Antimicrobial Activity of Catfish Gelatin Coating Containing Origanum (Thymus capitatus) Oil against Gram-Negative Pathogenic Bacteria

B.J. MIN AND J.-H. OH

ABSTRACT: The antimicrobial activity of origanum oil (OG) was determined against Salmonella Typhimurium and E. coli O157:H7. The efficacy of catfish gelatin coating containing selected concentrations of OG (0.5%, 1.0%, and 2.0%, v/v) was also investigated in catfish flesh irradiated, inoculated with both bacteria, and stored at selected temperatures. The antimicrobial activity of catfish gelatin coating was determined by cell counts during storage at 4 and 10 ºC for 12 d. The color properties of catfish flesh coated with catfish gelatin containing OG were also measured. In the media, the inhibitory effects of OG against S. Typhimurium were greater than the inhibition effects against E. coli O157:H7, exhibiting complete inhibition against S. Typhimurium at the concentration greater than 0.5% (v/v). The incorporation of OG into catfish gelatin coating also inhibited both S. Typhimurium and E. coli O157:H7 in catfish flesh stored at 4 ºC. The reduction of S. Typhimurium counts by gelatin coating containing 0%, 0.5%, 1%, and 2% (v/v) OG were 0.58, 0.73, 1.16, and 1.76 log CFU/g, respectively, exhibiting the greatest effect at 2% (v/v) OG (P < 0.05). During storage at 10 ºC, the reduction of S. Typhimurium counts by catfish gelatin coatings with 0%, 0.5%, 1%, and 2% (v/v) OG were 0.67, 0.91, 1.32, and 2.05 log CFU/g, respectively. The incorporation of OG into catfish gelatin coating exhibited greater antimicrobial activity against S. Typhimurium than against E. coli O157:H7 at both temperatures. The lightness and yellowness of catfish flesh was not altered; however, the redness of catfish gelatin was decreased by catfish gelatin coating containing OG.

Keywords: antimicrobial activity, catfish, coating, gelatin, origanum oil

Introduction

Biodegradable materials possess potential alternatives to replace synthetic plastics widely used in food packaging industry. Food packaging plays an important role in food and food products not only to protect the contents from physical damages, but also to provide effective barriers against moisture, oxygen, light, and microbial growth. Due to increased consumer demand for natural and safe foods and food products, natural food ingredients are gaining considerable attention in the market, and the functional properties of natural ingredients are being investigated extensively (Delaquis and others 1999; Ahn and others 2004; Souza and others 2007). Recently, natural compounds such as essential oils and herb extracts containing antimicrobial activities are applied to bio-active packaging systems as an alternative to synthetic antimicrobial agents in packaging films and materials (Suhr and Nielsen 2005; Zivanovic and others 2005; Seydim and Sarikus 2006).

Seafood products are vulnerable to foodborne pathogenic bacteria such as Listeria, Salmonella, and Escherichia coli (Adesiyun 1993; Embarek 1994). Fresh or frozen channel catfish products can be contaminated by pathogenic bacteria such as Salmonella, and coliforms were detected in the various processing steps (Andrews and others 1977; Suvanich and others 2000). Therefore, extensive preservation methods were used to process catfish fillets to prevent bacterial growth or extend shelf life of catfish fillet, including irradiation (Przybylski and others 1989), modified atmosphere packaging (Reddy and others 1997), and use of preservatives (Kim and Hearnsberger 1994; Williams and others 1995).

As natural resources to replace synthetic preservatives, a broad range of essential oils extracted from plant sources such as basil, clove, horseradish, garlic, mustard, oregano, sage, and thyme were tested (Ismaiel and Pierson 1990; Delaquis and others 1999; Suhr and Nielsen 2005; Zivanovic and others 2005; Seydim and Sarikus 2006). After testing the antimicrobial activities of essential oils in laboratory trials, a variety of essential oils were considered for substantial application in the food industry (Kim and others 1995; Zivanovic and others 2005; Rojas-Grau and others 2007). The application methods for essential oils to food and food products are broad. In general, essential oils are applied to inhibit bacterial growth by direct incorporation into foods as an ingredient (Shekarforosh and others 2007; Busatta and others 2008; Solomakos and others 2008), coating the food surface (Quattara and others 2001), or fabricating with edible films to wrap foods (Oussalah and others 2006, 2007).

