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Synergistic antimicrobial effect of pyrophosphate on *Listeria monocytogenes* and *Escherichia coli* O157 in modified atmosphere packaged and refrigerated seabass slices

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Abstract

Effect of pyrophosphate (PP) in combination with modified atmosphere (MAP) (80% CO_2, 10% O_2 and 10% N_2) on the survival of *Listeria monocytogenes* and *Escherichia coli* O157 inoculated on seabass slices stored at 4 °C was investigated. PP pretreatment showed the synergistic effect on microbiological inhibition with MAP as evidenced by the lowered TVC and LAB counts, compared with samples stored in air and those kept under MAP. Microbiological changes of seabass slices inoculated with different levels of *L. monocytogenes* or *E.coli* O157 (10^3 and 10^5 cfu/g) were monitored during storage. PP pretreatment reduced colony count of *E. coli* O157 and extended the lag phase of *L. monocytogenes*. Therefore, MAP in combination with PP pretreatment not only retarded microbiological deterioration of seabass slices but also reduced or inactivated some pathogenic bacteria to some extent.

Keywords: *Listeria monocytogenes*; *Escherichia coli* O157; Modified atmosphere packaging; Pyrophosphate; Seabass

1. Introduction

Modified atmosphere packaging (MAP) is widely used as a supplement to ice or refrigeration to delay spoilage and extend the shelf-life of fresh fishery products (Pedrosa-Menabrito & Regenstein, 1988; Reddy, Villanueva, & Kautter, 1995). MAP under various levels of CO_2 was used in the1930 s to extend the shelf-life of fresh fishery products kept in barrier package. The shelf-life increased as a result of lag phase extension of several aerobic spoilage bacteria and retardation of enzymatic spoilage (Pastoriza, Sampedro, Herrera, & Cabo, 1996; Ashie, Smith, & Simpson, 1996). However, MAP conditions create an environment which supports the growth of facultative anaerobic and psychrotrophic pathogens (Farber, 1991). Continuous hygiene is the most effective pathogen-intervention strategy available. However, occasional contamination still occurs.

Recently, *Listeria monocytogenes* and *Escherichia coli* O157:H7 outbreaks have attracted worldwide attention. *L. monocytogenes* is quite tolerant to high levels of sodium chloride and relatively low pH and refrigeration temperature (Wilkins, Bourgeosis, & Murray, 1972). Its psychrotrophic characteristic may result in its growth on meat at low temperature. This type of cross-contamination is the most common cause of outbreaks of listeriosis (Beuchat, 1995; Lambert, Smith, & Dolds, 1991). *E. coli* is a member of Enterobacteriaceae family that commonly occurs in fresh and frozen meats (Jay, 1992). Contamination of beef with the enterohemorrhagic *E. coli* O157:H7 resulted in two outbreaks with 48 cases and 4 death in 1984 (Bean, Griffin, Goulding, & Iveg, 1990) and several more outbreaks since then. *E. coli*

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O157 was also found in the bivalve shellfish (Guyon et al., 2000). The inhibition effect of CO_2 on bacterial growth has been widely reported for fixed population of spoilage microorganisms and pathogens in food (Kimura, Yoshiyama, & Fujii, 1999). Moreover, application of pyrophosphate prior to MAP has been shown to inhibit the growth of bacteria in fish (Kim, Hearnsberger, Vickery, White, & Marshall, 1995). Changes in pH and the ability to chelate metal ions essential in bacterial metabolism determine the antimicrobial effectiveness of various ortho-, pyro-, and polyphosphates (Molins, 1991). Recently, Masniyom, Benjakul, and Visessanguan (2002) found that MAP (80% CO_2, 10% O_2 and 10% N_2) effectively extended the shelf-life of seabass slices during refrigerated storage. However, no information regarding the efficacy of MAP in the reduction or inhibition of pathogenic bacteria on seabass slices has been reported. Thus, the objective of this study was to determine the combination effect of pyrophosphate and MAP on *L. monocytogenes* and *E. coli* O157 inoculated on seabass slices during refrigerated storage at 4 °C.

