UNIVERSITI PUTRA MALAYSIA

ANTIBACTERIAL ACTIVITY OF ORGANIC ACIDS ON THE GROWTH OF SELECTED BACTERIA IN MEAT SAMPLES

MOHAMMAD RAFTARI

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By

MOHAMMAD RAFTARI

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Chairman: **Associate Professor Fatimah Abu Bakar, PhD**

Faculty: **Food Science and Technology**

Meat can harbour a large variety of pathogenic and spoilage microorganisms which include mesophilic and psychrophilic bacteria, during slaughtering and further processing. These microorganisms may be sources of infection to human and spoilage of meat. Organic acids are generally recognized as safe antimicrobial agents and the low dilute solutions of organic acids are generally without affecting on the desirable sensory properties of meat; in addition, they do not create residual problems when used as carcass decontaminants. Spray wash treatments utilizing three concentrations (1, 1.5 and 2%) of acetic, lactic, propionic and formic acids (individually and/or in combination of two acids) were performed to evaluate their efficacy in reducing numbers of *Escherichia coli* O157:H7, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* Typhimurium and *Pseudomonas putida* on meat tissues stored at 4±1ºC. The procured beef pieces were decontaminated with hot water and then inoculated with *E. coli* O157:H7, *S. aureus, L. monocytogenes*, *S.* Typhimurium and *P. putida* seperately which then were spray washed with organic acids for 15 seconds either individually or in combination of two acids separately.

The population of *E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, *L. monocytogenes* and *P. putida* ($P<0.05$) were reduced statistically after being spray washed with all treatments at a range of 0.89-3.19 $\log_{10}$ cfu/ml. The inhibitory effect of all organic acids according to the concentration was 2% concentration > 1.5% concentration > 1% concentration. Mean log reductions of *E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, *L. monocytogenes* and *P. putida* showed that the antibacterial effect of formic acid > lactic acid > acetic acid > propionic acid. Combinations of two organic acids indicated a stronger inhibitory effect on selected bacteria compared to the effect of each acid alone. The combinations of acetic and formic, lactic and formic, and propionic and formic acids showed higher reductions effect at ranges of 0.22-1.67, 0.26-1.55, 1.43-1.56, 1.43-1.69 and 0.44-1.59 $\log_{10}$ cfu/ml for *E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, *L. monocytogenes* and *P. putida* respectively, more than combinations of acetic and lactic, acetic and propionic, and lactic and propionic acids. The combination of lactic and formic acids showed the highest reduction effect, where more than 3 $\log_{10}$ cfu/ml, of all bacterial species were reduced. The populations of *S. aureus* and *L. monocytogenes* as Gram-positive bacteria reduced more significantly ($P<0.05$) than the population of *E. coli* O157:H7, *S.* Typhimurium and *P. putida* as Gram-negative bacteria. The results of this study indicated that formic acid is a good antibacterial agent for decontaminating animals' carcass surfaces especially when mixed with lactic acid.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

AKTIVITI ANTIBAKTERIA ASID ORGANIK KE ATAS PERTUMBUHAN BAKTERIA TERPILIH DI DALAM SAMPEL DAGING

Oleh

MOHAMMAD RAFTARI

June 2009

Pengerusi: **Profesor Madya Fatimah Abu Bakar, PhD**

Fakulti: **Sains dan Teknologi Makanan**

Daging boleh mengandungi pelbagai patogen dan mikroorganisma perosak termasuk bakteria mesofilik dan psikrofilik, semasa proses penyembelihan dan proses seterusnya. Mikroorganisma ini mungkin menjadi punca jangkitan kepada manusia dan kerosakan daging. Asid organik secara amnya dikenali sebagai agen antimikrob yang selamat dimana penggunaan larutan asid organik pada pencarian rendah kebiasaannya tidak memberi kesan perubahan deria ke atas ciri-ciri daging dan tidak menyebabkan masalah apabila digunakan sebagai agen nyahkontaminasi. Kaedah semburan menggunakan tiga kepekatan (1, 1.5 dan 2%) asetik, laktik, propionik dan asid formik (secara sendirian atau gabungan dua asid) dijalankan untuk menilai kesan dalam penurunan bilangan *Escherichia coli* O157:H7, *Staphylococcus aureus, Listeria monocytogenes*, *Salmonella* Typhimurium dan *Pseudomonas putida* pada tisu daging yang disimpan pada 4±1°C. Kepingan daging yang diperolehi daripada haiwan yang baharu disembelih telah dicuci dengan air panas dan kemudian diinokulat dengan *E. coli* 0157:H7, *S. aureus*, *L. monocytogenes, S.* Typhimurium dan *P. putida* yang kemudiannya dicucisembur dengan asid organik selama 15 saat

