



**UNIVERSITI PUTRA MALAYSIA**

***ANTIMICROBIAL ACTIVITY OF NUTMEG (*Myristica fragrans* Houtt.)  
AND SENSORY ATTRIBUTE OF BEEF TREATED  
WITH THE NUTMEG EXTRACT***

***MAYA PUTERI MALINA BT. ZAKARIA***

**FSTM 2015 9**



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**By**

**MAYA PUTERI MALINA BT. ZAKARIA**

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

**May 2015**

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

**ANTIMICROBIAL ACTIVITY OF NUTMEG (*Myristica fragrans* Houtt.) AND  
SENSORY ATTRIBUTE OF BEEF TREATED WITH THE NUTMEG  
EXTRACT**

By

**MAYA PUTERI MALINA BT. ZAKARIA**

**May 2015**

**Chair: Assoc. Prof. Yaya Rukayadi, PhD**  
**Faculty: Food Science and Technology**

Antimicrobial and antioxidant of nutmeg were studied intensively in research and showed potential as antimicrobial and antioxidant agent apart. There were no report on antioxidant and antibacterial potential of nutmeg extract on any beef product. Beef muscles undergo several changes that can affect their safety (foodborne pathogens growth) and sensory attributes such as colour and flavor during storage. Therefore, there is a need to find an alternative method to control and maintain the safety and quality of raw beef during storage. The objective of this study is to evaluate the antimicrobial activity of nutmeg and to determine the effect of nutmeg extract towards the microorganism growth and sensory attributes of treated beef (lipid oxidation, colour, pH, texture, nutrition value). In this study, application of nutmeg (*Myristica fragrans* Houtt.) extract at different concentrations; 0.25%, 0.65%, 1.25%, 2.50% and 5.00% (g/ml) were used to treat raw beef (2.5 × 2.5 × 1.0 cm; 4 ± 0.5 g). Samples were then individually packed in overwrapped trays and stored for 3 weeks at -18 ± 2°C and 4°C ± 2°C. The treated raw beef were evaluated at 0, 1, 4, 7, 10, 14 and 21 days of storage. The results showed *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia* and *Listeria monocytogene* was susceptible to nutmeg extract. The antimicrobial activities of nutmeg extract were not significantly affected by pH (3, 7, and 11) and temperatures (121°C). There were significant differences on reduced number of bacterial count of beef with the concentrations of extract. Treatment at 5.00% showed the strongest bactericidal efficacy among all concentrations on *L. monocytogenes*, *E. coli*, by reductions of 2.10 and 4.54 log<sub>10</sub> CFU/g, respectively stored at -18 ± 2°C. Treated beef starting at concentration 1.25% and above resulted significantly different (p<0.05) on inhibition of microbial growth stored at 4 ± 2°C. This results show that nutmeg extract effectively has antimicrobial effect on beef, not because of the temperature effect. Lipid oxidation analysis was identified using TBARS method. There were significant difference (p<0.05) on TBARS value of beef with the concentrations of extract. Extract at concentration of 1.25% and above inhibited TBARS value able to maintain the lipid oxidation of beef at both temperature. 1.25% of extract was also able to maintain the redness (a\*) of treated beef compared to untreated beef during both temperatures. Treated beef with nutmeg extract able to maintain the pH value of normal conditions throughout the storage for both temperatures. There was a significant difference (p<0.05) on hardness with untreated and treated samples starting at 7<sup>th</sup> day of storage. There was no significant difference in term of protein

content in all treated or untreated samples. However, fat and moisture content were significantly different ( $p < 0.05$ ) by the concentration of nutmeg extract. Overall, 1.25% of nutmeg extract was chosen based on best combination of sensory evaluation scores together with all analysis result to maintain the safety and quality of beef as well as maintaining organoleptic properties. From this study indicated that nutmeg extract showed a potential preservation ingredient and increase shelf life of beef without changes in quality, nutritional and sensory characteristics.



