Effects of commercial marinade seasoning and a natural blend of cultured sugar and vinegar on *Campylobacter jejuni* and *Salmonella* Typhimurium and the texture of chicken breasts

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**ABSTRACT** Marination using various ingredients has been widely used to improve microbial safety and quality of chicken products at retail markets. The objective of this study was to investigate the effects of commercial marinade seasoning and cultured sugar/vinegar blend on *Campylobacter jejuni* and *Salmonella* Typhimurium populations during refrigerated storage. In addition, their effects on the texture of precooked chicken breasts during frozen and refrigerated storage was investigated. Chicken breasts inoculated with 4.5 to 5.0 log cfu/g of *C. jejuni* and *Salmonella* Typhimurium were treated with 3% cultured sugar/vinegar blend with and without 0.6% polish rub seasoning containing 32% herb content. Breasts were then vacuum-packaged and stored at 4 and 10°C. Survival and growth curves were fitted to the Baranyi equation to determine survival and growth kinetics of *C. jejuni* and *Salmonella* Typhimurium. In addition, the vacuum-packaged precooked chicken breasts with different marination treatments were subjected to 3 freeze-thaw cycles and shear force was measured. At 4°C, the populations of *C. jejuni* and *Salmonella* Typhimurium decreased, regardless of treatment group during storage. The greatest survival for *C. jejuni* was observed in untreated chicken breasts. At 10°C, the growth of *Salmonella* Typhimurium was completely prevented in precooked chicken breasts treated with 3% cultured sugar/vinegar blend, regardless of the presence of 0.6% seasoning. The 3% cultured sugar/vinegar blend also improved the tenderness of frozen chicken breasts and refrigerated, ready-to-eat chicken breast. Therefore, a natural blend of cultured sugar and vinegar can be used as antimicrobial and texture-modifying agents for poultry meat and poultry products.

**Key words:** *Campylobacter jejuni*, *Salmonella* Typhimurium, precooked chicken breast, antimicrobial agent, texture

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**INTRODUCTION**

Precooked and refrigerated ready-to-eat meat products are gaining popularity as consumers purchase convenient foods that do not require extensive home preparation (Ntzimani et al., 2011). During the pre-cooking process, the meat is partially heated until the core temperature of meat reaches 74°C, which results in reducing cook time at home. However, precooked products are not completely cooked, so they are at high risk for microbial spoilage. In addition, change of physical properties is a concern for the consumer.

*Campylobacter jejuni* are major bacterial contaminants in poultry products, along with *Salmonella* (Bryan and Doyle, 1995). *Campylobacter jejuni* causes more cases of foodborne gastroenteritis each year than any other bacterial pathogen worldwide and has become recognized as a leading cause of diarrheal disease and bacterial gastroenteritis. Despite many efforts to minimize contamination of poultry with *Campylobacter*, the overall number of reported human campylobacteriosis cases related to poultry meat consumption has not been reduced (EFSA, 2009, 2010). In 2012, there were 7,800 infections, 2,284 hospitalizations, and 33 deaths caused by *Salmonella* in the United States. For *Campylobacter* these numbers were reported as 6,793, 1,044, and 6, respectively (CDC, 2013).

Various techniques including marination have been studied to improve meat quality and microbial safety of chicken during storage at retail markets (Smith and Acton, 2001; Carroll and Alvarado, 2008). Cultured sugar and vinegar are natural alternatives to lactic acid salt compounds, are generally regarded as safe, and can be added to poultry and other meat products during processing to control food safety (Purac, 2008; Sullivan, 2011). A natural blend of cultured sugar and vinegar is produced by fermentation with specifically selected cultures and consists of fermentation products such as...
sugars, organic acids, peptides, and aromas. Glass and Sindelar (2010) reported that a cultured sugar and vinegar blend inhibited the growth of *Listeria monocytogenes* in ham, beef, and turkey. Birk et al. (2010) also reported wine vinegar (a fermentation product of wine containing a combination of organic acids such as acetic acid, tartaric acid, and citric acid, as well as other fermentation metabolites) was more effective in reducing the population of *C. jejuni* on chicken breasts than lemon juice, which had the lowest pH (2.7) tested.

