Wines as possible meat marinade ingredients possess antimicrobial potential against Campylobacter

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ABSTRACT This research studied the survival of high (7 log cfu/mL) and low (3 log cfu/mL) inoculum levels of Campylobacter in white and red wines and in grape and tomato juices, which could function as potential antimicrobial marinade ingredients. For comparison, survival was also studied in a commercial poultry meat marinade. White and red wines were shown to have very high bactericidal effects against Campylobacter. High counts were rapidly inactivated to undetectable numbers within 15 min in white wine and within 1 h in red wine, and low counts within 15 min in white wine and within 30 min in red wine. By contrast, grape and tomato juices did not possess high bactericidal effects against Campylobacter because even low counts were occasionally detected after 48 h. The commercial marinade had rather high bactericidal effects against Campylobacter; the high counts were inactivated in most cases within 48 h, and all the low counts were inactivated within 3 h. When testing chicken meat inoculated with Campylobacter and subsequently submerged in white or red wine, the antibacterial activity of the wine was largely reduced. Wines lowered the Campylobacter load inoculated on chicken meat by approximately 1 log cfu/mL over 48 h. The results suggest that wines could be used as antimicrobial ingredients together with the addition of further antimicrobial agents in meat marinades to reduce the numbers of Campylobacter in naturally contaminated poultry products, thus lowering the risk of Campylobacter cross-contamination and transmission through food.

Key words: Campylobacter, survival, chicken meat, wine, juice

INTRODUCTION

Campylobacter are the most common cause of foodborne bacterial gastroenteritis in humans worldwide (Samuel et al., 2004; European Food Safety Authority, 2010). The incidence of campylobacteriosis has increased steadily over the years, and it currently exceeds the number of cases of Salmonella infection (European Food Safety Authority, 2010). Important sources of Campylobacter infection are surface water, water from private wells, natural waters (via swimming), and unpasteurized milk (Park, 2002; Humphrey et al., 2007; Jacobs-Reitsma et al., 2008). However, the consumption and handling of improperly prepared poultry meat is considered one of the other major risk factors (Potter et al., 2003; Wingstrand et al., 2006; Jacobs-Reitsma et al., 2008).

A significant amount of poultry meat at the retail level is sold marinated in a wide range of flavors. Marinades are complex spiced, acidic water-oil emulsions typically containing salt, sugar, and sorbate, benzoate, or both. The high NaCl concentration, low pH, and addition of different spices to the marinades prevent the growth of spoilage bacteria, thus increasing the shelf life of meat products (Björkroth, 2005). Nevertheless, Campylobacter have been detected in different countries in both marinated and nonmarinated poultry products at the retail level, with a prevalence of between 10 and 100% and counts between <10 and 2.4 log cfu/g (Hanninen et al., 2000; National Veterinary and Food Research Institute, 2003; Kang et al., 2006; Scherer et al., 2006; Atanassova et al., 2007; Jacobs-Reitsma et al., 2008; Katzav et al., 2008; Pointon et al., 2008).

One potential approach to reducing the counts of Campylobacter that may be present in poultry meat could be to add substances with antimicrobial properties to the marinades used. Several studies have described the effect of the antimicrobial properties of wines on food pathogens such as Bacillus spp., Escheri-
Wines as Possible Meat Marinade

The white wine (Sauvignon Blanc 2006, Gallo Family Vineyards, Modesto, CA) had an alcohol content of 13% and contained sulfites. The red wine (Cabernet Sauvignon 2006, Gallo Family Vineyards) had an alcohol content of 13.5% and also contained sulfites. The grape juice (grape nectar, Valio Oy, Valio, Finland) was a 72% grape nectar juice made from juice concentrates containing grape juice, added vitamin C, and water, with no preservatives. The tomato juice (Natur tomato juice drink, Oy Marli Ab, Turku, Finland) was a 100% tomato juice drink manufactured from juice concentrates containing tomato juice, glucose-fructose syrup, sugar, salt, and water, with no preservatives. According to the manufacturer, the commercial poultry meat marinade contained water, turnip, rapeseed oil, honey- apple wine vinegar, maltodextrin, glucose, spices, salt (1.0%), stabilizers (E451, E450, E452), yeast extract, flavor enhancer (E621), thickeners (E412, E415), aromas, preservatives (E211, E202), and an acidity regulator (E330). The wines and juices were purchased from local shops, and the marinade was provided by a local poultry processor. All the liquids were stored at 4°C before the experiments and were used for inoculation immediately after opening the container. As a control, the survival of Campylobacter was studied in brain heart infusion (BHI) broth (LAB49, LabM, Lancashire, UK), which was prepared according to the manufacturer’s instructions.

