Journal of Food Safety

Journal of Food Safety ISSN 1745-4565

SYNERGISTIC EFFECT OF PROPOLIS AND HEAT TREATMENT LEADING TO INCREASED INJURY TO *ESCHERICHIA COLI* O157:H7 IN GROUND PORK

YOUN-HA KIM, SANG-A KIM and HYUN-JUNG CHUNG[1]

Department of Food and Nutrition, Inha University, Incheon 402-751, Korea

[1]Corresponding author.
TEL: (032)-860-8122;
FAX: (032)-862-8120;
EMAIL: hjchung@inha.ac.kr

Received for Publication June 27, 2013
Accepted for Publication November 11, 2013

doi: 10.1111/jfs.12088

ABSTRACT

This study was conducted to determine the thermal inactivation of *Escherichia coli* O157:H7 in the presence of propolis in culture and in ground pork. Overnight cultures (~10^6 cfu/mL) or inoculated ground pork (1.0 g, ~10^6 cfu/g) were heat treated at 57, 60 and 63 ± 0.1C for predetermined times. The surviving cell populations were enumerated on appropriate media, from which *D* and *z* values were determined. The *D* values for *E. coli* O157:H7 in broth were 7.33 ± 1.33, 1.34 ± 0.29 and 0.85 ± 0.04 min, respectively ($z = 6.4$C), whereas in the presence of propolis (8.98 mg/mL), the *D* values were 0.53 ± 0.02, 0.25 ± 0.00 and 0.17 ± 0.00 min ($z = 10.3$C). For ground pork, the *D* values of *E. coli* O157:H7 were 4.88 ± 0.23, 0.77 ± 0.00 and 0.37 ± 0.00 min, respectively ($z = 5.4$C), whereas the *D* values in the presence of propolis (35.92 mg/g) were 2.98 ± 0.02, 0.46 ± 0.03 and 0.26 ± 0.02 min ($z = 5.5$C). Thermal injury was more pronounced in the presence of propolis at all temperatures. These data suggest that propolis and heat treatment may have a synergistic effect that could have potential applications in the case of meat products.

PRACTICAL APPLICATIONS

The addition of propolis to *Escherichia coli* in broth and meat rendered the pathogen more sensitive to the lethal effects of heat at all temperatures, resulting in smaller *D* values than those obtained with *E. coli* heat treated alone in both culture and meat samples. These data suggest that propolis and heat treatment may have a synergistic effect that could have potential applications for meat products.

INTRODUCTION

Propolis is a hard resinous substance prepared by honeybees from plant exudates, beeswax and bee secretions. Propolis is a traditional medicine with known biological activities, and numerous studies have reported its versatile pharmacological activities, including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant and antitumor actions (Banskota *et al.* 2001; Yang *et al.* 2010). Its activity depends on its content and types of flavonoid, which are affected by different geographical and climatic zones as well as the extraction methods (Dimov *et al.* 1992; Cho *et al.* 2005; Orsi *et al.* 2005).

A number of studies have described the antimicrobial actions of propolis against foodborne pathogens including *Bacillus cereus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Enterococcus faecalis* and *Clostridium perfringens* (Cho *et al.* 2005; Ferreira *et al.* 2007; Erkmen and Özcan 2008). The mechanisms underlying the antimicrobial activities of propolis are complex and can be attributed to the synergistic actions of phenolic and flavonoid compounds, which uncouple the energy-transducing cytoplasmic membrane and inhibit bacterial motility (Krol *et al.* 1993; Mirzoeva *et al.* 1997).

Gram-negative bacteria are generally more resistant to hydrophobic antimicrobial compounds than gram-positive bacteria because of the presence of an outer membrane that acts as a permeability barrier (Nikaido 1976). Cho *et al.* (2005) reported that gram-positive bacteria are more susceptible to ethanol-extracted propolis than gram-negative

bacteria, including *Escherichia coli* O157:H7, but several other studies have reported that it exerts antimicrobial activities against both gram-positive and gram-negative bacterial species.

To minimize bacterial resistance to antimicrobial treatments, several studies have used hurdle technology, which applies a combination of two or more preservation methods with synergistic effects to improve the stability and quality of food products (Leistner 1985; Lee 2004). The major hurdles used in food preservation include temperature (high or low), water activity (aw), acidity (pH), redox potential (Eh), preservatives (e.g., nitrite, sorbate and sulfite) and competitive microorganisms (e.g., lactic acid bacteria) (Leistner 2000). Tsuchido *et al.* (1975) reported that *E. coli* cells heated at 52C for 5 min were sensitized to tylosin, a hydrophobic antibiotic, because of the destruction of the outer membrane. The sublethal heat treatment of *E. coli* cells induced blebbing and vesiculation of the outer membrane, causing the release of lipopolysaccharides from the outer membrane into the menstruum and an increase in cell surface hydrophobicity, suggesting substantial destruction of the outer membrane structure (Tsuchido *et al.* 1985; Tsuchido and Takano 1988). *E. coli* O157:H7 was inactivated by the combination of generally recognized as safe chemicals from cinnamon extract and heat treatment (Venkitanarayanan *et al.* 1999). Consistent with this, Knight and McKellar (2007) reported decreased heat resistance of *E. coli* O157:H7 in the presence of cinnamon oil. Juneja and Friedman (2008) also reported that carvacrol and cinnamaldehyde facilitated the thermal destruction of *E. coli* O157:H7 in ground beef. Recently, *Pseudomonas* and *Salmonella* were found to be inhibited by a combination of cinnamon and mace oils and by cinnamon and prikhom oils (Nanasombat and Wimuttigosol 2011).

