Research Note

Inhibitory Effect of Commercial Green Tea and Rosemary Leaf Powders on the Growth of Foodborne Pathogens in Laboratory Media and Oriental-Style Rice Cakes

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ABSTRACT

The antimicrobial effects of green tea and rosemary added to foods as antagonists to foodborne pathogens were determined in laboratory media and oriental-style rice cakes. The growth of each pathogen (Bacillus cereus, Salmonella Typhimurium, Enterobacter sakazakii, Escherichia coli O157:H7, Staphylococcus aureus, and Listeria monocytogenes) in tryptic soy broth or rice cake with or without addition of green tea or rosemary leaf powders before autoclaving or cooking, respectively, was investigated after inoculation. The addition of 1% green tea or rosemary produced similar results for inhibiting the growth of pathogens in tryptic soy broth. However, green tea was more effective than rosemary for inhibiting the growth of L. monocytogenes. Both botanicals had inhibitory effects against all pathogens tested in this study. Green tea was particularly effective against B. cereus, S. aureus, and L. monocytogenes, and rosemary was strongly inhibitory against B. cereus and S. aureus. The addition of 1 or 3% green tea or rosemary to rice cakes did not significantly reduce total aerobic counts; however, levels of B. cereus and S. aureus were significantly reduced in rice cakes stored for 3 days at room temperature (22°C). The order of antimicrobial activities against B. cereus in rice cake was 1% rosemary < 1% green tea < 3% rosemary = 3% green tea. These results indicate that the use of natural plant materials such as green tea and rosemary could improve the microbial quality of foods in addition to their functional properties.

Rice cakes with various ingredients are popular food items in Asia, and a variety of oriental-style rice cakes, i.e., Korean, Vietnamese, and Philippine, are available in markets in these countries (18). Rice cakes can be manufactured with a variety of bases, such as regular rice, sweet rice, white rice, and tapioca, and a variety of other ingredients, such as red beans, green beans, mung beans, and sesame seeds. Rice cakes are cooked by steaming, frying, or boiling in water; however, steam cooking is the most convenient and popular method for manufacturing a variety of rice cakes (17). Cooking of starch results in swelling and gelatinization of starch granules, which are then slowly reformed into a helical structure during cooling and storage. This process is known as staling or retrogradation (18). Retrogradation causes hardness in starch-based foods. The rate of starch retrogradation is highest at refrigeration temperature and declines with increasing temperature (27). Because of this characteristic, storage under refrigeration conditions is not recommended for rice cakes. Therefore, rice cakes for sale are held at room temperature for 1 day and are discarded the following day. The state of California permits the sale of Korean rice cakes held at room temperature for up to 2 h and requires that manufacturers provide the date and time when the rice cakes were made (2). Because rice cake characteristics such as water activity, pH, and nutrients favor microbial growth, the populations of pathogenic contaminants can increase during room temperature storage.

Herbs and spices are known for their antimicrobial and antioxidant properties. Because of increasing demand for natural food additives, herbs and spices have emerged as popular ingredients as replacements for synthetic antimicrobial and antioxidant agents (20). Green tea (Carnellia sinensis L.) is a popular beverage and has been reported to have several beneficial effects such as chemopreventive, anticarcinogenic, antiatherogenic, antioxidant, and antimicrobial activities (8, 21, 24, 32). Several authors have reported the antibacterial property of green tea extracts (1, 14, 15, 24, 29, 33). The beneficial effect of green tea catechin on human enteric microflora includes inhibition of the growth of harmful bacteria and promotion of the growth of useful bacteria (1). Rosemary (Rosmarinus officinalis L.) belongs to the Lamiaceae family of herbs, which in addition to their usefulness as food flavorings are known for their powerful antioxidant activity and antibacterial, antimutagenic, and chemopreventive properties (24). Because of its possible beneficial properties, rosemary is widely used today as a food ingredient either in ground form or as an extract (23).

