EFFECTS OF GRILLING OF BEEF AND CHICKEN SATAY ON THE SURVIVAL OF LISTERIA MONOCYTOGENES

SITI ZULAIHA BINTI HANAPI

FSTM 2006 26
EFFECTS OF GRILLING OF BEEF AND CHICKEN SATAY ON THE SURVIVAL OF *LISTERIA MONOCYTOGENES*

By

SITI ZULAIHA BINTI HANAPI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of the Master of Science

April 2006
EFFECTS OF GRILLING OF BEEF AND CHICKEN SATAY ON THE SURVIVAL OF LISTERIA MONOCYTOGENES

By

SITI ZULAIHA BINTI HANAPI

April 2006

Chairman : Associate Professor Zaiton bt Hassan, PhD

Faculty : Science and Food Technology

The isolation and enumeration of heat-injured and non-injured of Listeria monocytogenes and other Listeria species was studied in samples of chicken (40 samples) and beef (50 samples) satay isolated from 3 different vendors around Kajang and Bangi, Selangor. Samples of chicken satay were grilled by the vendors for 180 seconds while beef satay was grilled for 270 seconds. The internal temperature obtained was $64\pm 4^\circ C$ measured by probe. This resulted to reduce total viable count of chicken satay and beef satay by 3 log reduction. Three different methods namely, U.S. FDA (Canadian Version), Overlay method (Compendium Method for the Microbiological Examination of Food (1995) and Thin Agar Layer (TAL) (Kang and Fung, 1999) were evaluated for the recovery of Listeria species from raw and grilled chicken satay and beef satay. TAL method gives higher recovery rates compared with OV while U.S FDA method unable to allow the recovery of Listeria species in raw and grilled satay. Listeria species isolated from raw chicken and beef satay using TAL method were L. monocytogenes, L. innocua, L ivanovii and L. grayi, respectively. Similar Listeria species were also detected in grilled chicken and beef satay after grilled to 180 s.. L. welshimeri is the only
species was isolated in raw beef satay. On contrary, in OV agar, three species namely, *L. monocytogenes*, *L. innocua* and *L. ivanovii* were recovered in chicken satay with grilling time 150 seconds. However, prolonged heating to 180 seconds resulted in destruction of all *Listeria* species. Three different internal temperature (60°C, 70°C and 80°C) and 3 different storage temperature (4°C, 30°C and −20°C) were studied in chicken satay inoculated with *L. monocytogenes* (~10^6-7 CFU/g). The population of *L. monocytogenes* increased rapidly on chicken satay stored at 30°C for 24 hours. *L. monocytogenes* was also shown the ability to survive at refrigeration temperature for up to 7 days. In temperature storage at −20°C, the population of *L. monocytogenes* remained 14 days only. A study of hemolysin gene using specific Polymerase Chain Reaction (PCR) showed that *L. monocytogenes* was found resistance to internal temperature of 60 and 70°C but not at 80°C.

Results clearly demonstrated that the detection and enumeration of injured *Listeria* species was influenced by the medium used for the isolation. Microorganism that have been treated with a sub-lethal stress are considered injured if they fail to grow and produce colonies when they are plated on agar media containing selective agents. The interpretation results found that the pattern of growth of *Listeria* in satay (raw and grilled) was strongly influenced by the storage and internal grilling temperatures. Higher heating temperature resulted in destruction of *L. monocytogenes* in grilled satay.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

