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Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis

J.M.N. Marikkar [a], H.M. Ghazali [a], Y.B. Che Man [b], T.S.G. Peiris [c], O.M. Lai [a,*]

[a] *Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, DE, Malaysia*
[b] *Department of Food Technology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia, UPM 43400 Serdang, Selangor, DE, Malaysia*
[c] *Coconut Research Institute, Lunuwila, Sri Lanka*

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Abstract

Detection of animal fat adulterants in vegetable oils is of great importance from commercial and health perspectives. Distinguishable identification of lard contamination in some vegetable oils has been attempted in this study. Vegetable oils, namely palm oil (PO), palm kernel oil (PKO), and canola oil (CLO), were spiked with different proportions of animal fats, such as lard (GLD), beef tallow (BT), and chicken fat (CF). High-performance liquid chromatographic (HPLC) analyses were performed to monitor the triacylglycerol (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 a visual comparison of TAG profiles of PKO adulterated with different animal fats with those of the animal fats. This approach was not useful for PO and CLO. However, by subjecting liquid chromatographic data to multivariate procedures, distinguishable grouping of lard-contaminated samples was achieved for all three oils.

Keywords: Adulteration; Animal fat; High-performance liquid chromatography; Lard; Vegetable oils; Multivariate data analysis

1. Introduction

High-performance liquid chromatography (HPLC) is one of the major analytical techniques that has been widely used in food analysis. Its application for the analysis of contaminants or detection of adulteration in foods has attracted much attention since the technique itself has many advantages. The most important advantage is the fact that sample components that are not readily volatilized could be separated easily by HPLC. Therefore, it is applicable to highly polar, high molecular mass, strongly ionic and thermally unstable components in food systems. The other advantage of HPLC is that derivatization of analyte is not required so often as in gas-chromatographic analysis (Hawer & Kim, 1999; Lee et al., 2001).

Several studies on the use of HPLC for detection of adulterations in oils and fats can be found in the literature. In the past, most of the adulteration studies using HPLC were focused on developing an effective method for detecting adulteration of olive oil with other seed oils (Kapoulas & Andrikopoulos, 1986; Tsimidou, Macrae, & Wilson, 1987). The basis for detection of adulteration in those cases was the additional triacylglycerol (TAG) peak, coming from trilinoleoylglycerol (LnLnLn), which is a TAG species absent or present only in traces in olive oils. Sesame is another highly expensive oil, which is a candidate for adulteration with cheaper perilla oils. An HPLC study by Lee et al. (2001) showed that remarkable differences existed between sesame and perilla oils in the amounts of 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (LLO) and LnLnLn. Therefore, the ratio of LnLnLn/

* Corresponding author. Tel.: +60-389-468-347; fax: +60-389-423-552.
E-mail address: omlai@putra.upm.edu.my (O.M. Lai).

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LLO was found to be a useful index for discriminating authentic sesame oil from adulterated mixtures. The HPLC detection of adulteration in oils and fats could, however, become more difficult if the TAG composition of the adulterant becomes similar to that of the major oil (Salivaras & McCurdy, 1992).

Detection of lard in food systems by HPLC is of particular interest since there has been a need, in a number of countries, to establish methods for determining the presence of pork as the adulterant in processed beef, mutton and chicken products. Saeed, Ali, Rahman, and Sawaya (1989) have reported a method based on HPLC analysis of derivatized TAG. Pork fat, generally, has larger amounts of TAG-containing saturated fatty acids at the *Sn*-2 position than do fats of other meat origin. Therefore, in the event of any adulteration, the ratio of TAG-containing saturated fatty acids (SSU) and TAG-containing unsaturated fatty acids at the same (*Sn*-2) position (SUS) in a sample tended to increase compared to those of pure meats. This method was used to detect pork as an adulterant in processed beef and mutton mixtures. HPLC was also investigated to compare the common merits between genuine and randomized lard and it was suggested that certain TAG peak ratios could be used for the determination of lard and randomized lard in food systems (Rashood, Abou-Shaaban, Abdel-Moety, & Rauf, 1996). However, it has now been realized that qualitative analysis mainly of single component TAG species may be inadequate. Instead, quantitative data considering the whole TAG profile might serve better as a characteristic 'fingerprint' of the oil. If such profiles are to be used to study the effects caused by adulterations, powerful statistical methods to deal with multivariate data are required to consider differences taking place in the whole chromatogram. As such, the objective of this study was to explore the use of liquid chromatography, coupled with multivariate data analysis, as a means for distinguishing lard adulteration from other animal fat adulterations in some refined vegetable oils.