As a multifunctional protein, gelatin has been applied to broad areas in food, cosmetic, and pharmaceutical industries (Djagny and others 2001). Gelatins obtained from a wide range of sources were applied to develop biodegradable and edible films due to the excellent film-forming properties of gelatin. Compared to mammalian gelatins, fish gelatins possess potential application to unique food and food products with the issues of transmissible spongiform encephalopathy (TSE) and religious concerns (Gudmundsson 2002; Haug and others 2004; Muyonga and others 2004; Zhang and others 2007). With the unique physical and functional properties, catfish gelatin extract can be utilized for edible coating and film materials for food packaging (Yang and others 2007; Zhang and others 2007).
In addition, the incorporation of natural antimicrobial compounds into catfish gelatin extract will add functionality to catfish gelatin coating by improving shelf life of the catfish products. Origanum oil extracted from the perennial plant (*Thymus capitatus*) is an essential oil and generally used as flavoring agents similar to other essential oils (Bounatirou and others 2007). There is limited information available on origanum oil (*Thymus capitatus*) and antimicrobial activity (Kandil and others 1994). Therefore, the objectives of this study were (1) to determine the antimicrobial activity of OG against Gram-negative pathogenic bacteria in culture media and (2) to investigate the efficacy of OG incorporated into catfish gelatin for catfish flesh coating to extend the shelf life of catfish during storage at selected temperatures.

**Materials and Methods**

**Materials**

Frozen catfish skins for gelatin extract were obtained from the Harvest Select Inc. plant (Uniontown, Ala., U.S.A.). Frozen skinless whole catfish fillet was purchased in local groceries and thawed in a refrigerator for 18 h prior to use. Origanum (*Thymus capitatus*) oil (OG) was purchased from Sigma-Aldrich Co. (St. Louis, Mo., U.S.A.). The other reagents used in the experiments were analytical grade. Two Gram-negative pathogenic bacteria, *Salmonella Typhimurium* ATCC13311 and *E. coli* O157:H7 204 were used for the antimicrobial study. *S. Typhimurium* was provided by James Barbaree and Stuart Price Laboratories at Auburn Univ. (Auburn, Ala., U.S.A.) and *E. coli* O157:H7 was provided by the Center for Food Safety at Univ. of Georgia (Griffin, Ga., U.S.A.).

**Antimicrobial activity of origanum oil in culture media**

From the stock culture of *S. Typhimurium* and *E. coli* O157:H7, 1 colony of bacteria was transferred to 9.9 mL tryptic soy broth (TSB) and incubated for 16 to 18 h at 37 °C aerobically. Transferring to a new TSB was repeated using the same procedure. The cultured bacteria in TSB were centrifuged at 3000 × g for 10 min, and the pellets were diluted with 0.1% peptone solution. From the serial dilution with 0.1% sterile peptone water, bacterial population in tryptic soy agar (TSA) was counted and the initial population of bacteria in the peptone solution ranged from 8.0 to 9.0 log CFU/mL. For the antimicrobial test, the stock solution of OG (2.0%, v/v) with tween 80 (4.0%, w/v) in sterile distilled water in a 50-mL tube was prepared by shaking vigorously. Five selected concentrations of OG ranging from 0.1% to 2.0% (v/v) were diluted from the stock solution. An aliquot of 100 μL OG solutions of selected concentrations was placed into sterile petri dishes, and mixed with approximately 10 mL molten TSA containing bacteria at the level of 6.0 log CFU/mL. Therefore, the actual concentration of OG in the media was approximately 100-fold lower than the indicated concentration. The plates were incubated at 37 °C for 48 h, and the bacterial colonies were counted after incubation.

