UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL PROPERTIES OF DIFFERENT TYPES OF HONEY AND EFFECTS OF TUALANG HONEY AS A MARINATING AGENT AND VACUUM PACKAGING ON CHICKEN

KHADRA YOUSUF MOHAMED

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By

KHADRA YOUSUF MOHAMED

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

April 2018

DEDICATION

This thesis is dedicated to my mother Shifa Mohamed, my Father Yousuf Mohamed Fatah and my brother Gabriel Yousuf Mohamed for their endless love, support and encouragement!

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

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Chairman : Nor Khaizura Mahmud @ Ab Rashid, PhD
Faculty : Food Science and Technology

The antimicrobial activity of honey is mainly credited to its acidity, osmolarity and enzymatic generation of hydrogen peroxide via glucose oxidase. Additional honey components, such as aromatic acids or phenolic compounds, might also contribute to the overall antimicrobial activity. The level of antimicrobial activities found in honey varies with different types of honey, and these differences are mainly due to the composition, percentage as well as the nature of the sugars present in the honey. Chicken meat is widely consumed, and it is the most common and popular poultry species in the world. The diverse nutritional composition of chicken meat makes it an ideal environment for the growth and propagation of meat spoilage microorganisms and common foodborne pathogens. It is therefore essential that adequate preservation is applied to maintain its safety and quality. Due to the widespread of antibiotic resistance there is a need to replace modern chemical medicines and preservatives with safer natural remedies to increase the shelf life of chicken. The antimicrobial properties of local (Malaysia) honey are still very limited. Chicken is a perishable commodity, application of honey as a natural preservative could increase the shelf life. The aims of this study were therefore (i) to evaluate the antimicrobial activity of four types of honey namely Tualang Honey (TH_1), Tualang Honey (TH_2), Acacia Honey (AH) and Yemeni Sumur Honey (YSH), and (ii) to evaluate the effect of honey marinated chicken in different packaging on the microbiological and physicochemical characteristics. Nine bacterial strains were used. Disc Diffusion Assay, Well Diffusion Assay, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and time-kill methods were performed to reveal the antimicrobial potentials of the honey samples. The diameter of inhibition zone (DIZ) was between 11.17 mm to 35.50 mm for 12.5, 25 and 50% (w/v), respectively. The MIC ranged between 12.5 to 50% for both TH_1 and YSH while for TH_2, and AH it ranged between 25 to 50%. For MBC, it ranged from 25 to 50%. The time-kill with

TH_1 of *Staphylococcus aureus* (food isolate) was 6 h in 2 × MIC and for *S. aureus* ATCC 29737 was 3.84 log CFU/g at 6 h. For TH_2 and AH, the time-kill was decreased from 7 to 5 log CFU/g. While for YSH *S. aureus* (food isolate) at 2 × MIC was decreased from 6.42 to 3.68 log CFU/g in 6 h, for *S. aureus* ATCC 29737 at 1× MIC it was decreased from 6.66 to 5.16 log CFU/g and for the 2 × MIC it reached 4.26 log CFU/g at 6 h, for *Salmonella* Typhimurium ATCC 13311 at 2 × MIC was decreased from 6.56 to 3.83 lo CFU/g at 6 h. Physicochemical quality of honey resulted as follows: the pH of the honey samples were acidic ranging from 3.69 to 3.94, and the a_w of the honey samples were between 0.53 to 0.69. For colur analysis, maximum lightness and yellowness were seen in YSH, and maximum redness was seen in TH_1, while AH had minimum lightness, redness, and yellowness. For the marinated chicken at different temperatures (25, 10 and 5°C) overall 5°C with vacuum packaging showed increased shelf life and less microbial count for *S. aureus*, *Salmonella* Typhimurium and *Escherichia coli*. While *Listeria monocytogenes* was not detected in any of the marinated chicken. Therefore, honey marinated chicken with vacuum packaging (HCVP) treatment might be used as an alternative preservative method for storage of chicken and could be recommended to be used for other poultry. The remarkable inhibitory activity of honey might attribute them as potential antimicrobial agent and natural preservative.

