UNIVERSITI PUTRA MALAYSIA

DECONTAMINATION OF CHICKEN BREASTS USING ORGANIC ACIDS AND LAURICIDIN

DANIEL MENSAH ANANG

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By

DANIEL MENSAH ANANG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of Philosophy

December 2005

Dedicated to
my beloved wife (Nancy Anang)
and
daughter (Aniyanni Yehowahi Naa Ode Anang)

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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Chairman: **Professor Gulam Rusul Rahmat Ali, PhD**

Faculty: **Food Science and Technology**

Lauricidin, lactic and oxalic acids were evaluated for their effectiveness in reducing and inhibiting the growth of predominant spoilage and pathogenic microorganisms. Chicken breasts (150 – 200 g each) of freshly slaughtered chickens were purchased from a local wet market and analysed within 2 hr. Chicken breasts were dipped in 0, 0.5, 1.0, 1.5 and 2.0% solutions of lauricidin (w/v, containing 1% lactic acid and 1% ethanol), lactic acid (v/v) or oxalic acid (w/v) for 10, 20 and 30 min, then individually packed in oxygen-permeable polyethylene bags, and stored at 4°C. Aerobic plate counts (APC), populations of *Pseudomonas* spp. and *Enterobacteriaceae* on chicken breasts were determined before, after treatment and after storage for 1, 3, 7, 10, and 14 days at 4°C. Surviving aerobic organisms were isolated and identified from chicken breasts treated with lauricidin, lactic and oxalic acids. Dipping chicken breasts in solutions of lauricidin, lactic and oxalic acids caused significant ($P \leq 0.05$) reduction in APC and also retarded microbial growth throughout the 14 d storage period. APC on chicken samples treated with 0.5 to 2.0% lauricidin, lactic acid and oxalic acid solutions were significantly ($P \leq 0.05$) reduced by 0.92 - 1.2, 0.53-2.36 and 1.38-2.76 log CFU/g, respectively. Initial

Pseudomonas counts on samples treated with 0.5 to 2.0% lauricidin and lactic acid were in the range of 0.79 – 1.77 and 0.39 – 1.82 log CFU/g, respectively, which were significantly ($P \leq 0.05$) lower compared to fresh samples, and growth of *Pseudomonas* spp. was limited throughout the storage period. In chicken breasts treated with 0.5 to 2.0% lauricidin and lactic acid, *Enterobacteriaceae* counts decreased by 0.14–1.14 and 0.59-2.18 log CFU/g, respectively. Less than log 2 CFU/g *Enterobacteriaceae* and *Pseudomonas* counts were observed on samples treated with 1.0 – 2.0% oxalic acid for 10 to 30 min. *Enterobacter cloacae, Pseudomonas lundensis* and *Kocuria rhizophila* were the predominant aerobic organisms isolated from chicken breasts treated with 0.5 to 2.0% lauricidin, lactic acid and oxalic acid, respectively.

Lauricidin, lactic and oxalic acids were also evaluated for their effects on growth and survival of *Listeria monocytogenes* (L55), *Salmonella* Enteritidis (S552) and *Escherichia coli* O157:H7 (E19) inoculated onto raw chicken breasts. In chicken breasts treated with 0.5 to 2.0% lauricidin, initial counts of *L. monocytogenes, S.* Enteritidis and *E. coli* O157:H7 were significantly ($P \leq 0.05$) reduced by 2.90, 1.31 and 2.27 log CFU/g, respectively. *L. monocytogenes, S.* Enteritidis and *E. coli* O157:H7 counts on samples treated with lactic acid were significantly ($P \leq 0.05$) reduced by 1.97, 1.71 and 2.59 log CFU/g, respectively. Initial counts of *L. monocytogenes, S.* Enteritidis and *E. coli* O157:H7 in chicken samples treated with oxalic acid were significantly ($P \leq 0.05$) reduced by 2.87, 2.02 and 4.12 log CFU/g, respectively. Dipping of chicken breasts in higher concentrations of solutions of lauricidin, lactic and oxalic acids and longer dipping time gave additional benefit.