2. Materials and methods

2.1. Materials

Three different edible oils of plant origin were used in this study. Palm oil (PO) (slip melting point 30.5 °C; iodine value 54.0) and palm kernel oil (PKO, slip melting point 28.0 °C; iodine value 19.8) were purchased from a local refinery. Canola oil (CLO, iodine value 113) was purchased separately from a local super-market. Animal body fats, namely lard (GLD), beef tallow (BT), mutton tallow (MT) and chicken fat (CF) were obtained from adipose tissues of animals using a fat-rendering method as reported previously (Marikkar et al., 2001). The oils and fats were stored under refrigerated conditions 4 °C and melted at 60 °C prior to their use. All chemicals used in this experiment were of analytical or HPLC grade.

2.2. Blend preparation

PO, PKO and CLO were spiked with GLD, BT and CF in varying proportions, ranging from 2% to 20%. Altogether, 15 blends were prepared for each oil: 98:2, 95:5, 90:10, 85:15 and 80:20 (w/w), identified by the mass ratio of vegetable oil (VO) to animal fat (AF) (VO:AF). Only in the case of PO, one additional series of blends was prepared by spiking MT with the proportions shown above.

2.3. HPLC analysis of TAG composition

TAG composition was determined according to the method described by Haryati et al. (1998). The system used was a Shimadzu LC-10 AD liquid chromatograph, equipped with a Shimadzu SIL-10 AD auto injector, Shimadzu system controller SCL-10A, and RID-6A Shimadzu refractive index detector (Shimadzu Corporation, Kyoto, Japan). The analysis of TAG was performed on a LiChroCART 100-RP-18 (5 μm) column (12.5 cm × 4 mm i.d.; Merck, Darmstadt, Germany). The mobile phase was a mixture of acetone–acetonitrile (63.5:36.5) and the flow rate was 1 ml/min at 30 °C. The injector volume was 10 μl of 5% (w/w) oil in chloroform. Sensitivity of the detector was adjusted to 16×10^4 RI units. Each sample was chromatographed three times, and the data were reported as percentage areas.

2.4. Statistical analysis

Data were statistically analyzed by one-way analysis of variance (ANOVA) with the SAS (Version 6.0) software package (SAS, 1989). The Pearson correlation was applied to evaluate the relationship among variables. Canonical discriminate (CANDISC) analysis, by stepwise procedure, was used for distinguishing lard-adulterated samples from those adulterated with other animal fats. Variable selection for CANDISC was based on the multiple comparison test (LSD) on treatments.

3. Results and discussion

3.1. General

The adulterant animal fats; namely GLD, CF, BT, and MT were analyzed for TAG profiles in order to show the differences of lard from other common animal