KESAN PEMBAKARAN SATE LEMBU DAN AYAM KE ATAS KEUPAYAAN *LISTERIA MONOCYTOGENES*

Oleh

*SITI ZULAIHA BINTI HANAPI*

April 2006

Pengerusi : Profesor Madya Zaiton bt Hassan, PhD

Fakulti : Sains dan Tekologi Makanan


Hasil kajian menunjukkan kaedah dan media pemencilan mempengaruhi pengiraan dan pemencilan organisma. Mikroorganisma yang telah diberi tekanan separa kuat dikatakan tercedera sekiranya mereka gagal untuk hidup dan menghasilkan koloni apabila di tumbuhkan diatas media yang mengandungi agen-agen selektif. Keputusan yang diperolehi menunjukkan pertumbuhan Listeria didalam sate (mentah dan masak) dipengaruhi kuat oleh faktor penyimpanan dan suhu dalaman semasa...
pembakaran. Pembakaran pada suhu tinggi juga menyebabkan kemusnahan *L. monocytogenes* didalam sate yang telah dimasak.
This thesis submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

**Zaiton Bt Hassan, PhD**  
Associate Professor  
Faculty of Science and Food Technology  
Universiti Putra Malaysia  
(Chairman)

**Gulam Rusul Rahmat Ali, PhD**  
Professor  
Faculty of Science and Food Technology  
Universiti Putra Malaysia  
(Member)

**Russly Abd Rahman, PhD**  
Professor  
Faculty of Science and Food Technology  
Universiti Putra Malaysia  
(Member)

AINI IDERIS, PhD  
Professor/Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: 14th SEPTEMBER 2006
DECLARATION

I hereby declare that the thesis is based on my original work except for equations 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.

SITI ZULAIHA BINTI HANAPI

Date: 25th AUGUST 2006
# TABLE OF CONTENT

<table>
<thead>
<tr>
<th>TABLE OF CONTENT</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>vii</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>viii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>x</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xv</td>
</tr>
</tbody>
</table>

## CHAPTER

1 INTRODUCTION 1

2 LITERATURE REVIEW 6

2.1 General Characteristics of *L. monocytogenes* 6

2.2 Biochemical Characteristics of *Listeria* 6

2.3 Reservoir 9

2.4 Virulence and Pathogenesis 10

2.5 Symptoms of Listeriosis 12

2.6 Outbreak of Listeriosis 13

2.7 Prevalence of *Listeria monocytogenes* in Food 15

2.7.1 *Listeria* in Meat and Meat Product 16

2.7.2 *Listeria* in Seafood 16

2.7.3 *Listeria* in Dairy Product 18

2.7.4 Other Food 20

2.8 Factor Affecting Growth and Survival of *L. monocytogenes* 23

2.8.1 Temperature 23

2.8.2 pH 23

2.8.3 Salt 24

2.8.4 Water Activity 24

2.9 Detection of *Listeria* in Food 25

2.9.1 Detection of Natural Cells 25

2.9.2 Detection of Injured Cells 26

2.9.3 Principles of Developing a Method for Injured Cells 28

2.9.3.1 MPN Technique and Direct Plating Method 29

2.9.3.2 Overlay Method 29

2.9.3.3 Thin Agar Layer Method 30

2.9.3.4 Detection of *L. monocytogenes* by Specific Polymerase Chain Reaction 31

3 ISOLATION AND ENUMERATION OF *L. MONOCYTOGENES* AND *LISTERIA* SPECIES FROM CHICKEN AND BEEF SATAY 35

3.1 Introduction 35

3.2 Materials and Method 37

3.2.1 Samples Collection 37

3.2.2 Determination of Total Viable Count 39
3.2.3 Isolation of *Listeria* species and *L. monocytogenes* using Canadian Method 39
3.2.4 Enumeration of *Listeria* using OV Method 41
3.2.5 Enumeration of *Listeria* using TAL Method 41
3.2.6 Identification of *Listeria* species 44
3.2.7 Beta Hemolysis Test 44
3.2.8 CAMP Test 45
3.2.9 Statistical Analysis 45