secara berasingan. Populasi *E. coli* O157:H7, *S.* Typhimurium, *S. aureus, L. monocytogenes* dan *P. putida* menurun dengan ketara (P<0.05) selepas dicucisembur dengan kesemua rawatan pada lingkungan 0.89-3.19 $\log_{10}$ cfu/ml. Kesan kematian bagi semua asid organik mengikut kepekatan adalah kepekatan 2% > kepekatan 1.5% > kepekatan 1%. Purata log penurunan *E. coli* O157:H7, *S.* Typhimurium, *S. aureus, L. monocytogenes*, dan *P. putida* menunjukkan bahawa kesan antibakteria bagi asid formik > asid laktik > asid asetik > asid propionik. Gabungan dua asid organik menunjukkan kesan kematian yang lebih kuat ke atas bakteria terpilih. Gabungan asid asetik dan formik, laktik dan formik, dan propionik dan asid formik menunjukkan kesan antibakteria yang lebih baik pada lingkungan 0.22-1.67, 0.26-1.55, 1.43-1.56, 1.43-1.69 dan 0.44-1.59 $\log_{10}$ cfu/ml penurunan untuk *E. coli* O157:H7, *S.* Typhimurium, *S. aureus*, *L. monocytogenes* dan *P. putida* lebih daripada gabungan asid asetik dan laktik, asetik dan propionik, dan laktik dan propionic. Gabungan asid laktik dan formik menunjukkan kesan penurunan yang baik lebih daripada 3 $\log_{10}$ cfu/ml, ke atas populasi spesis bakteria yang dikaji. Populasi *S. aureus* dan *L. monocytogenes*, bakteria gram positif menurun lebih banyak (P<0.05) daripada populasi *E. coli* O157:H7, *S.* Typhimurium dan *P. putida* iaitu bakteria gram negatif. Keputusan bagi penyelidikan ini menunjukkan asid formik adalah agen antibakteria yang baik untuk membersihkan permukaan daging haiwan yang disembelih terutama apabila dicampur dengan asid laktik.

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I certify that an Examination Committee has met on 18th June 2009 to conduct the final examination of Mohammad Raftari on his Master of Science thesis entitled "Antibacterial Activity of Organic Acids on the Growth of Selected Bacteria in Meat Samples" in accordance with Universiti Pertanian Malysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the student be awarded the Master of Science degree.

Members of the Examination Committee were as follows:

Abdulkarim Sabo Mohammed, PhD
Lecturer
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Saleha Abdul Aziz, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Shuhaimi Mustafa, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Mohd Khan Ayob, PhD
Associate Professor
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 August 2009

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Fatimah Abu Bakar, PhD
Associate Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Son Radu, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Zamberi Sekawi, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOD. GHAZALI, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 September 2009

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

MOHAMMAD RAFTARI

Date: 15 August 2009

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LIST OF ABBREVIATIONS

AA	Acetic Acid
AAFA	Acetic Acid+Formic Acid
AALA	Acetic Acid +Lactic Acid
AAPA	Acetic Acid+Propionic Acid
ATCC	American Type Culture Collection
CDC	Centers of Disease Control and Prevention
CFU	Colony-forming unit
FA	Formic Acid
FDA	Food Drug Administration
LA	Lactic Acid
LAFA	Lactic Acid+Formic Acid
LAPA	Lactic Acid+Propionic Acid
PA	Propionic Acid
PAFA	Propionic Acid+Formic Acid
USDA	United States Department of Agriculture
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Demand for quality oriented foods of animal source has been affected by gradual rising in world population and changes in standard of living (Dubal *et al*., 2004). Meat and meat products besides being tasty are considered a very important part of any balanced and nutritious diet. They are rich in protein and valuable quantities of the B vitamins; hence, they play an important role in growth, repair and maintenance of body cells and are necessary for our everyday activities (Kalalou *et al*., 2004).