We previously demonstrated that propolis from Korea exerted antimicrobial effects against major gram-positive foodborne pathogens. However, gram-negative bacteria, including *E. coli* O157:H7, were not inhibited (Kim and Chung 2011). In this study, we used a combination of heat and propolis to control the gram-negative foodborne pathogen *E. coli* O157:H7 in culture and in foods for future applications. Therefore, the aims of this study were to investigate the effects of Korean propolis on the thermal inactivation of *E. coli* O157:H7 in culture and in ground meat using *D* and *z* values and to determine the effects of propolis on pathogen injury during thermal treatment.

MATERIALS AND METHODS

Microorganism

E. coli O157:H7 (ATCC 35150) was obtained from the Department of Food and Nutrition at Chung-Ang University. Stock cultures were made in tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) with 20% glycerol (v/v) and stored at −80C. Active cultures for experiments were prepared by streaking material from the frozen cultures onto trypticase soy agar (TSA; Difco Laboratories) plates, which were incubated for 24 h at 37C. Single colonies were transferred to TSB and incubated for 24 h at 37C, followed by a subsequent loop transfer into TSB and overnight incubation at 37C. Working cultures were maintained on a slant of TSA and stored at 4C.

Sample Preparation

Propolis (Kim and Chung 2011) was provided by Withealth Propolis Co. (Kyungnam, Korea) as an ethanol extract from 100% Korean propolis. The product is aqueous and could be used directly for analysis. Propolis was refrigerated until use (5C). Ground pork (20% fat) was obtained from the local supermarket and frozen (−18C) until needed. Prior to the experiments, the ground pork was thawed at 5C and then sterilized at 121C for 15 min.

Heat Inactivation of *E. coli* O157:H7 in Culture with or without Propolis

Overnight cultures of *E. coli* O157:H7 were diluted to ~10^6 cfu/mL. One milliliter of adjusted culture was placed in an aluminum can with an inside diameter of 18 mm and height of 4 mm, immersed in a water bath and held at 57 ± 0.1, 60 ± 0.1 and 63 ± 0.1C for predetermined times (Chung *et al.* 2008). For combination treatments, 1 mL of *E. coli* culture was mixed with propolis (8.98 mg/mL) and heat treated at 57 ± 0.1, 60 ± 0.1 and 63 ± 0.1C for predetermined times. After heat treatment, the tested samples were serially diluted and plated on TSA plates. The plates were incubated at 37C for 24 h, and the surviving cell population was counted and expressed as colony forming units (cfu/mL).

Heat Inactivation of *E. coli* O157:H7 in Ground Pork with or without Propolis

Ten grams of ground pork was aseptically weighed into a sterile bag and inoculated with 0.1 mL culture of *E. coli* O157:H7 to make a final concentration of ~10^6 cfu/g *E. coli* O157:H7. Inoculated ground pork was refrigerated for 1 h to allow *E. coli* to attach to the ground pork. One gram of the inoculated ground pork was then placed in an aluminum cell that was immersed in a water bath and held at 57 ± 0.1, 60 ± 0.1 and 63 ± 0.1C for predetermined times. For combination treatments, inoculated ground pork was mixed with propolis (35.92 mg/g), refrigerated for 1 h and heat treated at 57 ± 0.1, 60 ± 0.1 and 63 ± 0.1C for

predetermined time intervals. Surviving colonies were enumerated on TSA and eosin methylene blue agar (EMB; Difco Laboratories). *D* and *z* values were then calculated.

Calculation of *D* and *z* Values

D values (decimal reduction time) were calculated as the negative inverse slope of the linear portion of survival curves generated by plotting decimal logarithms of survival counts versus heating time. Linear regression lines were fitted to the linear portion of three sets of independent data (Miles and Mackey 1994). *z* values (the temperature necessary to reduce *D* value by 10-fold) were calculated as the negative reciprocal of the slope of the regression line between the logarithm of *D* values and treatment temperatures (Miles and Mackey 1994).

Determination of the Degree of Injury

The difference between selective (EMB) and nonselective (TSA) media gives an indication of cell injury during heat treatment. Therefore, the percentage injury can be calculated using the following equation by Duffy *et al.* (1999) and Hansen and Knochel (2001):

$$\%\ \text{injured cells} = 100 \times (\text{cfu}_{\text{TSA}} - \text{cfu}_{\text{EMB}})/\text{cfu}_{\text{TSA}}$$

Statistical Analysis

All experiments were repeated three times with duplicate samples. Data were analyzed using SAS (version 9.1; SAS Institute, Inc., Cary, NC) for analysis of variance to compare survivor curves obtained following heating experiments whether significant differences ($P < 0.05$) existed between the mean values of treatments.