3.3 Results 46
3.4 Discussion 64

4 SURVIVAL OF *L. MONOCYTOGENES* IN CHICKEN SATAY
4.1 Introduction 74
4.2 Materials and Methods 76
4.2.1 Source and Maintainance of Stock Culture 76
4.2.2 Culture Media 76
4.2.3 Isolate Confirmation 77
4.2.4 Preparation of Inoculum Cultures for Survival Studies of *L. monocytogenes* 77
4.2.5 Inoculation of *L. monocytogenes* in Raw Satay 78
4.2.6 Enumeration of Survivors 80
4.2.7 Grilling Procedure 80
4.3 Results 82
4.3.1 Total Viable Count of Chicken Satay stored at 30°C, 4°C and -20°C. 82
4.3.2 Growth of *L. monocytogenes* in Chicken Satay Stored At 30°C, 4°C and −30°C 82
4.3.3 Effect of Internal Temperature (60°C, 70°C and 80°C) During Grilling on *L. monocytogenes* inoculated in raw Chicken Satay at 30°C 88
4.3.4 Effect of Internal Temperature (60°C, 70°C and 80°C) During Grilling on *L. monocytogenes* inoculated in raw Chicken Satay at 4°C and −20°C 89
4.4 Discussion 89

5 DETECTION OF *L. monocytogenes* HEMOLYSIN GENE BY POLYMERASE CHAIN REACTION
5.1 Introduction 96
5.2 Materials and Methods 98
5.2.1 Bacteria Strain and Media 98
5.3.2 DNA Extraction 98
5.3.3 PCR Primer Sequence 99
4.3.4 Detection of Hemolysin Gene by Specific PCR 99
5.3 Results 103
5.4 Discussion 108

6 SUMMARY AND CONCLUSION 114

REFERENCES
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Differentiation of <em>L. monocytogenes</em> from other species of <em>Listeria</em>.</td>
<td>7</td>
</tr>
<tr>
<td>2.2</td>
<td>Prevalence of <em>L. monocytogenes</em> in Foods in Malaysia.</td>
<td>22</td>
</tr>
<tr>
<td>3.1</td>
<td>Frequency of sampling of raw and grilled satay from satay vendors in Kajang and Bangi.</td>
<td>37</td>
</tr>
<tr>
<td>3.2</td>
<td>Internal temperature of chicken satay grilled for 30, 60, 90, 120, 150 and 180 sec.</td>
<td>48</td>
</tr>
<tr>
<td>3.3</td>
<td>Temperature of beef satay grilled for 45, 90, 135, 180, 225 and 270 sec.</td>
<td>48</td>
</tr>
<tr>
<td>3.4</td>
<td>Total viable counts for raw chicken satay and satay after grilled for 30, 60, 90, 120, 150 and 180 sec.</td>
<td>49</td>
</tr>
<tr>
<td>3.5</td>
<td>Total viable counts raw beef satay and satay after grilled for 45, 90, 125, 180, 225 and 270 sec.</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>Presumptive (log_{10} CFU/g) <em>Listeria</em> count on TAL and Overlay (OV) agar in raw chicken and beef satay.</td>
<td>51</td>
</tr>
<tr>
<td>3.7</td>
<td>Prevalence of <em>Listeria</em> sp in raw chicken satay samples obtained from vendors A, B and C.</td>
<td>53</td>
</tr>
<tr>
<td>3.8</td>
<td>Prevalence of <em>Listeria</em> sp in raw beef satay samples obtained from vendors A, B and C.</td>
<td>53</td>
</tr>
<tr>
<td>3.9</td>
<td>Presumptive (log_{10} CFU/g) <em>Listeria</em> count on Thin Agar Layer (TAL) and Overlay (OV) agar in chicken satay grilled for 150 and 180 sec.</td>
<td>54</td>
</tr>
<tr>
<td>3.10</td>
<td>Presumptive (log_{10} CFU/g) <em>Listeria</em> count on Thin Agar Layer (TAL) and Overlay (OV) agar in beef satay grilled for 225 and 270 sec.</td>
<td>54</td>
</tr>
</tbody>
</table>
3.11 Recovery of *Listeria* species from grilled chicken satay samples using TAL method.