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

**AKTIVITI ANTIMIKROB BUAH PALA (*Myristica fragrans* Houtt.) DAN  
SIFAT- SIFAT SENSORI DAGING LEMBU YANG DIRAWAT DENGAN  
EKSTRAK BUAH PALA**

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Antimikrobiol dan antioksidan buah pala telah dikaji secara intensif dalam penyelidikan dan menunjukkan potensi sebagai agen antimikrobiol dan antioksidan. Tiada rekod penyelidikan mengenai kesan penggunaan ekstrak pala di dalam makanan atau bahan mentah. Otot daging lembu adalah sensitif semasa penyimpanan yang boleh memberi kesan kepada keselamatan (pertumbuhan mikroorganisma) dan kualiti (warna, rasa dan nutrisi). Oleh itu, cara mengawal dan mengekalkan kualiti daging lembu yang lebih efektif perlu dikaji. Objektif kajian ini adalah untuk mengkaji aktiviti antimikrobial buah pala dan mengkaji kesan penggunaan ekstrak pala terhadap pertumbuhan mikroorganisma dan ciri-ciri sensori daging lembu. Kajian ini menggunakan ekstrak pala (*Myristica fragrans* Houtt.) pada kepekatan yang berbeza; 0.25%, 0.65%, 1.25%, 2.50% and 5.00% (g/ml) telah digunakan untuk memerap daging lembu ( $2.5 \times 2.5 \times 1.0$  cm;  $4 \pm 0.5$  g). Sampel kemudiannya dibungkus secara individu di dalam bekas bungkusan dan disimpan selama 3 minggu pada suhu  $-18^{\circ}\text{C} \pm 1$  dan  $4 \pm 1^{\circ}\text{C}$ . Kesan ekstrak telah dinilai pada hari penyimpanan ke 0, 1, 4, 7, 10, 14 dan 21. Keputusan menunjukkan pertumbuhan *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia* dan *Listeria monocytogene* terjejas oleh ekstrak pala. Aktiviti antimikrobiol buah pala tidak terkesan oleh perubahan kondisi pH (3, 7 dan 11) dan suhu ( $121^{\circ}\text{C}$ ). Keputusan menunjukkan terdapat perbezaan kesan yang ketara ( $p < 0.05$ ) terhadap kepekatan ekstrak dalam pengurangan pertumbuhan mikroorganisma pada daging lembu. Pemerapan pada 5.00% menunjukkan pengurangan pertumbuhan *L. monocytogenes* dan *E. coli* paling besar sebanyak 2.10 dan 4.54  $\log_{10}$  CFU/g. Pada suhu penyimpanan  $4^{\circ}\text{C}$ , penggunaan 1.25% dan ke atas ekstrak pala menunjukkan keberkesanan terhadap anti-mikrobial pada daging lembu. Maka, keputusan ini menyimpulkan bahawa keberkesanan pengurangan pertumbuhan mikroorganisma dalam daging adalah daripada pemerapan ekstrak dan bukan disebabkan kesan suhu. Bagi ujian pengoksidaan lemak, kaedah TBARS value digunakan dalam kajian ini. Keputusan menunjukkan terdapat perbezaan kesan yang ketara ( $p < 0.05$ ) terhadap kepekatan ekstrak dalam hasil TBARS value. Kepekatan pada 1.25% dan ke atas ekstrak pala juga menunjukkan pengurangan penghasilan TBARS value dan ini bermaksud bahawa ekstrak dapat mengekalkan kestabilan pengoksidaan lemak daging lembu pada kedua-dua suhu. Pada kepekatan 1.25% dan ke atas ekstrak menunjukkan dapat mengekalkan warna merah ( $a^*$ ) daging lembu apabila dibandingkan dengan daging perap dan tidak diperap. Nilai pH bagi sampel yang diperap dan tidak diperap memberi perbezaan yang ketara ( $p < 0.05$ ). Bagi

