SPOILAGE MICROORGANISMS IN SOUTH EAST ASIA MEAT PRODUCTS AND CONTROL MEASURES WITH LACTATES/ACETATES AT LOW pH

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Abstract – This paper reports a survey of spoilage microorganisms in various South East Asian cooked meat products. Commercial meat samples from Asian producers were artificially spoiled at 10°C or 30°C for 2 – 30 days, were then plated on rich and selective media and typical colonies were identified by 16S rDNA gene sequencing. Members of lactic acid bacteria group were found to dominate the spoilage microflora and the top 3 are reported. *Leuconostoc* sp. was isolated most often from samples stored at 30°C and from vacuum products at 10°C; on the other hand, *Lactobacillus* sp. was prevalent in products aerobically stored at 10°C. Inclusion of salts of lactates or acetates at low pH (<6.0) were effective at reducing growth rate of those microorganisms.

Key Words – Lactic acid bacteria, lactic acid, acetate acid.

I. INTRODUCTION

Meat and meat products are rich sources of nutrients for microorganisms, thus, they are subject to spoilage caused by bacteria, especially lactic acid bacteria [1,2]. In Asia and especially South East Asia (SEA), the susceptibility of meat products to microbial spoilage can be exacerbated by high temperatures and humidity [3,4]. Meat producers often apply the "hurdle technology" concept to design preservative systems suitable for meat products. The hurdle concept makes use of several small antimicrobial characteristics e.g. pH, water activity, preservatives, of which the additive effects of all factors are equal or larger than a single one [5]. As a result, in order to design an effective preservative system i.e. to use correct antimicrobial agents and dosages, knowledge about prevalent spoilage species or groups of microorganisms is imperative. It is also essential to understand how various antimicrobial hurdles affect the growth of these microorganisms. This study reported a survey of Asian cooked meat products and determined the top spoilage microorganisms. Based on the obtained results, application of lactate/acetate salts at reduced pH was shown as a measureto control this cause of spoilage.

II. MATERIALS AND METHODS

IDENTIFICATION OF DOMINANT SPOILAGE MICROORGANISMS

A. Spoiling commercial products

Commercial cooked meat products, which included meat/fish ball, hotdogs and sausages in non-vacuum or vacuum packaging were obtained from supermarkets or meat producers in Indonesia, the Philippines, Thailand and Vietnam. The samples in original packages were artificially spoiled by placing the products in incubators for 2 days at 30°C or 30 days at 10°C. The spoilage condition was chosen based on our observation of the likelihood that products would be sold at ambient or refrigerated temperatures in the country of origin.

B. Identification of spoilage microorganisms

Spoiled samples were diluted with peptone saline water (PSW), homogenized and serially diluted with PSW. Diluted solutions were plated on rich and selective media including trypton soy agar for bacteria, malt extract agar for yeasts and moulds and de Man, Rogosa, Sharpe agar for lactic acid bacteria (LAB). Plates were incubated at 30°C for 2-5 days.

Distinct colonies grown on agar plates were selected and transferred to new agar until pure cultures were obtained.

Agar plates with pure cultures were then sent to BaseClear Group (Leiden, the Netherlands) for

identification using the 16S ribosomal RNA if they were bacteria or the D2-LSU gene if they were fungi. The top 3 spoilage species were established by counting the frequency at which a particular species was isolated from meat products.

EFFECTS OF LACTATES/ACETATES AT DIFFERENT pH ON MICROBIAL GROWTH

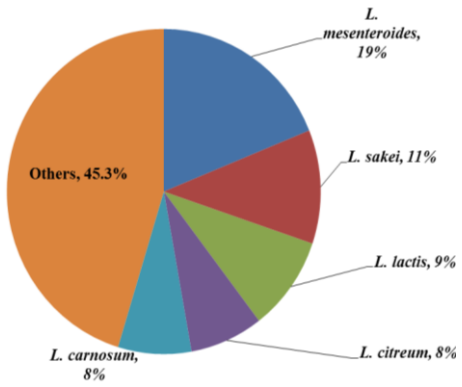
Two LAB species *Lactobacillus sakei* and *Leuconostoc mesenteroides* were taken from Corbion culture collection (Gorinchem, the Netherlands) for growth rate experiment. The microorganisms were grown in MRS broth solutions containing gradients 0 – 9 % of sodium acetate or sodium lactate. The pH of the solutions was corrected to 6.5, 5.8 or 5.0 with NaOH/HCl. Two hundreds (200) µL of every solution was transferred into the wells of a honeycomb plate of Bioscreen C. Each well was inoculated with 3 µL of the inoculum, resulting in a final concentration of 10_4 cfu/ml in the well. The bioscreen run was performed at 30°C, for 5 days in duplicates (Bioscreen C).The optical density (OD) was measured every 20 minutes with shaking before reading at wavelength between 420-580 nm.. Data points were fitted with logistic growth equation. Maximum specific growth rates (μ_{max}) and optimum growth rate (μ_{opt}) were determined from exponential phase. Dosage response curves were then formed by plotting relative growth rates against concentrations of lactic/acetic acids which represent the general forms of lactate/acetate salts [6].

III. RESULTS AND DISCUSSION

The top 5 spoilage microorganisms in cooked meat products all belong to the LAB group (Figure 1). The two LAB species *Leuconostoc* sp. and *Lactobacillus* sp. together made up 68% of the isolates. Specifically, *L. mesenteroides* (19%) was the top spoiler of the *Leuconostoc* sp. group, and *L. sakei* (11%) was the most frequent isolate in the *Lactobacillus* sp. group. *L. lactis* (9%) was the third most common isolate and *L. citreum* and *L. carnosum* were equally found (8%).

