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By

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

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ESPECIALLY DEDICATED TO MY BELOVED FAMILY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

CHARACTERIZATION, ANTIOXIDANT PROPERTIES AND AUTHENTICATION OF VIRGIN COCONUT OIL

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MARINA BINTI ABDUL MANAF

August 2009

Chairman: **Professor Yaakob bin Che Man, PhD**

Faculty: **Food Science and Technology**

A study on chemical properties and authentication of virgin coconut oil (VCO) was conducted. Chemical properties showed that commercial VCO had iodine value of 4.47 to 8.55, peroxide value of 0.21 to 0.57 meq oxygen/kg, free fatty acid of 0.15 to 0.25, saponification value of 250.07 to 260.67 mg KOH/g oil and anisidine value of 0.16 to 0.49. Lauric acid was the predominant fatty acid which ranged from 46.64 to 48.03%. Major triacylglycerol (TAG) were LaLaLa, LaLaM, CLaLa, LaMM and CCLa (La:lauric; C:capric; M:Myristic) which accounted for more than 80% TAG of the oil. Total phenolic content ranged from 7.78 to 29.18 mg GAE/g oil. VCO samples exhibited higher antioxidant activity (49.79 to 79.87%) compared to refined, bleached and deodorized (RBD) coconut oil (49.58%).

Comparison between different processing methods of VCO showed that VCO produced by fermentation method possessed the strongest scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) with the amount of oil necessary to decrease the initial DPPH radical concentration by 50% (EC_{50}) value of 1.24 mg/mL. VCO produced by fermentation method also exhibited the highest antioxidant activity of 71% while the highest reducing power of VCO produced by chilling method was 1.02 at 10 mg/mL. The results revealed that VCO produced by both fermentation and chilling method had higher antioxidant potency than RBD coconut oil. Total phenolic content was strongly correlated with radical scavenging capacity (r = 0.91) and reducing power (r = 0.96) while no correlation was observed for β-carotene bleaching test. Some phenolic acids found in VCOs were protocatechuic, vanillic acid, caffeic acid, syringic acid, coumaric acid and ferulic acid.

Rapid methods were developed to detect adulteration in VCO. First, Fourier transform infrared (FTIR) spectroscopy was used to detect adulteration of VCO with palm kernel olein. The results showed that FTIR was capable of detecting adulteration down to 1% adulteration level. Discriminant analysis using 10 principal components was able to classify pure and adulterated samples on the basis of their spectra. A partial least square (PLS) calibration demonstrated a good linear regression (R^2) of 0.9875 of actual value against FTIR predicted concentration of palm kernel olein. Discriminant analysis was also capable to distinguish between VCO and other vegetable oils.

Another rapid method, differential scanning calorimetry (DSC) was also used to determine adulteration of VCO with selected vegetable oils, namely soybean oil (SBO) from linolenic acid group, sunflower oil (SFO) from oleic-linoleic acid group and palm kernel oil (PKO) from lauric acid group. The heating curves of SBO and SFO adulterated samples demonstrated adulteration peaks appearing at the lower temperature region starting at 10% adulteration level. Regression analysis using stepwise multiple linear regressions (SMLR) was used to predict the percent of adulterant with R^2 of 0.9390 for SFO and 0.9490 for SBO. No adulteration peak was observed for PKO adulterated oils but a good relationship between the main exothermic peak height of PKO and percentage of adulteration was established with R^2 of 0.9454.

Finally, electronic nose with surface acoustic wave (SAW) sensor was used to detect adulteration in VCO with palm kernel olein. Qualitative analysis was made possible using VaporPrint™, which translated the sensor's response into visualized two dimensional image. Adulteration peaks were identified from chromatogram profile and the best relationship (R^2 = 0.9093) was obtained between adulterant peak F and the percentage of palm kernel olein added. Pearson correlation (r) of 0.92 was obtained between adulterant peak F and iodine value while correlation (r) of 0.89 was obtained between peroxide value and adulterant peak F. Principal component analysis (PCA) provided good separation of samples with 74% of the variation accounted for principal component 1 and 17% accounted for principal component 2. Excellent result

was obtained in the differentiation of pure and adulterated samples down to 1% detection limit.

In conclusion, this study provides references on chemical properties as well as presented the antioxidative potential of VCO. New methods were also developed for detection of adulteration of VCO with other oils using rapid analytical techniques.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENCIRIAN, SIFAT ANTIOKSIDA DAN AUTENTIKASI MINYAK KELAPA DARA

Oleh

MARINA BINTI ABDUL MANAF

Ogos 2009

Pengerusi: Profesor Yaakob bin Che Man, PhD

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Sifat kimia dan autentikasi minyak kelapa dara telah dikaji. Sifat kimia menunjukkan minyak kelapa dara komersil mempunyai nilai iodine 4.47 hingga 8.55, nilai peroksida di antara 0.21 sehingga 0.57 meq oksigen/kg, nilai asid lemak bebas di antara 0.15 sehingga 0.25, nilai saponifikasi di antara 250.07 sehingga 260.67 mg/KOH minyak dan nilai anisidin di antara 0.16 sehingga 0.49. Asid laurik merupakan asid lemak tertinggi dengan nilai kandungan daripada 46.64 hingga 48.03%. Triasilgliserida (TAG) paling tinggi terdiri daripada LaLaLa, LaLaM, CLaLa, LaMM dan CCLa (La: laurik, C: Kaprik dan M: Miristik) yang mewakili sebanyak 80% daripada keseluruhan TAG. Jumlah kandungan fenolik minyak kelapa dara di antara 7.78 sehingga 29.18 mg GAE/100g minyak. Minyak kelapa dara mempunyai aktiviti antioksidan (49.79 hingga 79.87%) lebih tinggi daripada minyak kelapa komersil (RBD) (49.58%).