ujian tekstur daging lembu keputusan menunjukkan terdapat perbezaan yang ketara dibandingkan pada sampel yang diperap dan tidak diperap bermula pada hari ke 7 penyimpanan. Pada kepekatan 0.65% dan ke atas ekstrak dapat mengekalkan tekstur semasa penyimpanan. Bagi analisis proksimat, tiada perbezaan ketara dari segi kandungan protein dalam semua sampel. Walaubagaimanapun, kepekatan ekstrak pala mempengaruhi kandungan lemak dan kelembapan dengan menunjukkan perbezaan ketara ( $p < 0.05$ ). Secara keseluruhan, kepekatan 1.25% ekstrak pala dipilih sebagai kombinasi terbaik untuk diaplikasikan kepada daging lembu berdasarkan skor tertinggi ujian penilaian deria dan semua hasil analisis bagi memelihara kualiti dan keselamatan daging dan juga mengekalkan sifat organoleptik.

## ACKNOWLEDGEMENTS

Bismillahirrahmanirrahim,

Alhamdulillah. Thanks to Allah SWT, who with His willing giving me the opportunity and strength to complete this research project entitled **Antimicrobial activity of Nutmeg (*Myristica fragrans* Houtt.) and sensory attribute of beef treated with the nutmeg extract.**

I would like to express my heartiest gratitude and appreciation to my supervisor, Associate Prof. Dr. Yaya Rukayadi, for his supervision, helpfull, advises, suggestions and effective comments throughout this research. Appreciation also is extended to my co-supervisor, Associate Prof. Dr. Faridah Abas towards the success in completing this study.

Sincere thanks to my laboratory mates, Nurul Syazwani, Lau Kah Yan, Ying Ling Nurul Husna, Sylvester, Aimi Syazana and Alyani for their generous help. Thank you to all laboratory assistants and staff from Biochemistry, Microbiology, Food Engineering and Processing lab, En. Zulkefli, Cik Asikin, Cik Fateahah, En. Azman, Pn. Hafizah, Pn. Rosmawati, Pn. Asmawati, En. Amran and Pn. Jamilah for their outstanding technical help.

My heartfelt and special gratitude to my parents, Prof. Dr. Zakaria bin Mohd Amin and Puan Marianna Istiati, family members and friends for their love, care, and understanding. Last but not least, I would like to thank my husband, Wan Muhammad Iqra', who offered me tremendous support, encouragement throughout my years in UPM.



I certify that a Thesis Examination Committee has met on **22 May 2015** to conduct the final examination of **Maya Puteri Malina bt. Zakaria** on her master thesis entitled **Antimicrobial activity of Nutmeg (*Myristica fragrans* Houtt.) and sensory attribute of beef treated with the nutmeg extract** in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [ P.U. (A) 106] 15 March 1998. Committee recommends that the student be awarded the Master of Science (Food Science).

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## LIST OF ABBREVIATIONS

|                         |                                         |
|-------------------------|-----------------------------------------|
| ANOVA                   | Analysis of Variance                    |
| ADP                     | Diphosphate                             |
| ATP                     | Triphosphate                            |
| CFU                     | Colony Forming Unit                     |
| <i>E. coli</i>          | <i>Escherichia coli</i>                 |
| GRAS                    | Generally Recognized Safe               |
| <i>L. monocytogenes</i> | <i>Listeria monocytogenes</i>           |
| <i>M. fragrans</i>      | <i>Myristica Fragrans</i>               |
| Mb                      | Deoxymyoglobin                          |
| Mbo                     | Oxymyoglobin                            |
| MetMb                   | Metmyoglobin                            |
| MIC                     | Minimum Inhibition Concentration        |
| MBC                     | Minimum Bactericidal Concentration      |
| MDA                     | Malonaldehyde                           |
| MHA                     | Mueller Hinton Broth                    |
| MHB                     | Mueller Hinton Agar                     |
| MS                      | Mass Analysis                           |
| TBARS                   | Thiobarbituric Acid Reactive Substances |
| TFC                     | Total Flavonoid Content                 |
| TPC                     | Total Plate Count                       |
| DIW                     | Deionized water                         |