The breakdown of the predominant spoilage microorganisms per product group showed variations in terms of species and their shares but the top 3 spoilers shared many common features (Table 1). For the meat products spoiled at ambient temperatures (30°C), the top 3 spoilage microorganisms included *L. mesenteroides* (20%), *L. plantarum* (15%), *L. lactis* (15%) (Table 1). The top 3 spoilers in vacuum packaged products at 10°C were *L. mesenteroides* (57%), *L. sakei* (14%) and *L. lactis* (14%). The spoilage flora found in non-vacuum packaged items at 10°C was dominated by *L. sakei* (40%), *L. mesenteroides* (20%) and *Leuconostoc carnosum* (20%).

Figure 1. Top spoilage microorganisms of SEA meat products



Although the most prevalent spoilage belong to the previously reported top five species in chilled, vacuum packaged cooked meat [8], the total percentage of the top 5 is different and smaller than those in the previous report. The differences may be directly linked to the higher temperature, which is typical in SEA markets, and different types of packaging analyzed in the present study. Apparently, in meat products diversity in environment can lead to certain diversity in microbial population.

Table 1 Top 3 spoilage microorganisms in different groups of SEA meat products

Product group	Microorganisms	Share (%)
Ambient temperature stored (30°C)		
	Leuconostoc mesenteroides	20
	Lactobacillus plantarum	15
	Lactobacillus lactis	15
Vacuum packaged (10°C)		
	Leuconostoc mesenteroides	57
	Lactobacillus sakei	14
	Lactobacillus lactis	14
Non-vacuum packaged (10°C)		
	Lactobacillus sakei	40
	Leuconostoc mesenteroides	20
	Leuconostoc carnosum	20

Antimicrobial effects of lactates and acetates at pH 6.5, 5.8 and 5.0 were investigated for two common LAB spoilage species, *L. mesenteroides* and *L. sakei*. The effect of lactic acid on their growth rate at 3 different pH levels were shown in Figure 2 and 3. Similar trends were observed for acetates (not reported here).

Figure 2. Dose response curve to lactates of *Leuconostoc mesenteroides*

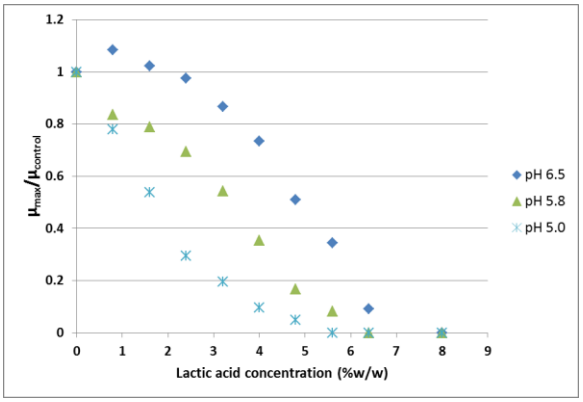
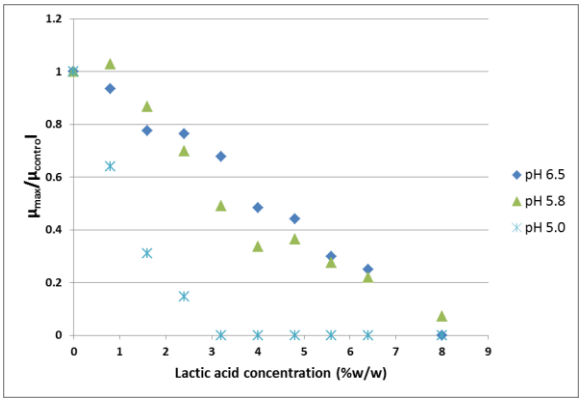


Figure 3. Dose response curve to lactates of *Lactobacillus sakei*



The inhibitory effects of lactates increased when concentrations increased and/or pH decreased. Concentrations at 3% or above generally reduced relative growth rate by half except in the case of the highest pH condition (pH 6.5). At the same concentration, decreasing pH from 6.5 to 5.8 could enhance the antimicrobial efficacy by about 10-30%. Further pH reduction to 5.0 could further double the inhibition, especially at intermediate concentrations 2-4%. The more inhibitory effect of lactic/acetic acids at low pH can be explained by a higher percentage of un-dissociated acid in the extracellular environment. The un-dissociated component passively diffuses through the plasma membrane of microbial cells, dissociates and lower the cellular internal pH. Microbial cells respond by expending energy (ATP) to export the acidic agents out of the cell and raise the pH, thus the energy cannot be spent for growth that leads to spoilage [7]. Therefore, combining lactates and/or acetates at lower pH can offer a solution to control LAB spoilage microorganisms without increasing dosage of the preservatives in meat products.

IV. CONCLUSION

The main spoilage microorganisms in cooked meat products in SEA were *Leuconostoc* sp. and *Lactobacillus* sp.. There were variations among the top spoilers per product group: *L. mesenteroides* in products stored/sold at ambient temperature and vacuum packed product at refrigerated temperature; *L. sakei* in non-vacuum packed products at refrigerated temperature. Salts of lactates and/or acetates at reduced pH offer solutions to control those microorganisms and extend shelf-life of meat products without increasing application dosage.

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