3.12 Recovery of *Listeria* species from grilled chicken satay samples using OV method.

3.13 Recovery of *Listeria* species from grilled beef satay samples using TAL method.

3.14 Recovery of *Listeria* species from grilled beef satay samples using OV method.

4.1 Period of sampling for inoculated raw satay stored at different storage temperature.

4.2 Total Viable Count for inoculated raw chicken satay samples after storage at 30°C, 4°C and -20°C.

4.3 Effect of grilling to different internal temperature (60°C, 70°C and 80°C) on *Listeria monocytogenes* inoculated to raw chicken satay stored at 30°C for 0 to 24 h.

4.4 Effect of grilling to different internal temperature (60°C, 70°C and 80°C) on *Listeria monocytogenes* inoculated raw chicken satay stored at 4°C for 1 to 7 d.

4.4 Effect of grilling to internal temperature (60°C, 70°C and 80°C) on *Listeria monocytogenes* inoculated raw chicken satay stored at -20°C for 7 to 21 d.

5.1 Primer sequences for Specific PCR


<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Grilling process for chicken and beef satay.</td>
<td>38</td>
</tr>
<tr>
<td>3.2</td>
<td>A Canadian version of the U.S. FDA <em>Listeria</em> isolation protocol for <em>Listeria</em>.</td>
<td>40</td>
</tr>
<tr>
<td>3.3</td>
<td>Procedure for isolating <em>Listeria</em> species from raw and grilled satay using OV method.</td>
<td>42</td>
</tr>
<tr>
<td>3.4</td>
<td>Procedure for isolating <em>Listeria</em> species from raw and grilled satay using TAL method.</td>
<td>43</td>
</tr>
<tr>
<td>3.5</td>
<td>Isolation of heat-injured <em>Listeria</em> species on TAL medium.</td>
<td>57</td>
</tr>
<tr>
<td>4.1</td>
<td>Preparation of inoculum for survival study of <em>L. monocytogenes</em>.</td>
<td>79</td>
</tr>
<tr>
<td>4.2</td>
<td>Satay griller and charcoal amber used for grilling satay in the survival study of <em>L. monocytogenes</em> study.</td>
<td>81</td>
</tr>
<tr>
<td>4.3</td>
<td>Growth of <em>L. monocytogenes</em> (CFU/g) on TAL medium after exposure to temperature storage of 30°C.</td>
<td>86</td>
</tr>
<tr>
<td>4.4</td>
<td>Growth of <em>L. monocytogenes</em> (CFU/g) on TAL medium after exposure to temperature storage of 4°C.</td>
<td>86</td>
</tr>
<tr>
<td>4.5</td>
<td>Growth of <em>L. monocytogenes</em> (CFU/g) on TAL medium after exposure to temperature storage of -20°C.</td>
<td>87</td>
</tr>
<tr>
<td>5.1</td>
<td>Procedure for detection of hemolysin gene.</td>
<td>102</td>
</tr>
<tr>
<td>5.2</td>
<td>Agarose gel electrophoresis of <em>Listeria monocytogenes</em> amplification products obtained from colonies isolated from raw chicken satay and samples grilled to internal temperature 60°C.</td>
<td>104</td>
</tr>
</tbody>
</table>
5.3 Agarose gel electrophoresis of *Listeria monocytogenes* amplification products obtained from colonies isolated from chicken satay samples grilled to internal temperature 70°C.

5.4 Agarose gel electrophoresis of *Listeria monocytogenes* amplification products obtained from colonies isolated from different samples of raw and grilled chicken satay products.