The colour and pH of chicken breasts dipped in solutions of lauricidin, lactic acid and oxalic acid were also evaluated. Sensory attributes of chicken breasts dipped in oxalic acid were determined. Oxalic acid residues in chicken breasts treated with oxalic acid were also determined. Dipping of chicken breasts in 0.5 to 2.0% lauricidin, lactic acid and oxalic acid caused significant ($P \leq 0.05$) decreased in pH, however, the decrease in pH was more pronounced in samples dipped in oxalic acid. Dipping chicken breasts in 0.5 to 2.0% lauricidin, lactic acid and oxalic acids caused slight darkening, as reflected by the increase in Hunter *L* values. Lauricidin caused a slight decrease in Hunter *a* value (decreased redness), and an increase in Hunter *b* value (increase in yellowness). Lactic acid also caused an increase in Hunter *a* values (increased redness) and Hunter *b* values (increased yellowness). Oxalic acid gave a more bleached chicken breasts compared to lauricidin and lactic acid. Higher concentrations of oxalic acid and longer dipping time caused more bleaching of the chicken breasts compared to lower concentrations and shorter dipping time. The maximum residue of oxalic acid in unwashed chicken breasts was 36 mg/100g (in chicken samples dipped in 2% oxalic acid for 30 min). Oxalic acid residue significantly ($P \leq 0.05$) decreased by more than 50% when the chicken breasts were washed and subsequently cooked. The maximum residue of oxalic acid in roasted chicken breast was 2 mg/100g. Results showed that lauricidin, lactic and oxalic acids have the potential to be used as a sanitizer in chicken carcasses during processing. Sensory evaluation of chicken breasts treated with oxalic acid demonstrated that, even though instrumental measurements may indicate deterioration in appearance, cooked chicken breasts were acceptable to consumers.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

DEKONTAMINASI DADA AYAM MENGGUNAKAN ASID ORGANIK DAN LAURICIDIN

oleh

DANIEL MENSAH ANANG

Disember 2005

Pengerusi: **Profesor Gulam Rusul Rahmat Ali, PhD**

Fakulti: **Sains dan Teknologi Makanan**

Penilaian terhadap keberkesanan lauricidin, asid laktik dan asid oksalik untuk mengurangkan dan merencatkan pertumbuhan mikroorganisma perosak pradominan dan patogenik telah dilakukan. Bahagian dada ayam (150 – 200 g setiap satu) daripada ayam yang baru disembelih dibeli daripada pasar tempatan dan dianalisis dalam masa 2 jam. Bahagian dada ayam dicelup ke dalam 0, 0.5, 1.0, 1.5 and 2.0% larutan lauricidin (w/v, mengandungi 1% asid laktik dan 1% etanol), asid laktik (v/v) atau asid oksalik (w/v) selama 10, 20, and 30 min, dan kemudian dibungkus secara berasingan di dalam beg polietilena yang telus oksigen dan disimpan pada suhu 4°C. Aerobic Plate Counts (APC), populasi *Pseudomonas* spp. dan *Enterobacteriaceae* pada dada ayam ditentukan sebelum dan selepas rawatan serta selepas disimpan selama 1, 3, 7, 10, dan 14 hari pada 4°C. Organisma yang dapat terus bertahan dan hidup pada dada ayam setelah dirawat dengan asid laktik, asid oksalik dan lauricidin telah diasingkan dan dikenalpasti. Dada ayam yang dicelup dalam lauricidin, asid laktik dan asid oksalik menyebabkan pengurangan bererti ($P \leq 0.05$) APC dan merencat pertumbuhan bakteria sepanjang 14 hari tempoh penyimpanan. APC pada sampel ayam yang telah dicelup dalam 0.5 hingga 2.0% larutan lauricidin, asid laktik

