**INTRODUCTION**

Fermented beef sausages are products made by mixing ingredients including raw meat, fat, cooked rice, salt, sugar, sodium nitrite and some spices such as garlic and pepper with or without the addition of starter cultures, and then the mixture is allowed to ferment under appropriate conditions until the pH drops to less than or equal to 4.5. During fermentation, lactic acid bacteria produce various inhibitory substances such as organic acids, ethanol, hydrogen peroxide, diacetyl and bacteriocins (De Vuyst and Vandamme, 1994). However, the survival of pathogenic bacteria in meat and fermented meat products has been reported (Nanasombat et al., 2002). Bacteria including *Staphylococcus*, *Salmonella*, *Listeria*, *Bacillus*, *Clostridium*, *Escherichia*, *Campylobacter*, *Acinetobacter*, *Aeromonas*, *Enterococcus*, *Moraxella*, *Psychrobacter* are common contaminants in raw meat (Jay et al., 2005). Some of the pathogenic bacteria such as *Salmonella* can be found in fermented meat products as this organism can tolerate or adapt to a wide variety of environmental stresses during meat fermentation (D’Aoust, 2001). Food poisoning...
outbreaks caused by the consumption of fermented meat products contaminated with *Salmonella* such as *S. Typhimurium* DT 124 and *S. Typhimurium* PT 193 in salami have been reported (Cowden et al., 1989; Pontello et al., 1998). *Salmonella* was detected in 81.25% of ‘nham’ (a Thai fermented pork sausage) samples tested (Nanasombat et al., 2002). In addition, *Staph. aureus* can grow and produce enterotoxin during meat fermentation (Varnam and Sutherland, 1995). Although these pathogenic bacteria can be destroyed by heat during cooking, some consumers prefer to consume raw or medium-cooked products. Therefore, this suggests the need to control these pathogenic bacteria in fermented sausages.

More natural foods have recently promoted the search for alternative antimicrobial agents due to increasing consumer awareness regarding synthetic, chemical preservatives and the demand for reduced additives in food products. Spice essential oils have received great interest for use as natural antimicrobials in food products and their application has been reported (Shelef et al., 1984; Koutsoumanis et al., 1999; Vrinda and Garg, 2001). Oregano and mint oils were found to be effective for growth inhibition of *Salmonella Enteritidis* in cod’s roe salad and in yogurt, respectively (Koutsoumanis et al., 1999). Furthermore, the previous study revealed that clove and sage oils inhibited *Bacillus cereus* and *Staph. aureus* in meat products (Shelef et al., 1984; Vrinda and Garg, 2001). Nanasombat and Wimuttigosol (2011) studied the antimicrobial activities of eight spice essential oils and revealed that cinnamon and mace oils had strong antibacterial activity. The combination of cinnamon oil with mace oil showed a synergistic effect against *Staph. aureus* and *Salmonella* Rissen with a fractional inhibitory concentration index of 0.32. Therefore, it may be possible to use cinnamon and mace oils as natural preservatives in fermented beef sausages for controlling the growth of *Staph. aureus* and *S. Rissen*.

**MATERIALS AND METHODS**

**Preparation of essential oils**

Dried spice materials of cinnamon bark (*Cinnamomum zeylanicum*) and mace (*Myristica fragrans* seed coats) were extracted by steam distillation for 3 h. The essential oil samples were collected and stored in the dark at 4°C until use.

**Bacterial strains and inoculum preparation**

Nine bacterial strains were used in the study. *Salmonella* Rissen DMST 7097, *Salmonella* Senftenberg DMST 7113 and *Listeria monocytogenes* DMST 11256 were obtained from the culture collection of the Department of Medical Science, Ministry of Public Health, Thailand whereas *Staphylococcus aureus* TISTR 118 and *Pseudomonas fluorescens* TISTR 358 were obtained from the Microbiological Resources Centre for Southeast Asian Region (Bangkok MIRCEN), Thailand. In addition, four lactic acid bacteria—*Pediococcus pentosaceus* P0805 isolated from raw pork (Treebavonkusol et al., 2008), *Lactococcus lactis* isolated from spotted featherback (Nanasombat et al., 2008), *Enterococcus faecalis* 4IS17 isolated from fermented shrimp and *Enterococcus faecium* 11S11 isolated from raw mussel (Jaichalad, 2009)—were used in this experiment.