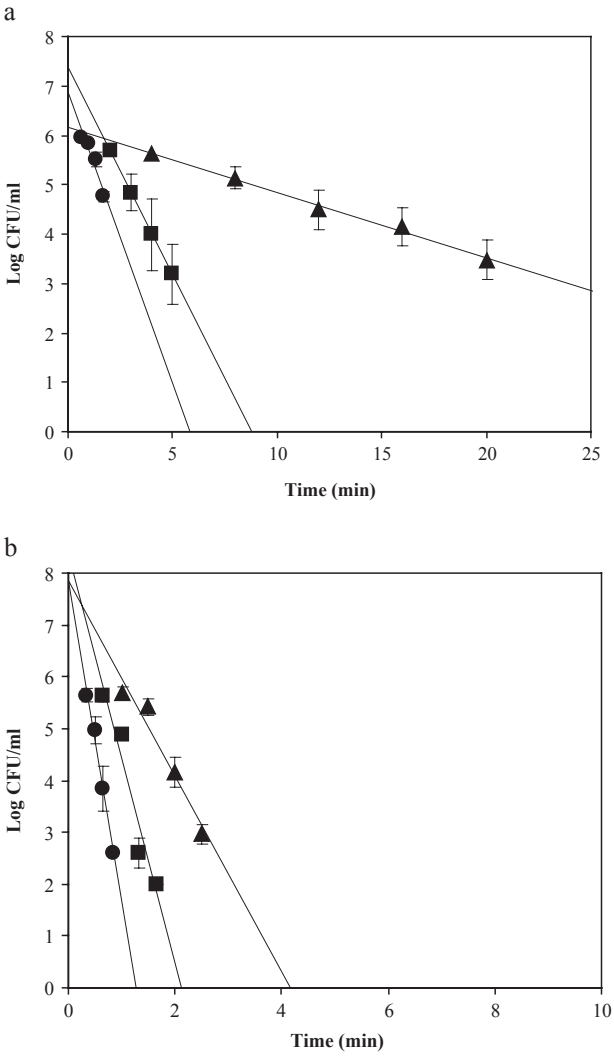

FIG. 1. THERMAL DEATH TIME CURVES OF *ESCHERICHIA COLI* O157:H7 (a) WITHOUT PROPOLIS AND (b) WITH PROPOLIS IN CULTURE. (▲) 57C, (■) 60C, (●) 63C

RESULTS AND DISCUSSION

Inactivation of *E. coli* O157:H7 in Culture by Heat and Propolis

In this study, the sensitivity of *E. coli* O157:H7 to the combination of propolis and heat treatment was evaluated by comparing *D* and *z* values obtained through thermal death time curves (Figs. 1 and 2). Figure 1 demonstrates representative survival curves of microorganisms after heat treatment, which was produced by plotting log survivors over time at 57 ± 0.1, 60 ± 0.1 and 63 ± 0.1C. The decimal reduction times were then calculated from the linear decrease in population (Table 1). In the absence of propolis, the destruction of *E. coli* O157:H7 by heat alone resulted in $D_{57} = 7.33 \pm 1.33$, $D_{60} = 1.34 \pm 0.29$, $D_{63} = 0.85 \pm 0.04$ min, demonstrating a linear decrease in the population against exposure time, and a gradual loss of the resistance of *E. coli* O157:H7 with increased temperatures. Kwon *et al.* (1997) reported *D* values of *E. coli* O157:H7 of $D_{50} = 129.2$, $D_{55} = 27.1$ and $D_{60} = 2.4$ min in the same temperature range of broth. The *D* values obtained in this study were within the same magnitude as these.

The destruction of *E. coli* O157:H7 by heat was significantly enhanced by cotreatment with propolis (8.98 mg/mL) at all temperatures. *D* values of $D_{57} = 0.53 \pm 0.02$, $D_{60} = 0.25 \pm 0.00$ and $D_{63} = 0.17 \pm 0.00$ min were obtained with rapid decreases in the population ($P < 0.05$) (Fig. 1 and Table 1). We previously demonstrated that *E. coli* O157:H7 was not sensitive to propolis at a concentration of

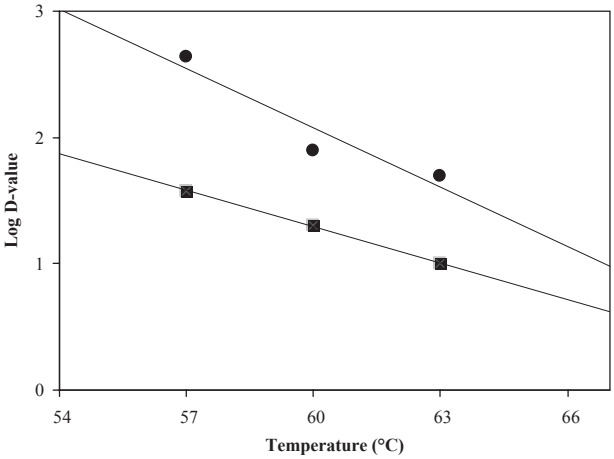

FIG. 2. THERMAL DEATH TIME CURVES (*Z* VALUES) OF *ESCHERICHIA COLI* O157:H7 WITHOUT (●) AND WITH PROPOLIS (■) IN CULTURE

8.98 mg/mL by the agar diffusion assay. However, when used as a cotreatment with mild heat, the addition of propolis increased the sensitivity of the pathogen to heat treatment. The *E. coli* O157:H7 cell membrane was sensitized by heat treatment, which facilitated diffusion of propolis to the cytoplasm and caused a rapid decrease in the population.