2. Materials and methods

2.1. Inoculum preparation

L. monocytogenes DMST 4553 and *E. coli* O157 DMST 4554 were obtained from the Department of Medical Sciences, Ministry of Public Health, Bangkok, Thailand. Each strain of bacteria was maintained on tryptic soy agar (TSA) (Merck, Germany) at 4 °C. Culture were reinoculated onto new media every 2 weeks and incubated at 37 °C for 48 h. Prior to the inoculum preparation, *L. monocytogenes* and *E. coli* O157 were inoculated into tryptic soy broth (TSB) (Merck, Germany) and incubated at 37 °C for 14 and 18 h, respectively, to obtain the late logarithmic phase. The number of each culture was estimated on the basis of absorbance at 660 nm against a standard curve and expressed as cfu/ml. The appropriate dilution was made and the suspension was inoculated into seabass slices to obtain the inoculum of 10^3 and 10^5 cfu/g of slices.

2.2. Preparation of fish

Seabass (*Lates calcalifer*) with an average weight of 1.5–2 kg were purchased from a farm in Koyo Island, Songkhla, Thailand. Fish were placed in crushed ice with a fish/ice ratio of 1:3 (w/w) and transported to the Department of Food Technology, Prince of Songkla University within 1–2 h after catching. They were then washed with tap water, filleted, deskinned and cut into slices with a thickness of 0.8–1 cm.

2.3. Phosphate pretreatment

Sodium pyrophosphate (PP) obtained from Univar (Seven Hill, Australia) was dissolved in the distilled water to obtain a final concentration of 2% (w/v). Seabass slices were soaked in five volumes of the solution (4 °C) for 10 min and drained for 10 min at 4 °C. Fish samples (25 g) were transferred into individual sterile Petri dishes. *L. monocytogenes* or *E. coli* O157 suspensions (250 μl) were inoculated randomly at five different spots selected on the fish slices. After inoculation, the Petri dishes containing seabass slices were placed in a vacuum bag (15 cm × 25 cm) with gas permeability (O_2 transmission rate of 46.6 cm^3/m day at 38 °C, 1 atm pressure) and was packaged with a fish/gas ratio of 1:3 (w/v) using a Henkovac type 1000 (Tecnovac, Italy). Gas mixture containing 80% CO_2, 10% N_2 and 10% O_2 was used. The control samples were prepared in the same manner, except that the samples were packed in air. All samples were stored at 4 °C for 21 days and taken at 0, 3, 6, 9, 12, 15 and 21 days for microbiological analyses.

2.4. Microbiological analyses

Fish slices (25 g) were collected aseptically in a stomacher bag and 10 volumes of 0.85% sterile saline solution were added. After homogenizing in a Stomacher M400 (Seward, UK), a series of ten-fold dilutions was made using 0.85% saline solution. Total viable count (TVC) was determined by plate count agar (PCA) with incubation at 35 °C for 2 days (Speck, 1976). Lactic acid bacteria (LAB) were counted in double-layer in man rogaso sharpe (MRS) agar and incubated at 35 °C for 3 days according to the method of Ordonez, Pablo, Castro, Asensio, and Sanz (1991). Cells of *L. monocytogenes* and *E. coli* O157 were enumerated using Polymyxin B Acriflavine HCl Lithium chloride Ceftazid e Aesculin D-mannitol agar (PALCAM) (Merck, Germany) and Sorbitol MacConkey agar (SMAC) (Merck, Germany), respectively, (Samelis, Sofos, Kendall, & Smith, 2001). Colonies on plates were incubated at 35 °C for 2 days. Microbial counts were expressed as log cfu/g. Colonies suspected to be *L. monocytogenes* were confirmed using the following test: gram stain reaction, catalase, tumbling motility and API Listeria (Bio Merieux, France). Colonies suspected to be *E. coli* O157 were confirmed by slide agglutination tests with *E. coli* O antiserum O157 (Difco, UK).

2.5. Statistical analysis

All experiments were run in triplicate. Data were subject to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences between mean (Steel & Torrie, 1980).