



UNIVERSITI PUTRA MALAYSIA

**ANTIMICROBIAL ACTIVITY OF CITRUS FRUITS AND EFFECTS OF KEY
LIME (*Citrus aurantifolia* Christm. & Panzer) JUICE EXTRACT ON
QUALITY CHANGES OF WHITE SHRIMP (*Penaeus vannamei* Boone)**

SELVI VELU

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

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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 CITRUS FRUITS AND EFFECTS OF
KEY LIME (*Citrus aurantifolia* Christm. & Panzer) JUICE EXTRACT ON
QUALITY CHANGES OF WHITE SHRIMP (*Penaeus vannamei* Boone)**

By

SELVI VELU

January 2014

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Faculty : Food Science and Technology

The demand for novel antimicrobial agents and natural preservatives from natural resources has increased worldwide particularly in the food preservation field. In this study, antimicrobial activity of musk lime (*Citrus microcarpa*), key lime (*Citrus aurantiifolia* Christm. & Panzer) and lemon (*Citrus limon*) extracts were evaluated against various food borne pathogens and spoilage bacteria using the disc diffusion test. The antimicrobial activity was evaluated at 50% and 100% concentration level using various extracts. Alternatively, no information was available regarding application of key lime (KL) juice extract as a preservation agent for white shrimp (*Penaeus vannamei* Boone). Hence, this study further aimed to investigate the effect of the key lime juice extract (100% concentration level) in combination with vacuum packaging (VP) and storage bag (SB) on microflora and biochemical changes in white shrimp stored at 2 ± 1 °C. Microbiological and biochemical analysis were carried out at 3 days interval till the 12th day of storage. Microbiological analyses were performed for the total mesophilic aerobic count, total mesophilic anaerobic count, total aerobic psychrotrophic count, proteolytic bacterial count, histamine, putrescine and cadaverine producer count. Proximate content on 0 and 12th day of storage, color changes of cephalothoraxes, pH and total volatile base nitrogen (TVBN) was the biochemical analysis performed. For the antimicrobial activity of various extraction solvent of musk lime, key lime and lemon, ethanol extracts exhibited remarkable diameter of inhibition zone (DIZ) compared to water and juice extract towards all of the tested microorganisms. Largest DIZ were obtained using ethanol extract of musk lime (39.7 mm), key lime (26.7 mm) and lemon (32.0 mm) particularly against *Aeromonas veronii* at 100% concentration level. As for juice extract, KL juice extracts revealed prominent antimicrobial activity compared to musk lime and lemon juice extracts. Accordingly, white shrimps treated with KL juice extract (100% concentration level) in combination with VP exhibited significant ($p<0.05$) lower count for all of the microbiological analysis performed. As for the total aerobic

mesophilic count, significant ($p<0.05$) lower counts obtained in VP+KL, VP and SB+KL samples. Prominent total proteolytic counts were obtained from the 9th day of storage onwards in VP+KL and SB+KL samples. Similarly putrescine and cadaverine former counts were significantly lower in VP+KL and SB+KL samples. On the whole, VP+KL treated samples revealed a lowest bacterial count in the range of 4.37 to 4.91 log cfu/ g at 12 day of storage. As for proximate content, VP+KL samples retained highest percentage of protein on the 12th day of storage. Moreover, VP+KL and VP samples showed significant ($p<0.05$) results for the color changes of cephalothoraxes starts from the 6th day of storage. Besides, significantly lower TVBN content was obtained in VP+KL (5.33 mg N/ 100g) followed by VP (6.03 mg N/ 100g) samples on the 12th day of storage. Therefore, VP+KL treatment might be used as an alternative preservative method for post mortem storage of white shrimp and may recommend for quality preservation of other fishery products. The remarkable inhibitory activity of musk lime, key lime and lemon extracts of various extraction solvents (ethanol, water and juice) may attribute them as potential antimicrobial agents and natural preservatives as well.

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

AKTIVITI ANTIMIKROB BUAH CITRUS DAN KESAN JUS LIMAU NIPIS
(*Citrus aurantiifolia* Christm. & Panzer) PADA PERUBAHAN KUALITI
UDANG PUTIH (*Penaeus vannamei* Boone)

