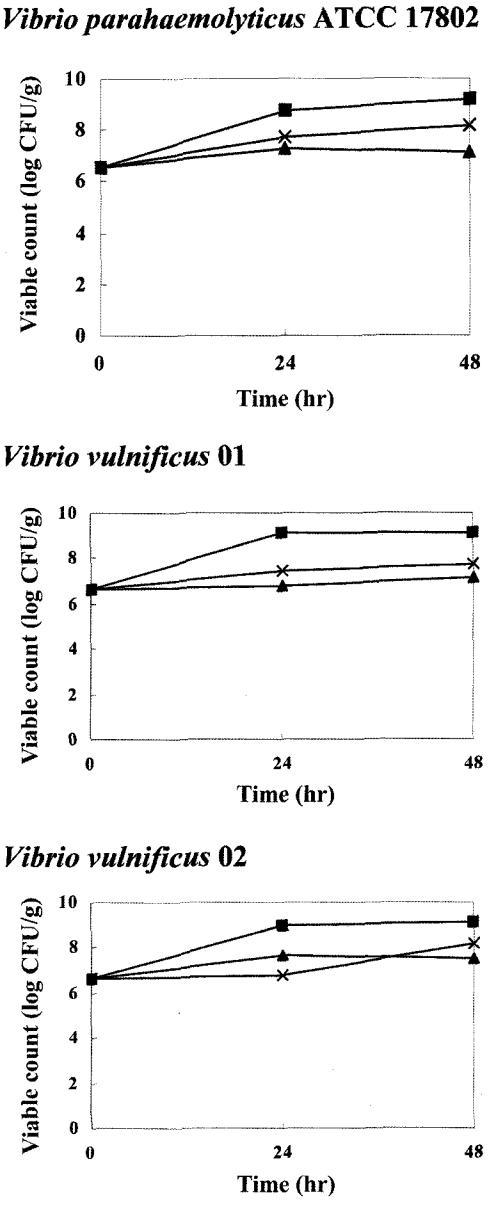


Fig. 3. Changes of viable cell counts of *Vibrio* species inoculated on sliced raw flatfish during storage at 20°C. -■-, sliced raw flatfish inoculated with *Vibrio* sp. (control); -▲-, natural mustard essential oil + inoculated with *Vibrio*; -×-, natural ginger essential oil + natural mustard essential oil (1:1) + inoculated with *Vibrio* sp.

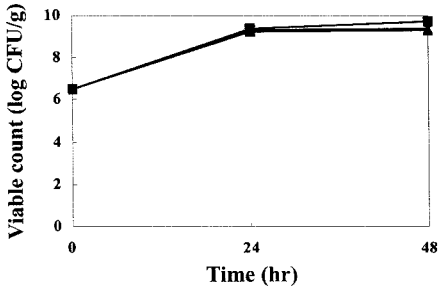
oil) increased to 8.74-9.10 log CFU/g and continued to increase up to 48-hr of storage.

During storage at 37°C (Fig. 4), the viable cell counts of *Vibrio* species drastically increased by 2.61-3.23 log CFU/g in all cases.

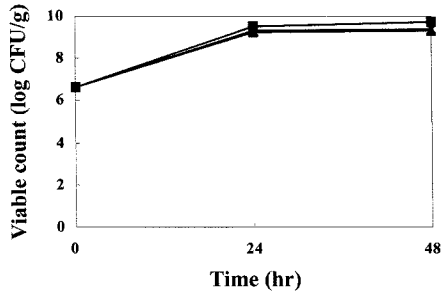
Faith *et al.* (14) reported that the fat content of pepperoni slices influenced the survival of *Escherichia coli* O157:H7 cells during the baking of frozen pizza, with higher fat pepperoni displaying greater D values at all three temperatures (135, 191, and 246°C). Venkitanarayaman *et al.* (16) reported that in 1% peptone medium, 50 and 100 mg of lactoferricin B per mL reduced *E. coli* O157:H7 populations by approximately 0.7 and 2.0 log CFU/mL, respectively, but no significant difference ($p<0.05$) in the total plate counts between treated and control samples (ground beef) stored at 4 and 10°C was observed. Therefore, we assumed that, due to the protective effect of food components on microorganisms and the volatility of essential oils, the antibacterial effect of these oils toward *Vibrio* species was reduced in sliced raw flatfish relative to agar medium.