A *z* value provides information on the relative resistance of an organism to different heating temperatures and allows for the calculation of equivalent thermal processes at different temperatures. Interestingly, the *z* value of *E. coli* O157:H7 was increased from 6.4C by heat treatment alone to 10.3C by the combined treatment of heat and propolis (Fig. 2). This is consistent with the observations of Steenstrup and Floros (2002), who studied the inhibition of *E. coli* O157:H7 in cider. The authors reported that the *z* value increased from 6.3 to 26.5C by the addition of varying concentrations of malic acid, potassium sorbate and sodium benzoate during heat treatment. Increased *z* values infer that microorganisms become less influenced by temperature changes. This is advantageous at lower temperatures because *D* values will increase slowly.

TABLE 1. *D* AND *Z* VALUES OF *ESCHERICHIA COLI* O157:H7 IN THE PRESENCE OR ABSENCE OF PROPOLIS IN CULTURE

Temperature (C)	*D* values (r^2)	
	Heat alone	Heat + propolis
57	7.33 ± 1.33† (0.99)‡	0.53 ± 0.02 (0.94)*
60	1.34 ± 0.29 (0.99)	0.25 ± 0.00 (0.94)*
63	0.85 ± 0.04 (0.89)	0.17 ± 0.00 (0.98)*
z value (r^2)	6.4C (0.90)	10.3C (0.99)

* Indicates that the value was significantly different ($P < 0.05$) in the independent sample *t*-test, at 5% significance level.

† *D* values shown are the means of three experiments, each performed in duplicate and expressed as mean ± standard deviation.

‡ Correlation coefficients in parentheses.

Inactivation of *E. coli* O157:H7 in Ground Meat by Heat and Propolis

To evaluate the effects of combined treatment with heat and propolis against *E. coli* O157:H7 in foods, *E. coli* O157:H7 was inoculated into ground pork and heated in the presence or absence of propolis. The *D* and *z* values were then calculated from the survival curves (Figs. 3 and 4).

In ground pork in the absence of propolis, the destruction of *E. coli* O157:H7 by heat alone resulted in *D* values

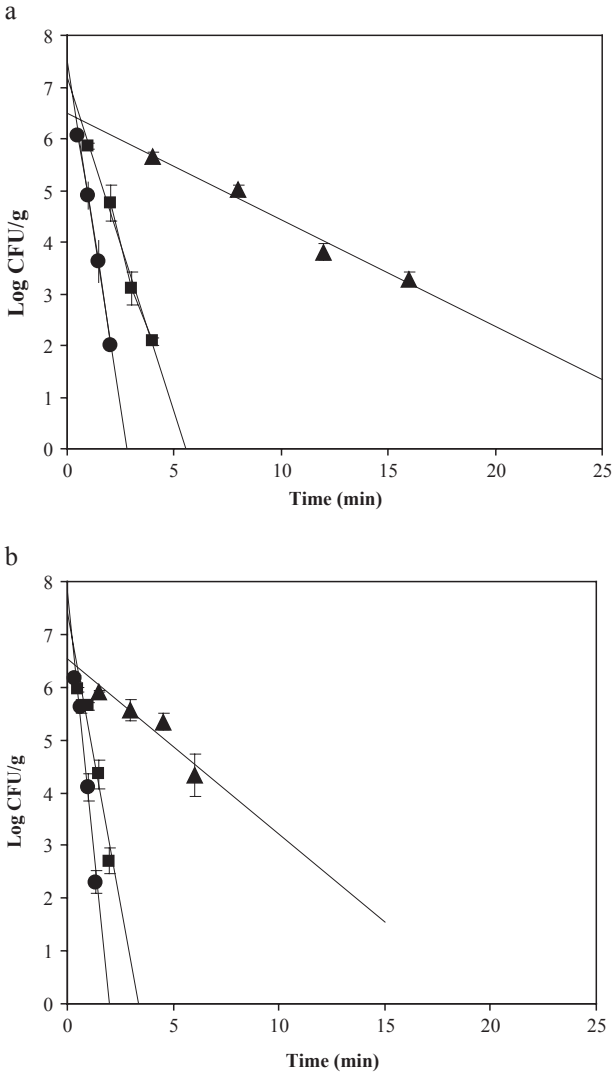

FIG. 3. THERMAL DEATH TIME CURVES OF *ESCHERICHIA COLI* O157:H7 WITHOUT PROPOLIS (a) AND WITH PROPOLIS (b) IN GROUND MEAT. (▲) 57C, (■) 60C, (●) 63C

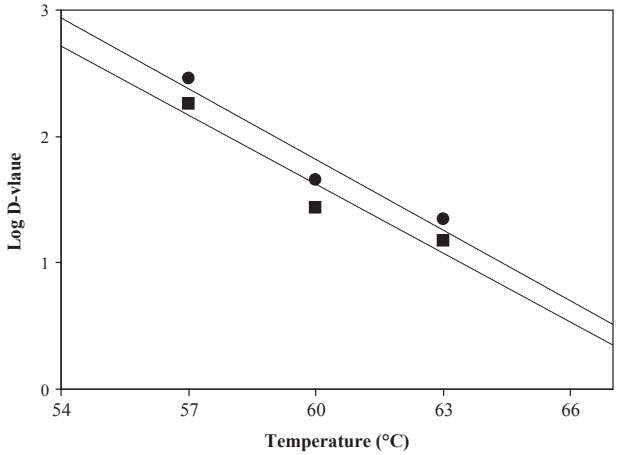

FIG. 4. THERMAL DEATH TIME CURVES (*Z* VALUES) OF *ESCHERICHIA COLI* O157:H7 WITHOUT (●) AND WITH PROPOLIS (■) IN GROUND MEAT