Oleh

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Januari 2014

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Permintaan untuk bahan antimikrob baru dan bahan pengawet semula jadi daripada sumber semula jadi telah meningkat di seluruh dunia terutamanya dalam bidang pengawetan makanan. Dalam kajian ini, limau kasturi (*Citrus microcarpa*), limau nipis (*Citrus aurantiifolia* Christm. & Panzer) dan lemon (*Citrus limon*) telah dinilai aktiviti antimikrob terhadap pelbagai patogen makanan dan bakteria perosak makanan menggunakan ujian penyebaran cakera. Aktiviti antimikrob telah dinilai pada tahap kepekatan 50% dan 100% menggunakan pelbagai pelarut pengekstrakan' etanol (gred makanan), air dan jus. Selain itu, tiada maklumat boleh didapati mengenai penggunaan ekstrak jus limau nipis (KL) sebagai bahan pengawetan udang putih (*Penaeus Vannamei* Boone). Oleh itu, kajian ini selanjutnya telah bertujuan untuk mengkaji kesan utama ekstrak jus limau nipis (tahap kepekatan 100%) kombinasi dengan pembungkusan vakum (VP) dan beg penyimpanan (SB) pada perubahan mikroflora dan biokimia dalam udang putih yang disimpan pada $2 \pm 1^\circ\text{C}$. Analisa mikrobiologi dan biokimia telah dijalankan pada 3 hari selang hingga ke hari ke-12 penyimpanan. Analisis mikrobiologi telah dijalankan untuk jumlah kiraan mesofil aerob, jumlah kiraan anaerob mesofil, jumlah kiraan psikotrof aerob, kiraan bakteria proteolitik, histamin, putrescin dan cadaverin. Kandungan proksimat pada hari 0 dan 12, perubahan warna cephalothoraxes, pH dan jumlah nitrogen asas meruap (TVBN) adalah analisis biokimia yang telah dilakukan. Untuk aktiviti antimikrob dengan penggunaan pelbagai pelarut kasturi limau, limau nipis dan lemon, ekstrak etanol telah mempamerkan diameter zon perencutan yang luar biasa berbanding dengan ekstrak air dan jus terhadap semua mikroorganisma yang diuji. Diameter zon perencutan yang terbesar telah ditunjukkan oleh ekstrak etanol daripada limau kasturi (39.7 mm), limau nipis (26.7 mm) dan lemon (32.0 mm) terutamanya terhadap *Aeromonas veronii* pada tahap kepekatan 100%. Bagi jus ekstrak pula, ekstrak jus limau nipis telah mendedahkan aktiviti antimikrob yang menonjol berbanding ekstrak jus limau kasturi dan lemon. Oleh itu, udang putih yang telah dirawat dengan ekstrak jus limau nipis (paras kepekatan 100%) dengan

kombinasi pek vakum (VP) telah mempamerkan keputusan yang signifikan ($p<0.05$) yang lebih rendah untuk semua analisis mikrobiologi. Bagi jumlah kiraan mesofil aerob, bilangan yang signifikan ($p <0.05$) lebih rendah telah diperolehi dalam sampel udang putih dalam pek VP+KL, VP dan SB+KL. Jumlah kiraan proteolitik terkemuka diperolehi dari hari ke-9 dan seterusnya dalam sampel udang putih di pek VP+KL dan SB+KL. Begitu juga putrescin dan cadaverin, kiraan bakteria adalah lebih rendah dalam sampel di VP+KL dan SB+KL. Secara keseluruhannya, sampel udang putih yang telah dirawat dalam keadaan VP+KL telah menunjukkan kiraan bakteria paling rendah dalam julat $4.37 - 4.91 \log \text{cfu/g}$ pada hari ke 12 penyimpanan. Bagi kandungan proksimat, sampel dalam VP+KL telah mengekalkan peratusan tertinggi protein pada hari ke-12 penyimpanan. Selain itu, sampel VP+KL dan VP telah menunjukkan hasil yang signifikan ($p <0.05$) bagi perubahan warna cephalothoraxes bermula dari hari ke 6 penyimpanan. Selain itu, kandungan TVBN jauh lebih rendah telah diperolehi dalam VP+KL (5.33 mg N / 100g), diikuti oleh VP (6.03 mg N / 100g) sampel pada hari ke-12 penyimpanan. Oleh itu, rawatan udang putih dalam keadaan VP+KL boleh digunakan sebagai satu kaedah pengawet alternatif untuk penyimpanan udang putih dan boleh mencadangkan untuk pemuliharaan kualiti produk perikanan yang lain. Aktiviti perencutan luar biasa daripada ekstrak limau kasturi, limau nipis dan lemon melalui pelbagai pelarut pengekstrakan (etanol, air dan jus) boleh menyifatkan mereka sebagai bakal agen antimikrob dan juga sebagai bahan pengawet semulajadi.

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I certify that a Thesis Examination Committee has met on 15 January 2014 to conduct the final examination of Selvi a/p Velu on her thesis entitled "Antimicrobial Activity of Citrus Fruits and Effects of Key Lime (*Citrus aurantifolia* Christm. & Panzer) Juice Extract on Quality Changes of White Shrimp (*Penaeus vannamei* Boone)" 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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DECLARATION

Declaration by the student

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