## CHAPTER 1

### INTRODUCTION

#### 1.1 Background

Beef is a highly perishable food and need special protection to extend its shelf life. Microbial growth is a major concern for consumer and food producer because microorganisms are potentially can cause foodborne illness. Foodborne pathogens can grow in food due to its sufficient nutrients that can support many types of microorganism. These microorganisms can contribute undesirable reactions changes such as in odour, flavor, colour and textural properties (Ferguson et al., 2001). Storage condition such as temperature, water content and absence or presence of oxygen can be factors affecting the growth of microorganism in foods (Malikarjuna and Mittal, 1996).

Rancidity and deterioration of colour are the most common problems during storage (Delaquis et al., 1999; Tan and Chen, 2005). Rancidity is lipid oxidation reaction that occurs when the meat is stored at extended time in the presence of oxygen. During distribution and display, beef meat is exposed to the oxidation; oxymyoglobin to metmyoglobin which leads to the discoloration of beef meat (Mancini and Hunt, 2005). Beef colour influences consumer preferences of purchasing beef product where bright red looking fresh beef is indicator of wholesomeness.

Several preservations techniques have been used to improve the beef freshness including heat treatment, salting, and acidification (Davidson and Taylor, 2007). However, these techniques can cause deterioration of nutrient value and safety of the food (Annalisa et al., 2012). Nitrate is one of the common additives in beef meat to provide some benefits such as reducing microbial growth and enhance the red color of the meat. Regardless of benefits, nitrate can react with amines under circumstances of low pH and high temperature, which then form carcinogenic compound, nitrosamines (Bingham et al., 2002). In recent years, demands of minimal processing and free-synthetic preservatives are increasing because of growing concern among consumers regarding the safety issues of additives in food industry. Thus, food treated with natural preservatives has turn to very popular to inhibit microorganism. There are several ways of use of natural additives, can be directly added in product formulation such as coating, spraying or dipping on its surface of the food (Valeria and Pamela, 2011).

Natural active compound are mainly derived from plants such as bay leaves, lemongrass, clove and basil, and also from animals sources. Plant essential oil has gained high interest in food industry for their potential antimicrobial agent as they are highly accessible, generally safe and free from chemicals (Burt, 2004; De Oliveira et al., 2011). The active compounds in plants essential oil had already been established and frequently studied from time to time due to broad scope of antimicrobial which against foodborne pathogens and spoilage bacteria.

Nutmeg (*Myristica fragrans* Houtt.) is a dried seed kernel, commonly used as spices in cooking for flavoring and aroma. Traditionally, nutmeg has been used as traditional remedy herbs for kidney stones, muscle pain and dental carries (Ashish et al., 2013). Antimicrobial and antioxidant properties were studied as reported nutmeg contain macelignan, myristicin, eugonel,  $\alpha$ -pinene,  $\beta$ -pinene (Dorman et al., 2000; Rukayadi et al., 2008a). The main reason to choose nutmeg for this study is that, in spite of its cooking use as well as its antimicrobial and antioxidant properties that were evaluated, there are no analysis on antioxidant and antibacterial potential of nutmeg extract on raw beef or any beef product. Therefore, the aim of this study is to evaluate the effect of nutmeg extract on safety and quality of beef during storage at -18°C and 4°C.

## 1.2 Objectives

1. To determine the susceptibility of nutmeg (*Myristica fragrans* Houtt.) extract on eight species foodborne pathogens and microbial growth of beef at different concentration and storage.
2. To determine the effect of nutmeg (*Myristica fragrans* Houtt.) extract on sensory attributes of beef meat at different concentration and storage.
3. To examine the acceptance of consumer towards nutmeg (*Myristica fragrans* Houtt.) applied on treated beef meat at different concentration via sensory evaluation test.

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