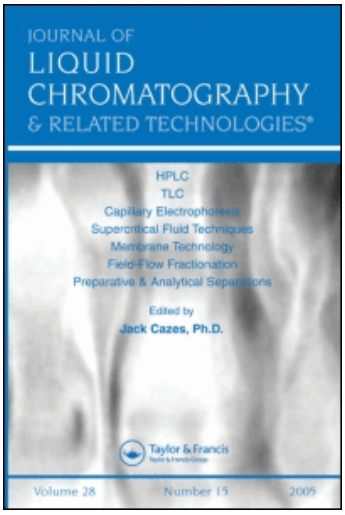

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Triacylglycerols-Profiling by High Performance Liquid Chromatography: A Tool for Detection of Pork Fat (Lard) in Processed Foods

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TRIACYLGLYCEROLS-PROFILING BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY: A TOOL FOR DETECTION OF PORK FAT (LARD) IN PROCESSED FOODS

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ABSTRACT

A rapid high-performance liquid chromatographic procedure for separation of the triacylglycerols (TG) of animal fats using refractive index detection is described. A LiChrospher-100 RP-18 (5 μm) column was used for the TG-profiling of pork, beef, mutton, chicken and turkey fats. Detection of pork fat in processed foods and lard in fat-admixtures is also discussed. TG-separation and checking genuinity and adulteration was achieved isocratically in $\sim$15 min. by using CH_3CN/CH_2Cl_2 (58:42, v/v) at ambient temperature.

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INTRODUCTION

Fraudulent substitution of expensive flesh with cheaper ones in meat products is objectionable to the consumer for different reasons; such as, medical requirements of a person who may have specific food allergies, economics and religious dietary restrictions. Inter-species meat adulteration is common in many parts of the world. Meat of similar pigmentation, beef and mutton or pork and poultry, are difficult to distinguish by eye once they have been frozen or cooked. In order to detect any possible substitution or admixing adulterations and to ensure that processed foods comply with quality restrictions, identification of the component lipid part of the food has become an important area of research to many analysts.

Several methods have been published on detection of lard in processed foods. Saeed *et al.* (1) selected the presence of 11,14-eicosadienoic acid as an indicator for lard. However, this acid has been claimed in beef and mutton samples (2). Pork fat has mainly 2-palmityl acylglycerols (3). Palmitic acid enrichment factor (PAEF), i.e. percent ratio of palmitic acid in the 2-monoacylglycerols to that of original triacylglycerols has been taken as a detection merit for lard (3,4). However, positional distribution affected by randomization. The use of enzyme-linked immunosorbent assay (ELISA) in food analysis has been also reported (5).

Reversed phase high-performance liquid chromatography (RP-HPLC) is a good separation technique and has application to the analysis of animal and vegetable fats and oils (6-10). Detection of TG is complicated as the ester bond absorbs at low wavelength which limits the selection of suitable mobile phase for UV-detection. Generally, refractive index (RI), (11), ultraviolet (UV) (12), for highly unsaturated TG,

and light-scattering detectors (13) are used for the analysis of TG. Refractive index detection is generally adopted becuase it doesn't have the drawback of low sensitivity and of different responses towards saturated and unsaturated TG like in case of UV-detection. HPLC-profiling of derivatized TG have been reported for the detection of pork in beef (14), but major disadvantage of the method is lengthy and needs tedius sample preparation.

This report describes a rapid RP-HPLC screening procedure for the profiling of the component TG of animal fat extracted from unprocessed and processed meat products. Also, TG-profiling of fat-admixtures are included. Such profiling characterization is of value for the genuinity testing and checking the adulteration in fatty products.

EXPERIMENTAL

Chemicals

All solvents were HPLC-grade (Merck, Darmstadt-Germany) and were filtered through a 0.22 μm Millipore filter. Standard simple and mixed TG ($\sim$99% purity), namely, trilaurin, tirpalmitin, tristearin, triolein and trilinolein were obtained from NU CHEK PREP, INC (Elysian, MN-USA) and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (OPO), & 1-palmitoyl -2-oleoyl-3-stearoyl-*rac*-glycerol (OSP) were purchased from Sigma Chemical Co. (St. Louis, MO-USA).