5.5 Agarose gel electrophoresis of *Listeria monocytogenes* amplification products obtained from colonies isolated from different samples of raw and grilled beef satay products.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AOAC</td>
<td>Association Official Analytical Chemistry</td>
</tr>
<tr>
<td>AP-PCR</td>
<td>Arbitrary Primered-Polymerase Chain Reaction</td>
</tr>
<tr>
<td>CAMP</td>
<td>Christie-Atkins-Munch-Peterson</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>d</td>
<td>day (s)</td>
</tr>
<tr>
<td>dNTPs</td>
<td>deoxyribonucleotide triphosphates</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra-acetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assays</td>
</tr>
<tr>
<td>EtBr</td>
<td>Ethidium bromide</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>H</td>
<td>hour (s)</td>
</tr>
<tr>
<td>LLO</td>
<td>Listeriolysin O</td>
</tr>
<tr>
<td>LEB</td>
<td><em>Listeria</em> enrichment broth</td>
</tr>
<tr>
<td>L-PALCAMY</td>
<td>Polymyxin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol Egg Yolk Broth</td>
</tr>
<tr>
<td>min</td>
<td>minute (s)</td>
</tr>
<tr>
<td>ml</td>
<td>mililiter</td>
</tr>
<tr>
<td>μg</td>
<td>microgram</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>μl</td>
<td>microliter</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
</tbody>
</table>
PFGE  Pulsed-field gel electrophoresis
RTE  Ready to eat food
RAPD  Random Amplification of Polymorphic DNA
RNA  ribonucleic acid
rRNA  ribosomal ribonucleic acid
SDS  Sodium dodecyl sulphate
s  second
sp  Species
Taq  Thermus aquaticus DNA (polymerase)
TAL  Thin Agar Layer
TBE  Tri-Borate EDTA electrophoresis buffer
Tris  Tris (hydroxymethyl) methylamine
TSA  Tryptone Soy Agar
TSB  Tryptone Soy Broth
OV  Overlay
U.S  United States
USDA  United States Food and Drug Administration
UV  Ultraviolet
UVM  University of Vermont
WHO  World Health Organisation
w/v  weight/volume
>  more than
° C  degree Celcius
CHAPTER 1

INTRODUCTION

*L. monocytogenes* is a highly adaptable, asporogenous, motile, Gram-positive bacterium and appreciably more heat resistant than many other non-sporing pathogens associated with foods such as *Salmonella* and *Campylobacter* (Mackey and Bratchell, 1989; Farber and Peterkin, 1991). Its ability to grow at wide range of temperature especially at refrigerator temperature makes it particularly troublesome to the food industry (Knabel et al., 1990; Dykes, 1999; Fung et al., 2004; Kim et al., 2005; Matagaras et al., 2006).

*L. monocytogenes* can be isolated from many sources in the environment including soil, water, sewage and many animal species (Patel et al., 1994; Kang and Fung, 1999; Diane et al., 1991). *L. monocytogenes* was also been isolated from a wide range of foods, including dairy products, seafood, raw fruits and vegetables, poultry and raw and processed meats (Schlech et al., 1983; Bula et al., 1995; Linnan et al., 1988). Foodborne transmission of listerios is has been recognized since 1981 when an outbreak in Canada was associated with consumption of coleslaw made from contaminated cabbages (Schlech et al., 1983). Since then, epidemiologic investigation have implicated whole and 2% milk (Flemming et al., 1985), Mexican–style cheese (Linnan et al., 1988), liver pate (McLauchin et al., 1991), jellied pork tongue (Jacquet et al., 1995), smooked mussels (Brett et al., 1998), frankfurters (CDC, 1999) and pigs tongue in aspic (FDA, 2001).
The first reported case of human listeriosis was made by Nyfeldt in 1929, where the blood of patients with an infections mononucleosis-like disease was positive with *L. monocytogenes* (Gray *et al.*, 1966). Following the year in 1936, *L. monocytogenes* was a cause of perinatal infection in humans (McLauchlin, 1987). Subsequently, *Listeria* was found to cause abortion in cattle and sheep, septicemia in fowl, encephalitis in sheep and mastitis in cows (Wehr, 1987). Potel provided the first description of foodborne listeriosis in humans with a direct-link to animal in 1953 (Hird, 1987). In this instance, *L. monocytogenes* was isolated from a cow with listerial mastitis and from stillborn twins of a woman who had ingested raw milk from the same animal.