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

SIFAT ANTIMIKROB TERHADAP PELBAGAI JENIS MADU DAN KESAN MADU TUALANG SEBAGAI PERAPAN AYAM DALAM PEMBUNGKUSAN KEDAP UDARA

Oleh

Khadra Yousuf Mohamed

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Pengerusi : Nor Khaizura Mahmud @ Ab Rashid, PhD
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Aktiviti antimikrob madu sebahagian besar datang dari keasidan, keosmolaran dan penjanaan enzim hidrogen peroksida melalui glukosa oksidase. Komponen tambahan seperti asid aromatik ataupun sebatian fenolik juga menyumbang kepada aktiviti antimikrob tersebut. Tahap aktiviti antimikrob berbeza mengikut jenis madu dan perbezaan ini bergantung kepada komposisi, peratusan dan juga kandungan gula semulajadi di dalam madu. Daging ayam digunakan secara meluas dan merupakan jenis daging yang paling popular serata dunia. Kandungan nutrisi yang pelbagai di dalam daging ayam menjadikannya tapak tumbesaran yang sesuai bagi mikroorganisma perosak daging dan patogen bawaan makanan. Maka, pengawetan adalah sangat penting untuk mengawal kualiti dan keselamatan daging ayam. Oleh kerana penyebaran rintangan antibiotik, wujud keperluan untuk menggantikan ubatan dan pengawet berasaskan kimia dengan alternatif yang lebih selamat dan semulajadi. Sifat antimikrobial madu tempatan (Malaysia) masih lagi sangat terhad. Ayam merupakan komoditi yang mudah rosak maka penggunaan madu sebagai bahan pengawet semulajadi boleh meningkatkan jangka hayat tersebut. Tujuan kajian ini ialah (i) untuk mengkaji aktiviti antimikrob empat jenis madu iaitu Madu Tualang (TH_1), Madu Tualang (TH_2), Madu Akasia (AH) dan Madu Yemeni Sumur (YSH), dan (ii) untuk mengkaji kesan penggunaan madu sebagai perapan dan pembungkusan vakum ke atas ciri-ciri mikrob dan kimia fizik ayam mentah. Sembilan bakteria telah digunakan. Kaedah *Disk Diffusion Assay*, *Well Diffusion Assay*, *Minimum Inhibitory Concentration* (MIC), *Minimum Bactericidal Concentration* (MBC) dan *time-kill* dijalankan untuk mendedahkan potensi antimikrob di dalam madu. Diameter Zon Perencatan (DIZ) adalah di antara 11.17 mm ke 35.50 mm untuk 12.5, 25 dan 50%. Julat MIC adalah di antara 12.5 ke 50% untuk TH_1 dan YSH. Manakala untuk TH_2 dan AH di antara 25 ke >50%. Julat MBC adalah di antara 25 ke 50%. *Time-kill S. aureus* (sampel makanan) di dalam TH_1 menunjukkan jumlah perencatan pada jam ke-6 pada 2 × MIC dan *S. aureus* ATCC 29737 adalah 3.84 log CFU/g. TH_2 dan AH menunjukan penurunan dari 7 ke 5 log CFU/g. Manakala, YSH

S. aureus (sample makanan) di 2 × MIC menurun dari 6.42 ke 3.68 log CFU/g pada jam ke-6, *S. aureus* ATCC 29737 di 1 × MIC berkurang dari 6.66 ke 5.16 log CFU/g. 2 × MIC mencapai 4.26 log CFU/g pada jam ke-6, S. Typhimurium ATCC 13311 di 2 × MIC berkurang dari 6.56 ke 3.83 log CFU/g. Kualiti kimia fizik madu adalah seperti berikut: pH madu adalah di antara 3.69 ke 3.94, a_w di antara 0.53 ke 0.69. Bagi analisis warna, tahap kekuningan maksimum dapat dilihat pada YSH, manakala tahap kemerahan maksimum dapat dilihat pada TH_1. Manakala, AH menunjukkan tahap kecerahan, kemerahan dan kekuningan yang minimum. Untuk ayam perapan madu pada suhu yang berbeza (25, 10 dan 5°C), keseluruhan menunjukkan ayam yang dibungkus mengunakan pembungkus vakum pada suhu 5°C menunjukkan peningkatan jangka hayat dan kurang bilangan mikrob seperti *S. aureus*, *Salmonella* Typhimurium dan *Escherichia coli*. *Listeria monocytogenes* langsung tidak dapat dikesan di dalam semua ayam yang diperap. Oleh itu, ayam yang diperap bersama madu mengunakan pembungkus vakum (HCVP) mungkin dapat digunakan sebagai alternatif untuk menyimpan ayam dan dapat diaplikasikan pada jenis daging yang lain. Aktiviti antimikrob madu yang tinggi membuatkan madu berpotensi sebagai agen antimikrob dan pengawet semulajadi.

