UNIVERSITI PUTRA MALAYSIA

DETECTION AND QUANTIFICATION OF ALCOHOL COMPOUNDS IN FOODS AND BEVERAGES USING STATIC HEADSPACE-GAS CHROMATOGRAPHY/ MASS SPECTROMETRY.

LAW SE VERN

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By

LAW SE VERN

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

February 2012

Dedicated to my beloved parents, siblings and friends for their endless love and support

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: Professor Fatimah Abu Bakar, PhD

Institute: Halal Product Research Institute

Foods serve as a basic requirement for human survival and play an important role in society from religious and cultural perspectives. In Islam, the cardinal rule is that any substance capable of intoxicating human when consumed is unlawful (*haram*) be it in small or large quantity.The objective of the present study wasto develop a standard method for quantification of ethanol and other alcohols using Static Headspace-Gas Chromatography/ Mass Spectrometry (HS-GC/MS) in complex food matrices. The study also examined the ethanol contentsfrom various types of food and beverage available atthe local market. Ethanol land higher alcohols in alcoholic beverages were also analyzed and quantified.An incubation temperature of 80 ^{o}C for 15 minutes (for 5 mL of liquid samples) and 30 minutes for 5 g solid samples gave an optimum headspace extraction. The extraction

efficiency of samples with different sugar, salt and acid concentrations were determined. Headspace extraction efficiency showed no significant difference ($p<0.05$) between standard ethanol solution and solutions containing less than individually 5 % salt, sugar and acid. The method for determination of ethanol and higher alcohols contents in liquid and solid food samples using Static HS-GC/MS was also validated. For liquid samples, the internal standard calibration method was used and linearity ($r > 0.99$) in the range from 5 to 4,000 µg/mL of ethanol was observed. Results obtained indicated that the method was reliable with good recoveries (an average of 99.3%) and relative standard deviation (RSD) < 5.2. The standard addition method was used in the quantification of ethanol from solid foods. Ethanol concentrations from samples were classified into four; trace, low, medium, and high. In this study, three spiked levels within the linearity range for each level were added tothe samples. The concentration of ethanol spiked into solid food samples ranged from 1 mg/kg sample to 5240 mg/kg sample. RSD of 2.39 and 3.94 were obtained for intraday and interday repeatability tests. A total of 154 samples including fermented foods, carbonated drinks, juices and cordials, tea and coffee, energy drinks, colouring and flavourings, vinegars and sauces were analyzed. Of the total number of samples screened, 106 were found to contain ethanol which ranged from 5 to 33919 ppm. Twenty-five commercial (industrially manufactured and taxed) and two home-made alcohol products in Malaysia

were also collected and analyzed for volatile compounds. The quantification of higher alcohols including 1-propanol, 2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol were carried out. The concentrations of higher alcohols in alcoholic beverages ranged from 5 to 331 ppm. The presence of ethanol in a food product is either through fermentation or added as processing aid.The standard method for quantification of low ethanol concentration in a complex food matrix is crucial for the development of *halal* food analysis. The results of this study also provided a database of the ethanol contents in various foods and beverages. The presence of the other higher alcohols in *khamr*is also highlighted.The issue of contamination of prohibited substances such as *khamr* has to be resolved through utilizing advanced scientific techniques as tools for inspection and analysis where even trace amounts of contamination is essential and crucial to assure that *halal* requirements are met. This will in turn increase competitiveness of Malaysian food exporters as well as protect the rights of consumers.

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

PENENTUAN KANDUNGAN ALKOHOL DALAM PELBAGAI MAKANAN DAN MINUMAN DENGAN KAEDAH PENGEKSTRAKAN RUANG KEPALA KROMATOGRAFI GAS-SPEKTROMETER JISIM.

Oleh

LAW SE VERN

Februari 2012

Chairman: Professor Fatimah Abu Bakar, PhD

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Sepertimana yang diketahui, makanan bukan sahaja merupakan keperluan asasi bagi manusia, malah memainkan peranan yang penting dalam agama, social dan budaya sesebuah masyarakat. Undang-undang Islam mengharamkan pengambilan khamr iaitu sesuatu bahan yang boleh memabukkan termasuk minuman keras, dadah, minuman tapai kismis, dan lain-lain. Tujuan kajian ini adalah untuk mengoptimumkan parameter dalam pengekstrakan ruang kepala static dan untuk membangunkan dan mengesahkan satu kaedah piawai untuk menentukan kandungan etanol dalam pelbagai matriks sampel makanan. Kajian turut dijalankan bagi mengenalpasti kandungan etanol pelbagai jenis makanan dan minuman

yang terdapat di pasaran tempatan. Alkohol-alkohol alifatik rendah dalam minuman keras juga dianalisis dan diukur.