Consumers have become more critical of the use of artificial additives used to preserve food or enhance characteristics such as color, flavor, and nutritional value (3).
Interest in the development of effective natural antimicrobials as food preservatives has increased because of concerns regarding the safety of synthetic compounds (22). Many researchers have reported the antimicrobial effects of green tea and rosemary (13, 32). However, most of these researchers used the extracted forms even though these herbs are commercially used as dried leaves or powders. Therefore, this study was conducted to investigate the antibacterial effects of a dried powder form of green tea and rosemary on the survival and growth of foodborne pathogen in laboratory media and oriental-style rice cakes. For general manufacturing procedures, steam cooking of rice cakes will completely destroy the vegetative cells of various pathogens. However, spore-forming bacteria such as Bacillus cereus can survive the cooking procedures and grow after germination of spores during storage. Cooked rice cakes also can be contaminated with foodborne pathogens during slicing, handling, or packaging. The possible sources or conditions of contamination during postcooking processing are numerous. Therefore, the antibacterial effect of green tea and rosemary powders against various foodborne pathogens were evaluated in laboratory media. In a second study, the effects of green tea and rosemary powders on the growth of B. cereus (spore-forming bacteria) and Staphylococcus aureus (indicator organisms for post-processing contamination) on rice cakes were evaluated.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** Three strains of pathogens obtained from the Food Science and Human Nutrition bacterial culture collection at Washington State University (Pullman) were used in this study: B. cereus ATCC (American Type Culture Collection, Manassas, VA) 10876, ATCC 13061, and W-1; Salmonella Typhimurium ATCC 43174; Enterobacter sakazakii ATCC 12868; S. aureus ATCC 12600, ATCC 12692, and ATCC 49444; Escherichia coli O157:H7 ATCC 35150; and Listeria monocytogenes ATCC 7644. Individual strains were cultured in tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) at 37°C for 24 h, and cells were harvested by centrifugation at 4,000 × g for 20 min at 4°C and washed three times with buffered peptone water (Difco, Becton Dickinson). Final pellets were resuspended in buffered peptone water corresponding to approximately 10⁸ to 10⁹ CFU/ml. When multiple strains of a single pathogen were used, cells were pooled to construct a mixed culture cocktail.

**Inhibitory effect of green tea and rosemary in laboratory media.** Dry green tea leaves (product of Korea) and rosemary leaves (product of France) were purchased from a local grocery store in Seoul, Korea, and were ground finely in a mortar. One percent milled leaf powder was added into 10 ml of TSB, and the tubes were autoclaved for 15 min at 121°C. The autoclaved TSB tubes with or without 1% (wt/vol) green tea or rosemary leaf powder were inoculated with each pathogen using prepared culture cocktails. Inoculated tubes were incubated at 37°C, and populations of pathogens were enumerated after 0, 6, 12, and 24 h of growth on tryptic soy agar (Difco, Becton Dickinson).

**Inhibitory effect of green tea and rosemary on oriental-style rice cakes.** To investigate the inhibitory effect of green tea and rosemary leaves against total aerobic bacteria and pathogens such as S. aureus and B. cereus, oriental-style rice cakes (Sulgid-duk) were prepared with or without the addition of each botanical as follows. Fifty grams of regular rice flour (Icheon, Korea), 6.25 g of sterile water, 5 g of sugar, and 0.5 g of salt were mixed thoroughly with gloved hands. To some of the rice cakes, 1 or 3% (wt/wt) ground green tea or rosemary leaves were added, and cakes with and without the added botanicals were placed in a sieve lined with cheesecloth over a pot of boiling water and steam cooked for 30 min. After cooking, the rice cakes were cooled to room temperature and inoculated with S. aureus or B. cereus as a diluted culture cocktail in buffered peptone water corresponding to approximately 10⁴ to 10² CFU/g. After inoculation, the rice cakes were placed in UV-sterilized plastic Zip bags (G. T. Bag Company, Novato, CA) and stored at room temperature (22°C) for 72 h.

**Bacterial enumeration.** Inoculated stored rice cakes (50 g) were placed in a stomacher bag containing 100 ml of buffered peptone water and homogenized for 2 min with a stomacher (BagMixer 400, Interscience Laboratory Inc., St. Nom, France). After homogenization, 1-ml aliquots were serially diluted in 9 ml of sterile buffered peptone water, and 0.1 ml of sample or diluent was spread plated onto aerobic plate count Petrifilm (3M, St. Paul, MN) or each selective medium. Baird-Parker agar (Difco, Becton Dickinson) supplemented with 25 ml/liter egg yolk tellurite (Oxoid, Basingstoke, UK) was used for enumeration of S. aureus, and B. cereus selective agar (Oxoid, Ogdenburg, NY), which contains 25 ml/liter egg yolk emulsion and 10⁵ units/liter B. cereus selective supplements, was used for enumeration of B. cereus. The aerobic plate count Petrifilm was incubated at 32°C for 48 h, and the selective agars were incubated at 37°C for 24 to 48 h under aerobic conditions before enumeration.