Polish rub seasoning is a commercially available marinade dry seasoning and has been widely used for precooked chicken breast products. It contains herbs, including crushed mustard seed, polish blend, crushed red pepper, whole basil, whole thyme, parsley leaf, black pepper whole, and oleoresin nutmeg as flavor ingredients, and also includes refined salt, garlic flare, brown sugar, α-corn, crust breading, soybean oil, and oleoresin paprika (Kim, 2006). Chicken meat products spread with dry seasoning have become common and they are sold routinely by convenience stores in Korea. However, effects of commercial marinade seasonings and cultured sugar/vinegar blend on chicken breast as antimicrobial and texture modifying agents have not been studied.

In the present study, we examined the effects of commercial marinade seasoning containing 32% herbs and a natural blend of cultured sugar and vinegar as antimicrobial agents to control *C. jejuni* and *Salmonella Typhimurium* during refrigerated storage at retail markets. We also studied their effects on the texture of chicken breasts during refrigerated and frozen storage.

**MATERIALS AND METHODS**

**Preparation of Bacterial Cultures**

Strains of *C. jejuni* (ATCC 33291) and *Salmonella Typhimurium* (ATCC 13311) were purchased from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea) and maintained at −80°C in Brucella broth (BD, Sparks, MD) with 0.16% agar and brain heart infusion broth (BD) containing 20% glycerol (Sigma-Aldrich, St. Louis, MO), respectively. For each experiment, stock cultures of *C. jejuni* and *Salmonella Typhimurium* were thawed at room temperature and 0.01 mL of each was inoculated into 25-mL Erlenmeyer flask containing 10 mL of sterile Brucella broth with 0.16% agar or brain heart infusion broth, respectively, which was then sealed with a silistopper (Korea Ace Scientific, Seoul, Korea). *Campylobacter jejuni* cultures were placed in a microaerophilic jar that contained CampyGen sachet (Oxoid, Hampshire, UK) producing 5% O2, 10% CO2, and 85% N2 at 37°C for 24 h at 100 rpm. *Salmonella Typhimurium* cultures were incubated aerobically at 37°C for 24 h at 140 rpm. Viable cell counts for *C. jejuni* and *Salmonella Typhimurium* at the end of the incubation period ranged from 8.8 to 9.4 log cfu/mL. One milliliter of the stationary phase overnight culture was transferred into 9 mL of 0.1% sterilized peptone water (BD), which was serially diluted before inoculation into the chicken breasts.

**Antimicrobial Agents**

Two different seasoning ingredients were used either alone or as a mixture, for treatment of precooked chicken breasts. The first one was a commercially available marinade dry seasoning (polish rub seasoning) that has been widely used for precooked chicken breasts products. The polish rub seasoning containing 32% herb components was obtained from Harim (Iksan, Jeollabuk-do, Korea). The level of polish rub seasoning (0.6%) used in this study was the same level as used in commercial chicken products. Polish rub seasoning was dried in a vacuum oven (DF-102V2, Vision Scientific Co., Ltd., Daejeon, Korea) at 110°C for 2 h to control the background of microorganisms including thermophilic bacteria. After drying process, no background microorganisms were detected. The second seasoning was a natural blend of cultured sugar and vinegar (Purac verdad NV55), obtained from Purac (Lincolnshire, IL). The 3% level used was determined by the manufacturer’s recommendation.

**Preparation and Inoculation of Chicken Breasts for Microbial Analysis**

Boneless, skinless chicken breasts were purchased from a local retail market. Chicken breasts were sliced in half (6 × 12 × 1.5 cm) and steamed for about 3 min in a steamer until core temperature reached 74°C. The internal temperatures of chicken breasts were determined using meat thermometers (Testo 925, Testo AG, Lenzkirch, Germany), which were inserted into the thickest portion of each chicken breasts. After steaming, no background microorganisms were detected. Steamed chicken breasts were cut into 10-g portions, and aseptically transferred to sterile Petri dishes. Challenge studies were initiated by inoculating breasts with both 0.1 mL of *C. jejuni* and 0.1 mL of *Salmonella Typhimurium* simultaneously, using a sterile repeat pipette to obtain a target population of approximately 4.5 to 5.0 log cfu/g. Then 0.6% of polish rub seasoning with or without 3% cultured sugar/vinegar blend (Purac verdad NV55) was spread over the sample using a sterile pipette. The treated samples were individually vacuum-packaged (polyethylene and nylon) and incubated at 4 and 10°C for 30 d.

