Inactivation of murine norovirus and feline calicivirus during oyster fermentation

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Abstract

Fermented seafood is popular in Asian countries. This study examined the survival of feline calicivirus (FCV) and murine norovirus (MNV) during oyster fermentation. Oysters spiked with FCV and MNV were fermented with 5% or 10% salt at 18 °C for 15 days, and MNV and FCV titers, lactic acid bacteria (LAB) populations, pH, and enzymatic activity were measured at 0, 1, 3, 5, 7, 10, and 15 days post-fermentation (DPF). Reductions in MNV and FCV were greater in 5% NaCl-supplemented oysters than in 10% NaCl-supplemented oysters. In 5% NaCl oysters, MNV and FCV titers significantly decreased by 1.60 log and 3.01 log, respectively, at 15 DPF. Populations of LAB increased from 3.62 log10 colony-forming units/g at 0 DPF to 8.77 log10 colony-forming units/g at 15 DPF during oyster fermentation supplemented with 5% NaCl supplementation, and the pH decreased gradually from 5.38 at 0 DPF to 4.17 at 15 DPF. During oyster fermentation, α-amylase, proteinase, and lipase were produced at higher levels in 5% salted oysters than in 10% salted oysters (P < 0.01). We concluded that many of the antimicrobial factors produced in fermented oysters could contribute to a reduction in foodborne viruses.

Keywords:
Murine norovirus
Feline calicivirus
Oyster
Fermentation
Lactic acid bacteria

1. Introduction

Fish and vegetable fermentation have been used in Asian countries since 300 BC (Hutkins, 2006; Lee et al., 1993). In South Korea, many types of fermented foods are made from agricultural and marine products. Approximately 150 different types of fermented seafood products, called jeot or jeotgal, are produced and consumed in Korea (Lee, 2001; Lee et al., 1993; Suh, 1987). Because jeotgal is made of fish, fish eggs, shrimp, and shellfish, including salted oysters, it develops a distinct savory taste related to the endogenous enzymes produced by the seafood and salt-tolerant bacteria during curing (Lee, 1997). Among these foods, fermented oysters, called eoriguljeot, are a popular and representative Korean fermented food. These oysters are fermented with 5%–10% salt at room temperature for approximately 2 weeks and then marinated with hot pepper (Lee et al., 1993; Suh, 1987).

Oysters are the main ingredient of eoriguljeot (Suh, 1987). Although many recent norovirus (NoV) outbreaks have been associated with the consumption of raw oysters (Dowell et al., 1995; Webby et al., 2007), fermented oysters have not yet been reported as a causative agent of NoV outbreaks in Korea. Oysters filter enormous amounts of seawater, and NoV is easily concentrated in their digestive glands, the mucous of their gills, and other tissues (Greening, 2006; Lees, 2000). In humans, NoV is transmitted through person-to-person contact, airborne routes, and the consumption of contaminated food and water (Doyle et al., 2009; Greening, 2006; Kim et al., 2005). As few as 10–100 NoV particles can cause nonbacterial gastroenteritis in humans (Barker et al., 2004; Cault, 1994). NoVs are highly resistant to environmental surface and sanitizing treatments such as heat, ether, acid, and chlorine (Barker et al., 2004; Keswick et al., 1985).

To date, few in vitro culture systems have been developed for the study of NoV or animal models of NoV infection (Duizer et al., 2004). Although a three-dimensional cell culture model for NoV was recently reported, this in vitro cultivation method is nonstandard for NoV (Straub et al., 2007). Therefore, the NoV surrogates feline calicivirus (FCV) and murine NoV (MNV) are used to investigate the survival and infectivity of NoV under specific environmental and sanitation conditions (Cannon et al., 2006; Wobus et al., 2006).

Lactic acid bacteria (LAB) reduce the viability of foodborne pathogens during the fermentation process (Charernjiratragul et al., 2010; Hernández et al., 2005). The antimicrobial effects of
fermented foods are attributed to the low pH produced by lactic acid and bacteriocins such as nisin as well as to other factors such as salt concentration, temperature, and curing time (Cho et al., 2011; Lee et al., 2012; Noonpakdee et al., 2003; Yateem et al., 2008). LAB produced from fermented products such as fish sausage, cereal gruels, dairy, and meat products mainly inhibit Salmonella spp., Escherichia coli, and Staphylococcus aureus (Adams and Nicolaides, 1997; Aryanta et al., 1991; Cho et al., 2011; Noonpakdee et al., 2003; Nout et al., 1989). Although the reduction of foodborne pathogens in fermented foods has been well studied, the survival of foodborne viruses in fermented foods is not well understood. To our knowledge, Lee et al. (2012) were the first to report the inactivation of FCV and MNV during vegetable fermentation in dong-chimi kimchi.