*S. Rissen*, *S. Senftenberg*, *L. monocytogenes*, *Staph. aureus* and *P. fluorescens* were cultured at 37°C for 24 h on Mueller Hinton Agar (MHA; Difco Laboratories; Detroit, MI, USA) and transferred to Mueller Hinton Broth (MHB; Difco Laboratories; Detroit, MI, USA) while *P. pentosaceus*, *L. lactis*, *E. faecalis* and *E. faecium* were grown in deMan Rogosa Sharpe agar (MRS; Difco Laboratories; Detroit, MI, USA) and transferred to MRS broth. After incubation at 37°C overnight, cells were harvested by centrifugation at 3,000×g, 4°C for 15 min, washed twice and resuspended with 0.1% peptone water. Cell turbidity was adjusted with 0.1% peptone
water to match the turbidity of the 5-Mcfarland standard, except for lactic acid bacteria where the 2-Mcfarland standard was used. The final cell concentration was approximately \(1 \times 10^8\) CFU mL\(^{-1}\).

**Antibacterial activity of cinnamon and mace oils on different food model media**

Cinnamon and mace oils were determined for minimum inhibitory concentrations (MICs) by the agar dilution method (Collin et al., 2001) on three different food model media against nine bacterial strains as mentioned above. The food model media used were starch agar (3 g beef extract, 10 g soluble starch and 15 g agar per 1,000 mL), meat agar (30 g beef extract, 10 g beef fat and 15 g agar per 1,000 mL, modified from Gutierrez et al. (2008)) and sausage agar (30 g beef extract, 10 g beef fat, 12 g sodium chloride, 3 g sodium tri-polyphosphate, 10 g glucose and 15 g agar per 1,000 mL, modified from Gutierrez et al. (2008) and Swewiwiwathana et al. (1999)). The pH values of all media were adjusted to 5.5 using 85% lactic acid.

To determine the MICs by the agar dilution method, a stock solution of each essential oil in 10% dimethyl sulphoxide was diluted with water and a molten suitable agar medium (starch agar, meat agar and sausage agar) to obtain a final concentration ranging from 0.0078 to 22 mg mL\(^{-1}\). After surface drying, 5 µL of cell suspension (\(1 \times 10^8\) CFU mL\(^{-1}\)) were inoculated at the center of each agar plate. Inoculated plates were incubated at 37°C for 24 h. After incubation, plates were evaluated for the presence or absence of colonies. The MIC was defined as the lowest concentration of the essential oil which inhibited the growth of microorganisms.

**Effect of mixed cinnamon and mace oils against *Staphylococcus aureus* and *Salmonella Rissen* in fermented beef sausage**

To evaluate the antimicrobial activity of mixed cinnamon and mace oils in a food system, beef sausage was prepared and tested using five distinct essential oil combinations with respect to the results of the MICs from previous experiments.