Perbandingan di antara kaedah pemprosesan menunjukkan bahawa minyak kelapa dara yang dihasilkan daripada kaedah fermentasi mempunyai kuasa pelunturan ke atas DPPH paling tinggi dengan nilai EC_{50} 1.24 mg/mL. Minyak kelapa dara yang dihasilkan daripada kaedah fermentasi juga menunjukkan aktiviti antioksidan paling tinggi iaitu 71% manakala kuasa penurunan paling baik didapati pada minyak kelapa dara yang menggunakan kaedah pendinginan, dengan nilai pencerapan di antara 1.02 sehingga 10 mg/mL. Hasil kajian menunjukkan bahawa minyak kelapa dara yang dihasilkan daripada kedua-dua kaedah fermentasi dan pendinginan mempunyai potensi antioksidan lebih tinggi daripada minyak kelapa RBD. Korelasi juga menunjukkan jumlah kandungan fenolik mempunyai perhubungan yang kuat dengan kuasa pelunturan DPPH dan kuasa penurunan manakala korelasi tinggi ditunjukkan oleh ujian pelunturan β-karotena dan jumlah asid fenolik. Beberapa asid fenolik yang dijumpai di dalam minyak kelapa dara ialah asid vanillik, asid kaffeik, asid syringik, asid koumarik dan asid ferulik.

Seterusnya, kaedah pantas dibangunkan untuk mengesan penambahan bahan asing ke dalam minyak kelapa dara. Pertama, spektroskopi fourier transform Infra Merah (FTIR) spektroskopi telah digunakan untuk mengesan penambahan olein isirung sawit ke dalam minyak kelapa dara. Hasil kajian menunjukkan FTIR dapat mengesan kehadiran olein isirung sawit sehingga 1%. Analisis diskriminasi menggunakan 10 komponen utama berjaya memisahkan minyak kelapa dara asli dan minyak kelapa dara bercampur olein isirung sawit berdasarkan spektrum

minyak tersebut. Kalibrasi menggunakan 'Partial Least Square' menunjukkan persamaan linear yang baik dengan nilai R^2 bersamaan 0.9875. Analisis diskriminasi juga dapat membezakan di antara sampel minyak kelapa dara dan lain-lain minyak sayuran.

Satu lagi kaedah pantas, kalorimeter pengimbas pembezaan (DSC) juga digunakan untuk mengesan pencampuran di antara minyak kelapa dara dengan minyak sayuran terpilih, iaitu minyak kacang soya, yang merupakan minyak sayuran daripada kumpulan asid linolenik, minyak bunga matahari, yang merupakan minyak sayuran daripada kumpulan oleik-linoleik dan minyak isirung sawit daripada kumpulan asid laurik. Keluk pemanasan sampel yang dicampur minyak kacang soya dan minyak bunga matahari menunjukkan satu puncak baru (puncak pencampuran) muncul pada suhu rendah pada pencampuran 10%. Analisis regressi 'stepwise multiple linear regression (SMLR)' digunakan untuk meramalkan peratus pencampuran dengan nilai R^2 bersamaan 0.9390 dan 0.9490 untuk minyak kacang soya dan minyak bunga matahari. Tiada puncak baru diperhatikan pada minyak kelapa dara yang dicampur minyak isirung sawit tetapi terdapat korelasi yang tinggi di antara ketinggian puncak eksotermik utama dengan peratus pencampuran, dengan nilai R^2 bersamaan 0.9454.

Dalam kajian akhir, hidung elektronik dengan pengesan gelombang permukaan akustik (SAW) telah digunakan untuk mengesan pencampuran minyak kelapa dara dengan olein isirung sawit. Analisis kualitatif dapat diperhatikan dengan

menggunakan VaporPrint^{TM}, yang dapat menunjukkan tindak balas pengesan secara visual. Beberapa puncak yang menunjukkan pencampuran telah dikenalpasti dan perhubungan yang kuat diperolehi di antara puncak F dan peratus olein isirung sawit dengan nilai R^2 bersamaan 0.9093. Terdapat kolerasi yang tinggi (r) 0.92 di antara puncak F dan nilai iodin manakala korelasi (r) di antara puncak F dan nilai peroksida adalah 0.89. Analisis komponen utama (PCA) menghasilkan pemisahan yang baik di antara sampel minyak kelapa dara asli dan sampel yang dicampuri olein isirung sawit dengan 74% variasi diperuntukkan untuk komponen utama 1 dan 17% diperuntukkan untuk komponen utama 2. Tahap minima pengesanan pencampuran adalah sehingga 1%.

Sebagai kesimpulan, kajian ini memberi rujukan tentang sifat kimia dan menunjukkan potensi antioksidan yang terdapat pada minyak kelapa dara. Tambahan pula, kaedah pengesanan pencampuran minyak kelapa dara dengan minyak sayuran lain telah dibangunkan menggunakan teknik analitikal pantas.

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I certify that an Examination Committee has met on 11th August 2009 to conduct the final examination of Marina Binti Abdul Manaf on her thesis entitled "Characterization, Antioxidant Properties and Authentication of Virgin Coconut Oil" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be rewarded the Doctor of Philosophy

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirements for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follows:

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Date: 16 October 2009

DECLARATION

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

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MARINA BINTI ABDUL MANAF

Date: 24 August 2009

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