Ensuring the microbial safety of fermented seafood including *eoriugleot* is critical because oysters can be easily contaminated with *NoV*. Therefore, the aims of this study were to examine the survival of MNV and FCV and investigate changes in pH and enzyme production during oyster fermentation.

2. Materials and methods

2.1. Oyster fermentation and experimental design

MNV-1 was kindly provided by Dr. Skip Virgin from the University of Washington (Seattle, WA, USA). FCV strain P9, RAW264.7 cells and Crandell Rees feline kidney (CRFK) cells were purchased from the American Type Culture Collection (Manassas, VA, USA).

Oysters produced in Tongyeong, South Korea, were purchased from a local market. Excess seawater in the oyster package was drained completely, and the oysters were washed with sterile artificial seawater. Approximately 20 g of oysters was divided into 50-mL tubes. Then, 5% or 10% (w/w) salt was added to each tube. The tubes were spiked with 6.39 ± 0.45 log10 plaque-forming units (PFU)/g of MNV or FCV. Four experimental groups were devised as follows: (1) FCV-spiked oysters fermented with 5% NaCl (5FCV), (2) FCV-spiked oysters fermented with 10% NaCl (10FCV), (3) MNV-spiked oysters fermented with 5% NaCl (5MNV), and (4) MNV-spiked oysters fermented with 10% NaCl (10MNV). Each sample was fermented under traditional conditions at 18 °C. Fermented oysters were collected at 0, 1, 3, 5, 7, 10, and 15 days post-fermentation (DPF). Juice collected from each fermented oyster sample was sequentially filtered for virus titration using syringe filters with 5-, 1.2-, 0.8-, and 0.45-µm pore sizes to prevent cloaking.

2.2. Titration of MNV and FCV

MNV-1 and FCV titrations were carried out as previously described (Su et al., 2010; Wobus et al., 2004) with slight modifications. For MNV, RAW264.7 cells and Crandell Rees feline kidney (CRFK) cells were purchased from the American Type Culture Collection (Manassas, VA, USA).

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For FCV, CRFK cells cultured in 24-well plates were inoculated with juice collected from the fermented oysters serially diluted 10-fold. Each well was treated for 2 h at 37 °C in a 5% CO2 incubator and overlaid with DMEM containing 0.75% agarose (Sigma, St. Louis, MO, USA), 5% fetal bovine serum, and 1% penicillin–streptomycin (Hyclone Laboratories). CRFK cells were maintained for 24 h at 37 °C in a 5% CO2 incubator. FCV titers were measured after staining with neutral red solution.

2.3. α-Amylase activity

α-Amylase assays were conducted according to previously published methods (Suzuki et al., 1989; Yoo et al., 1987) with slight modifications. α-Amylase activity was assayed by measuring changes in iodine coloration, which occur during dextrinization of starch by α-amylase. Aliquots of juice collected from the fermented oysters (100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000 µL) were prepared in test tubes. Each aliquot was mixed with 1 mL of 0.1 M acetate buffer solution. Five milliliters of 1% soluble starch preheated to 40 °C was then added, and the samples were incubated at 40 °C for 30 min. Next, 2 mL of 2N acetic acid was added to each test tube to terminate the enzymatic reaction, and the reaction mixture was colorized by adding 1 mL of 0.01 N iodine solution. α-Amylase activity was defined as the volume of 1% soluble starch hydrolyzed from 1 mL of enzyme solution that achieved a light purple color.

2.4. Proteinase activity

Proteinase activity was assayed using the casein-Folin method (Hiraga et al., 2005; Ji et al., 2012). One milliliter of juice collected from the fermented oysters was mixed with 5 mL of 0.6% casein solution preheated to 30 °C. After incubation at 30 °C for 10 min, the reaction was stopped with 5 mL of 0.44 M trichloroacetic acid at 30 °C for 30 min. Two milliliters of the supernatant was mixed with 5 mL of 0.55 M sodium carbonate and 1 mL of Folin reagent. After incubation at 30 °C for 30 min, the optical density of the color was measured at 660 nm. One unit of proteinase activity was defined as the amount of enzyme that catalyzed casein to produce color equivalent to 1.0 µg of tyrosine per minute at 30 °C.