**Statistical analysis.** All experiments were repeated three times with duplicate samples. Data were processed with the Statistical Analysis System (SAS Institute, Cary, NC) for a one-way analysis of variance and Duncan’s multiple range tests to determine whether significant differences (P < 0.05) existed between mean values for treatment groups or storage times.

**RESULTS AND DISCUSSION**

Results in Table 1 show changes in bacterial populations of foodborne pathogens in laboratory medium (TSB) with or without 1% green tea or rosemary powder during storage at room temperature (22 ± 2°C) for 24 h. Initial levels of pathogens ranged from 1.65 to 3.34 log CFU/ml. In TSB without botanical powder (control), these levels increased significantly during storage at room temperature (P ≤ 0.05) and resulted in high levels of 6.83 to 8.25 log CFU/ml after 12 h of storage and 7.37 to 9.24 log CFU/ml after 24 h of storage. Addition of 1% green tea or rosemary powder into TSB was effective for inhibiting or delaying the growth of all tested pathogens, although the effectiveness of each botanical differed depending on the type of bacteria.

Among all tested bacteria, B. cereus was most effectively inhibited by both green tea and rosemary. Levels of B. cereus remained unchanged or even decreased during storage when green tea or rosemary powder was added. After 24 h of storage, levels of B. cereus in TSB with 1% green tea and rosemary were 1.75 and 0.95 log CFU/ml, respectively, compared with 7.37 log CFU/ml in the control.

Both botanicals also were effective at delaying the
TABLE 1. Populations of foodborne pathogens in tryptic soy broth without or with 1% (wt/vol) green tea or rosemary during storage at room temperature (22 ± 2°C)