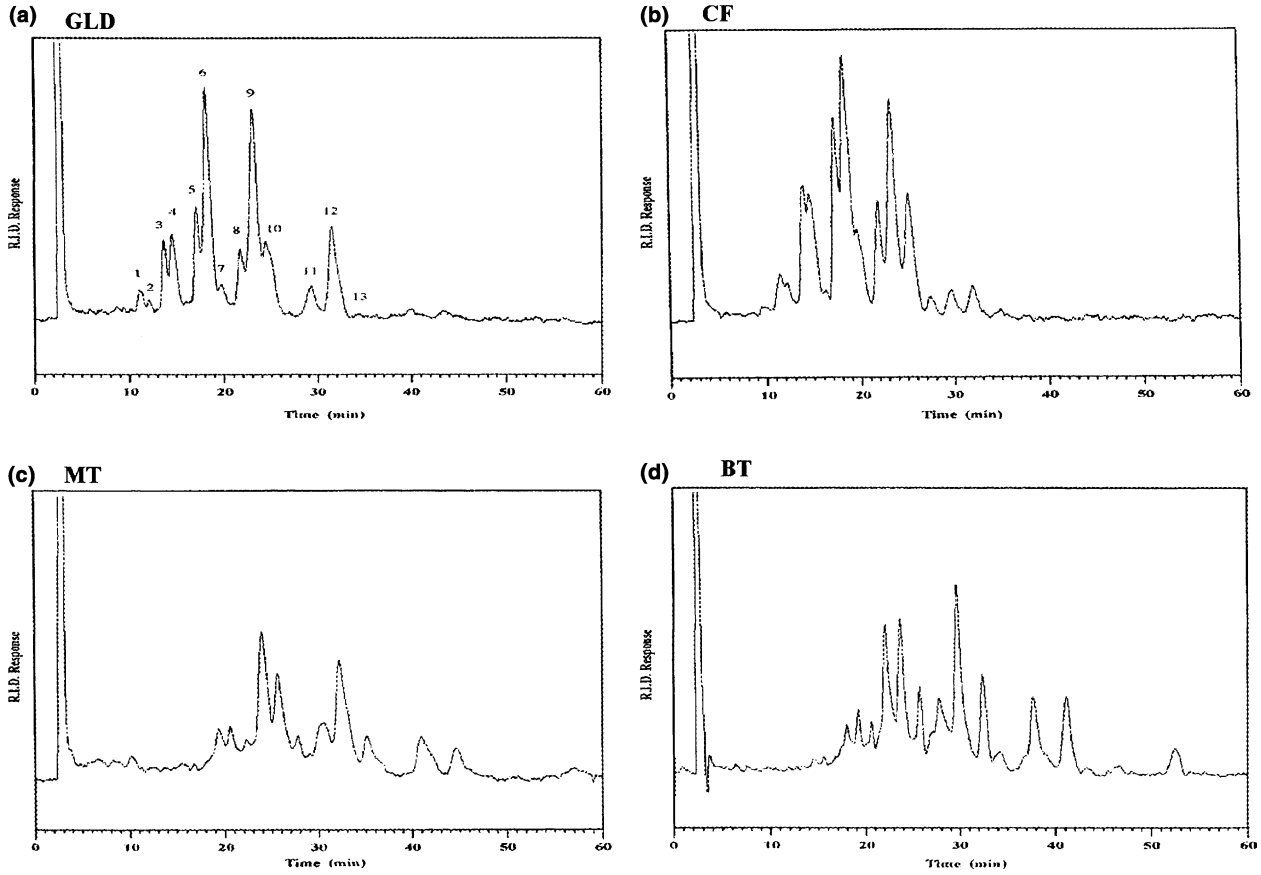

Fig. 1. HPLC chromatograms of: (a) genuine lard (GLD); (b) beef tallow (BT); (c) chicken fat (CF); (d) mutton tallow (MT).

fats (Fig. 1). In fact, variation in the nature of TAG (and their distribution) is the principal factor that makes animal fats different from one another and subsequently these variations affect the TAG separation by HPLC. Usually, the degree of unsaturation of TAG species present in an oil or fat sample has a great influence on the TAG separation in a reverse-phase silica column. As a result, the retention time of SSU TAG species could be higher than that of a SUU species (Tan and Che Man, 2000). It is obvious from Fig. 1, despite minor differences, that the TAG profiles of BT and MT look apparently similar but show significant differences from that of lard. The amount of fully saturated TAG (SSS) and disaturated (SUS/SSU) TAG species are relatively higher for BT/MT than for GLD (Marikkar et al., 2002). CF TAG profile, on the other hand, shows a number of features that are comparable to those of GLD. However, according to previous studies, the predominant TAG species of lard are SSU and USU with USU being the highest while UUS and UUU are the dominant TAG species present in CF (de Man, 1999). Consequently, this background has provided a basis for the discrimination of adulterant animal fats of different origin when they are present in admixtures. PO, PKO and CLO are the three oils selected for this study since they cover three major classes of edible oils, namely palmitic, lauric and oleic oils.

3.2. TAG analysis of adulterated samples of PO by HPLC

A sample chromatogram of PO is presented in Fig. 2(a). The identification of TAG peaks of PO is based on the previous study of Haryati et al. (1998). Accordingly, peaks were identified as 1:MMM (X_1), 2:PLL (X_2), 3:MPL (X_3), 4:OOL (X_4), 5:PLO (X_5), 6:PPL (X_6), 7:OOO (X_7), 8:OOP (X_8), 9:PPO (X_9) 10:PPP (X_{10}), 11:OOS (X_{11}), 12:POS (X_{12}), 13:PPS (X_{13}) and 14:SOS (X_{14}) where M stands for myristic, P for palmitic, O for oleic, L for linoleic, and S for stearic. The changes in the chromatographic profile of PO, due to the increasing level of adulteration with GLD, are shown in Table 1. According to the data analysis obtained from chromatographic profiles, GLD adulteration did not cause any additional peak indicative of lard adulteration in PO. This may be due to the fact that the elution-range of the GLD–TAG profile falls within the same range as that of the PO–TAG profile. Therefore, the adulterant caused changes only in the existing peaks. As a general rule, increase of any one TAG peak in the profile must