Beef sausage was manufactured according to a formula: 51.5% beef (round part), 28.1% beef fat, 14% cooked rice, 4.6% garlic, 1.3% salt and 0.5% sugar. The raw materials were washed, comminuted and weighed separately. Then, they were thoroughly mixed in a decontaminated bowl mixer (kitchenAid model 5KSS; Large Appliances; Benton Harbor, MI, USA) for 3 min. The sausage batter was divided into five equal portions by weight. Each portion of the sausage mixture was inoculated with cell suspensions of two pathogenic bacteria (*Staph. aureus* TISTR 118 and *S. Rissen* DMST 7097) and a starter bacterium, *P. pentosaceus* P0805 to get a final concentration of \(1 \times 10^7\), \(1 \times 10^7\) and \(1 \times 10^4\) CFU g\(^{-1}\), respectively. Then, each portion of sausage mixture was added with mixed cinnamon and mace oils at five different combinations as follows: treatment 1 (C, control), no oil added; treatment 2 (EO1), 126 ppm cinnamon oil mixed with 1,375 ppm mace oil; treatment 3 (EO2), 252 ppm cinnamon oil mixed with 1,375 ppm mace oil; treatment 4 (EO3), 504 ppm cinnamon oil mixed with 1,375 ppm mace oil; and treatment 5 (EO4), 1,008 ppm cinnamon oil mixed with 1,375 ppm mace oil.

Uninoculated sausage mixture was tested for the presence or absence of *Salmonella* and *Staph. aureus* using the method of the Bacteriological Analytical Manual (Andrews and Hammack, 2007; Bennett and Lancette, 2001) to ensure that all raw materials were free from these pathogenic bacteria. The beef sausage mixture was tightly packed in polyethylene plastic bags. The sausages (200 g per treatment) were fermented at 30°C for 96 h. Total viable counts of *Staph. aureus* and *S. Rissen* were determined at 0, 24, 48, 72 and 96 h of fermentation by spread
plating onto Baird-Parker agar (BPA; Difco Laboratories; Detroit, MI, USA) supplemented with egg yolk and potassium tellurite, and xylose lysine deoxycholate (XLD; Difco Laboratories; Detroit, MI, USA) agar, respectively. The data were expressed as the percentage of survival cell number, where % survival = viable cell number (CFU.g⁻¹) at each storage time divided by initial cell number (CFU.g⁻¹) × 100. The number of total lactic acid bacteria (LAB) was determined by pour plating with MRS agar. The pH values of sausage samples were measured using a pH meter (Testo 205; Testo AG.; Lenzkirch, Germany).

**Statistical analysis**

Data from three replications were analyzed using analysis of variance to determine if significant differences (P ≤ 0.05) existed between mean values and using Duncan’s multiple range test to compare between treatment means. All data were analyzed using the SPSS 17.0 version statistical package (SPSS Inc; Chicago IL, USA).

**RESULTS AND DISCUSSION**

**Antibacterial activity of cinnamon and mace oils on food model media**

The pathogenic bacteria, S. Rissen, S. Senftenberg, *Staph. aureus* and *L. monocytogenes* were more sensitive to cinnamon oil on most of the food model media compared to mace oil (Table 1). These bacteria were sensitive to cinnamon oil on sausage agar (0.063 mg.mL⁻¹ MIC), but quite resistant on meat agar, except for *S. Senftenberg* which had high resistance on starch agar (14 mg. mL⁻¹ MIC). However, the spoilage bacteria (*P. fluorescens*) was susceptible to cinnamon oil on meat agar (0.015 mg.mL⁻¹ MIC). Unlike the inhibitory effect of cinnamon oil, all the pathogenic bacteria were more resistant to mace oil on sausage agar (14–22 mg.mL⁻¹ MIC) than the other two media. Among all pathogenic bacteria, *S. Rissen* was the most resistant to mace oil on sausage agar with an MIC of 22 mg.mL⁻¹. Most of the lactic acid bacteria tested were more resistant to cinnamon oil than the pathogenic bacteria. Compared to the other two media, *P. pentosaceus* and *E. faecium* had higher resistance to cinnamon oil on starch agar (4 mg.mL⁻¹ MIC) whereas *L. lactis* and *E. faecalis* were more resistant to this oil on meat agar (2 mg.mL⁻¹ MIC). However, *P. pentosaceus*, *E. faecalis* and *E. faecium* were the most resistant to mace oil on sausage agar (22 mg.mL⁻¹ MIC).