**Enumeration of *C. jejuni* and *Salmonella Typhimurium***

At selected times postinoculation, depending on the incubation temperature, the marinated chicken breasts were homogenized (Pulsifier, Microgen Bioproducts, Surrey, UK) for 15 s in 90 mL of 0.1% sterilized peptone water. Two samples were processed per each treatment.
One hundred microliters was spiral plated (Whitley automatic spiral plater, Don Whitley Scientific, West Yorkshire, UK) onto selective media, modified charcoal cefoperazone deoxycholate agar plates (Oxoid, Hampshire, UK), for enumeration of *C. jejuni* and onto selective media, xylose lysine deoxycholate plates (Oxoid), for enumeration of *Salmonella* Typhimurium. Modified charcoal cefoperazone deoxycholate agar plates were incubated in a 42°C chamber (miniMACS, Miltenyi Biotech, Auburn, CA) under microaerophilic conditions (5% O₂, 10% CO₂ and 85% N₂) for 2 to 3 d. Xylose lysine deoxycholate plates were incubated aerobically at 37°C for 24 h. Colonies on duplicate plates for each sample were counted with an automated colony counter (Scan 1200, Interscience, Saint Nom, France). The mean of the duplicate plates was graphed at each sampling time to generate growth curves for *C. jejuni* and *Salmonella* Typhimurium as a function of time.

**Survival and Growth Curves for C. jejuni and Salmonella Typhimurium on Precooked Chicken Breasts**

Survival or growth representing viable cell counts (log cfu per gram) of *C. jejuni* and *Salmonella* Typhimurium as a function of time were iteratively fit to the Baranyi equation using the DM Fit 1.0 curve-fitting program (Institute of Food Research, Norwich, UK). The equation used was as follows (Baranyi and Roberts, 1994):

\[
y = y_0 + \frac{\mu_{max} \ln(10)}{\ln(10)} - \frac{1}{\ln(10)} \ln \left[ 1 + \frac{e^{\mu_{max}A - 1}}{10^{(\mu_{max} - y_0)}} \right] \\
A = t + \frac{1}{\mu_{max}} \ln \left( \frac{e^{\mu_{max}t} + y_0}{1 + y_0} \right) \\
t_{lag} = \frac{\ln \left( 1 + \frac{1}{y_0} \right)}{\mu_{max}},
\]

where *y* is the logarithm of the cell numbers (log cfu per gram), *y₀* is the initial cell number, *yₘₐₐₓ* is the final cell number, *A* is the time variable, *μₘₐₐₓ* is the specific growth rate or inactivation (log per day), *q₀* is the physiological state of the inoculum; *t_{lag}* is the lag time, which in the case of inactivation curves will be regarded as the shoulder period, or survival period, and *t* is the sampling time. Each experiment was replicated twice. The goodness of fit of the data was evaluated based on the coefficient of determination (R²), which was calculated using DM Fit software (Institute of Food Research, Norwich, UK).

**Preparation of Samples for Texture Analysis**

For texture analysis, samples were prepared and shear force was measured similar to methods described by Lyon and Lyon (1998). Whole chicken breasts (6 × 12 × 2.8 cm) were steamed in a steamer for 7 min until core temperature reached 74°C. The internal temperature of the chicken breasts was determined as described above. The chicken breasts were weighed and treated with 0 or 0.6% polish rub seasoning with or without 3% cultured sugar/vinegar blend. After treatments, samples were vacuum-packaged and stored at 10°C for 20 d. To investigate the effect of treatments on the texture of chicken breasts during frozen storage, samples were also subjected to 3 freeze-thaw cycles for accelerated frozen storage (1 freeze-thaw cycle: frozen at −18°C for 3 d and thawed at 4°C for 1 d).