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Treatment</th>
<th>0 h</th>
<th>6 h</th>
<th>12 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cereus</td>
<td>Control</td>
<td>1.65 ± 0.29 A a</td>
<td>4.91 ± 0.85 B a</td>
<td>6.84 ± 0.35 C a</td>
<td>7.37 ± 0.22 C a</td>
</tr>
<tr>
<td></td>
<td>Green tea</td>
<td>1.65 ± 0.30 A a</td>
<td>1.73 ± 0.32 B b</td>
<td>1.83 ± 0.28 B b</td>
<td>1.75 ± 0.48 B b</td>
</tr>
<tr>
<td></td>
<td>Rosemary</td>
<td>1.65 ± 0.29 A a</td>
<td>0.95 ± 0.01 A b</td>
<td>0.95 ± 0.00 A b</td>
<td>0.95 ± 0.00 A b</td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td>Control</td>
<td>3.13 ± 0.06 A a</td>
<td>5.33 ± 0.97 B a</td>
<td>8.25 ± 0.48 C a</td>
<td>9.24 ± 0.06 C a</td>
</tr>
<tr>
<td></td>
<td>Green tea</td>
<td>3.13 ± 0.09 A a</td>
<td>3.42 ± 0.64 A b</td>
<td>4.00 ± 0.70 A b</td>
<td>6.56 ± 1.42 B a</td>
</tr>
<tr>
<td></td>
<td>Rosemary</td>
<td>3.13 ± 0.08 A a</td>
<td>3.20 ± 0.92 A a</td>
<td>4.53 ± 1.66 A a</td>
<td>6.39 ± 1.53 A a</td>
</tr>
<tr>
<td>E. sakazakii</td>
<td>Control</td>
<td>3.24 ± 0.07 A a</td>
<td>6.04 ± 1.08 B a</td>
<td>7.86 ± 0.46 B c</td>
<td>8.82 ± 0.08 C a</td>
</tr>
<tr>
<td></td>
<td>Green tea</td>
<td>3.24 ± 0.03 A a</td>
<td>4.36 ± 0.57 A a</td>
<td>6.47 ± 0.54 B a</td>
<td>8.38 ± 0.26 C a</td>
</tr>
<tr>
<td></td>
<td>Rosemary</td>
<td>3.24 ± 0.04 A a</td>
<td>3.97 ± 0.64 A a</td>
<td>6.67 ± 1.02 B a</td>
<td>8.65 ± 0.17 B a</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Control</td>
<td>2.96 ± 0.10 A a</td>
<td>5.88 ± 0.70 B a</td>
<td>7.13 ± 1.37 B c</td>
<td>9.01 ± 0.05 C a</td>
</tr>
<tr>
<td></td>
<td>Green tea</td>
<td>2.96 ± 0.09 A a</td>
<td>2.72 ± 0.34 B b</td>
<td>3.26 ± 0.63 B b</td>
<td>5.03 ± 1.02 B b</td>
</tr>
<tr>
<td></td>
<td>Rosemary</td>
<td>2.96 ± 0.12 A a</td>
<td>2.38 ± 0.32 B a</td>
<td>2.38 ± 0.60 A b</td>
<td>5.76 ± 1.20 A b</td>
</tr>
<tr>
<td>E. coli O157:H7</td>
<td>Control</td>
<td>2.76 ± 0.27 A a</td>
<td>5.64 ± 0.60 B a</td>
<td>7.92 ± 0.47 C a</td>
<td>8.73 ± 0.43 C a</td>
</tr>
<tr>
<td></td>
<td>Green tea</td>
<td>2.76 ± 0.20 A a</td>
<td>4.06 ± 0.40 A a</td>
<td>4.17 ± 0.60 A b</td>
<td>6.97 ± 1.11 B a</td>
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<tr>
<td></td>
<td>Rosemary</td>
<td>2.76 ± 0.12 A a</td>
<td>3.82 ± 0.73 B a</td>
<td>5.68 ± 1.25 A b</td>
<td>7.47 ± 1.40 B a</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>Control</td>
<td>3.34 ± 0.10 A a</td>
<td>5.12 ± 0.46 A a</td>
<td>6.83 ± 0.42 B a</td>
<td>9.09 ± 0.24 d a</td>
</tr>
<tr>
<td></td>
<td>Green tea</td>
<td>3.34 ± 0.08 A a</td>
<td>3.43 ± 0.31 A a</td>
<td>5.01 ± 0.28 B b</td>
<td>7.31 ± 0.45 C b</td>
</tr>
<tr>
<td></td>
<td>Rosemary</td>
<td>3.34 ± 0.14 A a</td>
<td>2.72 ± 0.31 A a</td>
<td>2.82 ± 0.10 A c</td>
<td>2.60 ± 0.23 C c</td>
</tr>
</tbody>
</table>

* Each pathogen was enumerated on tryptic soy agar. Values are the mean ± standard deviation of three replicated experiments. For each pathogen, means within a column with the same uppercase letter are not significantly different (P > 0.05). For each pathogen, means within a row with the same lowercase letter are not significantly different (P > 0.05).

FIGURE 1. Growth of total aerobic bacteria in oriental-style rice cakes without (●) or with 1% (○) or 3% (▼) green tea and 1% (▲) or 3% (■) rosemary at room temperature (22 ± 2°C). Values are the means of three replicated experiments.
organisms on rice cakes without or with 1 or 3% green tea or rosemary during storage at room temperature. Initial populations of microorganisms ranged from 1.00 to 1.56 log CFU/ml, and there was no significant difference among samples \( (P > 0.05) \). After 12 h of storage, levels of total aerobic microorganisms started to increase. Among all samples, the sample with 3% rosemary had the lowest populations of microorganisms, whereas samples with 3% green tea or rosemary had the lowest, although the differences were not significant. Although rice cakes with green tea or rosemary had lower levels of total aerobic microorganisms than did control cakes during storage for 72 h, the differences were not significant. These results indicate that the addition of green tea and rosemary into rice cakes did not inhibit the growth of total aerobic bacteria.