The pH of each liquid type was measured before initiation of the studies by using a standard pH meter (MeterLAB, Radiometer Analytical, Copenhagen, Denmark). To check the sterility of the liquids before the tests, 100 μL of each liquid was spread on casein-peptone soybean meal-peptone agar (CASO, Merck, Darmstadt, Germany) with 5% bovine blood and was incubated aerobically at 37°C for 24 h.

Preparation of Bacterial Suspension

To prepare the bacterial suspension used for inoculations, the cells from the frozen stock culture were plated onto a Campylobacter blood-free selective medium [modified CCDA-Preston (mCCDA), CM0739, Oxoid, Hampshire, UK] containing CCDA selective supplement (SR0155, Oxoid) and incubated at 42°C for 48 h under microaerobic conditions obtained by CampyGen (CN0035, Oxoid). Between 1 and 3 single colonies from the growth were suspended into 5 mL of BHI broth (LabM) that had been prewarmed at 42°C, and the suspension was incubated at 42°C for 24 h under microaerobic conditions obtained as described above. After incubation, the optical density of the suspension at 600 nm was adjusted to 0.1, corresponding to approximately 8 log cfu/mL. The log colony-forming units per milliliter of bacterial suspension were counted by serial
Preparation of Chicken Meat

Chicken breast fillets were purchased from a local retail outlet and immediately tested for the absence of Campylobacter spp. Testing was done according to method ISO 10272-1:2006 (International Organization for Standardization, 2006). Portions of approximately 10 g were cut aseptically, put in separate stomacher bags (BA6041, Seward, Worthing, UK), and stored at 18°C until use. Only Campylobacter-negative meat samples were included in the study.

Inoculation

Inoculation of the Liquids. For the experiments on the liquids, all 4 Campylobacter strains (C. jejuni strain RefCJ, C. jejuni strains RetCJ29 and SlaCJ26, and C. coli strain RetCC27) were used. To inoculate the liquids with high bacterial counts, 1 mL of the original suspension, containing approximately 8 log cfu/mL, was suspended into 9 mL of the liquid type studied to give a bacterial concentration of approximately 7 log cfu/mL. To inoculate the liquids with low bacterial counts, 1 mL from a 10−4 dilution tube (used earlier for counting the original log colony-forming units per milliliter of suspension) containing approximately 4 log cfu/mL was suspended in 9 mL of the liquid type studied to give a bacterial concentration of approximately 3 log cfu/mL. The inoculated liquids were always vortexed immediately after inoculation and before plating.

Inoculation of the Chicken Meat. For the experiments on chicken meat, 2 Campylobacter strains, RefCJ and RetCC27, were used. Frozen chicken meat samples were thawed at ambient temperature for 60 min. For each test, a 10-g meat sample was used. To inoculate the meat samples with high bacterial counts, 100 μL of the original suspension, containing approximately 7 to 8 log cfu/mL, was applied to the separate meat pieces. To inoculate the liquids with low bacterial counts, 100 μL from the 10−3 dilution tube containing approximately 5 log cfu/mL was applied to the separate pieces of meat. Inoculated pieces of meat were kept at room temperature for 20 min to allow possible attachment and diffusion. To each meat sample, 10 mL of the corresponding liquid [white wine, red wine, or phosphate PBS (Oxoid)] was added.