**Extraction of catfish gelatin**

Extraction of catfish gelatin was performed following procedures established in the previous study (Zhang and others 2007). Approximately 40 g of cleaned catfish skin were pretreated by soaking in 240 mL of 0.09 M acetic acid at 4 °C for 1 h, and washed with 4 °C distilled water 5 times, followed by soaking in 240 mL of 0.25 M sodium hydroxide at 4 °C for 1 h. The pretreated catfish skins were placed into a flask with 120 mL distilled water at 50 °C for 3 h in a water bath for main extraction. The catfish gelatin extracts were filtered through glass wool, and freeze-dried prior to further use. Irradiation of catfish flesh

The frozen catfish fillet flesh was thawed in a refrigerator for 18 h, trimmed, cut into squares (approximately 2.54 × 2.54 cm), vacuum packed in plastic film, and then stored at −70 °C. The average weight of catfish flesh was 10 g. The frozen catfish flesh was irradiated by exposing to gamma rays from a 60Co source at the dose of 0.0882 KGY/min for 245 min at Auburn Univ. to achieve complete inactivation of preexisting microorganisms in the catfish fillet. Sterilization of catfish was confirmed by checking the bacterial growth in TSA. The irradiated catfish flesh was stored at −70 °C in a deep freezer until use.

**Catfish flesh coating with catfish gelatin extracts containing origanum oil**

The irradiated catfish flesh was thawed at 4 °C for 18 h, and excess water was removed by sterile paper towel before bacterial inoculation. Catfish flesh was submerged into the bacterial solution containing *S. Typhimurium* or *E. coli* O157:H7 for 10 min, and then dried for 1 h on the sterile screen under the bio hood. Six grams of catfish gelatin extracts were dissolved in 200 mL distilled water and boiled. After cooling to approximately 50 °C, 0.5%, 1%, and 2% (v/v) OG with tween 80 (v/w; 1:2) was incorporated into the catfish gelatin solution to prepare the coating solution. The inoculated catfish flesh was coated with the catfish gelatin solution containing OG by dipping the catfish flesh for 10 s. The coated catfish flesh was dried for 1 h on the sterile screen under the bio hood (Contamination Control Inc., Landale, Pa., U.S.A.).

**Bacterial counts during storage**

The coated catfish flesh was placed in a sterile sampling bag (Labplas, Quebec, Canada) and stored at both temperatures of 4 and 10 °C for 12 d. During storage, the bacterial cell counts were performed with 3-d intervals. Catfish flesh (approximately 10 g) was mixed with 90 mL of 0.1% sterile peptone water. The mixtures were blended for 1 min in a Stomacher. Further dilution was done by 0.1% sterile peptone water. Diluted solution was plated in TSA agar and bacterial numbers were counted after incubation at 37 °C for 48 h. The initial loads of *S. Typhimurium* and *E. coli* O157:H7 onto catfish flesh before gelatin coating were approximately 4.2 and 4 log CFU/g, respectively.

**Color measurement**

The colors of catfish flesh before and after coating with catfish gelatin containing OG were measured using a minolta Chromameter (CR-300, Minolta Co., Osaka, Japan). The CIE (Commission Internationale de l’Eclairage—the Intl. Commission on Illumination) *L*′, *a*′, *b*′ color space values were used to characterize the surface color.

**Statistical analysis**

Each experiment was performed 3 times in duplicates. Data were analyzed using the general linear models procedure in SAS software program (SAS Inst. Inc., Cary, N.C., U.S.A.). Multiple mean comparisons among the log cell counts of uncoated and coated catfish flesh samples were carried out by Duncan’s multiple range tests at 5% significance level (P < 0.05).