Samples

Genuine and processed pork were obtained from the Amon Factories for pork and their processed foods, Shoubra, Cairo-Egypt, Some of the luncheon meat and

sausages were imported from USA and Bahrain. Processed samples of beef, mutton, chicken and turkey from various origin were obtained from the local markets. Genuine samples of beef and mutton fats have been freshly prepared from the underskin fat's caracass and adipose tissues (various parts) of freshly slaughtered animals at local slaughter house. For chicken and turkey fats, their skin and visible fats were removed from the flesh and homogenised. In all cases, lipids were extracted from the ground samples by modified Folch procedure (15) using $CHCl_3/CH_3OH$ (2:1, v/v) as the solvent. Fat extraction and storage were performed under nitrogen atmosphere to prevent oxidation of the unsaturated fatty acids.

Isolation of Triacylglycerols

TG were isolated from fats by column chromatography. Small chromatographic column (14 cm × 2 cm, ϕ) was packed with a slurry of 6 gm of silica gel 100 (70-230 mesh) in n-C_6H_{14}. After settle down of the silica, 0.5 g of the dired, melted fat in 0.5 ml of n-C_6H_{14} was introduced carefully into the top of the column and sample was allowed to adsorb on the surface of silica. Column was eluted with 20 ml of n-C_6H_{14} followed by 5% $(C_2H_5)_2O$ in n-C_6H_{14} till TG were completely eluted. Fractions, 20-ml each, were collected and the solvent was removed under vacuum. The purity of TG-fraction was checked on TLC-plate utilizing n-$C_6H_{14}/(C_2H_5)_2O/CH_3COOH$ (90:10:1, by volumes) as the developing system. Visualization of the spots was made by charring the plates after spraying with 20% $HClO_4$ (aq.)

If isolated fats from the processed foods containing only traces of free fatty acids, mono- and diacylglycerols and/or coloring matter, it is advisblae to use fat directly for analysis.

RP-HPLC/RI Analysis

The liquid chromatographic analysis of TG was performed on LiChrospher 100RP-18 (5 μm) column (12.5 cm $\times$ 4mm, ϕ, E. Merck, Darmstadt-Germany) and a refractive index detector (Waters-differential refractrometer R401). The mobile phase contained CH_3CN/CH_2Cl_2 (58:42, v/v) was used as an isocratic mixture at a flow rate of 1 ml/min^{-1} at ambient temperature. HPLC-system of Shimadzu (Kyoto, Japan), consisting of a solvent delivery module LC-10AD with a double plunger reciprocating pump, a DGU-3A degasser, an injector valve (20-μl loop), column embeded in a CTO-10A column oven and C-R4A Chromatopac multi-functional data processor, was used.

RESULTS AND DISCUSSION

Figure 1 shows the HPLC profile for a reference mixture containing four triacylglycerol standards, while, figure 2 represents the TG-separattion of genuine animal meat fats (beef, lard, mutton and chicken turkey). The complete elution of all the molecular species of genuine animal fat samples was under 15 minutes. The reproducibility of the retention times of the eluted peaks was very good for standard and other lipid samples.

Few molecular species of animal fats were characterized by comparing retention times of sample to that of analyzed standard. If three stereospeific position on glycerol molecule were assumed to be equivalent and isomers are not separated, then for seven fatty acids, the total possible molecular species was calculated to be 84 by using the formula $(n^3 + 3n^2 + 2n)/6$; (where n = number of fatty acids) (16). However, practically observed value for TG is always less than calculated number (17).

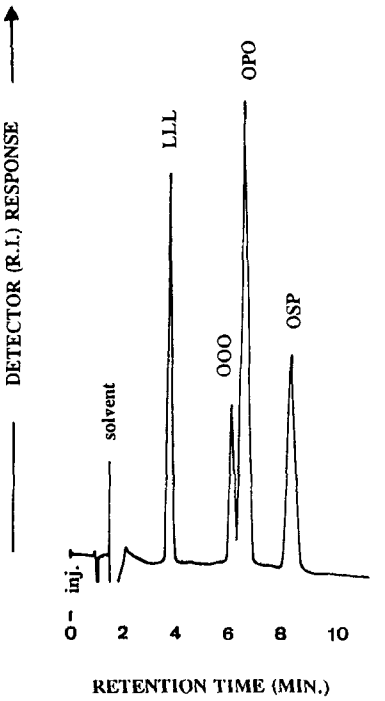

Figure 1: Separation of simple and mixed standard triacylglycerols.

The TG-profile of lard (figure 2) shows two most characteristic peaks with retention times, t_R-values, of about 5.60 and 8.66 min. Clear differences were observed in the overall profiles of beef, mutton; chicken and turkey which differentiate them from lard. Such fingerprints can be quickly used to check the presence or absence of lard (pork fat) in meat and meat products, even without making effort to obtain quantitative data.