dan asid oksalik masing-masing berkurangan secara bererti ($P \leq 0.05$) sebanyak 0.92 - 1.2, 0.53-2.36 dan 1.38-2.76 log CFU/g. Rawatan dengan lauricidin telah dapat mengurangkan kiraan *Pseudomonas* sebanyak 0.79 – 1.77 log CFU/g manakala rawatan dengan asid laktik mengurangkan kiraan *Pseudomonas* sebanyak 0.39 – 1.82 log CFU/g, di mana pertumbuhan ini sangat terhad sepanjang tempoh simpanan dan berkurangan secara bererti ($P \leq 0.05$) jika dibandingkan dengan pertumbuhan pada sampel ayam segar. Kiraaan *Enterobacteriaceae* pada sampel ayam yang dicelup dalam 0.5 hingga 2.0% larutan lauricidin berkurangan sebanyak 0.14–1.14 log CFU/g manakala kiraan pada sampel ayam yang dicelup dalam 0.5% hingga 2.0% larutan asid laktik berkurangan sebanyak 0.59-2.18 log CFU/g. Kiraan *Enterobacteriaceae* dan *Pseudomonas* telah berkurangan sebanyak kurang daripada log 2 CFU/g apabila sampel ayam dicelup dalam larutan 1.0 – 2.0% asid oksalik selama 10 hingga 30 min. Bakteria yang mendominasi bahagian dada ayam yang telah dicelup dalam 0.5 hingga 2.0% lauricidin ialah *Enterobacter cloacae. Pseudomonas lundensi* pula mendominasi bahagian dada ayam yang telah dicelup dalam 0.5 hingga 2.0% asid laktik manakala *Kocuria rhizophila* mendominasi bahagian dada ayam yang telah dicelup dalam 0.5 hingga 2.0% asid oksalik.

Keberkesanan lauricidin, asid laktik dan asid oksalik untuk merencat pertumbuhan *Listeria monocytogenes* (L55), *Salmonella* Enteritidis (S552) dan *Escherichia coli* O157:H7 (E19) yang telah diinokulatkan ke atas dada ayam diuji. Kiraan awal *L. monocytogenes* berkurangan secara bererti ($P \leq 0.05$) sebanyak 2.90 log CFU/g, kiraan awal *S.* Enteritidis berkurangan secara bererti ($P \leq 0.05$) sebanyak 1.31 log CFU/g dan kiraan awal *E. coli* O157:H7 berkurangan secara bererti ($P \leq 0.05$) sebanyak 2.27 log CFU/g apabila sampel ayam dirawat dengan 0.5 hingga 2.0%

larutan lauricidin. Asid laktik mengurangkan kiraan awal *L. monocytogenes*, *S.* Enteritidis dan *E. coli* O157:H7 secara bererti ($P \leq 0.05$) sebanyak 1.97, 1.71 dan 2.59 log CFU/g masing-masing. Kiraan awal *L. monocytogenes*, *S.* Enteritidis dan *E. coli* O157:H7 pada sampel ayam yang dicelup dalam larutan asid oksalik berkurangan secara bererti ($P \leq 0.05$) sebanyak 2.87, 2.02 dan 4.12 log CFU/g masing-masing. Dada ayam yang dicelup di dalam larutan lauricidin, asid laktik dan asik oksalik yang berkepekatan tinggi dan tempoh mencelup yang lebih lama dapat memberikan kesan yang lebih positif.