**Results and Discussion**

**Antimicrobial activity of origanum oil**

The antimicrobial activity of OG was determined in the culture medium, and the bacterial counts affected by OG with selected
Catfish gelatin coating with origanum oil against S. Typhimurium and E. coli O157:H7 at the level of 6 log CFU/ml are presented in Table 1. The bacterial counts of both bacteria containing less than 0.5% (v/v) OG exhibited too numerous to count (TNTC), presumably indicating no significant inhibitory effect at the ranges of concentrations. However, OG inhibited the growth of S. Typhimurium at the concentration of 0.5% (v/v), exhibiting a greater inhibition than against E. coli O157:H7. OG concentrations greater than 1% inhibited both bacteria. It was reported that the antibacterial activities of essential oils are dependent on the concentrations. The antimicrobial activity of thyme oil at the concentrations of 0.3, 0.6, and 0.9% (v/v) against E. coli O157:H7 in broth systems was greatly dependent on the concentrations of thyme oil (Solomakos and others 2008). In general, antimicrobial activities of essential oils vary among various research studies even for the identical essential oils, because the antibacterial activities of essential oils can be affected by numerous factors such as region of growth, genotype, harvest time, drying methods, and so on (Oussalah and others 2007). Therefore, based on the effective concentrations of OG against S. Typhimurium and E. coli O157:H7, 3 concentrations of 0.5%, 1%, and 2.0% (v/v) OG were selected for incorporation into catfish gelatin coating solution for further study.

Antimicrobial activities of catfish gelatin coating containing origanum oil against S. Typhimurium

Figure 1 presents the antimicrobial activity of catfish gelatin coatings containing OG against S. Typhimurium in catfish flesh during storage at 4 °C. Coating with catfish gelatin containing OG resulted in significant reduction in cell counts of S. Typhimurium by approximately 0.5 log CFU/g, compared to uncoated or coated with catfish gelatin only at initial day (P < 0.05). As the storage time at 4 °C increased, the cell counts of entire catfish flesh groups decreased, even in the uncoated catfish flesh. The slight reduction in bacterial growth in uncoated catfish during storage may be due to the low storage temperature of 4 °C. Oussalah and others (2006) reported similar trends that the growth of S. Typhimurium without antimicrobial film treatment decreased in beef muscle, when stored at 4 °C. Another similar reduction of S. Typhimurium stored at 4 °C was observed in untreated beef products, when compared with beef products treated with herb extracts (Cutten 2000).

Catfish flesh coated with gelatin containing OG exhibited greater inhibition effects against S. Typhimurium than catfish flesh coated with gelatin only during entire storage time period. The inhibitory effect of OG against S. Typhimurium was the greatest at 2% (v/v) concentration of OG (P < 0.05). At the end of storage (day 12), the cell counts significantly reduced by gelatin coating containing 0%, 0.5%, 1%, and 2% (v/v) OG were 0.58, 0.73, 1.16, and 1.76 log CFU/g, respectively (P < 0.05).

There is no available research on the antimicrobial activity of OG extracted from Thymus capitatus applied to seafood to compare. Coating with thyme oil, a similar type to OG, reduced the growth of aerobic bacteria and Pseudomonas putida in both irradiated and unirradiated cooked shrimp (Ouattara and others 2001). The inhibitory effect of coating with thyme oil was dependent on the concentrations of thyme oil, exhibiting the greatest inhibitory effect from the highest concentration of 1.8%. Zivanovic and others (2005) also reported that oregano essential oil alone or incorporated into chitosan films was effective to inhibit Listeria monocytogenes and E. coli O157:H7, and the inhibitory effects were dependent on the increased concentrations between 1% and 4% (v/v). The growth of L. monocytogenes in zero and low-fat hotdogs was reduced by thyme oil suspension at three concentrations of 1, 5, and 10 mL/L (Singh and others 2003).

Table 1 – Bacterial counts of Salmonella Typhimurium and E. coli O157:H7 inhibited by selected concentrations of origanum oil.

<table>
<thead>
<tr>
<th>Bacterial count (CFU/mL)</th>
<th>Concentration of origanum oil (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>TNTC</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>TNTC</td>
</tr>
</tbody>
</table>

*TNTC = too numerous to count.

*ESPC = estimated standard plate count based on a plate size of 65 cm².