* S. Rissen, *Staph. aureus*, *L. monocytogenes*, *L. lactis* and *E. faecalis* were resistant to cinnamon oil on meat agar. This was probably due to the protective effect of proteins,

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cinnamon oil</th>
<th>Mace oil</th>
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<tbody>
<tr>
<td></td>
<td>Starch agar</td>
<td>Meat agar</td>
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<tr>
<td><em>Salmonella Rissen</em></td>
<td>0.063</td>
<td>1</td>
</tr>
<tr>
<td><em>Salmonella Senftenberg</em></td>
<td>14</td>
<td>1</td>
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<tr>
<td><em>Staphylococcus aureus</em></td>
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<td>1</td>
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<tr>
<td><em>Listeria monocytogenes</em></td>
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</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
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<td>0.015</td>
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<tr>
<td><em>Pediococcus pentosaceus</em></td>
<td>4</td>
<td>2</td>
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<tr>
<td><em>Lactococcus lactis</em></td>
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<td>2</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
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<td>2</td>
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<tr>
<td><em>Enterococcus faecium</em></td>
<td>4</td>
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ND⁺ = The minimum inhibitory concentration value was not detected.
the main nutritional component in beef; they may have provided a protective effect to microbial cells (Jay et al., 2005). Juven et al. (1993) reported that bovine serum albumin supplemented in nutrient agar and minimal medium could protect Salmonella Typhimurium from the thymol of thyme oil as their amino and hydroxyl amine groups bound with this phenolic compound. In the current study, some bacteria were more resistant to mace oil on sausage agar than other media. This was probably because of the higher fat content in the sausage agar. Singh et al. (2003) reported that 1 mL.L\(^{-1}\) thyme oil effectively reduced the bacterial population in non-fat and low-fat hotdogs, but was less effective in full-fat hotdogs. Similarly, thyme oil at 10 mL.L\(^{-1}\) reduced the bacterial population by more than 1.3 log CFU.g\(^{-1}\) in non-fat hotdogs, but this oil was less effective in low-fat and full-fat hotdogs.

Cinnamon oil exhibited high antimicrobial activity on sausage agar. The effectiveness of this oil may have been due to its main active compounds such as cinnamaldehyde (Gupta et al., 2008). The constituents, structures and functional groups of essential oils are important to antimicrobial activity, especially phenolic groups which were the most effective constituents. Furthermore, the intrinsic properties of food (sodium chloride, pH, protein and fat) can influence the inhibitory activity of essential oils (Gutierrez et al., 2008). Tassou et al. (1995) reported that mint oil at a level of 1% in food with sodium chloride showed a synergistic effect against Salmonella Enteritidis.

**Survival of Staph. aureus and S. Rissen in fermented beef sausage**

The number of viable Staph. aureus cells in the sausage samples of all treatments decreased as the fermentation time increased. After 96 h of fermentation, the sausages added with cinnamon oil at the highest concentration (1,008 ppm) had significantly lower \((P < 0.05)\) survival of Staph. aureus cells compared to the other treatment samples (Figure 1A). Similarly, the survival number of S. Rissen in all treatments of sausage samples added with mixed essential oils significantly \((P < 0.05)\) decreased compared to those in the control sample at 48–96 h of fermentation (Figure 1B). The sausage samples added with cinnamon oil (1,008 ppm) had the

![Figure 1](image-url)  
*Figure 1* Survival of Staphylococcus aureus (A) and Salmonella Rissen (B) in fermented beef sausages added with mixed cinnamon and mace oils during fermentation at 30°C. (C = control (no oil added); EO1 = 126 ppm cinnamon oil mixed with 1,375 ppm mace oil; EO2 = 252 ppm cinnamon oil mixed with 1,375 ppm mace oil; EO3 = 504 ppm cinnamon oil mixed with 1,375 ppm mace oil; EO4 = 1,008 ppm cinnamon oil mixed with 1,375 ppm mace oil.)
lowest survival population of S. Rissen (1.48 log CFU.g⁻¹) after 48 h of fermentation, but this bacterium could not be detected in sausages added with cinnamon oil at 252, 504 and 1,008 ppm when the fermentation time increased to 96 h.