Based on our results, we propose that the growth of *V. parahaemolyticus* and *V. vulnificus* on sliced raw flatfish at 5°C could be effectively inhibited by treatment with essential oils from ginger and mustard. However, the effects of such treatment at 20°C and above do not merit their use in sliced raw flatfish at these temperatures.

***Vibrio parahaemolyticus* ATCC 17802**



***Vibrio vulnificus* 01**



***Vibrio vulnificus* 02**

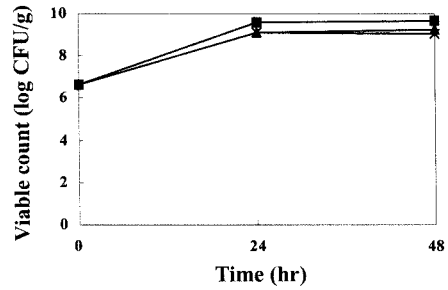


Fig. 4. Changes of viable cell counts of *Vibrio* species inoculated on sliced raw flatfish during storage at 37°C. -■-, sliced raw flatfish inoculated with *Vibrio* sp. (control); -▲-, natural mustard essential oil + inoculated with *Vibrio*; -×-, natural ginger essential oil + natural mustard essential oil (1:1) + inoculated with *Vibrio* sp.

References

1. Kim YS, Shin DH. A review - researches on the volatile antimicrobial compounds from edible plants and their food application. Korean J. Food Sci. Technol. 35: 159-165 (2003)
2. Hao YY, Brackett RE, Doyle MP. Inhibition of *Listeria monocytogenes* and *Aeromonas hydrophila* by plant extracts in refrigerated cooked beef. J. Food Protect. 61: 307-312 (1998)
3. Delaquis PJ, Ward SM, Holley RA, Cliff MC, Mazza G. Microbiological, chemical and sensory properties of precooked roast beef preserved with horseradish essential oil. J. Food Sci. 64: 519-524 (1999)
4. Kim YS, Ahn ES, Shin DH. Extension of shelf life by treatment with allyl isothiocyanate in combination with acetic acid on cooked rice. J. Food Sci. 67: 274-279 (2002)
5. Kim YS, Oh BC, Shin DH. The extension of the shelf life of cooked rice by the treatment with the plant extracts and their volatile constituents. Food Sci. Biotechnol. 13: 519-522 (2004)
6. Ahn ES, Kim YS, Shin DH. Observation of bactericidal effect of allyl isothiocyanate on *Listeria monocytogenes*. Food Sci. Biotechnol. 10: 31-35 (2001)
7. Kim YS, Kim HH, Yoo MJ, Shin DH. Bactericidal effect of the extracts of *Polygonum cuspidatum* on *Bacillus cereus*. Food Sci. Biotechnol. 13: 430-433 (2004)
8. Chang DS, Shin DH, Chung DH, Kim CM, Lee IS. Food Hygiene. Jungmunkag. Seoul, Korea. pp. 76-83 (2002)
9. Shelef LA, Naglik OA, Bogen DW. Sensitivity of some common food-borne bacteria to the spices sage, rosemary, and allspice. J. Food Sci. 45: 1042-1044 (1980)
10. Shelef LA. Antimicrobial effects of spices. J. Food Safety 6: 29-44 (1983)
11. Giese J. Spices and seasoning blends: A taste for all seasons. Food Technol. 48: 88-98 (1994)
12. SAS Institute, Inc. SAS User's Guide. Statistical Analysis Systems Institute, Cary, NC, USA (1998)
13. Yoo MJ. Comparative study on antibacterial effects of essential oils against *Vibrio* species. MS thesis, Chonbuk National University, Jeonju, Korea (2004)
14. Faith NG, Wierzba RK, Ihnot AM, Roering AM, Lorang TD, Kaspar CW, Luchansky JB. Survival of *Escherichia coli* O157:H7 in full- and reduced-fat pepperoni after manufacture of sticks, storage of slices at 4°C or 21°C under air and vacuum, and baking of slices on frozen pizza at 135, 191 and 246°C. J. Food Protect. 61: 383-389 (1998)
15. Ahn YS, Shin DH, Kim YS. Inhibitory effect of major food components on the activity of antimicrobial active substance from *n*-hexane fraction of *Mallotus japonicus* Muell on *Listeria monocytogenes*. Korean J. Food Sci. Technol. 32: 469-476 (2000)
16. Venkitanarayaman KS, Zhao T, Doyle MP. Antibacterial effect of lactoferricin B on *Escherichia coli* O157:H7 in ground beef. J. Food Protect. 62: 747-750 (1999)