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I certify that a Thesis Examination Committee has met on 18 April 2018 to conduct the final examination of Khadra Yousuf Mohamed on her thesis entitled "Antimicrobial Properties of Different Types of Honey and Effects of Tualang Honey as a Marinating Agent and Vacuum Packaging on Chicken" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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LIST OF ABBREVIATIONS

%	Percentage
a*	Redness
AH	Acacia Honey
ANOVA	Analysis of Variance
a_w	Water activity
b*	Brightness
CFU/g	Colony Forming Unit/gram
DIZ	Diameter of Inhibition Zone
FDA	Food and Drug Administration
g/mL	Gram Per Milliliter
GMP	Good Manufacturing Practice
GRAS	Generally Recognized as Safe
HCNP	Honey marinated chicken with normal packaging
HCVP	Honey marinated chicken with vacuum packaging
HE	Hektoen Enteric Agar
HMF	Hydroxymethylfurfural
L*	Lightness
MBC	Minimum Bactericidal Concentration
MGO	Methylglyoxal
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
TBARS	2-thiobarbituric acid reactive substances
TH_1	Tualang Honey
TH_2	Tualang Honey
TPC	Total Plate Count

UMF	Unique Manuka Factor
VP	Vacuum Packaging
VRE	Vancomycin Resistant Enterococci
w/v	Weight Per Volume
WHO	World Health Organization
YSH	Yemeni Sumur Honey

CHAPTER 1

INTRODUCTION

Honey is the oldest sweetener in the world. It was used since the ancient times. Honey is a sweet a viscous fluid that has pleasant aroma and taste which is produced by bees from flower nectar or plant secretion. Honey bees gather, modify and also add other specific substances of their own to ripen and mature the honey which then be eaten by humans and some animals as an energy food since the honey's simple sugars can be directly absorbed into the bloodstream without further digestion (Bogdanov, 1997). The color and flavor of honey are impacted by the flower source. The colors range from pale yellow to dark amber. In general, the darker the honey color, the stronger its flavor. Honey is a super saturated solution of sugars (mainly fructose and glucose), acids, vitamins, minerals and other minor component (Al-Nahari et al., 2015). Other sugars like sucrose and maltose are also present but in lesser quantity (Khalil et al., 2010).

According to previous report, the chemical composition of honey is complex which includes sugars, vitamins, minerals, proteins, 5-hydroxymethylfurfural (HMF), enzymes, phenolic acids, flavonoids and volatile compounds (Khalil et al., 2010). Honey also contains a variety of phenolic compounds which are good sources of antioxidants, thereby making honey a functional additive and increasing its potential use in medicine. The antimicrobial or the antibacterial activity of honey is mainly attributed to its acidity, osmolarity, hydrogen peroxide and phenolic compounds (Mundo et al., 2004). Honey also has been used for pain relief and healing of burn victims. The sugars in honey nourish healthy cells and help support the development of new white blood cells. Honey's antioxidants, amino acids, and vitamins play a role in reducing inflammation. The antibacterial compounds of honey rapidly kill the pathogens that cause typhoid fever, bacterial pneumonia, strep throat (streptococcal pharyngitis) and bacterial dysentery (David, 2005). The quality and safety of honey are mainly influenced by the presence of microorganisms in it. Bee products, including honey, are contaminated through various sources.

The environmental contaminants could be bacteria, heavy metals, pesticides and radioactive materials. The microorganisms found in honey and honeycomb are bacteria such as *Citrobacter, Clostridium, Bacillus, Pseudomonas, Escherichia coli, Klebsiella, Corynebacterium, Acinetobacter* and *Enterobacter*; yeasts such as *Saccharomyces* and *Torulopsis*; and molds such as *Aspergillus* and *Penicillium* (Olaitan et al., 2007; Snowdon & Cliverb, 1996). Due to its antimicrobial property and its ability to inhibit foodborne pathogens and food spoilage microorganisms (Mundo et al., 2004; Taormina et al., 2001), honey can serve as a natural food preservative in combatting microbial spoilage of foods. In terms of chemical spoilage, it has also been stated by many researchers that honey could reduce the enzymatic browning of fruits, and also prevent the lipid oxidation in meat (Taormina et al., 2001; Mundo et al., 2004).

Raw chicken meats are especially prone to microbial contamination and spoilage. Inadequate storage of poultry meat by the consumers is also linked to the occurrence of foodborne human infections since poultry products are highly perishable foods. Depending on the degree of processing following slaughter, their spoilage varies between four and ten days under refrigeration. Quality, including taste, color, freshness and tenderness of chicken meat are the major components of consumer satisfaction and the major marketing focus point by chicken processors. Therefore, honey which has an antimicrobial activity is seen as capable of improving the quality attributes and extending the shelf life of raw poultry products (Dave & Ghaly, 2011).

Therefore, the problem statement, hypothesis and objectives of this study are stated as follows;

Problem statement

- The antimicrobial properties of local (Malaysia) honey are still very limited
- Chicken is a perishable commodity, application of honey as a natural preservative could increase the shelf life

Hypothesis

- A certain concentration of honey leads to decreased in bacterial count and increased in shelf life of chicken meat

Objectives of this study

- To determine the *in vitro* antimicrobial activity of Tualang Honey 1 (TH_1), Tualang Honey 2 (TH_2), Acacia Honey (AH) and Yemeni Sumur Honey (YSH) against foodborne pathogens and bacteria
- To evaluate the effect of honey marinated chicken in different packaging on the microbiological and physicochemical characteristics of the chicken

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