**Texture Measurement**

The texture of refrigerated samples at 10°C was measured at 0, 5, 10, 15, and 20 d of storage after reheating in a microwave (RE-452R, Samsung, Suwon, Gyeonggi-do, Korea) at 700 W for 50 s to a final internal temperature of 77°C according to industry guidelines. After freeze-thaw cycles, samples were also reheated completely using the above method. Three pieces (1.9 cm wide and 1.9 cm high per piece) were obtained from reheated chicken breasts for texture analysis.

A Warner-Bratzler (WB) blade attached to a TA-XT Express texture analyzer (Stable Micro Systems, Surrey, UK) was used to shear the samples across the muscle fibers after the sample was equilibrated at room temperature (20°C) for 2 h. The rectangular blade was made of stainless steel with an inverted V cut in the bottom edge (70 mm wide, 70 mm high, and 3 mm thick). The height of the TA-WB blade was set so that the apex of the notch was 40 mm from the base. The blade traveled at 2 mm/s into the sample that was positioned on the slotted platform with muscle fibers perpendicular to the blade. As the blade traveled down and through the slot, the samples were sheared and maximum force was recorded. Results are expressed as shear force (g). Three measurements were performed on each sample to obtain mean and SE calculations. The instrument settings were maximum cell load, 5 kg; sensitivity, 0.1 g; probe pretest speed, 5 mm/s; test speed, 2 mm/s; post speed, 2 mm/s; cutting distance, 16 mm.

**Statistical Analysis**

Experiments were replicated twice on different occasions with different chicken breast samples for microbiological testing. For texture study, experiments were done in triplicate. Three samples were analyzed per treatment. Data were analyzed using the GLM procedures of SAS (version 9.3, SAS Institute Inc., Cary, NC). Comparisons of means among antimicrobial treatment or storage time were performed using Duncan’s multiple range test at the level of *P* < 0.05. The *t*-test was also used to determine the significant differences in the shear force between before frozen and after 3 freeze-thaw cycles.
RESULTS AND DISCUSSION

Antimicrobial Effects of Commercial Seasoning and Cultured Sugar/Vinegar Blend

Table 1 shows the survival or growth kinetics of \textit{C. jejuni} and \textit{Salmonella Typhimurium} on precooked chicken breasts treated with commercial seasoning and a cultured sugar/vinegar blend and stored at 4 and 10°C. In the \textit{C. jejuni} survival curve, only the control had a lag time of 13.6 d at 4°C. Treatment with 0.6% seasoning plus 3% cultured sugar/vinegar blend significantly increased the specific death rate (SDR) and maximum log reduction (MLR) of \textit{C. jejuni}.

At 4°C, no significant difference was observed in the average MLR of \textit{C. jejuni} between 0.6% seasoning (0.474 log cfu/g) and control (0.552 log cfu/g), whereas the 3% cultured sugar/vinegar blend significantly increased MLR compared with the control (1.150 log cfu/g; \(P < 0.05\)). The mixture of 0.6% seasoning plus 3% cultured sugar/vinegar blend was more effective in preventing the survival of \textit{C. jejuni} than 0.6% seasoning or the 3% cultured sugar/vinegar blend alone at 4°C, indicating a synergistic effect toward reducing the \textit{C. jejuni} population. It has been shown that organic acids are effective in controlling \textit{C. jejuni} populations (Riedel et al., 2009; Birk et al., 2010; Coşansu and Ayhan, 2010) or total microbial load (Tangkham et al., 2012). Glass and Sindelar (2010) reported that the growth of \textit{L. monocytogenes} on uncured turkey treated with 3% cultured cane sugar/vinegar blend was delayed for an additional 4 wk compared with the control at 4°C. In a similar cultured sugar/vinegar blend, wine vinegar (which is a fermentation product of wine and contains a combination of acetic acid, tartaric acid, citric acid, and other fermentation metabolites) was found to be more effective in reducing the population of \textit{C. jejuni} on chicken breasts fillets at 4°C than lemon juice, which had the lowest pH (2.7; Birk et al., 2010).

