

**DETECTION OF LARD AND INTERESTERIFIED LARD AS ADULTERANTS
IN SOME VEGETABLE OILS AND SELECTED FRIED FOOD PRODUCTS**

By

J. M. MOHAMMED NAZRIM MARIKKAR

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Faculty : Food Science and Biotechnology

Mixing of pork and lard in food systems is an important food safety issue. In view of the religious restrictions and risks associated with consumption of pork and lard, there is a growing need for lard detection methods in food systems. In fact, it is very essential to distinguish lard adulteration from other common animal fat adulterations so that misleading conclusions could be avoided in analytical determinations. Therefore, in this project we attempted to explore the potential of analytical techniques such as gas liquid chromatography (GLC), high performance liquid chromatography (HPLC), differential scanning calorimetry (DSC), and Fourier transform infrared (FTIR) spectroscopy for the detection of lard contamination in some vegetable oils and some fried food products.

In the initial stage, compositional and thermal profiles of genuine lard (GLD) were compared with those of beef tallow (BT), mutton tallow (MT) and chicken fat (CF).

The GLC studies showed that percent palmitic acid enrichment factor [PAEF (%)] for GLD was unusually high when compared to other animal fats. According to HPLC, triacylglycerol (TAG) profile of GLD was quite different from that of BT/MT but seemed to have a close resemblance to that of CF. However, the TAG peak ratio LOO/SPO of GLD was found to be a useful parameter to differentiate GLD from CF. In the DSC analysis, GLD was found to have cooling and heating profiles which were completely different from those of other animal fats and therefore easy to identify. Similarly, compositional and thermal characteristics of genuine lard (GLD) were also compared with chemically interesterified lard (CLD) and enzymatically interesterified lard (ELD) in order to find common merits between them. Even though GLD and interesterified lard (GLD/ELD) were similar in overall fatty acid composition, they differed in their *sn*-2 positional distribution of fatty acids. Particularly, PAEF was no longer a common characteristics between GLD and CLD. Drastic differences were also noted with respect to their TAG and DSC thermal profiles.

With this background study, investigations were scheduled to determine the presence of lard as an adulterant in the admixtures of palm oil (PO), palm kernel oil (PKO), and canola oil (CLO) using four major analytical techniques, namely GLC, HPLC, DSC, and FTIR spectroscopy. In these studies, special attention was paid to discriminate lard adulteration from other animal fat adulterations when they were present in admixtures.

Investigations conducted with GLC showed that it was very difficult to distinguish lard adulteration from other animal fats adulterations based on the overall FA data.

However, more promising results were obtained for FA compositional changes in the 2-position of TAG isolated for each adulterated sample of vegetable oil. Additionally, mathematical equations obtained by simple regression analysis could be used for quantification of lard content in admixtures.

HPLC was the next analytical technique used for detection of lard. HPLC analyses were performed to monitor the TAG compositional changes in the oil samples before and after adulteration. The results showed that qualitative determination of lard contamination in PKO was possible by visual comparison of HPLC profiles of PKO with those adulterated with different animal fats whereas this approach was not found useful for detection of lard in PO and CLO. However, by subjecting liquid chromatographic data into multivariate statistical analysis, it was shown that distinguishable grouping for lard-contaminated samples could be possible for all the three oils investigated in this study.

Investigations conducted with DSC showed that it was very difficult to locate an adulteration peak corresponding to lard for PKO adulterated series since the changes caused by GLD and CF were similar. In PO, DSC heating thermogram did not provide any positive indication for lard detection whereas DSC cooling profiles of adulterated PO samples showed an adulteration peak corresponding to lard in the low temperature region. In CLO, adulteration peak corresponding to GLD was found to emerge in the higher temperature region of the DSC heating thermogram.

FTIR spectroscopy was the last technique employed to detect lard in vegetable oils. According to the results, FTIR/ATR technique seemed to be very useful analytical

tool for detection of AF adulterations in general since it clearly highlights the deviations taking place in different spectral regions of vegetable oils. However, using this technique it was not possible to pinpoint any characteristic features peculiar to lard occurring in all three vegetable oils since the changes caused by all AF in many regions of the spectra were similar. Therefore, FTIR/ATR may not be a suitable technique to detect the presence of lard in vegetable oils.