Figure 1 — Antimicrobial activity of catfish gelatin coating containing origanum oil against Salmonella Typhimurium stored at 4 °C. (□) = Uncoated catfish; (■) = catfish coated with gelatin only; (♦) = catfish coated with gelatin containing 0.5% origanum oil; (△) = catfish coated with gelatin containing 0.8% origanum oil; and (▲) = catfish coated with gelatin containing 1.0% origanum oil. Bars represent standard deviation of the mean.
Temperature is one of the critical factors to control microbial growth during display or storage, and temperature abuse should be considered to protect food products from the bacterial growth by initial contamination or postcontamination (Baker and others 1990). The bacterial counts in the catfish flesh samples stored at 10 °C exhibited dramatic increases during storage, as presented in Figure 2. The growth of S. Typhimurium increased in both uncoated catfish flesh and coated flesh with catfish gelatin only during storage, exhibiting no significant difference (P > 0.05). However, the incorporation of OG greater than 0.5% (v/v) into catfish gelatin coatings exhibited significant inhibitory effects against S. Typhimurium from the initial day, similar to the inhibitory effects from 4 °C storage (P < 0.05). The inhibitory effects of OG with entire concentrations persisted until 9-d storage, exhibiting significant smaller increases in log CFU/g than uncoated catfish flesh or catfish flesh coated with gelatin only (P < 0.05). However, only OG with 2% (v/v) maintained the antimicrobial activity against S. Typhimurium after 9-d storage (P < 0.05). Compared to uncoated catfish, the bacterial reductions by coating with 0, 0.5, 1, and 2% (v/v) OG were 0.67, 0.91, 1.32, and 2.05 log CFU/g at the end of storage 10 °C, respectively. There was also a significant reduction of S. Typhimurium by coated catfish with 1.5% and 2.0% (v/v) OG (P < 0.05).

Antimicrobial activities of catfish gelatin coating containing origanum oil against E. coli O157:H7

The antimicrobial activity of catfish gelatin coating containing OG against E. coli O157:H7 at 4 °C is presented in Figure 3. Coating with catfish gelatin containing OG inhibited the growth of E. coli O157:H7 during the entire storage time, compared to uncoated catfish flesh and catfish flesh coated with gelatin only. Catfish flesh coated with catfish gelatin only exhibited apparent inhibitory effect; however, the inhibitory effect was not significant. The cell counts of E. coli O157:H7 at 4 °C including the uncoated catfish flesh were reduced during storage, exhibiting a similar trend with S. Typhimurium stored at 4 °C (Figure 1). The growth of E. coli O157:H7 in vacuum-packaged raw beef without antimicrobials was reduced at 4 °C during 28-d storage (Mustapha and others 2002). Cagri and others (2002) also reported a decrease in bacterial counts of E. coli O157:H7 in sausage without antimicrobial film during storage at 4 °C. It was speculated that the reduction in bacterial counts at low-temperature storage might be attributed to the combination effects from low temperature and antimicrobial activity of OG. At the end of storage time at 4 °C, catfish gelatin coatings, regardless of the concentrations of OG, significantly reduced the growth of E. coli O157:H7 by 0.17 to 0.46 log CFU/g (P < 0.05). The greatest inhibitory effects were obtained in the catfish gelatin coating with OG 2% (v/v), maintaining the antimicrobial activity against E. coli O157:H7 for 9-d storage (P < 0.05).

![Figure 2: Antimicrobial activity of catfish gelatin coating containing origanum oil against Salmonella Typhimurium stored at 10 °C.](image2)

![Figure 3: Antimicrobial activity of catfish gelatin coating containing origanum oil against E. coli O157:H7 stored at 4 °C.](image3)
coating containing 2.0% (v/v) OG. Busatta and others (2008) reported that there was a significant reduction in E. coli growth in fresh sausages by the incorporation of marjoram essential oil into the sausages, and the antimicrobial effects of the marjoram oil were greatly dependent on the concentration of the oil. However, the authors also reported that E. coli was not completely inhibited even by the highest concentration of 2.5 times of minimum inhibition concentration.

During storage at 10°C, the growth of E. coli O157:H7 in uncoated catfish flesh reached approximately 10 log CFU/g (Figure 4). The bacterial counts of E. coli O157:H7 in catfish flesh coated with gelatin containing OG did not significantly reduce the bacterial counts between 3- and 9-d-storage time periods. However, catfish gelatin coating containing 2% (v/v) OG exhibited a significant reduction in bacterial counts at 12-d storage ($P < 0.05$).