Though intact meat from healthy animals is sterile, it may be contaminated by microorganisms present on the exterior parts of the living animals during skinning, and/or from the environment (Sofos *et al*., 1999). *Salmonella* Typhimurium, *Staphylococcus aureus, Escherischia coli* O157:H7, *Listeria monocytogenes*, are some of the major pathogenic bacteria associated with meat and meat products. Meat pathogens can cause self-limiting human enteric diseases or systemic and fatal infections of the immunocompromised among the elderly, and the young (Marshall and Bal'a, 2001). Members of the genera *Pseudomonas* display the fastest growth rates and hence the greatest spoilage potential, when fresh meat is chill-stored aerobically (Davies and Board, 1998).

Most foodborne outbreaks have been attributed to foods from cattle-derived origins especially the ground beef (Adams and Moss, 2000). Many researchers indicated that meat is one of the main sources of pathogenic bacteria, which can cause foodborne

diseases and food poisoning in humans (Buchholz *et al*., 2005; Jay *et al*., 2005; Marshall & Bal'a, 2001; Adams & Moss, 2000; Grein *et al*., 1999).

Diseases caused by foodborne pathogens have been a serious threat to public health and food safety for decades and remain one of the major concerns of our society (Yang & Bashir, 2008). Mead *et al*. (1999) estimated that foodborne illness hospitalizations and foodborne pathogen-related deaths in the United State are respectively followed by *Salmonella* spp. causing 26% and > 30%, *Listeria* spp. accounting for 4% and 28%, *Campylobacter* spp. causing 17% and > 5%, and *E. coli*, both O157 and non-O157, accounting for 5% and > 4%.

E. coli O157:H7 causes around 73,000 cases of illness and 61 deaths per year. Besides that, *Salmonella* caused 40,000 reported cases with an estimated actual number of 20 times more than the reported number. More than 1000 deaths occur each year due to *Salmonella* infections, making it the most harmful foodborne pathogen. Ground beef products are commonly associated with outbreaks of *Salmonella* and *E. coli* O157:H7 (CDC, 2005).

As mentioned earlier, different pathogenic and spoilage microorganisms may be introduced onto the meat during slaughtering and processing, which cause foodborne illness, rapid spoilage and great loss of valuable protein (Dubal *et al*., 2004; Marshall & Bal'a, 2001). Therefore, it is very important to prevent and/or reduce the growth of pathogenic or spoilage bacteria on animals' carcass surfaces.

Several intervention strategies have been developed to reduce the level of bacteria on surface of animals' carcasses such as washing and sanitizing with chilled water, hot water, chlorinated water, food grade acids alone or in combination. All these sanitizers act differently on different types of microorganisms, but the information about the action of these sanitizers on artificially inoculated specific microorganisms in meat is still limited (Dubal *et al*., 2004; Smulders & Greer, 1998).

Chemical techniques of decontamination have lately received much attention (Acuff, 2005). Chemical preservatives are defined as "substances capable of inhibiting, retarding or arresting the growth of microorganisms" (Adams & Moss, 2000). Chemical preservatives can act as bactericidal or bacteriostatic agents. Most of the studies have been on the use of organic acids, which appear to be the most acceptable form of chemical decontamination (Acuff, 2005).

Organic acids have a long history of being applied as food additives and preservatives for preventing food deterioration and extending the shelf life of perishable food ingredients (Cherrington *et al*., 1991b). They are generally identified as safe antibacterial agents. The dilute solutions of organic acids (1–3%), when used as a carcass decontaminant, are generally without effect on the desirable sensory properties of meat (Smulders & Greer, 1998).

According to Acuff (2005), acid decontamination of meat surfaces may provide a means of reducing microbial populations of pathogenic and spoilage bacteria, thereby providing a product with reduced potential for foodborne illness and increased shelf life. In recent years, reducing foodborne bacteria and increasing the

shelf life of meat is followed by spraying of carcasses and cuts with acid sprays, which have been employed to decontaminate meat surfaces.

Due to the health problems and economic loss, which are caused by bacterial species on meat, the obligation to reduce initial load of bacteria should be taken into serious consideration. This study is an attempt to examine the antibacterial effects of four common food grade organic acids with low concentrations on important species of bacteria on meat. Determining the ability of organic acids to control bacteria will finally indicate the best type of acid to be applied for commercial purposes.

To the best of our knowledge, this is the first study, which simultaneously investigates such a large number of treatments for controlling different types of bacteria on meat.