of $D_{57} = 4.88 \pm 0.23$, $D_{60} = 0.77 \pm 0.00$ and $D_{63} = 0.37 \pm 0.00$ min, with a *z* value of 5.4C (Table 2). When cotreatment with propolis (8.98 mg/g) was assessed, propolis did not have any additional effect on destroying *E. coli* O157:H7 compared with heat treatment alone (data not shown). Thus, there were no evident differences in heat resistance in the presence or absence of propolis. When cells were treated with higher concentrations of propolis (35.92 mg/g), smaller *D* values ($D_{57} = 2.88 \pm 0.02$, $D_{60} = 0.46 \pm 0.03$, $D_{63} = 0.26 \pm 0.02$ min) were obtained with a *z* value of 5.5C ($P < 0.05$). The *D* values were significantly smaller than those treated with heat alone at all tested temperatures. The effects of propolis on *E. coli* O157:H7 in ground pork were similar to those in culture, although a higher concentration of propolis was required. This is consistent with a number of previous reports that suggested that the composition of the heating medium had a significant influence on the derived *D* values (Ahmed *et al.* 1995). Factors such as increased fat content and the presence of high levels of competitive flora also had a protective effect against pathogenic microflora during heat treatment (Ahmed *et al.* 1995; Duffy *et al.* 1995).

TABLE 2. *D* AND *Z* VALUES OF *ESCHERICHIA COLI* O157:H7 IN THE PRESENCE OR ABSENCE OF PROPOLIS IN GROUND MEAT

Temperature (C)	*D* values (r^2)	
	Heat alone	Heat + propolis
57	4.88 ± 0.23† (0.98)‡	2.98 ± 0.02 (0.89)*
60	0.77 ± 0.00 (0.99)	0.46 ± 0.03 (0.93)*
63	0.37 ± 0.00 (0.99)	0.26 ± 0.02 (0.95)*
z value (r^2)	5.4C (0.94)	5.5C (0.92)

* Indicates that the value was significantly different ($P < 0.05$) in the independent sample *t*-test, at 5% significance level.

† *D* values shown are the means of three experiments, each performed in duplicate and expressed as mean ± standard deviation.

‡ Correlation coefficients in parentheses.

When considering the *z* values, the effect of propolis on the sensitivity of *E. coli* O157:H7 to changing temperature in foods was smaller than in broth. The *z* values of *E. coli* O157:H7 obtained with or without propolis in ground meat (Fig. 4) were comparable with the values obtained in culture (Fig. 2). The *z* values obtained in this study were similar to those reported by Kotrola *et al.* (1997) for heating *E. coli* O157:H7 in turkey frankfurters (17% fat) (5.38C). It is feasible to compare published data on the heat resistance of *E. coli* O157:H7 by choosing common test temperatures. Doyle and Schoeni (1984) reported a D_{60} of 0.75 for the *E. coli* O157:H7 strain 932 inoculated into ground beef containing 17–20% fat, using Pyrex test tubes (Lake Charles Manufacturing, Lake Charles, LA) capped with rubber stoppers. Ahmed *et al.* (1995) studied the effects of fat on the survival of *E. coli* O157:H7 and reported that the product composition affected the sensitivity of *E. coli* O157:H7 to heat treatment. D_{60} values ranged from 0.45 to 0.47 in beef (7, 10 and 20% fat), 0.37–0.55 in pork sausage (7, 10 and 30% fat), 0.38–0.55 in chicken (3 and 11% fat) and 0.55–0.58 in turkey (3 and 11% fat). The D_{55} and D_{50} values were respectively longer, and the *z* values ranged from 4.4 to 4.8C. Overall, higher fat levels in all products resulted in higher *D* values, and *E. coli* O157:H7 in the lowest fat products had lower *D* values than in traditional beef and pork products ($P < 0.05$). However, the differences between the *D* values reported by different studies may be attributed to different *E. coli* O157:H7 strains, the physiological condition of the cells, meat with different fat contents and the experimental methodology used. These data suggest that, although propolis exerted no antimicrobial activity against gram-negative pathogens at low concentrations, a combination of propolis and heat treatment resulted in a greater reduction in the pathogen population than heat treatment alone. In this study, the significant decrease in the *D* values of *E. coli* O157:H7 in culture and pork suggests that there are synergistic effects of heat and propolis against gram-negative pathogens in culture and meat, indicating a potential application in foods.

When evaluating thermal destruction, it is crucial to recognize potentially injured cells during thermal inactivation to achieve accurate results because microorganisms subjected to heat treatment are not always killed. Injury involves damage to the cell membrane, which leads to a significant loss of internal solutes and increased sensitivity to unfavorable chemicals (Abee and Wouters 1999; Besse 2002). In reality, a large number of bacteria survive physiological injury and show only temporary structural damage (Hurst 1977; Smith and Archer 1988; Besse 2002).