The inhibitory effects of OG in catfish flesh were greater against S. Typhimurium than against E. coli O157:H7 at both temperatures. The essential oils obtained from plants exerted antimicrobial activity in the real food products, due to numerous factors such as food composition, ingredient, storage condition, and so on. Our results support that the inhibitory effects of OG were dependent on the concentration, storage temperature, and test bacteria.

The growth of both S. Typhimurium and E. coli O157:H7 was inhibited by OG. The antimicrobial activity of OG was greater against S. Typhimurium than against E. coli O157:H7. The incorporation of OG into catfish gelatin solution for coating catfish flesh inhibited both bacteria at the temperatures of 4 and 10°C. The antimicrobial activity of OG gelatin coatings against S. Typhimurium was greater than the antimicrobial activity against E. coli O157:H7 at both temperatures. The redness of catfish flesh coated with catfish gelatin extract containing OG was lower than redness of uncoated catfish flesh. The results demonstrate the antimicrobial activity of OG against Gram-negative pathogenic bacteria, and suggest that catfish gelatin extract containing OG can be applied to improve the safety of food products.

![Figure 4](image-url)  
*Figure 4—Antimicrobial activity of catfish gelatin coating containing origanum oil against E. coli O157:H7 stored at 10°C. (•) = Uncoated catfish; (○) = catfish coated with gelatin only; (♦) = catfish coated with gelatin containing 0.5% origanum oil; (▲) = catfish coated with gelatin containing 1.0% origanum oil; and (■) = catfish coated with gelatin containing 2.0% origanum oil. Bars represent standard deviation of the mean.*

### Table 2 — Color properties of catfish flesh coated with catfish gelatin containing selected concentrations of origanum oil.

<table>
<thead>
<tr>
<th></th>
<th>$L^*$</th>
<th>$a^*$</th>
<th>$b^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncoated catfish</td>
<td>108.0 ± 2.2*a</td>
<td>4.7 ± 1.1*a</td>
<td>14.3 ± 2.1*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin only</td>
<td>110.9 ± 3.9*a</td>
<td>1.0 ± 0.3*</td>
<td>15.0 ± 2.7*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin + 0.5% OG</td>
<td>109.4 ± 3.8*a</td>
<td>−1.7 ± 0.2*c</td>
<td>13.0 ± 2.5*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin + 1.0% OG</td>
<td>109.4 ± 3.2*a</td>
<td>−0.2 ± 0.1*a</td>
<td>13.4 ± 2.6*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin + 2.0% OG</td>
<td>108.3 ± 2.6*a</td>
<td>0.4 ± 0.2*c</td>
<td>13.0 ± 1.5*a</td>
</tr>
<tr>
<td>Uncoated catfish</td>
<td>106.4 ± 2.4*a</td>
<td>5.5 ± 1.1*a</td>
<td>14.5 ± 2.7*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin only</td>
<td>108.5 ± 3.9*a</td>
<td>1.5 ± 0.2*</td>
<td>13.0 ± 2.7*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin + 0.5% OG</td>
<td>109.0 ± 4.2*a</td>
<td>−0.9 ± 0.2*c</td>
<td>11.8 ± 2.2*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin + 1.0% OG</td>
<td>109.8 ± 3.3*a</td>
<td>−0.6 ± 0.2*c</td>
<td>12.4 ± 2.4*a</td>
</tr>
<tr>
<td>Catfish coated with gelatin + 2.0% OG</td>
<td>109.4 ± 1.5*a</td>
<td>−0.6 ± 0.2*c</td>
<td>12.4 ± 2.8*a</td>
</tr>
</tbody>
</table>

*aOG = origanum oil.

Means within a column with different superscripts are significantly different ($P < 0.05$).
Acknowledgments

The authors would like to thank Dr. Yifeng Wang at Auburn Univ., Auburn, Ala., U.S.A., for providing catfish skins for this study.

References