2.5. Lipase activity

Lipase activity was assayed as previously described (Ohnishi et al., 1994) with slight modifications. Briefly, 5 mL of olive oil emulsion and 4 mL of 0.1 M sodium phosphate buffer solution (pH 7.0) preheated to 30 °C were mixed with 1 mL of juice collected from the fermented oysters and incubated at 30 °C for 50 min. The mixture was treated with 20 mL aceton–ethanol (1:1, v/v) to terminate the reaction. The fatty acids liberated from the olive oil were titrated with 0.05 N NaOH solution. Lipase activity was routinely measured by titrating free fatty acids during the enzymatic hydrolysis of olive oil.

2.6. Measurement of LAB

Juice collected from the fermented oysters was sampled at each time point, diluted 10-fold with 0.2% peptone water (BD Difco, USA), and plated on selective de Man, Rogosa, and Sharpe agar (Oxoid, Basingstoke, Hampshire, England). After each plate was incubated at 37 °C for 48 h, populations of LAB were counted.

2.7. pH

Juice samples were collected from fermented oysters at each time point and centrifuged at 3743 × g for 2 min using a VS-15000N centrifuge (Vision, Kyounghi, Korea). The pH values of the fermented oyster samples in each tube were measured using a standard benchtop pH meter (Thermo Electron Corporation, USA).
2.8. Statistical analysis

All experiments in this study were conducted in triplicate. Virus titers, pH, LAB, and enzyme activity data obtained during fermentation were analyzed with one-way analysis of variance and Duncan’s multiple range test by using Statistical Analysis System software (version 9.1; Cary, NC, USA). Differences were considered significant when P values were less than 0.05.

3. Results

3.1. Inactivation of MNV and FCV

We observed a significant gradual reduction in MNV and FCV titers during oyster fermentation (Fig. 1). FCV titers decreased from 6.39 ± 0.1 log_{10} PFU/g at 0 DPF to 5.17 ± 0.27 log_{10} PFU/g at 10 DPF and reached a minimum of 3.38 ± 0.17 log_{10} PFU/g at 15 DPF in the 5FCV group (P < 0.01). Additionally, MNV titers decreased from 6.44 ± 0.04 log_{10} PFU/g at 0 DPF to 5.76 ± 0.24 log_{10} PFU/g at 3 DPF and reached a minimum of 4.84 ± 0.31 log_{10} PFU/g at 15 DPF in the 5MNV group (P < 0.01). In the 10FCV group, FCV titers decreased from 6.43 ± 0.07 log_{10} PFU/g at 0 DPF to 5.82 ± 0.1 log_{10} PFU/g at 7 DPF and 5.29 ± 0.13 log_{10} PFU/g at 15 DPF (P < 0.01). In the 10MNV group, MNV titers decreased from 6.44 ± 0.02 log_{10} PFU/g at 0 DPF to 5.90 ± 0.12 log_{10} PFU/g at 3 DPF and 5.45 ± 0.24 log_{10} PFU/g at 15 DPF (P < 0.01).

3.2. Oyster fermentation

LAB populations gradually increased during fermentation (Table 1). In the 5FCV and 5MNV groups, LAB populations increased from 3.62 ± 1.37 log_{10} colony-forming units (CFU)/g at 0 DPF to 8.35 ± 0.66 log_{10} CFU/g at 7 DPF and 8.77 ± 0.23 log_{10} CFU/g at 15 DPF (P < 0.01). In the 10FCV and 10MNV groups, the LAB populations increased from 3.11 ± 1.15 log_{10} CFU/g to 6.29 ± 0.09 log_{10} CFU/g at 7 DPF and 6.96 ± 0.71 log_{10} CFU/g at 15 DPF (P < 0.01). pH values significantly decreased from 5.38 ± 0.27 to 4.17 ± 0.56 at 15 DPF in the 5FCV and 5MNV groups (see Table 1). Although the pH decreased from 5.40 ± 0.22 to 4.96 ± 0.25 at 15 DPF in the 10FCV and 10MNV groups (see Table 1), the change was not statistically significant.

3.3. α-Amylase activity

α-Amylase activity in all experimental groups gradually increased during fermentation (see Table 1). In the 5FCV and 5MNV groups, α-amylase activity increased from 4.66 ± 1.48 U/mL at 0 DPF to 10.17 ± 0.84 U/mL at 3 DPF and 17.91 ± 1.69 U/mL at 15 DPF (P < 0.01). In the 10FCV and 10MNV groups, α-amylase activity increased from 5.53 ± 0.73 U/mL at 0 DPF to 8.58 ± 0.82 U/mL at 5 DPF and 10.91 ± 1.08 U/mL at 15 DPF (P < 0.01). Statistically significant differences in α-amylase activity were identified between the following groups: 5FCV versus 10FCV and 5MNV versus 10MNV from 5 DPF to 15 DPF, respectively (P < 0.01).