TG-profile of admixed fats may depends principally on the mixing proportions of each fat component and follows the additivity rules. In order to check the validity

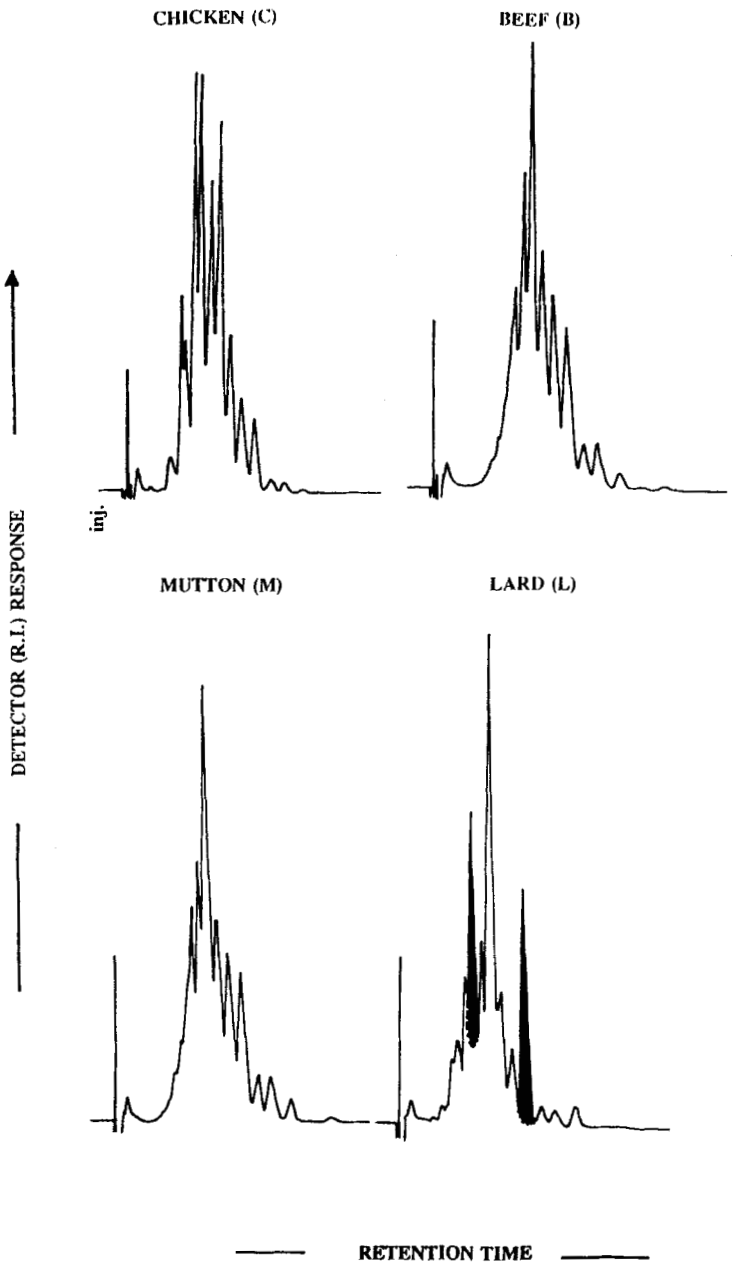

Figure 2: Component triacylglycerols of genuine beef, mutton, chicken and lard.