In the case of \textit{Salmonella Typhimurium}, no lag time was observed in all treatment groups. No significant differences were observed in the SDR or MLR in the sample with 0.6% seasoning or 3% cultured sugar/vinegar blend compared with the control at 4°C (\(P < 0.05\)). However, treatment with a mixture of 0.6% seasoning and 3% cultured sugar/vinegar blend significantly increased SDR and MLR of \textit{Salmonella Typhimurium} compared with the other treatment groups at 4°C (\(P < 0.05\)). At 10°C, the population of \textit{Salmonella Typhimurium} in the control was increased by approximately 2.316 log cfu/g after 10 d (SGR, 0.229 log cfu/d) and remained constant during the rest of the storage period. The growth of \textit{Salmonella Typhimurium} on precooked chicken breasts with 0.6% seasoning was also observed and these populations of \textit{Salmonella Typhimurium} increased from 15 to 20 d and remained at the highest populations similar to the control. However, the SGR was slower than that of the control, whereas no signifi-
The control and the sample with 0.6% seasoning. As shown in Figure 2, growth of *Salmonella* Typhimurium was observed at 10°C in both the control sample (Figure 2A) and precooked chicken breasts treated with 0.6% seasoning (Figure 2B). However, the number of *Salmonella* Typhimurium on precooked chicken breasts was decreased by 1.5 to 2.0 log cfu/g at the end of storage at 10°C, when samples were treated with either 3% cultured sugar/vinegar blend (Figure 2C), or a mixture of 0.6% seasoning and 3% cultured sugar/vinegar blend (Figure 2D). However, no synergistic effect between 0.6% seasoning with 3% cultured sugar/vinegar blend was observed in this study, as shown in Table 1.

Among the antimicrobial agents tested in the present study, the mixture of 0.6% seasoning and 3% cultured sugar/vinegar blend was the most effective antimicrobial agent to control the survival of *C. jejuni* and *Salmonella* Typhimurium on precooked chicken breasts at 4 and 10°C. The 0.6% commercial marinade seasoning containing 32% herb content showed a more mild inhibition of *C. jejuni* and *Salmonella* Typhimurium growth at 10°C than at 4°C. Polish rub seasoning contains various herbs and also includes refined salt, garlic flake, brown sugar, α-corn, crust breading, soybean oil, and oleoresin paprika (Kim, 2006). Antimicrobial compounds in plant materials are commonly found in the essential oil fraction of herb leaves (rosemary, sage, basil, oregano, thyme, and marjoram), flowers or buds (clove), bulbs (garlic and onion), seeds (caraway, fennel, nutmeg, and parsley), rhizomes (asafoetida), fruits (pepper and cardamom), or other parts of plants (Nychas and Skandamis, 2003; Gutierrez et al., 2008). Plant essential oils are generally more inhibitory against gram-positive than gram-negative bacteria (Marino et al., 2001; Chorianopoulos et al., 2004; Gutierrez et al., 2008). In this study, 0.6% polish rub seasoning was not strong enough to prevent growth of gram-negative, *Salmonella* Typhimurium in precooked chicken breasts at refrigeration temperatures.

**The Effects of Commercial Seasoning and Cultured Sugar/Vinegar Blend on Texture**

The effect of commercial marinade seasoning and cultured sugar/vinegar blend on the texture of precooked chicken breasts were investigated after 3 freeze-thaw cycles (Table 2) and 20 d of refrigerated storage at 10°C (Table 3). As shown in Table 2, the lowest increase in shear force was demonstrated by precooked chicken breasts treated with 3% cultured sugar/vinegar blend among the treatments. There were no observed synergistic effects between 0.6% commercial marinade seasoning and 3% cultured sugar/vinegar blend on texture softening of chicken breasts (*P* > 0.05). However, the changes in shear force were not significant after 3 freeze-thaw cycles, regardless of different treatment. Similar results have been reported in previous studies.
There is general agreement in the literature that the tenderness of meat increases with freezing and thawing (Wheeler et al., 1990; Shanks et al., 2002; Farouk et al., 2004; Lagerstedt et al., 2008) due to the loss of membrane strength caused by ice crystal formation, reducing the force needed to shear the meat (Liu et al., 2010). It has also been found that the increase in tenderness is correlated with the length of frozen storage and the degree to which the meat was aged before freezing. The tenderizing effect of freezing seems to be negated when the meat has been sufficiently aged before freezing (Vieira et al., 2009). However, the slight increase of shear force observed in the current study was likely caused by changes in muscle tissue due to fiber shrinkage following freezing and thawing, which has also been reported by Hale and Waters (1981) and Van Laack and Solomon (1994).