Warna dan pH dada ayam yang telah dicelup di dalam larutan lauricidin, asid laktik dan asik oksalik juga dinilai. Penilaian deria dan sisa asid oksalik pada dada ayam yang telah dicelup dengannya turut ditentukan. Mencelup dada ayam di dalam 0.5 hingga 2.0% larutan lauricidin, asid laktik dan asik oksalik memberi kesan yang siknifikan ($P \leq 0.05$) pada pengurangan pH tetapi pengurangan pH lebih ketara pada dada ayam yang dicelup dengan asid oksalik. Rawatan menggunakan 0.5 hingga 2.0% larutan lauricidin, asid laktik dan asik oksalik menyebabkan warna menjadi sedikit gelap seperti yang ditunjukkan pada pertambahan nilai Hunter L. Larutan lauricidin menyebabkan terdapat sedikit pengurangan pada nilai Hunter a (pengurangan warna merah), dan peningkatan nilai Hunter b (peningkatan warna kuning). Larutan asid laktik menyebabkan peningkatan nilai Hunter a (peningkatan warna merah) dan nilai Hunter b (peningkatan warna kuning). Larutan asid oksalik menghasilkan produk yang lebih putih jika dibandingkan dengan larutan lauricidin dan larutan asid laktik. Kepekatan asid oksalik yang tinggi dan masa yang lebih lama semasa mencelup menghasilkan produk yang lebih putih jika dibandingkan dengan kepekatan asid oksalik yang rendah dan masa yang singkat semasa mencelup. Sisa

maksimum asid oksalik pada bahagian dada ayam yang belum dibasuh ialah 36 mg/100 g (sample ayam yang dicelup dalam 2% asid oksalik selama 30 min). Sisa asid oksalik berkurangan bereti 50% ($P \leq 0.05$) atau lebih apabila sampel dibasuh dan seterusnya dimasak. Sisa maksimum asid oksalik di dalam ayam yang telah dipanggang ialah 2mg/100g. Keputusan menunjukkan bahawa lauricidin, asid laktik, dan asid oksalik mempunyai potensi untuk digunakan sebagai bahan sanitasi untuk mengurangkan populasi mikroorganisma perosak dan patogenik yang wujud secara semulajadi semasa pemprosesan ayam mentah. Penilaian deria menunjukkan bahawa pengguna masih boleh menerima ayam yang telah dicelup di dalam asid oksalik walaupun sukatan menggunakan instrumentasi mungkin telah menunjukkan kemerosoton nilai dari segi rupa luaran.

Acknowledgements

First and foremost, I wish to express my Thanks and Praises to the Almighty God for giving me life and strength to undertake my PhD research programme.

I wish to express my profound gratitude and appreciation to my supervisor, Professor Dr. Gulam Rusul Rahmat Ali, for his much effortless guidance, advice, support and constructive criticism he provided during my studies in UPM and for that matter Malaysia. I am also much indebted to Professor Dr. Son Radu and Professor Dr. Jamilah Bakar, who were members of my supervisory committee, for their guidance, advice, encouragement and support. Appreciation also goes to Associate Professor Dr. Foo Hooi Ling, Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, and Dr. Nazimah Sheikh Abdul Hamid, Department of Food Science, Faculty of Food Science and Technology.

Recognition is given to the Malaysian and Ghanaian governments for support and sponsorship through the Malaysia Commonwealth Scholarship and Fellowship Plan, which enabled me to carry out my PhD research programme.

I also wish to express my sincere thanks to Ayamas Food Corporation Bhd, for donating the chicken breast used in the sensory evaluation part of this research.

My appreciation also goes to staff of the Microbiology Lab of Faculty of Food Science and Technology; especially En. Zulklifi Nordin and Puan Jamilah; Staff of Biochemistry Lab; particularly En. Halim; and Ms. Poh Lian Neo (ITMA). Special thanks also goes to my Lab mates (Food Safety Lab); Henie Parillon, Kqueen,

Yousr, Yin Sze Lim, Sia Yen Yap, Suwaibah Ghaffar, Tunnung, Marlina, Rani, Bambi, Aniza, Khaizura and Chandrika. I also wish to acknowledge all my Malaysian friends who contributed to make my life comfortable and enjoyable during my stay in Malaysia. Appreciation also goes to Dr. Ahmed Harun Osman, Abdulkarim Sabo, Melcoil Ambunda, Andreas Malyenge, Ignatius Hinjou and Biliyamin Ibetoye.