The detection and enumeration of heat-treated pathogens can be carried out using selective and nonselective

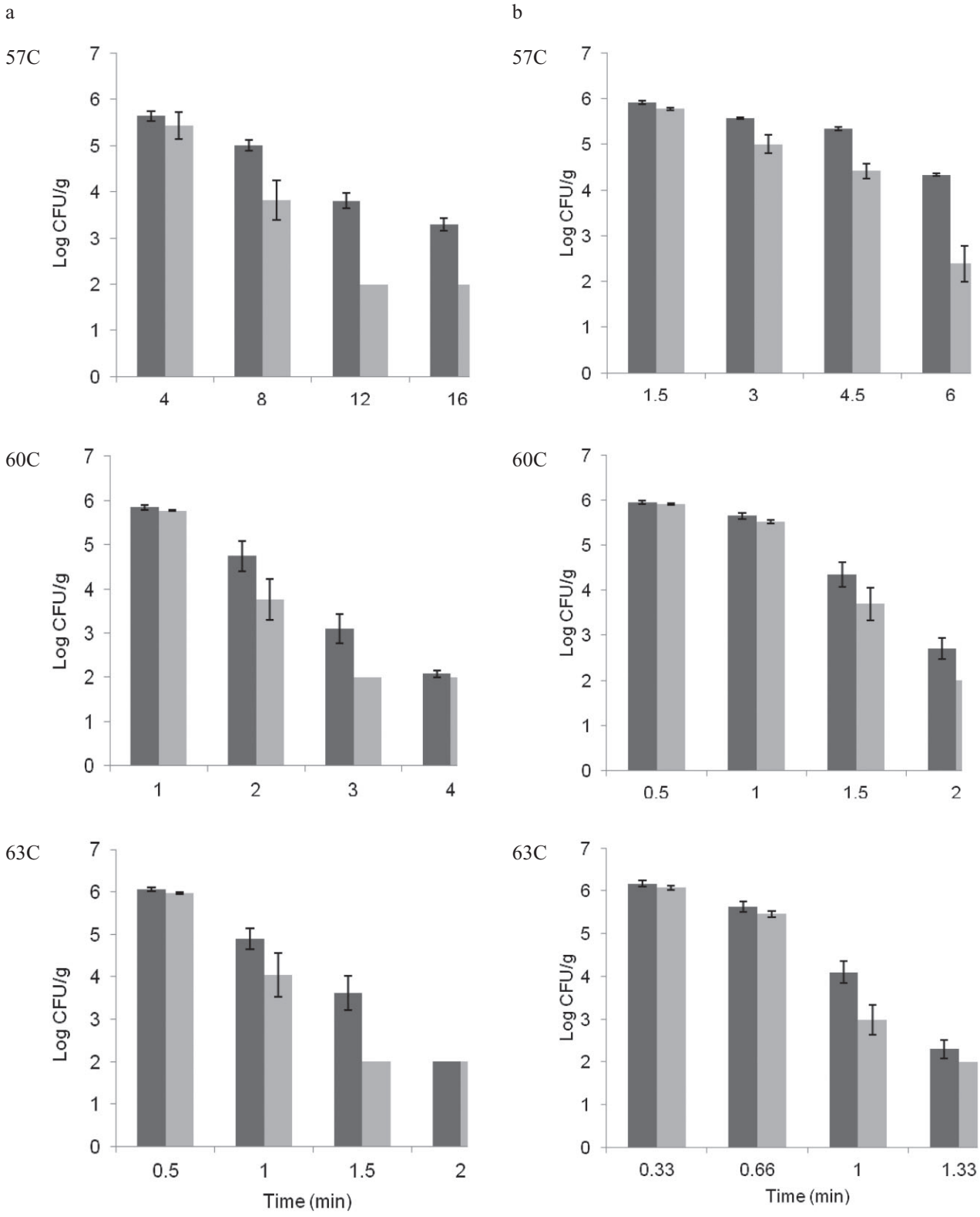

FIG. 5. DEGREE OF INJURY CAUSED BY HEAT (a) AND COMBINED TREATMENT OF HEAT AND PROPOLIS (b) ON TRYPTICASE SOY AGAR (TSA) AND EOSIN METHYLENE BLUE AGAR (EMB). (■) TSA, (■) EMB

culture media. Nonselective media contain ample nutrition that allows injured cells to undergo repair and become functionally normal. However, in selective media, injured cells suffer additional stresses because of the presence of inhibitory components and therefore fail to repair the initial damage (Smith and Archer 1988). Although there is a lack of information concerning the quantification of injured cells and/or their ability to recover from stresses, it is important to identify the degree of injury because sublethally damaged bacteria may recover and regain their pathogenicity. Duffy *et al.* (1999) and Hansen and Knochel (2001) expressed the degree of heat injury in terms of the percentage of injured cells. The rate at which a population of injured cells undergoes repair also varies with incubation temperature, pH and salt concentration in the medium (Chawla *et al.* 1996).