3.4. Lipase activity

Lipase activity in all experimental groups gradually increased during fermentation (see Table 1). In the 5FCV and 5MNV groups, lipase activity increased from 0.79 ± 0.06 U/mL at 0 DPF to 2.25 ± 0.21 U/mL at 7 DPF and reached 2.86 ± 0.10 U/mL at 15 DPF (P < 0.01). In the 10FCV and 10MNV groups, lipase activity increased from 0.73 ± 0.04 U/mL at 0 DPF to 1.35 ± 0.15 U/mL at 3 DPF and reached 1.98 ± 0.69 U/mL at 15 DPF (P < 0.01). During oyster fermentation from 5 DPF to 15 DPF, lipase activity was statistically higher in the 5FCV and 5MNV groups than in the 10FCV and 10MNV groups (P < 0.01).

3.5. Proteinase activity

Proteinase activity in all experimental groups gradually increased during the fermentation process (see Table 1). In the 5FCV and 5MNV groups, proteinase activity increased from 0.004 ± 0.002 U/mL at 0 DPF to 0.013 ± 0.002 U/mL at 5 DPF and reached 0.033 ± 0.001 U/mL at 15 DPF (P < 0.01). In the 10FCV and 10MNV groups, proteinase activity increased from 0.005 ± 0.001 U/mL at 0 DPF to 0.016 ± 0.002 U/mL at 7 DPF and reached 0.026 ± 0.005 U/mL at 15 DPF (P < 0.01). Unlike α-amylase and lipase activity, statistically significant differences in proteinase activity in oysters fermented with 5% and 10% NaCl were not observed at any time point.

4. Discussion

To the best of our knowledge, this study is the first to show a reduction in MNV and FCV titers in fermented oysters (called eor-iguljeot in Korean). Because oysters are easily contaminated with NoVs (Greening, 2006; Lees, 2000), the inhibition of NoV surrogates during oyster fermentation is crucial. Although titers of both FCV and MNV were significantly reduced in our study, oysters supplemented with 5% NaCl exhibited higher reductions than those fermented with 10% NaCl. Thus, salt concentration seemed to be an
important factor in reducing NoV surrogate titres during oyster fermentation. Lee et al. (2008) have found that the level of MNV reduction depends on salt concentration. By contrast, reductions in MNV and FCV titers in this study were observed in oysters fermented with 5% rather than 10% salt supplementation. In a previous study, the traditional fermented food kimchi was optimally fermented at a high temperature in the presence of a low salt concentration (Mheen and Kwon, 1984), supporting the conditions for optimal oyster fermentation obtained in the present study.

Many previous studies have reported the inhibition of foodborne bacteria in fermented foods (Charernjiratrangul, et al, 2010; Cho et al, 2011; Hernández et al, 2005). Among them, Lactobacillus plantarum TF711 reduces Bacillus cereus, Clostridium sporogenes, S. aureus, Shigella sonnei, and Klebsiella pneumoniae in Tenerife goat cheese (Hernández et al, 2005). Additionally, L. plantarum PSU-LAB71 isolated from fermented pork inhibits the pandemic strain of Vibrio parahaemolyticus (Charernjiratrangul et al, 2010). However, the survival of foodborne viruses in fermented foods has rarely been reported. Lee et al. (2012) have shown a significant reduction in NoV surrogates in the fermented vegetable dongchimi kimchi.

More than 15 novel species of bacteria and archaea have been identified in Asian fermented seafood products (Euzéby, 1997; Roh et al., 2010). The predominant LAB in fermented oysters is the genera Lactobacillus at pH 4.5; however, fermented oysters also contain a small number of Weissella spp. (Roh et al., 2010). Lactobacillus and Weissella spp. are found in fermented foods and produce organic acids—mostly lactic acid—with decreasing pH (Adams and Nicolaides, 1997, Lee et al., 1997). Fermented foods generally exhibit a wide range of pH values (3.5–4.5), and low pH conditions generally inhibit bacteria that grow optimally between pH 6 and pH 7 (Adams and Nicolaides, 1997). Acidity, low pH, organic acids, bacteriocins, CO2, hydrogen peroxide, diacetyl, ethanol, low redox potential, and nutrient depletion are known antimicrobial factors associated with LAB fermentation (Adams and Nicolaides, 1997).