On the other hand, significantly lower shear force was observed for chicken breasts treated with 3% cultured sugar/vinegar blend, as well as with the mixture of 0.6% seasoning and 3% cultured sugar/vinegar blend, as compared with samples treated with 0.6% seasoning or the control, when stored at 10°C for 20 d.

![Figure 1. Comparison of the death curves of Campylobacter jejuni on precooked chicken breasts marinated with 0.6% seasoning plus 3% cultured sugar/vinegar blend at 4 and 10°C. A) 4°C, control; B) 4°C, 0.6% seasoning and 3% cultured sugar/vinegar blend; C) 10°C, control; D) 10°C, 0.6% seasoning and 3% cultured sugar/vinegar blend.](image)

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<th>Table 2. Comparison of shear force of precooked chicken breasts with different treatments after 3 freeze-thaw cycles1</th>
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<td>Treatment</td>
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<td>0.6% seasoning + 3% cultured sugar/vinegar</td>
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$^A,B$ Different superscripts in the same column denote a significant difference ($P < 0.05$).

1 Results are presented as means ± SD (n = 9).

2 Derived from $t$-test.
After 5 d of storage at 10°C, the shear force observed in precooked chicken breasts treated with 3% cultured sugar/vinegar blend, or with the mixture of 0.6% seasoning and 3% cultured sugar/vinegar blend, was significantly decreased compared with nonfrozen sample ($P < 0.05$) and remained during the rest of 20 d of refrigerated storage. However, no significant change in shear force was observed in the control or precooked chicken breasts treated with 0.6% seasoning during these 20 d (Table 2). Similar results have been reported in previous studies. The change in shear force was not significant in precooked, vacuum-packaged turkey rolls produced by the cook-in-bag process during 87 d of storage (Smith and Alvarez, 1988) or in cooked chicken breasts during 14 d in storage at 4°C (Yoon, 2003).

Overall, treatment with 0.6% polish rub seasoning containing NaCl, spices, and flavoring compounds did not affect the tenderizing of precooked chicken breasts stored at 10°C after 3 freeze-thaw cycles in the present study.

Textural characteristics are important in the production of processed meat products because they affect consumer acceptability (Chan et al., 2011). The results of this study indicated that the 3% cultured sugar/vinegar blend prevented hardness in precooked chicken breasts that were subjected to 3 freeze-thaw cycles, and

![Figure 2. Comparison of the growth and death curves of Salmonella Typhimurium on precooked chicken breasts marinated with 0.6% seasoning, 3% cultured sugar/vinegar blend, and 0.6% seasoning plus 3% cultured sugar/vinegar blend at 10°C. A) Control, B) 0.6% seasoning, C) 3% cultured sugar/vinegar blend, D) 0.6% seasoning and 3% cultured sugar/vinegar blend.](image-url)
improved tenderness of refrigerated precooked chicken breasts. This suggests an increase in water-holding capacity caused by the 3% cultured sugar/vinegar blend. The reduction in shear force can be attributed to weakening of the myofibrillar structure due to increasing water-holding capacity.

Conclusions

Precooked and refrigerated ready-to-eat meat products are gaining popularity as consumers purchase convenient foods that do not require extensive home preparation. In this work, we examined the effect of commercial marinae seasoning containing herbs and a cultured sugar/vinegar blend as antimicrobial and texture-modifying agents. Among the antimicrobial agents, a combination of the cultured sugar/vinegar blend and commercial seasoning may be valuable ingredients to control _C. jejuni_ and _Salmonella_ Typhimurium in poultry products during refrigerated storage. Additionally, the 3% cultured sugar and vinegar blend prevented texture change in frozen chicken breasts and increased tenderness of refrigerated, ready-to-eat chicken breasts, which is often cited as the most important quality of both refrigerated and frozen chicken breasts. Therefore, cultured sugar and vinegar can be used in the manufacturing of natural and organic precooked chicken products, where the ingredients listed must be recognizable to the consumer.

REFERENCES