The inhibitory effect of cinnamon and mace oils against Staph. aureus and S. Rissen in fermented beef sausages may have been due to their antimicrobial compounds. López-Malo et al. (2005) reported that phenolic compounds such as cinnamaldehyde in cinnamon oil, thymol in thyme oil or eugenol in clove oil possessed antimicrobial activity. The phenolic compounds are able to disturb the cell membrane which leads to leakage of the cell contents. However, not only the effect of essential oils, but other inhibitory substances produced by lactic acid bacteria during fermentation such as lactic acid, hydrogen peroxide, diacetyl and bacteriocins could cause a decrease in the Staph. aureus and S. Rissen viable populations (Holzapfel, 1998) and change the lactic acid bacterial population as well as the pH value of the fermented sausage. Lactic acid, a weak acid, is the main product of fermentation which reduces the pH of fermented sausages and inhibits the growth of pathogenic bacteria such as Salmonella, L. monocytogenes, Staph. aureus, Bacillus cereus and Clostridium botulinum. Its undissociated form diffuses across the cell membrane and dissociates within the cytoplasm, thereby leading to cell acidification (Holzapfel, 1998; Brul and Coote, 1999). This reason is relevant to the results of the current study which found that the viable counts of Staph. aureus and S. Rissen in all sausage samples decreased as the pH decreased.

Holley and Patel (2005) reported that the pH of food is an important factor affecting the activity of essential oils. At low pH, the hydrophobicity of some essential oils increases. They can dissolve in the lipid phase of food and the cell membrane, thus enhancing antimicrobial activity. In the current study, viable counts of S. Rissen decreased more rapidly than those of Staph. aureus. This may have been due to the difference in their cell wall structures. S. Rissen (a Gram-negative bacterium) has more lipid in its cell walls, compared to the cell walls of Staph. aureus (a Gram-positive bacterium). Thus, a high amount of essential oil may be dissolved into S. Rissen cells under low pH conditions in fermented sausage. This may have been the reason why S. Rissen was more sensitive to essential oil than Staph. aureus.

Growth of lactic acid bacteria and pH change

The lactic acid bacterial population in fermented beef sausages under all treatments rapidly increased by 0.92 to 1.74 log CFU.g⁻¹ after 24 h of fermentation (Table 2) while pH values in these sausages decreased from 5.31–5.34 to 4.55–4.60 (Table 3). Then, the number of total lactic acid bacteria increased slightly throughout the fermentation period. At the end of fermentation, the total lactic acid bacterial counts were 11.60–12.33 log CFU.g⁻¹ and the pH values of all sausages were in the range 4.23–4.28.

Lactic acid bacteria in all sausage samples could grow during fermentation. However, no significant difference was observed between the total viable lactic acid bacteria in the control samples and those in the samples of the other treatments. This indicated that they were resistant to mixed cinnamon and mace oils added. Jay et al. (2005) reported that lactic acid bacteria are the most resistant Gram-positive bacteria to spices. Similarly, Darmadji et al. (1993) found that garlic and coriander had an inhibitory effect against Bacillus subtilis, Staph. aureus, E. coli and Pseudomonas fragi, but no inhibitory effect against P. pentosaceus and L. plantarum was found.

In conclusion, the combination of cinnamon and mace oils in fermented beef sausages effectively inhibited the growth of Staph. aureus and S. Rissen. The combination of 1,008
ppm cinnamon oil and 1,375 ppm mace oil showed the best results, but the high concentration of cinnamon oil added in food may not be acceptable to consumers from the sensory aspect. Therefore, it would be interesting to investigate the combined application of mixed essential oils and other additives such as organic acid salts or bacteriocins for the optimization of antimicrobial effects with sensory acceptability in future research.

**LITERATURE CITED**


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