The objectives of this study are as follows:

1. To study the antibacterial effect of each individual and in combinations of two organic acids at 1, 1.5 and 2% concentrations on selective bacteria species inoculated on meat stored at 4±1°C
2. To compare the response of Gram-positive and Gram-negative bacteria against the organic acids

CHAPTER 2

LITERATURE REVIEW

2.1 Meat Microflora

The contamination of sterile animal muscle used as food is a direct consequence of slaughtering and dressing of animal carcasses. Various microorganisms from diverse sources are transferred onto moist muscle surfaces that are rich in nutrients. It is argued that only a small portion (10%) of these microorganisms is able to survive and grow during storage, distribution and retail sales of meat (Marshall & Bal'a, 2001; Sofos *et al*., 1999).

Meat can harbour a large number of pathogenic and spoilage microorganisms during primary and further processing. Pathogens include *Clostridium perfringens, S. aureus, Salmonella* spp., pathogenic *E. coli, Campylobacter* spp., *Yersinia enterocolitica, L. monocytogenes,* and *Aeromonas hydrophila* (Jay *et al.,* 2005, Gill & Jones, 1995; Gill & Bryant, 1993; Rogers *et al*., 1992).

Spoilage of meat is largely dependent on initial microbiological quality and subsequent storage conditions. *Pseudomonas* spp. dominate in chilled air-stored meat (Gennari & Dragotto, 1992), Enterobacteriaceae in temperature-abused meat (Lindberg *et al.,* 1998), lactic acid bacteria and Micrococcaceae in meat packaged with preservatives (Leisner *et al.,* 1995; Makela *et al.,* 1992), and *Brochothrix thermosphacta* in vacuum- and modified atmosphere-packaged products (Sheridan *et al.,* 1997).

2.1.1 Meat Contamination

The microbiological profile of meat products presented to consumers is the sum total of slaughtered animal health, conditions under which it was reared, quality of slaughtering, processing, packaging and conditions under which the meat was stored (Marshall & Bal'a, 2001).

Gill (1998) reviewed the potential sources of meat contamination during slaughtering and butchering of food animals. Animal health, hide, feces, oral microflora and carcass handling are all potential sources of cross contamination of sterile muscle during dressing operations. The major source of initial meat contamination is the animals' hide or fleece (Mies *et al.,* 2004; Gill, 1998; Hadley *et al.,* 1997). These sources are exposed to soil, feces, water and oral microorganisms during animal rearing (Van Donkersgoed *et al*., 1997). Animal hides not only introduce spoilage bacteria such as *Pseudomonas, Acinetobacter,* and *Moraxella*, but also may introduce potential pathogens such as *C. perfringens, S. aureus, Salmonella* spp., *E. coli, Campylobacter* spp., *Y. enterocolitica, L. monocytogenes* and *A. hydrophila* (Gill & Jones, 1995; Rogers *et al*., 1992).

2.2 *Escherichia coli*

2.2.1 Characteristics

For the first time, *E. coli* was identified in 1885 by German pediatrician Theodore Escherich. This bacterium belongs to the Enterobacteriaceae family. *E. coli* is a Gram-negative, facultative anaerobe, non-sporeforming rod shape bacterium (Adams & Moss, 2000). It is a typical mesophile bacterium which can grow at temperatures ranging from 7-8°C up to 46°C with an optimum growth rate around 37°C (Meng *et*

al., 2007). It has optimum growth at pH near neutral, but growth is also possible as low as pH 4. Optimum Aw for growth of *E. coli* is 0.995, but it can also grow as low as 0.95. *E. coli* is serotyped according to three main antigens on the surface, which are O (lipopolysaccharide somatic), H (flagella) and K (capsule) (Jay *et al.,* 2005; Adams & Moss, 2000).

One part of the normal enteric flora of humans and warm-blooded animals' intestines is non-pathogenic strains of *E. coli*, which live as commensals in the bowel and are the major facultative anaerobe microorganism in the human gastro-intestinal tract, but some are pathogenic and cause diarrheal illness. The main source for this environmentally ubiquitous microorganism is the intestinal tract. However, it is considered an indication of fecal infectivity and suggests the possible existence of enteric pathogens when it is found somewhere else in the environment (Meng *et al*., 2007).

2.2.2 Types of *Escherichia coli*

Based on virulence properties, mechanisms of pathogenicity, clinical syndrome and distinct O:H serogroups, diarrheagenic *E. coli* isolates are classified into specific groups. These serogroups are enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffuse-adhering *E. coli* (DAEC), enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC) (Meng *et al*., 2007). The mechanisms by which diarrhea is produced, based on the attachment of bacteria to the intestinal cells, invasion and production of enterotoxins is varied for each type of *E. coli* (Fratamico *et al*., 2002).