The Centres of Disease Control (CDC) in 1999(a), reported that all the foodborne pathogens tracked by CDC, *L. monocytogenes* had the second highest case fatality rate (20%) and the highest hospitalisation (90%) with approximately 2500 cases of listeriosis occur annually in the United States (Mead *et al.*, 1999). Individuals at highest risk of listeriosis include those with decreased immune function caused by disease or medications; pregnant woman who usually have clinically mild illness; and their infants who may be infected before or during delivery, resulting in stillbirth or neonatal infection. The LA country outbreak, which occurred between January 1 and August 15, 1985, resulted in 142 cases and 48 (34%) deaths. Ninety-three (65.5%) of the infections were in pregnant women or their neonates. Thirty-two (32%) of those cases ended in death. At least, 6% of human maybe asymptomatic gastrointestinal carries of *L. monocytogenes* without ill effects (Rocourt, 1996). The substance that is responsible for hemolysis of erythrocytes and the destruction of
phagocytic cells has been designated as Listeriolysin O (LLO). This hemolysin gene can be detected using the specific Polymerase Chain Reaction (PCR).

Early work by the Food and Drug Administration, which attempted to resolve the *Listeria* heat resistance issue as a result of the 1983 Massachusetts outbreak, determined that *L. monocytogenes* had a $D_{71.7^\circ C}$ value of 0.9 s (Bradshaw *et al.*, 1985). Thus, in order to survive high-temperature, short time pasteurisation ($17.1^\circ C, 15$ sec) populations of $>10^{15} Listeria$ /ml would need to be present in milk. The maximum population to which *Listeria* can grow in milk is $10^9$/ml (Donnelly and Brigss, 1986). Data for other food are less comprehensive but there have been several reports that *L. monocytogenes* can survive in red meat or poultry cooked to temperature as high as $82^\circ C$ (Karaioannoglou and Xenos, 1980; Carpenter and Harrison, 1989). These results suggest that *L. monocytogenes* is much more resistant in meat than in milk.

*Listeria* can be sublethally injured by a variety of processing treatments such as heating, drying and freezing (Martin and Katz, 1993). Microorganisms that have been treated with a sublethal stress are considered to be injured if they fail to grow and produce colonies when they are plated on agar media containing selective agents such as bile salts, Sodium chloride, antibiotics and others (Smith, 1990). Such treatment would cause the loss of membrane integrity, degrading RNA, reducing enzymatic activity and changing transport kinetics of cells. The temporary loss of tolerance for specific condition by microorganism needs a procedure called resuscitation to allow the organism to recover and regain growth and virulence potential (Hill and Gahan, 2000). If the injured microorganisms can escape the
stressed environment at if the stress is removed, they may, under the proper conditions, repair the damage induced by the stress, divide and potentially as dangerous as their uninjured counterparts (Alex et al., 1999; Jung et al., 2003).

Although many culturing methods have been developed or proposed for \textit{L. monocytogenes}, most are time consuming and not ideal for use by the food industry. Frequently, perishable foods are consumed before analysis for \textit{L. monocytogenes} is completed. Many plating methods have been developed for this purpose and no one medium has emerged as being optimal for all foods. Efficacy of medium to allow recovery and enumeration of \textit{L. monocytogenes} depends on the physical state of the cells, the type of food being analysed and the presence of contaminating microflora (Brackett et al., 1990). Other factors such as incubation temperature, composition of recovery medium and the incubation atmosphere also play important roles (Taormina and Beachaut, 2002). Less inhibitory media are necessary when analysing foods, which contain injured \textit{L. monocytogenes} cells. Kang and Fung (2000) reported that the recovery of injured cells is superior in the absence of oxygen.