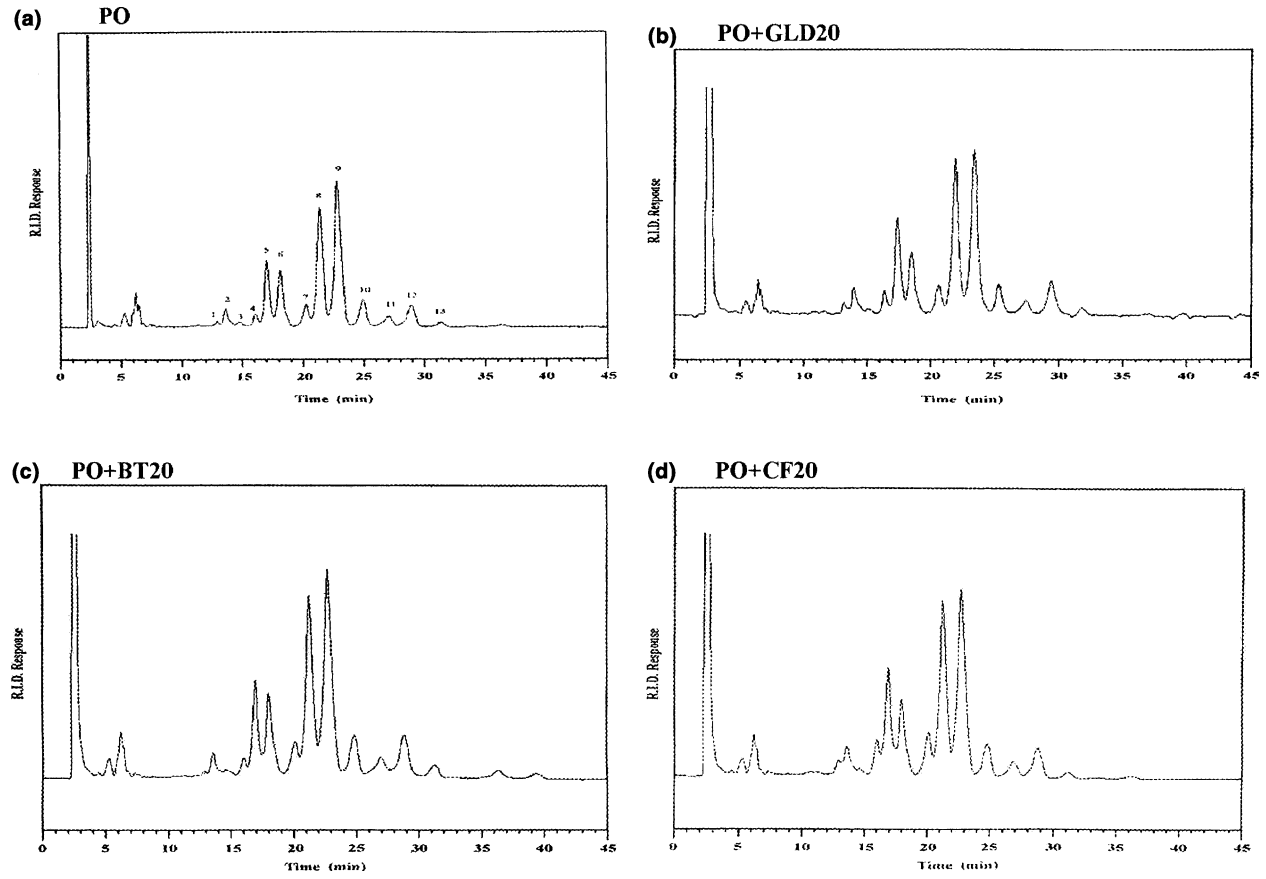

Fig. 2. HPLC chromatograms of: (a) palm oil (PO); (b) PO adulterated with 20% GLD; (c) 20% BT; (d) 20% CF. Abbreviations: see Fig. 1.