In the last part of the project means of identifying lard contamination in fried food products was investigated by employing GLC, HPLC and DSC techniques. Results showed that PAEF calculated from GLC analysis of fatty acid methyl esters seems to be a useful parameter in determining lard contamination in all four fried products. Using TAG profiling by HPLC, it was possible to detect lard in fried- chicken and fried-tempeh while DSC cooling and heating traces were found to be useful for lard detection in fried-tempeh, fried-chicken and fried-beef products.

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

**PENGESANAN LEMAK KHINZIR DAN LEMAK KHINZIR YANG TELAH
DIINTERESTERIFIKASI SEBAGAI BAHAN ASING DALAM SEBAHAGIAN
MINYAK SAYUR-SAYURAN DAN BAHAN MAKANAN GORENG YANG
TERTENTU**

Oleh

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Pencampuran daging dan lemak khinzir dalam system makanan adalah satu isu keselamatan makanan yang penting. Oleh kerana halangan agama dan risiko yang berkaitan dengan penggunaan daging dan lemak khinzir, keperluan untuk suatu cara pengesanan lemak khinzir dalam sistem makanan semakin bertambah. Sememangnya, adalah mustahak untuk membezakan kehadiran lemak khinzir daripada lemak binatang lain supaya keputusan penentuan secara analitikal yang tidak betul dapat dielakkan. Oleh yang demikian, matlamat utama projek ini adalah untuk mengkaji potensi teknik analisa seperti kromatografi gas cecair, kromatografi cecair berprestasi-tinggi, permeteran kalori pengimbasan kebezaan, dan spektroskopi infra-merah penukaran fourier (FTIR) untuk mengesan kehadiran lemak khinzir dalam sebahagian minyak sayur-sayuran dan bahan makanan goreng yang tertentu.

Pada peringkat awal, jujukan dan profil terma lemak khinzir tulen (GLD) telah dibandingkan dengan lemak lembu, lemak kambing dan lemak ayam. Kajian daripada kromatografi gas-cecair menunjukkan bahawa factor perkayaan acid palmitik (PAEF) bagi lemak khinzir adalah luar biasanya lebih tinggi bila dibandingkan dengan lemak haiwan yang lain. Berdasarkan kromatografi cecair berprestasi tinggi, profil trigliserida bagi lemak khinzir tulen adalah berbeza dengan lemak lembu dan kambing, tetapi hampir sama dengan lemak ayam. Akan tetapi, nisbah puncak trigliserida LOO/SPO daripada lemak khinzir adalah satu parameter yang berguna untuk membezakan lemak khinzir daripada lemak ayam. Daripada analisa permeteran kalori pengimbasan kebezaan, didapati lemak khinzir tulen mempunyai profil penyejukan dan pemanasan yang langsung berbeza daripada lemak haiwan lain, oleh itu ianya mudah dikenalpasti. Dengan cara yang sama, ciri-ciri jujukan dan terma lemak khinzir yang tulen telah dibandingkan dengan lemak khinzir yang diinteresterifikasikan secara kimia dan enzim untuk menyelidik kesamaan antara mereka. Walaupun lemak khinzir tulen dan lemak khinzir yang telah diinteresterifikasi adalah sama dari segi jujukan asid lemak keseluruhan, mereka adalah berbeza antara satu sama lain dari segi pengagihan asid lemak di kedudukan sn-2. Terutamanya, faktor perkayaan asid palmitik bukan lagi satu ciri yang biasa antara lemak khinzir tulen dan lemak khinzir yang diinteresterifikasikan secara kimia. Perbezaan yang ketara dari segi profil trigliserida dan profil terma daripada permeteran kalori pengimbasan kebezaan juga didapati.

Dengan kaji selidik ini, ujian telah dilaksanakan bagi mengenalpasti kehadiran lemak khinzir sebagai bahan asing dalam campuran, minyak sawit, minyak isirong sawit, dan minyak kanola dengan menggunakan empat kaedah analitik yang utama, iaitu kromatografi gas-cecair, kromatografi cecair berprestasi tinggi, permeteran kalori pengimbasan kebezaan dan spektroskopi infra-merah penukaran fourier. Dalam kajian

ini, tumpuan khas telah diberikan untuk mendiskriminasikan kehadiran lemak khinzir daripada lemak haiwan lain yang turut terkandung dalam sesuatu campuran.