Determination of Reductions in the Liquids and in Chicken Meat-Liquid Samples

The survival of all 4 Campylobacter strains in each liquid type was monitored at <1 min, 15 min, 30 min, 1 h, 3 h, 24 h, and 48 h after inoculation. The survival of the Campylobacter strains in each meat-liquid type was monitored at 10 min, 15 min, 30 min, 1 h, 3 h, 24 h, and 48 h after inoculation. The inoculated liquids and meat-liquid samples were kept at room temperature until the 3-h time point and were then stored at 4°C. The meat-liquid samples were homogenized in a laboratory blender (Stomacher 400 Circulator, Seward) before Campylobacter cell count. The reductions in the high and low inoculum levels were determined with serial 10-fold dilutions in BHI broth (LabM) and by preparing 2 spread plates (mCCDA, Oxoid) from each dilution. All plates were incubated at 42°C for 48 h under microaerobic conditions obtained as described above, and the colonies were identified by colony morphology and Gram staining. The results were counted and expressed as log colony-forming units per milliliter based on averages from the duplicate spread plates. In the liquids, the detection limit was 1 log cfu/mL, and in the chicken meat-liquid samples, it was 2 log cfu/mL. The tests were replicated twice for each liquid type and 3 times for each meat-liquid sample, and the results of the test replications were averaged.

Statistical Analysis

The data from each time point and the statistical differences between the main effects of bacterial strains and liquid types were all determined separately. Because the data were not normally distributed, significance was determined by a nonparametric Kruskal-Wallis test. Analyses were performed by means of the statistical package SPSS 16.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Survival of Campylobacter in White and Red Wines, Grape and Tomato Juices, and the Commercial Marinade

Table 1 presents the survival of high and low inoculum levels of the 4 different Campylobacter strains in white and red wine, grape and tomato juice, and the commercial marinade after different exposure times. Of all the liquids, white wine had the strongest antibacterial effect on all 4 Campylobacter strains. High counts of all strains were inactivated within 15 min, and the low inoculum levels of RetCC27 and RefCJ were inactivated to undetectable numbers within <1 min in white wine. In red wine, high counts of all Campylobacter strains were reduced to low counts within 30 min and were inactivated within 1 h. The grape and tomato juices were less bactericidal than the wines. In the grape and tomato juices, the high inoculum levels of SlaCJ26, RetCJ29, and RefCJ were still detected after 48 h of exposure. The low counts of strains RefCJ and RetCJ29 in grape juice and of strain RetCJ29 in tomato juice were still detected after 48 h of exposure. In the commercial marinade, the high counts of most of
the Campylobacter strains were inactivated within 48 h of exposure, and all the low counts were inactivated to undetectable numbers within 3 h.

**Survival of Campylobacter in Meat-Wine Samples**

Figure 1 shows the survival of high and low inoculum levels of *C. jejuni* (RefCJ) and *C. coli* (RetCC27) in the meat-wine samples compared with survival of these strains in the meat-PBS samples. Red wine (Figure 1A and 1C) and white wine (Figure 1B and 1D) moderately reduced the Campylobacter load on chicken meat over the sampling period, by approximately 1 log cfu/mL, regardless of the Campylobacter species inoculated or the type of wine used. Nonetheless, Campylobacter were still detectable in higher numbers after 48 h of exposure to white and red wine when inoculated on meat.

**Survival of Campylobacter in BHI Broth and Sterility and pH Measurements of the Liquids**

The initial pH was 3.20 ± 0.07 in white wine, 3.79 ± 0.05 in red wine, 3.62 ± 0.07 in grape juice, 4.11 ± 0.07 in tomato juice, 4.16 ± 0.03 in the commercial marinade, and 7.36 ± 0.03 in the BHI broth control solution. No changes were observed in counts of the Campylobacter strains studied in the BHI broth (LabM) control solution within 48 h at either of the inoculum levels (data not shown). The sterility of each liquid type was checked before the studies and no contaminants were found in any of the liquids, except in the case of the commercial marinade, in which diverse background flora were observed on the plates.