This present study will focused on the efficiency of 3 different methods; Thin Agar Layer (TAL), Overlay (OV) and USDA methods to isolate and enumerate \textit{L. monocytogenes} and other \textit{Listeria} species in raw and grilled chicken and beef satay samples. In this study, the survival of \textit{L. monocytogenes} was evaluated at three different temperatures storage (4°C, 30°C and –20°C) and grilling at temperatures of 60°C, 70°C and 80°C. A storage of 4°C was selected because most of foods are kept in refrigeration temperature. A storage of 30°C was selected because many foods are handled at this temperature, and often \textit{L. monocytogenes} maybe present on surfaces
and utensils and be a major source of contamination. A storage of \(-20^\circ\text{C}\) was selected because many foods are stored at this temperature as a stock for a period of time. Satay was chosen as a model because satay is the favourite foods not only in South East Asia but also in Europe and can be easily found in the market. The customers who eat satay always come in a group with different ages including the young and elderly personal. Nowadays, satay has been sold commercially in a market including ready to eat or in frozen pack. Therefore, the monitoring program and research should be carried out to provide knowledge on the source of contamination and the presence of \textit{L. monocytogenes} and other pathogenic bacteria in satay. Thereby, the control option can be recommended.

The objectives of this study are

1. To compare the selectivity and efficiency of Thin Agar Layer Method (TAL), Overlay Method (OV) and USDA for the enumeration of injured and non-injured \textit{Listeria monocytogenes} in chicken satay and beef satay.
2. Identification of \textit{Listeria monocytogenes} isolated from raw and grilled chicken and beef satay.
3. To study the survival of \textit{L. monocytogenes} inoculated in raw chicken satay stored at \(4^\circ\text{C}\), \(30^\circ\text{C}\) and \(-20^\circ\text{C}\) and the resistance to grilling at internal temperatures of \(60^\circ\text{C}\), \(70^\circ\text{C}\) and \(80^\circ\text{C}\).
CHAPTER II

LITERATURE REVIEW

2.1 General Characteristics of *L. monocytogenes*

*L. monocytogenes* is a member of the genus *Listeria* and is characterized as a gram positive, non-sporeforming bacterium with regular rods 0.4-0.5 μm with rounded ends (Rocourt, 1999). They are both anaerobic and facultative anaerobic bacteria, belong to low G+C content gram-positive bacteria and is closely related to the *Bacillus* and *Streptococcus* genera (Miller *et al.*, 1992). They are motile with tumbling or slightly rotating fashion, best expressed at 20-25°C. The cells are sometimes almost coccoid, occurring singly or in short chains, often presenting ‘V’ shape. In old and rough cultures, 6-20 μ filaments may develop (Sutherland, and Porrit, 1997). When cultures grown on a clear medium, such as tryptic soy agar, are viewed under magnification using obliquely transmitted light as described by Henry (1933), colonies of *L. monocytogenes* appear bluish-green and have a finely textured surface. Colonies will vary in diameter between 0.3 and 1.5mm, depending on the number of colonies present on the agar surface (Ryser and Marth, 1991).

2.2 Biochemical Characteristics of *Listeria*

*Listeria* spp. can be identified according to the biochemical test listed in Tables 2.1. The identification of *Listeria* species based on a limited number of biochemical test
Table 2.1: Differentiation of *L. monocytogenes* from other species of *Listeria*.

<table>
<thead>
<tr>
<th></th>
<th><em>monocytogenes</em></th>
<th>ivanovii</th>
<th>seeligeri</th>
<th>innocua</th>
<th>grayi</th>
<th>welshimeri</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-haemolysis</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>CAMP test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>R. equi</em></td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Nitrate reduction</strong></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Voges-Proskauer</strong></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>Acid from</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hippurate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

1 Weak reaction only

(Source: Varnam and Evans, 1991)