Ujian yang dijalankan dengan kromatografi gas-cecair menunjukkan adalah sukar untuk membezakan kehadiran lemak khinzir daripada lemak haiwan yang lain berdasarkan data asid lemak secara keseluruhan. Walaubagaimanapun, keputusan yang lebih menyakinkan telah diperolehi berdasarkan perubahan komposisi asid lemak pada kedudukan kedua di trigliserida yang diasingkan daripada minyak sayuran yang telah dicampuraduk. Tambahan pula, persamaan matematik yang dihasilkan daripada analisis regresi mudah juga boleh digunakan untuk menghitung kandungan lemak khinzir dalam sesuatu campuran.

Teknik analisa yang seterusnya untuk mengesan kehadiran lemak khinzir adalah kromatografi cecair berprestasi-tinggi. Analisa menggunakan kromatografi cecair berprestasi-tinggi telah dijalankan bagi mengawasi perubahan komposisi trigliserida dalam contoh minyak sebelum dan selepas dicampuraduk dengan lemak khinzir. Keputusan menunjukkan bahawa penentuan secara kualitatif pencemaran lemak khinzir dalam minyak isirong sawit boleh dilakukan dengan membuat perbandingan secara visual profil trigliserida dengan minyak yang dicemari oleh lemak haiwan yang lain; manakala kaedah ini tidak boleh dilakukan untuk minyak sawit dan minyak kanola. Akan tetapi, perbezaan secara berkumpulan bagi contoh yang dicemari lemak khinzir boleh didapati untuk ketiga-tiga jenis minyak yang dikaji sekiranya data daripada kromatografi cecair berprestasi-tinggi dianalisa selanjutnya dengan statistik varian berbagai.

Ujikaji yang dijalankan dengan permeteran kalori pengimbangan kebezaan menunjukkan ianya adalah amat sukar untuk mengenalpasti puncak yang dicemari dengan lemak khinzir dalam minyak isirong sawit disebabkan perubahan yang diakibatkan oleh lemak khinzir tulen dan lemak ayam adalah sama. Bagi minyak sawit pula, termogram pemanasan daripada permeteran kalori pengimbangan kebezaan tidak dapat memberikan indikasi yang positif untuk mengesan lemak khinzir, manakala profil penyejukan daripada permeteran kalori pengimbangan kebezaan menunjukkan satu puncak pencemaran akibat lemak khinzir pada tahap suhu rendah. Dalam minyak kanola pula, puncak pencemaran berkait dengan lemak khinzir tulen muncul di tahap suhu yang lebih tinggi dalam termogram pemanasan daripada permeteran kalori pengimbangan kebezaan.

Spektroskopi infra-merah penukaran fourier merupakan kaedah terakhir yang digunakan untuk mengesan lemak khinzir dalam minyak sayur-sayuran. Berdasarkan kepada keputusan yang diperolehi teknik FTIR/ATR merupakan satu alat analitikal yang amat berguna untuk mengesan pencemaran AF secara amnya kerana ia dapat mempamerkan dengan jelas perbezaan yang berlaku di kawasan spektral yang berlainan dalam minyak sayur-sayuran. Walaubagaimanapun, teknik ini tidak dapat mengesan sebarang ciri-ciri yang bersangkutan dengan lemak khinzir dalam ketiga-tiga minyak sayuran ini disebabkan perubahan AF yang berlaku di kebanyakan kawasan dalam spektra ini adalah sama. Oleh demikian, FTIR/ATR bukanlah suatu teknik yang sesuai untuk mengesan kehadiran minyak khinzir dalam minyak sayur-sayuran.

Pada bahagian akhir projek ini, cara-cara untuk mengenalpasti pencemaran lemak khinzir dalam makanan goreng telah dikaji dengan menggunakan GLC, HPLC, dan

DSC. Keputusan menunjukkan bahawa faktor perkayaan asid palmitik yang dihitung daripada analisa metil ester asid lemak secara kromatografi gas cecair merupakan suatu parameter yang berguna untuk menentukan pencemaran lemak khinzir dalam empat produk goreng yang dikaji.

Dengan menggunakan profil triasilgliserida daripada kromatografi cecair berprestasi tinggi, kehadiran lemak khinzir dalam ayam goreng dan tempeh goreng boleh ditentukan; manakala kesan penyejukan dan pemanasan daripada permeteran kalori pengimbasan kebezaan didapati berguna untuk mengesan lemak khinzir dalam tempeh goreng, ayam goreng dan produk lembu yang digoreng.

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

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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 in UPM or other institution.

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