



UNIVERSITI PUTRA MALAYSIA

**EXTRACTION OF PHENOLIC COMPOUNDS FROM *Citrus hystrix*
LEAVES AND THEIR ANTIOXIDATIVE EFFECTS ON OIL AND MINCED
MEAT OF SARDINE (*Sardinella lemuru Bleeker*)**

MOHAMED ABDULKADIR GEDI

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By

MOHAMED ABDULKADIR GEDI

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

April 2011

DEDICATION

This work is dedicated to the soul of my mother, the secret behind all my achievements “may Allah’s peace, mercy and blessing be upon her”. The work is also dedicated to the love of my life, my dear wife for her continuing love and encouragements. You live deep inside me, I love you so much.



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

EXTRACTION OF PHENOLIC COMPOUNDS FROM *Citrus hystrix* LEAVES AND THEIR ANTIOXIDATIVE EFFECTS ON OIL AND MINCED MEAT OF SARDINE (*Sardinella lemuru* Bleeker)

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April 2011

Chairman: Prof. Dr. Jamilah Bakar, PhD

Faculty: Food Science and Technology

Optimization of extraction methods for extracting antioxidant and phenolic rich compounds from *Citrus hystrix* leaves was carried out by supercritical carbon dioxide (SC-CO₂) using response surface methodology (RSM). The conventional EtOH extraction was used as the control. The effects of CO₂ flow rate (15-25 g/min), extraction pressure (100-300 bar) and extraction temperature (40-60 °C) on yield, total phenolic content (TPC) and Diphenyl-picrylhydrazyl (DPPH-IC₅₀) were determined. Optimized extracts by SC-CO₂ and that of ethanol were analyzed by HPLC for the recovery of bioactive phenolic acids (Vanillic, *p*-Coumaric, *m*-Coumaric, Sinapic, Benzoic and trans-Cinnamic acid). The extracted phenolics were used as natural antioxidants to retard sardine (*Sardinella lemuru*) lipid oxidation. Different

concentrations of the extracts (0.1%, 0.2%, 0.3%, and 0.4%), control (without antioxidant) and positive control (BHT-0.02%) were added to the extracted oil and refrigerated (4 °C) mince of sardine. Their peroxide values (PV) as well as thiobarbituric acid (TBA) values were determined. Among the three extraction variables studied, extraction pressure was the most critical factor which influenced the yield, TPC and DPPH-IC₅₀ ($p < 0.001$) of the extracts, followed by CO₂ flow rate ($p < 0.05$) and extraction temperature ($p < 0.05$). The optimum conditions for extractions were pressure at 267 bars, CO₂ flow rate at 18g/ml and temperature at 50 °C. Their corresponding responses for yield, TPC and DPPH-IC₅₀ were 5.046%, 116.558 mg GAE/g extract and 0.064 mg/mL, respectively. These values were reasonably close to their predicted values ($p > 0.05$) with high overall coefficient of determination ($R^2 > 0.8$). R^2 values for yield, TPC, and DPPH-IC₅₀, were 0.935, 0.95, and 0.96, respectively. Better DPPH-IC₅₀ and TPC were obtained in the extracts using SC-CO₂ extraction method whereas higher yield and phenolic acids were obtained with the ethanol extraction. A significant effect ($p < 0.05$) of *C. hystrix* leaves extracts from SC-CO₂, in retarding fish lipid oxidation was apparent. Oxidation stability was linear with increasing concentrations of *C. hystrix* leaf which were extracts from 0.1 to 0.4%. There were no significant differences in oxidation retardation between the addition of 0.1% and/or 0.2% of the extracts in the storage period; however, both were significantly ($p < 0.05$) different from the control in some cases. Likewise, 0.3 and 0.4% exhibited similar antioxidant inhibitory effects which were remarkably better than those of 0.1 and 0.2% and comparable to those of the commercial antioxidant

(BHT).The SC-CO₂ optimized *C.hystrix* leaf extracts may thus be considered as a probable promising new source of antioxidants in marine lipids.



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

**PENGEKSTRAKAN SEBATIAN FENOLIK DARI DAUN LIMAU PURUT
DAN KESAN ANTIOKSIDANYA KE ATAS MINYAK DAN ISIIKAN SARDIN
(SARDINELLA LEMURU BLEEKER)**

Oleh

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April 2011

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Kaedah pengekstrakan sebatian yang kaya dengan antioksidan dan fenolik dari daun limau purut melalui (*supercritical carbon dioxide*) (SC-CO₂) dioptimumkan dengan menggunakan *response surface methodology* (RSM). Perbandingan (antara) pelarut EtOH yang biasa digunakan untuk pengekstrakan dijalankan. Pengaruh kelajuan aliran CO₂ (15-25 g / minit), tekanan ekstrak (100-300 bar) dan suhu ekstrak (40-60 °C) terhadap hasil, jumlah kandungan fenolik (TPC) dan *Diphenyl-picrylhydrazyl* (DPPH-IC₅₀) ditentukan. Ekstrak yang dioptimumkan melalui SC-CO₂ dan etanol dianalisis dengan menggunakan *HPLC* untuk mendapatkan semula asid fenolik bioaktif (Vanillic, *p*-Coumaric, *m*-Coumaric, Sinapic, Benzoic and trans-Cinnamic acid). Fenolik yang telah diekstrak digunakan sebagai antioksidan bagi