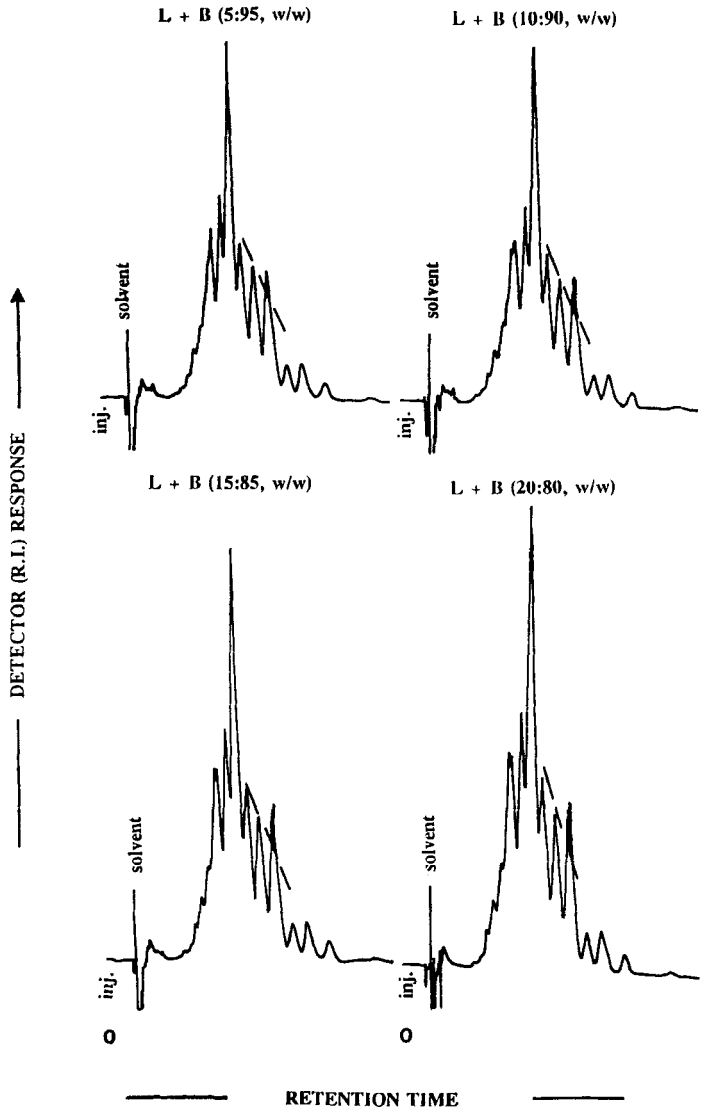

Figure 3: TG-profilings of different lard (L) beef (B) admixtures (5-20%, w/w).

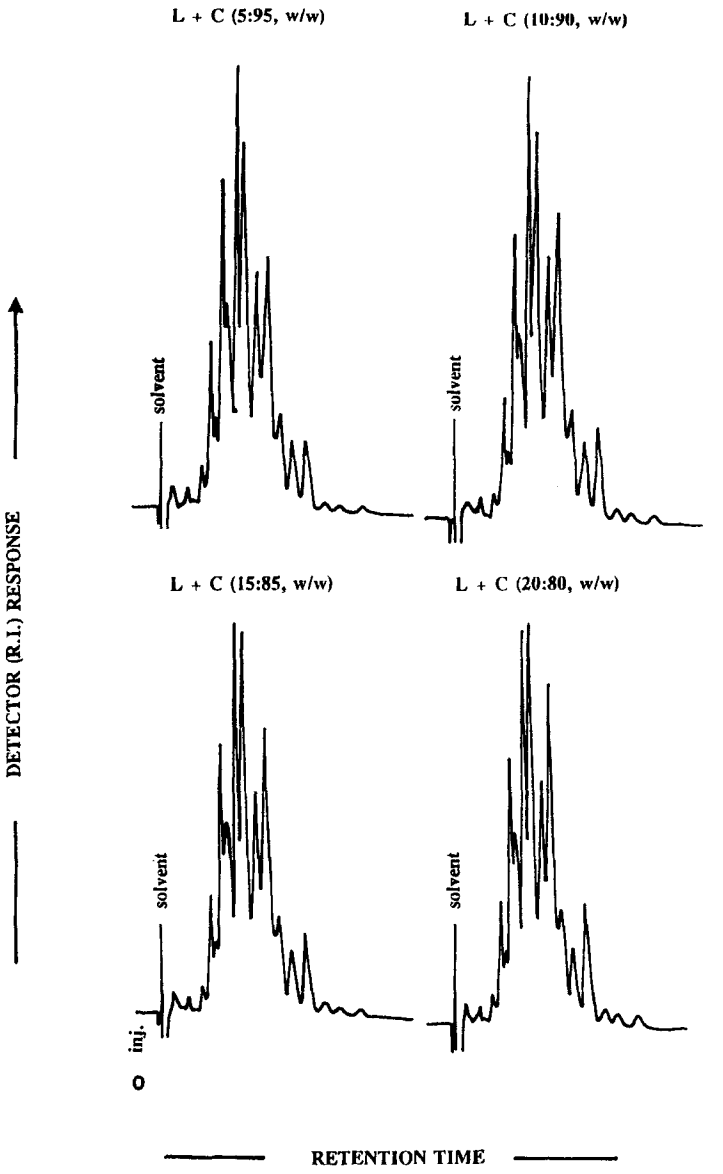

Figure 4: TG-profilings of different lard (L) chicken (C) admixtures (5-20%, w/w).