In this study, the treated *E. coli* O157:H7 was cultured on both nonselective (TSA) and selective media (EMB) to evaluate cellular damage caused by heat or combined treatments in ground pork. Figure 5a,b shows the survivors from cultures of heat-treated *E. coli* O157:H7 in the absence or presence of propolis, respectively, at different temperatures in meat. A comparison of the percentage of injured *E. coli* O157:H7 by heat treatment in the absence or presence of propolis was made based on Hansen and Knochel's (2001) equation. At 57C, heat treatment alone caused only 1.8% injury at 4 min, whereas combination treatment caused 17.2% injury at 4.5 min. Although the time of heat treatment was not exactly the same, there was a clear increase in injury because of the presence of propolis. In addition, the increased differences in the number of survivors grown on TSA and EMB suggest additional injury in *E. coli* O157:H7 by heat treatment in the presence of propolis (Fig. 5b). After treatment at 60C for 2 min, heat treatment alone caused 20.6% injury, compared with 26% injury by heat treatment in combination with propolis. At 63C for 1 min, 17% injury caused by heat alone was increased to 27% with cotreatment of heat with propolis. Therefore, different degrees of heat stimulated injury to *E. coli* O157:H7, which was further increased by the presence of propolis at all tested temperatures.

On EMB agar, the destruction of *E. coli* O157:H7 by heat alone resulted in $D_{57} = 2.85 \pm 0.15$, $D_{60} = 0.76 \pm 0.04$ and $D_{63} = 0.25 \pm 0.02$ min with a z value of 5.71C, whereas the combination of heat and propolis resulted in $D_{57} = 1.42 \pm 0.11$, $D_{60} = 0.37 \pm 0.01$ and $D_{63} = 0.25 \pm 0.01$ min with a z value of 7.6C in ground pork. Compared with the results on TSA (Table 2), the D values on EMB are smaller at all tested temperatures in the presence or absence of propolis. This study showed that injured *E. coli* O157:H7 could be present but may escape detection as they do not grow in selective media and have the capacity for repair. Duffy *et al.* (1999) reported that the percentage cell injury in the total cell population was dependent on the heating temperature, the strain of *E. coli* O157:H7, and the recovery and counting agars used. The combination treatment with propolis showed reduced thermal resistance of *E. coli* O157:H7, an increased injury rate and synergistically increased sensitivity to propolis.

CONCLUSION

The addition of propolis to *E. coli* O157:H7 in culture and ground pork rendered them more sensitive to the lethal effects of heat at all the temperatures tested, resulting in smaller D values than heat treatment alone in both cultures and pork with increased injury. The results of this study suggest that propolis enhanced the inactivation of *E. coli* O157:H7 in culture and in ground meat by decreasing the heat resistance of the pathogen. Therefore, these data suggest that the addition of propolis may be applicable during the heat treatment of foods to control foodborne pathogens.

ACKNOWLEDGMENTS

This study was supported by an Inha University research grant.

REFERENCES

ABEE, T. and WOUTERS, J.A. 1999. Microbial stress response in minimal processing. Int. J. Food Microbiol. *50*, 65–91.

AHMED, N.M., CONNER, D.E. and HUFFMAN, D.L. 1995. Heat-resistance of *Escherichia coli* O157:H7 in meat and poultry as affected by product composition. J. Food Sci. *60*, 606–610.

BANSKOTA, A.H., TEZUKA, Y., ADNYANA, I.K., ISHII, E., MIDORIKAWA, K., MATSUSHIGE, K. and KADOTA, S. 2001. Hepatoprotective and anti-*Helicobacter pylori* activities of constituents from Brazilian propolis. Phytomedicine *8*, 16–23.

BESSE, N.G. 2002. Influence of various environmental parameters and of detection procedures on the recovery of stressed *L. monocytogenes*: A review. Food Microbiol. *19*, 221–234.

CHAWLA, C.S., CHEN, H. and DONNELLY, C.W. 1996. Mathematically modeling the repair of heat-injured *Listeria monocytogenes* as affected by temperature, pH, and salt concentration. Int. J. Food Microbiol. *30*, 231–242.

CHO, J.S., KIM, Y.H. and KWON, M.-S. 2005. Antibacterial effects of propolis extracts on pathogenic bacteria. J. East Asian Soc. Dietary Life *15*, 457–464.

CHUNG, H.J., BARLA, S. and TANG, S. 2008. Performance evaluation of aluminum test cell designed for determining the heat resistance of bacterial spores in foods. LWT – Food Sci.Technol. *41*, 1351–1359.

DIMOV, V., IVANOVSKA, N., BANKOVA, V. and POPOV, S. 1992. Immunomodulatory action of propolis: IV. Prophylactic activity against gram-negative infections and adjuvant effect of the water-soluble derivative. Vaccine *10*, 817–823.

DOYLE, M.P. and SCHOENI, J.L. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. *48*, 855–856.

DUFFY, G., ELLISON, A., ANDERSON, W., COLE, M. and STEWART, G.S.A.B. 1995. Use of bioluminescence to model the thermal inactivation of *Salmonella typhimurium* in the presence of a competitive microflora. Appl. Environ. Microbiol. *61*, 3463–3465.

DUFFY, G., RIORDAN, D.C.R., SHERIDAN, J.J., EBLEN, B.S., WHITING, R.C., BLAIR, I.S. and MCDOWELL, D.A. 1999. Differences in thermotolerance of various *Escherichia coli* O157:H7 strains in a salami matrix. Food Microbiol. *16*, 83–91.

ERKMEN, O. and ÖZCAN, M.-M. 2008. Antimicrobial effects of Turkish propolis, pollen, and laurel on spoilage and pathogenic food-related microorganisms. J. Med. Food *7*, 587–592.