**Statistical Analysis**

The statistical differences in the effects of the different liquid types at each time point were examined using the data acquired from the Campylobacter strains with both high and low inoculum levels. Statistically significant differences (*P* ≤ 0.001) were observed in the survival of Campylobacter strains in the different liquid types at every time point, except at the 48-h time point with low inoculum levels. No statistically significant differences (*P* ≥ 0.05) were observed between the different Campylobacter strains in each of the liquid types studied with either of the inoculum levels. The observed differences in Campylobacter counts of meat-wine samples compared with meat-PBS samples were not statistically significant.

**DISCUSSION**

White and red wines had a very high bactericidal effect against all 4 Campylobacter strains studied, but they differed in their antimicrobial potential. In white wine, high counts of the strains were rapidly inactivated to undetectable numbers within 15 min and low counts of 2 strains were inactivated within <1 min. In red wine, high counts could not be detected after 1 h and low counts could not be detected after 30 min (Table 1). Carneiro et al. (2008) found that *C. jejuni* was inactivated within 30 s in red wine (initial counts, 6 to 7 log cfu/mL). Differences in inactivation rates may be due to differences in the strains and growth phases, cultivation media, and condition of and variations in the composition of the wine. Birk and Knechel (2009) showed that *C. jejuni* survived for 15 min at 4°C in undiluted red wine, but when the marinating temperature was raised to 42°C, the bacterium was not detectable after 1 min. Sources of wine have been shown to differ in their potential to inactivate bacteria. Gan et al. (2009) found, in contrast to our study, that red and rosé wines were more effective than white wine against *C. jejuni*. The combination of ethanol, phenolic compounds, certain organic acids, sulfur dioxide, and low pH (≤3.0) have been reported to be responsible for reducing the bacterial counts of various food-borne pathogens (Moretró and Daeschel, 2004; Waite and Daeschel, 2007; Carneiro et al., 2008; Gan et al., 2009; Birk et al., 2010). In the current study, grape and tomato juices did not reduce the Campylobacter counts as effectively as wines (Table 1). Previous studies have shown that both juices possess antimicrobial activity against different food pathogens such as *Listeria* spp., *Salmonella* menston, *E. coli*, *S. aureus*, *Yersinia enterocolitica*, and *Bacillus cereus*, with different survival times reported (Harding and Maidment, 1996; Eribo and Ashenafi, 2003; Rhodes et al., 2006; Hakovirta, 2008). Our findings are in agreement with those of Just and Daeschel (2003), who showed that bacteria survive longer in grape juice than in red wine. Because the 2 liquids in the present study had a similar pH value (3.79 and 3.62), it seems that in addition to the ethanol in wine, the type of acid and the specific composition of the liquid played a significant role in the survival of Campylobacter.