Finally, I wish to express appreciation and relentless gratitude to my family, particularly my spouse, Nancy Anang and daughter Aniyanni Yehowahi Naa Ode Anang, for their support, love and understanding. Even though I had to leave them for these four years to further my studies, they strongly stood by me.

Daniel Mensah Anang
November 2005

I certify that an Examination Committee met on 30th December 2005 to conduct the final examination of Daniel Mensah Anang on his Doctor of Philosophy thesis entitled "Decontamination of Chicken Breasts Using Organic Acids and Lauricidin" in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and University Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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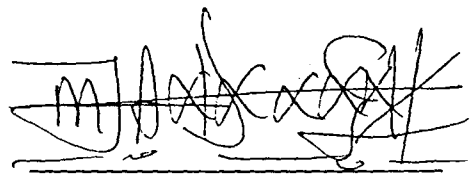
Date: 09 MAR 2006

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



DANIEL MENSAH ANANG

Date: 17th January 2006

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CHAPTER 1

INTRODUCTION

Chicken forms about two thirds of the total world poultry meat production (Anon, 1996). Poultry meat generally, is perceived as cheap, wholesome, and nutritious, being high in protein and low in fat. There are no religious constraints on consumption, unlike pork and beef (Mead, 2000). These characteristics and other factors has made poultry meat by far the most popular food product worldwide (Mulder, 1999). Poultry meat production has increased rapidly worldwide over the years and this increase is associated with equivalent increase in intensive animal production with both increase in the number of farms and flock size (Bolder, 1998). It was reported by the United States Food and Agricultural Policy Research Institute (FAPRI) (2005) that major disease outbreaks in both beef and pork industries shifted consumption in many countries toward poultry. In the long run, however, lower cost and health considerations are driving the increase in world poultry consumption. The per capita consumption of poultry for 2003 were estimated as 50.1 kg in the USA, 35.9 kg in New Zealand, 34.2 kg in Australia, 22.7 kg in Western Europe, 37.1 kg in Canada and 32.5 kg in Malaysia (year 2002) (MAFnet, 2003; USDA, 2002). FAPRI (2005) projects that over the next 10 years, per capita consumption, production, and broiler net trade will grow at annual rates of 2.1, 3.2 and 3.7 percent, respectively. It was estimated that global trade in broiler meat will increase to nearly 6500 thousand metric tons by 2010 from the current of 5392.73 thousand metric tons (FAPRI, 2005).

The poultry processing industry has also seen extensive development in the last 30-45 years, to meet the increased demand and has brought the food meat, which was available to limited class of consumer, to the popular, cheap and wholesome meat within everyone's budget (Mulder, 1999). Primary processing of poultry, however, consists of a number of operations, which have microbiological implications. From the farm gate, when birds are transported, to the processing plant, up to chilling and packaging, have all been implicated in some form of microbial cross-contamination. This problem is compounded with the high throughput of modern poultry processing plants, which leaves little room for effectively sanitizing of equipment. Growth of bacteria on meat results in the production of 'off' odours and flavours or an unacceptable appearance to the consumer. This is generally termed microbial spoilage. Microbial spoilage is delayed, but not prevented, by storage of meat at temperatures between -1^{o} to $5^{o}C$.

Epidemiological reports from all over the world incriminate poultry meat as a source of outbreaks of human food poisoning which has resulted in great economic loss to nations including the loss of human life. Most of these illnesses have been attributed to cross-contamination with human pathogenic organisms such as *Salmonella* spp, *Escherichia coli, Listeria monocytogenes* and *Campylobacter* spp. Several bacterial pathogens have been associated with poultry-borne human illnesses; however, *Salmonella* and *Campylobacter* have been of primary concern. The FAO/WHO (2002) report on foodborne diseases in Europe indicated that about 26% of foods in outbreaks involved poultry and poultry products (including eggs). *Salmonella* serovars are the most frequently reported causal agent of outbreaks in the European region, being responsible for 77.1% of outbreaks. Of these, more than one third were