melambatkan proses pengoksidaan lipid sardin (*Sardinella lemuru*). Kepekatan ekstrak yang berlainan (0.1%, 0.2%, 0.3%, dan 0.4%), kawalan (tanpa antioksida) dan kawalan positif (BHA-0.02%) ditambah ke dalam minyak sardin yang telah diekstrak dan sardin cincang yang didinginkan (4 °C). Nilai perokisda (PV) serta asid thiobarbituric (TBA) telah ditentukan. Antara ketiga-tiga pembolehubah ekstrak tersebut, tekanan ekstrak merupakan faktor yang paling penting mempengaruhi hasil, TPC dan DPPH-IC₅₀ ($p < 0.001$), diikuti dengan kadar aliran CO₂ ($p < 0.05$) dan suhu ekstrak ($p < 0.05$). Keadaan optimum pengekstrakan adalah 267 bar (tekanan), kadar aliran CO₂ 18g/ml dan suhu 50 °C. Kesan terhadap hasil, TPC dan DPPH-IC₅₀ masing-masing ialah 2.53g, 116.53 mg GAE/g ekstrak dan 0.063 mg/mL. Nilai ini hampir sama dengan nilai yang dijangkakan ($p > 0.05$) dengan penentuan pekali keseluruhan yang tinggi ($R^2 > 0.8$). Nilai R² untuk hasil, TPC, dan DPPH-IC₅₀ adalah masing-masing 0.935, 0.95 dan 0.96. DPPH dan TPC-IC₅₀ yang lebih baik diperolehi dalam ekstrak yang menggunakan kaedah ekstrak SC-CO₂ manakala hasil dan asid fenolik yang lebih tinggi didapati dalam ekstrak etanol. Kesan yang ketara ($p < 0.05$) SC-CO₂ ekstrak daun *C.hystrix* dalam melambatkan proses pengoksidaan lipid ikan diperhatikan. Kestabilan pengoksidaan adalah selari dengan peningkatan kepekatan ekstrak daun *C.hystrix* iaitu dari 0.1-0.4%. Dalam tempoh simpanan, tiada perbezaan yang ketara dalam kerencatan pengoksidaan dengan penambahan 0.1% dan/atau 0.2% ekstrak; namun dalam sesetengah keadaan, kedua-duanya mempunyai perbezaan yang ketara ($p < 0.05$) daripada kawalan. Demikian juga, 0.3 dan 0.4% menunjukkan kesan antioksida yang sama malah lebih baik daripada

kepekatan 0.1 dan 0.2% serta antioksidan yang komersial (BHT). Ekstrak daun *C.hystrix* yang dioptimumkan melalui SC-CO₂ dianggap sangat berpotensi sebagai sumber baru antioksidan bagi lipid hidupan laut.



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APPROVAL

I certify that the Thesis Examination Committee has met on 20 April 2011 to conduct the final examination of Mohamed Abdulkadir Gedi on his thesis entitled, "Extraction of Phenolic Compounds from *Citrus hystrix* leaves and their antioxidative effects on oil and minced meat of sardine (*sardinella lemuru Bleeker*)" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra [P.U.(A)106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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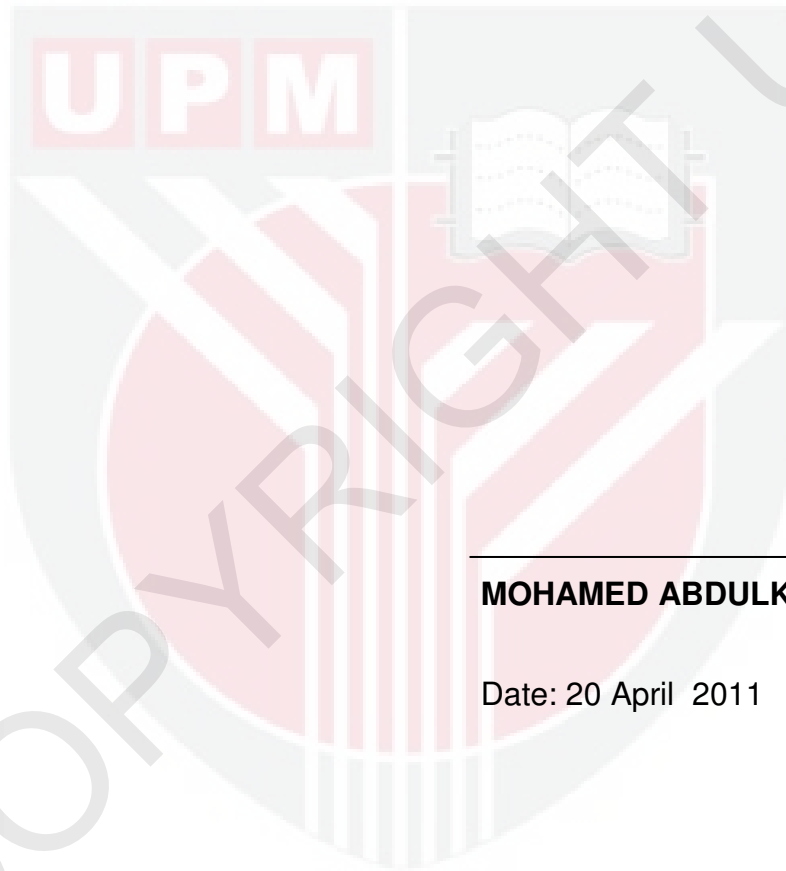
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DECLARATION

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 not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.



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Date: 20 April 2011

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