The commercial marinade inactivated most of the high Campylobacter counts within 48 h of exposure and all the low counts within 3 h of exposure (Table 1). This observed bactericidal effect of the marinade is concordant with the results of Perko-Mäkelä et al. (2000), who found, in a plain marinade (pH 4.5), that a mixture of 7 *C. jejuni* strains was not detectable after 48 h (initial counts, 5.4 log cfu/mL). However, *C. jejuni* survived for 7 to 10 d in marinated and nonmarinated chicken meat, depending on the level of inoculum used (between log 1.1 and log 5.3 cfu/mL; Perko-Mäkelä et al., 2000). Thus, marinating did not decrease the survival of *C. jejuni* in chicken meat stored at 4°C. In our study, the antimicrobial effect of wine on Campylobacter was also largely reduced when the Campylobacter inoculated on meat was exposed to wine. The Campylobacter load on chicken meat submerged in white or red wine was re-
<table>
<thead>
<tr>
<th>Liquid type</th>
<th>Exposure time</th>
<th>RefCJ</th>
<th>RetCJ29</th>
<th>RetCC27</th>
<th>SlaCJ26</th>
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<tr>
<td></td>
<td></td>
<td>High</td>
<td>Low</td>
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<td>Red wine</td>
<td>0 min</td>
<td>7.2 ± 0.1</td>
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<td></td>
<td>&lt;1 min</td>
<td>6.8 ± 0.5</td>
<td>2.6 ± 0.1</td>
<td>6.2 ± 0.4</td>
<td>2.3 ± 0.5</td>
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<td></td>
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<td>&lt;1.0</td>
<td>3.0 ± 4.3</td>
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<td>30 min</td>
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<td>1.4 ± 2.0</td>
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<td>1 h</td>
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<td>White wine</td>
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<td>7.1 ± 0.3</td>
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<td>2.8 ± 0.2</td>
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<td></td>
<td>&lt;1 min</td>
<td>2.2 ± 3.1</td>
<td>&lt;1.0</td>
<td>4.8 ± 1.6</td>
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<td>15 min</td>
<td>&lt;1.0</td>
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<tr>
<td>Grape juice</td>
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<td>7.4 ± 0.1</td>
<td>3.4 ± 0.1</td>
<td>7.2 ± 0.0</td>
<td>3.2 ± 0.0</td>
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<td></td>
<td>24 h</td>
<td>6.4 ± 0.3</td>
<td>2.5 ± 0.1</td>
<td>6.5 ± 0.2</td>
<td>2.8 ± 0.8</td>
</tr>
<tr>
<td>Tomato juice</td>
<td>0 min</td>
<td>7.2 ± 0.1</td>
<td>3.2 ± 0.1</td>
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<td>3.1 ± 0.1</td>
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<td></td>
<td>24 h</td>
<td>6.7 ± 0.4</td>
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<td>48 h</td>
<td>5.9 ± 0.4</td>
<td>&lt;1.0</td>
<td>6.2 ± 0.8</td>
<td>1.1 ± 1.6</td>
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<td>Marinade</td>
<td>0 min</td>
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<td>3.2 ± 0.0</td>
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</tr>
<tr>
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<td>1 h</td>
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<td>3.0 ± 0.3</td>
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<td></td>
<td>24 h</td>
<td>3.7 ± 0.4</td>
<td>&lt;1.0</td>
<td>2.0 ± 0.0</td>
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<td>48 h</td>
<td>1.0 ± 1.4</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
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</table>

1Mean ± SD (n = 2). Detection limit was 1 log cfu/mL.

2The following *Campylobacter* strains were used: a sequenced clinical human isolate *Campylobacter jejuni* NCTC 11168 strain (RefCJ; Health Protection Agency, London, UK); a *C. jejuni* strain (RetCJ29) and a *Campylobacter coli* strain (RetCC27), both isolated from honey-marinated retail poultry meat in a previous study by Katzav et al. (2008; RetCJ29 from Finnish poultry meat and RetCC27 from Brazilian poultry meat); and a *C. jejuni* strain (SlaCJ26) isolated at a Finnish slaughterhouse from turkey cecum content (laboratory collection).
duced by approximately 1 log cfu/mL after storage for 48 h at 4°C. With results corresponding to ours, Birk et al. (2007) found that red wine reduced the numbers of *C. jejuni* inoculated on chicken meat by only 0.5 log units (initial count, 7 log cfu/mL) after 3 d of storage at 4°C.

Based on results on the liquids only, the exposure of *Campylobacter* to white and red wines significantly reduced the number of viable cells. However, the antimicrobial activity of wines against *Campylobacter* was strongly reduced when their effects were tested on *Campylobacter* inoculated on chicken meat. The results suggest that wines could be used as antimicrobial ingredients in meat marinades together with further bactericidal agents, to reduce the numbers of *Campylobacter* in naturally contaminated poultry products. Björkroth (2005) has speculated that the buffering capacity of meat might neutralize the acidic components in marinades, leading to a decreased antimicrobial effect; however, the retail shelf life of refrigerated marinated chicken products is approximately 10 d (Perko-Mäkelä et al., 2000). Thus, even more effective results might be gained after the last time point used in this study (48 h).

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