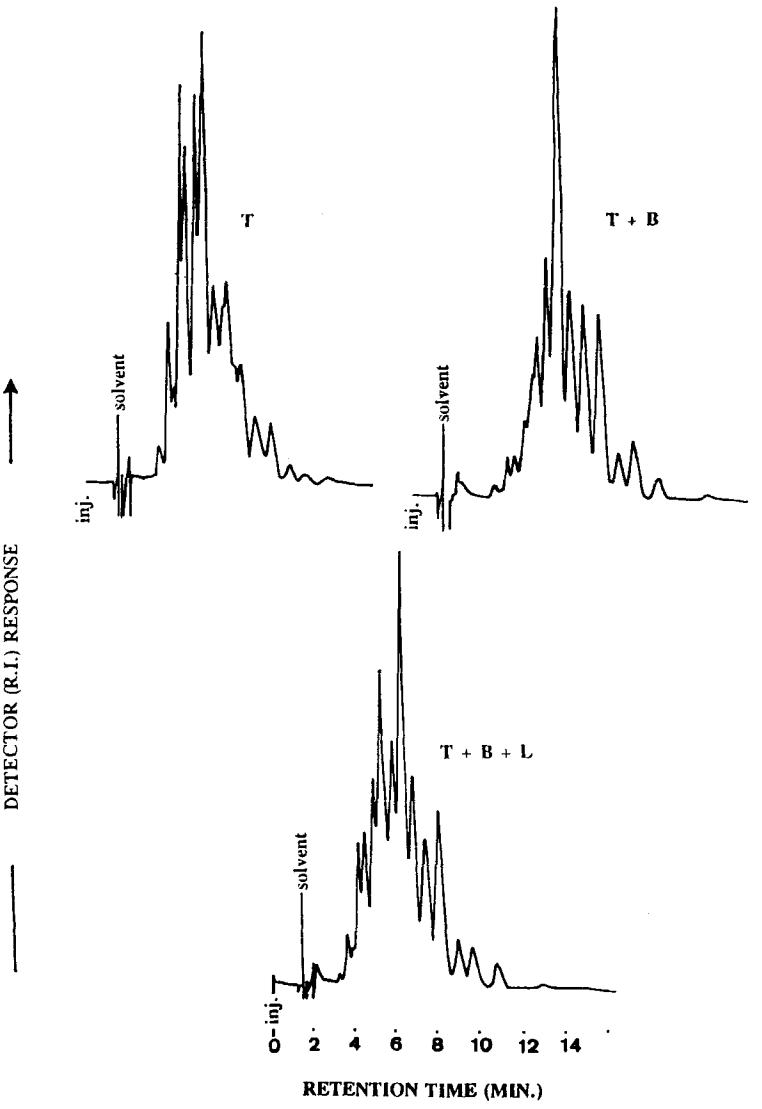

Figure 5: TG-profiling of genuine turkey fat (T), turkey fat + beef (B), and ternary admixture of turkey fat, beef and lard (L).

of the additivity measures in case of fat-admixtures containing lard and beef, binary labroatory admixtures of the individual genuine fats containing 5 to 20% lard and beef (w/w) have been prepared. The TG-profilings of such laboratory admixed fats at different proportions are represented in figure 3. Proportional increase in the peak intensity and net area count of that characteristic peaks of lard, *i.e.* dark shaded, can be clearly noticed. The indicative value of the justified peak for lard detection if admixed with beef is of great value and the additivity proportionality is quite rectilinear. Similarly, figure 4 illustrates the resolved triacylglycerols of laboratory prepared lard-chicken fat admixtures, where obvious differences could be identified. It is clear that the additivity rule is obeyed clearly on suggestive increasing of the lard portions added to chicken. Furthermore, it is clear that the addition of lard is easily detectable by observing the proportional increases in the intensity of the peak characteristic for it (figure 5).

The investigated TG-profiling method was also applied to triacylglycerols isolated from the processed meat products (salamy, sausages, luncheon) containing or free from pork of different origins. Clear detection of lard/pork can be easily concluded just by matching the obtained TG-profiles.

CONCLUSION

TG-profiling of animal fats and fats extracted from genuine meat specimens and from processed fatty foods is of great value for the detection of pork in processed foods samples. The detection limit for pork fat was observed to be about 5% admixtures based on the fat basis. The method is easy, simple, rapid and needs no derivatization,

and it allows also no artifact formation and confirms the presence of lard singly or admixed in food samples.

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