In this study, the population of LAB in oysters fermented with 5% NaCl was 1.81 log10 CFU/g higher than that in oysters fermented with 10% NaCl. In previous studies investigating antiviral LAB, metabolites produced by Lactobacillus spp. and Bifidobacterium spp. were found to display antiviral activity against the vesicular stomatitis virus (Botic et al., 2007; Collbre–Garapín et al., 2007; Nagata et al., 2011). Intake of probiotic bacteria improves the symptoms of viral gastroenteritis caused by human NoV and rotavirus (Collbre–Garapín et al., 2007; Nagata et al., 2011). Transmissible gastroenteritis virus and rotavirus are also inhibited by Lactobacillus casei strain Shirota and L. plantarum PCA236, which can attach to human and animal intestinal epithelial and immune cells (Maragkoudakis et al., 2010). In terms of lactic acid production, Leuconostoc mesenteroides isolated from oysters produces more lactic acid than that isolated from other shellfish (Kang et al., 2012). Lactobacillus rhamnosus MH22, one of the 83 Lactobacillus strains isolated from oysters, has antipathogenic effects against Vibrio alginolyticus and V. proteolyticus (Lee et al., 2010). Therefore, if LAB isolated from fermented oysters has antiviral activity, as has been shown in previous studies, they could be excellent resources for reducing the risk of NoV infection during oyster fermentation.

In this study, MNV and FCV titers decreased by 1.60 log and 3.01 log, respectively, at pH 4.18. During oyster fermentation, the reduction in FCV titers was more prominent than that of MNV titers. This result is consistent with a prior observation that FCV titers are reduced more than MNV titers under conditions of low pH when the pH value is decreased by lactic acid produced during dongchimi fermentation (Lee et al., 2012). Like other enteric viruses, including hepatitis A virus and human enteroviruses, MNV is resistant under conditions of high and low pH stress (Cannon et al., 2006). By contrast, a separate study has shown that FCV is rapidly inactivated because of instability under conditions of low or high pH stress (Cannon et al., 2006). Compared with those of a previous study (Cannon et al., 2006), our results demonstrated that MNV and FCV titers in fermented oysters were reduced more than that observed under environmental conditions by 0.5 log versus 1.6 log for MNV and 2.3 log versus 3.0 log for FCV.

Many organic acids are widely used as antimicrobial agents; for example, salicylic, pyrogallatit, benzoic, lactic, citric, acetic, fumaric, and malonic acids have been used to inactivate rhinovirus (Davidson et al., 2002; Turner et al., 2004). Poschetto et al. (2007) have reported that 0.5–1 h treatments with a 4%–5% commercial organic acid product can reduce human NoV genogroup II and FCV by 3 log. Organic acids are known to have virucidal activity against enveloped and nonenveloped viruses (Haas et al., 1995; Jeffrey, 1995; Pavlova et al., 2003). Hansman et al. (2012) recently showed that treatment with citrate diminished the affinity of human NoV capsid protein for histo-blood group antigens by analyzing saturation transfer difference using nuclear magnetic resonance. The affinity change of the capsid protein after treatment with organic acids is thought to be an antiviral mechanism because several organic acids are produced during fermentation. Thus, the relationship between organic acids and the structure of viral capsid proteins should be investigated in future studies.

Halophilic bacteria, such as Bacillus subtilis, L. mesenteroides, Pedicoccus halophilus, and Sarcina litoralis, play major roles in the fermentation of oysters, clams, and squid (Lee et al., 1993). Among them, B. subtilis generates highly active proteinase in fish sauce, producing greater levels of proteinase under low-salt conditions (5% NaCl) than at 10%, 15%, 20%, 25%, or 30% NaCl (Kim and Kim, 2005). These data are consistent with those in our present study, which demonstrated that enzyme activities, such as those of proteinase, lipase, and α-amylose, were higher in oysters fermented with 5% NaCl than in those fermented with 10% NaCl. Because triglycerides are hydrolyzed into antimicrobial fatty acids and