Suhu pengekstrakan 80 ^{o} C selama 15 minit (untuk 5 mLsampe lcecair) dan 30 minit (untuk 5 g sampel pepejal) dibuktikan member kadar pengekstrakan ruang kepala yang optimum untuk etanol dalam sampel makanan. Kecekapan pengekstrakan kaedah ruang kepala static pada sampel dengan kepekatan gula, garam dan asid yang berbeza juga dikaji. Pengekstrakan etanol dengan ruang kepala static tidak menunjukkan sebarang perbezaan yang ketara di antara larutan piawai etanol dan larutan piawai etanol yang mengandungi peratus garam, gula dan asid kurang daripada 5 %masing masing, pada $p < 0.05$.

Kaedah untuk menentukan kandungan etanol dalam sampel makanan cecair dan pepejal dengan menggunakan pengekstrakan ruang kepala static kromatografi gas-spektrometer jisim turut dibangunkan. Kaedah penentukuran piawaian dalaman telah digunakan untuk sampel cecair. Kelinearan ($r > 0.99$) dapat diperhatikan dalam julat dari 5 hingga 4000 µg/ml etanol. Keputusan kajian membuktikan bahawa kaedah yang dibentangkan adalah berkesan dan menyakinkan, dengan kadar pemulihan yang baik (purata 99.3%) dan sisihan piawai relative <5.2. Selain itu, kaedah penambahan piawai digunakan untuk penentuan kandungan etanol dalam

sampel makanan pepejal. Kajian mengkategorikan kepekatan etanol dalam sampel kepada empat tahap, mengesan, rendah, sederhana dan tinggi. Dalam kajian tersebut, tiga larutan piawai dalam julat kelinearan dengan isipadu yang meningkat ditambah kepada sampel dalam vial. Kepekatan larutan piawai etanol yang ditambahkan ke dalam sampel pepejal adalah dari 1.24mg/kg sampel kepada 5240 mg/kg sampel. Kaedah penambahan piawai menunjukkan sisihan piawai relative 2.39 dan 3.94 masing- masing bagi ujian kebolehulangan harian dan kebolehulangan dalam 3 hari berturut-turut,.

Sejumlah 154 sampel termasuk makanan diperam, minuman berkarbonat, jus, the dan kopi, minuman bertenaga, pewarna dan perasa, cuka dan sos telah dianalisis dalam kajian ini. Daripada jumlah sampel diperiksa, 106 didapati mengandungi etanol. Kandungan etanol dalam sampel dikenalpasti dalam lingkungan 5 - 33,919 ppm. Dua puluh tujuh minuman beralkohol komersial di Malaysia telah dikumpul dan dianalisis bagi sebatian yang tidak menentu. Penentuan kandungan sebatian alifatik alkohol yang rendah termasuk methanol, 1-propanol, 2-metil-1-propanol, 2-metil-1-butanol dan 3-metil-1-butanol telah dijalankan. Kandungan alifatik alcohol dalam minuman keras didapati dalam lingkungan 5-331 ppm.

Kehadiran etanol dalam produk makanan sama ada berlaku secara semulajadi melalui penapaian atau ditambah untuk membantu dalam pemprosesan. Kaedah yang dibangunkan dalam kajian ini mampu mengesah dan menentukan kandungan etanol yang rendah dalam pelbagai matriks makanan. Keputusan kajian menyediakan pangkalan data kandungan etanol dalam makanan dan minuman yang dijual di pasaran tempatan. Kehadiran alcohol alifatik yang lain dalam arak turut dikesan dalam kajian ini. Isu makanan halal dikontaminasi oleh unsur-unsur haram mampu diatasi dengan mengaplikasikan kemajuan sains dan teknologi dalam pengesanan dan penentuan kandungan kontaminan, yang mana aplikasi ini adalah penting untuk memastikan kesahihan status halal.

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I certify that aThesis Examination Committee has met on ……. to conduct the final examination of Law Se Vern on herthesis entitled "Detection and Quantification of Alcohol Compounds in Foods and Beverage Using Static Headspace Gas Chromatography/Mass Spectrometry" 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 degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is nor concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

LAW SE VERN

Date: 13 February 2012

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