FERREIRA, F.B.A., TORRES, S.A., FERREIRA, C.M. and MARCUCCI, M.C. 2007. Antimicrobial effect of propolis and other substances against selected endodontic pathogens. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. *104*, 709–716.

HANSEN, T.B. and KNOCHEL, S. 2001. Factors influencing resuscitation and growth of heat injured *Listeria monocytogenes* 13–249 in sous vide cooked beef. Int. J. Food Microbiol. *63*, 135–147.

HURST, A. 1977. Bacterial injury: A review. Can. J. Microbiol. *23*, 935–944.

JUNEJA, V.K. and FRIEDMAN, M. 2008. Carvacrol and cinnamaldehyde facilitate thermal destruction of *Escherichia coli* O157:H7 in raw ground beef. J. Food Prot. *71*, 1604–1611.

KIM, Y.H. and CHUNG, H.J. 2011. The effect of Korean propolis against foodborne pathogens and transmission electron microscopic examination. New Biotechnol. *28*, 713–718.

KNIGHT, K.P. and MCKELLAR, R.C. 2007. Influence of cinnamon and clove essential oils on the D-and z-values of *Escherichia coli* O157:H7 in apple cider. J. Food Prot. *70*, 2089–2094.

KOTROLA, J.S., CONER, D.E. and MIKEL, W.B. 1997. Thermal inactivation of *Escherichia coli* O157:H7 in cooked turkey products. J. Food Sci. *62*, 875–877.

KROL, W., SCHELLER, S., SHANI, J., PIETSZ, G. and CZUBA, Z. 1993. Synergistic effect of ethanolic extract of propolis and antibiotics on the growth of *Staphylococcus aureus*. Arzneimittelforschung *43*, 607–609.

KWON, O.J., YOOK, H.S., KIM, S.A. and BYUN, M.W. 1997. Effects of gamma-ray and heat treatment on sterilization of *Escherichia coli* O157:H7. Korean J. Food Sci. Technol. *29*, 1016–1020.

LEE, S. 2004. Microbial safety of pickled fruits and vegetables and hurdle technology. Int. J. Food Safety *4*, 21–32.

LEISTNER, L. 1985. Hurdle technology applied to meat products of the shelf stable product and intermediate moisture food types. In *Properties of Water in Foods in Relation to Quality and Stability* (D. Simatos and J.L. Multon, eds.) pp. 309–329, Martinus Nijhoff Publishers, Dordrecht, Netherlands.

LEISTNER, L. 2000. Basic aspects of food preservation by hurdle technology. Int. J. Food Microbiol. *55*, 181–186.

MILES, C.A. and MACKEY, B.M. 1994. A mathematical analysis of microbial inactivation at linearly rising temperatures: Calculation of the temperature rise needed to kill *Listeria monocytogenes* in different foods and methods for dynamic measurements of D and z-values. J. Appl. Bacteriol. *77*, 14–20.

MIRZOEVA, O.K., GRISHANIN, R.N. and CALDER, P.C. 1997. Antimicrobial action of propolis and some of its components: The effects on growth, membrane potential and motility of bacteria. Microbiol. Res. *152*, 239–246.

NANASOMBAT, S. and WIMUTTIGOSOL, P. 2011. Antimicrobial and antioxidant activity of spice essential oils. Food Sci. Biotechnol. *20*, 45–53.

NIKAIDO, H. 1976. Outer membrane of *Salmonella typhimurium*. Transmembrane diffusion of some hydrophobic substances. Biochim. Biophys. Acta *433*, 118–132.

ORSI, R.O., SFORCIN, J.M., FUNARI, S.R. and BANKOVA, V. 2005. Effects of Brazilian and Bulgarian propolis on bactericidal activity of macrophages against *Salmonella typhimurium*. Int. Immunopharmacol. *5*, 359–368.

SMITH, J.L. and ARCHER, D.L. 1988. Heat-induced injury in *Listeria monocytogenes*. J. Ind. Microbiol. *3*, 105–110.

STEENSTRUP, L.L. and FLOROS, J.D. 2002. Statistical modeling of D- and z- value of *E. coli* O157:H7 and pH in apple cider containing preservatives. J. Food Sci. *67*, 793–796.

TSUCHIDO, T. and TAKANO, M. 1988. Sensitization by heat treatment of *Escherichia coli* K12 cells to hydrophobic antibacterial compounds. Antimicrob. Agents Chermother. *32*, 1680–1683.

TSUCHIDO, T., OZAWA, O. and SHIBASAKI, I. 1975. Growth inhibition of heated cells of *Escherichia coli* by tylosin. J. Ferment. Technol. *53*, 363–371.

TSUCHIDO, T., KATSUI, N., TAKEUCHI, A., TAKANO, M. and SHIBASAKI, I. 1985. Destruction of the outer membrane permeability barrier of *Escherichia coli* by heat treatment. Appl. Environ. Microbiol. *50*, 298–303.

VENKITANARAYANAN, K.S., ZHAO, T. and DOYLE, M.P. 1999. Inactivation of *Escherichia coli* O157:H7 by combinations of GRAS chemicals and temperature. Food Microbiol. *16*, 74–82.

YANG, S., PENG, L., CHENG, Y., CHEN, F. and PAN, S. 2010. Control of citrus green and blue molds by Chinese propolis. Food Sci. Biotechnol. *19*, 1303–1308.