Populations of \( S. aureus \) and \( B. cereus \) in rice cakes without or with green tea or rosemary during storage at room temperature are presented in Figures 2 and 3, respectively. Initial populations of \( S. aureus \) inoculated onto rice cakes ranged from 1.00 to 2.02 log CFU/ml. Levels in the control increased dramatically during storage for 72 h, reaching 8.32 log CFU/g (Fig. 2). There were no significant differences in \( S. aureus \) populations among samples during storage for 48 h \( (P > 0.05) \). However, after 72 h of storage levels of \( S. aureus \) in samples containing green tea or rosemary ranged from 5.98 to 6.52 log CFU/g compared with 8.32 log CFU/g in the control (Fig. 2). The addition of green tea and rosemary did not have a strong inhibitory effect on the growth of \( S. aureus \) on rice cakes during storage. Figure 3 shows the population of \( B. cereus \) in rice cakes. Initial populations of \( B. cereus \) inoculated onto rice cakes ranged from 1.00 to 1.02 log CFU/ml. After 12 h of storage, populations of \( B. cereus \) increased, but no significant differences were detected among samples. After 24 of h storage, populations of \( B. cereus \) increased in some samples. Controls had higher counts of \( B. cereus \) (3.14 log CFU/g) than did samples containing green tea or rosemary. After 72 h of storage, there were significant differences in levels of \( B. cereus \) between controls and samples containing both botanicals \( (P \leq 0.05) \). Levels of \( B. cereus \) in samples containing 3% green tea and rosemary were 1.56 and 1.70 log CFU/g, respectively, compared with 4.96 log CFU/g in the control. Therefore, the addition of 3% green tea or rosemary effectively suppressed the growth of \( B. cereus \) in rice cake during storage for 72 h.

From results of experiments in laboratory medium, both green tea and rosemary were effective for inhibiting the growth of \( B. cereus, S. aureus, \) and \( L. monocytogenes \) but were not significantly effective against \( S. Typhimurium, E. sakazakii, \) or \( E. coli \) O157:H7. Therefore, it appears that both botanicals were more effective at inhibiting gram-positive bacteria than inhibiting gram-negative bacteria. Other researchers have reported similar results \((4, 7, 9, 11, 16, 25, 28, 30)\). Campo et al. \((4)\) investigated the antimicrobial effect of rosemary extracts in ethanol against several pathogens and found that the rosemary extract had no effect on gram-negative bacteria such as \( E. coli, Salmonella Enteritidis, \) and \( Erwinia carotovora \), whereas growth of gram-positive bacteria such as \( S. aureus, L. monocytogenes, B. cereus, \) \( Leuconostoc mesenteroides, \) and \( Streptococcus mutans \) was inhibited. Kim et al. \((16)\) investigated the antibacterial effect of 10% (wt/vol) water-soluble tea extracts, including green tea, on foodborne pathogens and found that green tea extract suppressed the growth of \( S. aureus \) and \( L. monocytogenes \) but did not inactivate \( E. coli \) O157:H7 and \( S. Typhimurium \). Roh et al. \((25)\) also reported that 500 and 1,000 ppm of green tea extract was more effective for inhibiting gram-positive bacteria such as \( Bacillus \) spp. and \( S. aureus \) than for inhibiting \( S. Typhimurium \).

Although steam cooking during rice cake manufacturing should completely destroy vegetative cells of pathogen-
ic microorganisms, steam cooking may not be effective for destroying spore-forming bacteria such as *B. cereus* (18). Surviving bacterial spores can germinate, grow, and produce toxin during storage of rice cakes at room temperature, causing illness in consumers. Therefore, it is important to develop a way to inhibit the growth of spore-forming bacteria such as *B. cereus* in rice cakes. Other researchers also have reported the antimicrobial effects of herb extracts on spore-forming bacteria. Friedman et al. (12) found that green tea catechins and black tea theaflavins (flavonoids) had antimicrobial activity against *B. cereus* at nanomolar levels, and Sakanaka et al. (26) reported that tea polyphenols had an antibacterial effect on *Bacillus stearothermophilus* and reduced the heat resistance of *Clostridium thermoaceticum* spores.

These results indicate that the addition of powdered botanicals such as green tea and rosemary could be helpful for improving the microbial quality of rice cakes by inhibiting the growth of spore-forming bacteria and their toxin production and for improving the sensory qualities of the cakes, such as color, texture, nutrients, and flavor. However, further studies on the inhibitory activity of these botanicals in other types of rice cakes or other food products and on their organoleptical properties are required before these additives can be recommended for commercial application.

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**REFERENCES**