Table 1

<table>
<thead>
<tr>
<th>Days post-fermentation</th>
<th>1</th>
<th>3</th>
<th>5</th>
<th>7</th>
<th>10</th>
<th>15</th>
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<tr>
<td>5% NaCl</td>
<td>LAB (log CFU/g)</td>
<td>3.62 ± 1.37</td>
<td>4.87 ± 1.50</td>
<td>6.23 ± 2.01</td>
<td>6.78 ± 1.94</td>
<td>8.35 ± 0.66</td>
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<tr>
<td>pH</td>
<td>5.38 ± 0.27</td>
<td>5.22 ± 0.17</td>
<td>5.07 ± 0.09</td>
<td>4.85 ± 0.26</td>
<td>4.41 ± 0.42</td>
<td>4.39 ± 0.38</td>
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<tr>
<td>α-Amylase (U/mL)</td>
<td>4.66 ± 1.48</td>
<td>6.59 ± 1.13</td>
<td>10.17 ± 0.84</td>
<td>11.29 ± 0.62</td>
<td>12.51 ± 0.46</td>
<td>12.74 ± 0.49</td>
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<tr>
<td>Lipase (U/mL)</td>
<td>0.79 ± 0.06</td>
<td>1.41 ± 0.09</td>
<td>1.76 ± 0.21</td>
<td>2.07 ± 0.29</td>
<td>2.25 ± 0.21</td>
<td>2.56 ± 0.21</td>
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<tr>
<td>Proteinase (U/mL)</td>
<td>0.004 ± 0.02</td>
<td>0.009 ± 0.002</td>
<td>0.01 ± 0.003</td>
<td>0.01 ± 0.003</td>
<td>0.02 ± 0.001</td>
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<tr>
<td>10% NaCl</td>
<td>LAB (log CFU/g)</td>
<td>3.11 ± 1.15</td>
<td>4.41 ± 1.17</td>
<td>5.12 ± 1.45</td>
<td>5.86 ± 1.13</td>
<td>6.29 ± 0.99</td>
</tr>
<tr>
<td>pH</td>
<td>5.40 ± 0.22</td>
<td>5.23 ± 0.23</td>
<td>5.13 ± 0.10</td>
<td>5.08 ± 0.05</td>
<td>5.04 ± 0.07</td>
<td>4.99 ± 0.24</td>
</tr>
<tr>
<td>α-Amylase (U/mL)</td>
<td>5.53 ± 0.73</td>
<td>5.67 ± 0.94</td>
<td>8.46 ± 1.29</td>
<td>8.58 ± 0.82</td>
<td>8.60 ± 0.47</td>
<td>8.87 ± 0.71</td>
</tr>
<tr>
<td>Lipase (U/mL)</td>
<td>0.73 ± 0.04</td>
<td>1.19 ± 0.23</td>
<td>1.35 ± 0.15</td>
<td>1.41 ± 0.26</td>
<td>1.48 ± 0.40</td>
<td>1.59 ± 0.42</td>
</tr>
<tr>
<td>Proteinase (U/mL)</td>
<td>0.005 ± 0.001</td>
<td>0.008 ± 0.003</td>
<td>0.007 ± 0.005</td>
<td>0.01 ± 0.004</td>
<td>0.02 ± 0.002</td>
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Letters indicate statistical difference (P < 0.01). CFU, colony-forming units.
monoglycerides by lipases, treatment with lipases inactivates herpes simplex virus-1 in infant formulas (Isaacs et al., 1992). Because hepatitis C virus can infect host cells through lipoprotein-dependent metabolism, treatment with lipoprotein lipase blocks the entry step via lipolytic action on triglyceride-rich lipoprotein and its bridging function between hepatitis C virus-associated lipoproteins and cell surface heparin sulfate (Maillard et al., 2011).

Although previous studies have shown that enveloped viruses are affected by enzymatic treatment, including treatment with lipase, enteric viruses can become resistant under conditions of environmental stress. Because enteric viruses lack a lipid envelope and are not metabolically active (Adams and Nicolaides, 1997), the effects of digestive enzymes on nonenveloped viruses should be investigated in future studies.

To our knowledge, this study is the first to demonstrate a significant reduction in MNV and FCV titers during oyster fermentation. Although antiviral activity against human NoV was not confirmed, our data suggested that titers of NoV surrogates were significantly reduced in fermented oysters. As in studies with other fermented foods, pH values and organic acids produced by LAB seemed to be important antimicrobial factors in this study. While several digestive enzymes produced by salt-tolerant microorganisms increase the savory taste of fermented oysters, they may also be involved in conferring antiviral activity in this food. Therefore, further studies are needed to determine the mechanisms through which digestive enzymes reduce NoV surrogates.

Acknowledgments

This study was funded by a grant from the Cooperative Research Program for Ministry of Food and Drug Safety (Project No. 12162-012), Republic of Korea.

References