Table 1
TAG compositional changes in PO after adulteration with different concentrations (%) of GLD[a]

Peak No.	Adulteration level					
	0	2	5	10	15	20
1 (MMM)	0.54 ± 0.03	0.59 ± 0.02	0.63 ± 0.05	0.71 ± 0.00	0.86 ± 0.02	1.17 ± 0.05
2 (PLL)	2.74 ± 0.04	2.91 ± 0.05	2.94 ± 0.04	3.07 ± 0.05	3.25 ± 0.03	3.53 ± 0.07
3 (MPL)	0.71 ± 0.02	0.76 ± 0.00	0.69 ± 0.05	0.55 ± 0.07	0.63 ± 0.01	0.73 ± 0.00
4 (OOL)	1.81 ± 0.11	1.89 ± 0.09	2.00 ± 0.00	2.24 ± 0.02	2.55 ± 0.08	2.71 ± 0.06
5 (PLO)	10.5 ± 0.13	10.8 ± 0.04	11.2 ± 0.05	11.8 ± 0.07	12.3 ± 0.14	12.8 ± 0.17
6 (PPL)	10.4 ± 0.11	10.2 ± 0.03	10.1 ± 0.09	9.73 ± 0.00	9.41 ± 0.03	9.05 ± 0.08
7 (OOO)	4.19 ± 0.03	4.23 ± 0.12	4.32 ± 0.07	4.32 ± 0.00	4.33 ± 0.02	4.50 ± 0.05
8 (OOP)	23.2 ± 0.02	23.3 ± 0.00	23.3 ± 0.01	23.5 ± 0.01	23.6 ± 0.00	23.5 ± 0.03
9 (PPO)	31.3 ± 0.00	31.1 ± 0.02	30.6 ± 0.06	29.6 ± 0.10	28.4 ± 0.14	27.2 ± 0.17
10 (PPP)	5.38 ± 0.03	5.25 ± 0.03	5.31 ± 0.01	4.94 ± 0.09	4.77 ± 0.03	4.66 ± 0.05
11 (OOS)	2.30 ± 0.02	2.38 ± 0.00	2.56 ± 0.07	2.47 ± 0.04	2.60 ± 0.02	2.69 ± 0.04
12 (POS)	5.29 ± 0.03	5.41 ± 0.05	5.50 ± 0.08	5.84 ± 0.07	5.99 ± 0.03	6.20 ± 0.04
13 (PPS)	0.97 ± 0.01	1.08 ± 0.03	0.84 ± 0.00	0.92 ± 0.02	0.98 ± 0.03	1.02 ± 0.02
14 (SOS)	0.44 ± 0.02	0.18 ± 0.03	0.11 ± 0.00	0.22 ± 0.03	0.24 ± 0.07	0.20 ± 0.06

[a] Each value in the table represents the mean ± SD of triplicate analyses. Abbreviations: TAG, triacylglycerol; PO, palm oil; GLD, genuine lard; M, myristic; P, palmitic; O, oleic; L, linoleic; S, stearic.

be complemented by decrease of another TAG peak. As such, peak increases were noticed for peaks l (MMM), 2 (PLL), 4 (OOL), 5 (PLO), 7 (OOO), 8 (OOP) and 12 (POS) while peaks 6 (PPL), 9 (PPO) and 10 (PPP) underwent decreases. Hence, in general, the lard adulteration of PO if it is by GLD, caused a slight increase in oleic-acid-predominating TAGs while the palmitic acid-containing TAGs decreased slightly. In accordance with the objective mentioned earlier, similar adulteration studies were also carried out with BT, MT and CF in

order to find out ways of discriminating lard adulteration from other animal fat adulterations. The nature of changes in TAG profiles caused by each different animal fat adulterant are compared in Fig. 2, taking the chromatogram of 20% adulterated sample from each series.

With regard to the other animal fat-adulterated series, the increasing concentration of BT and MT caused additional peaks in the latter part of the chromatogram (results of MT not shown). Although this feature qualitatively helps to distinguish lard adulteration from adulteration due to BT and MT, it may not be sufficient to rule out the presence of lard in PO. In the case of CF adulteration, TAG compositional changes are apparently similar to those caused by lard-adulteration in PO (data not shown). Hence, a cursory examination of these data does not reveal any obvious characteristics that would help to discriminate samples adulterated with lard from those adulterated with other animal fats. Therefore, multivariate data analysis is suggested as an alternative approach. Multivariate data analysis, using canonical discrimination (CANDISC), helps to establish linear combinations of quantitative variables that best summarize the differences among samples originating from different groups. Consequently, quite subtle compositional differences between samples can lead to good discrimination (Dyszel, 1993; Dyszel and Baish, 1992). In the present context, also, multivariate data analysis could be exploited, since each sample analysis involves the determination of changes in a series of TAG peaks where each TAG peak could be considered as a variable.

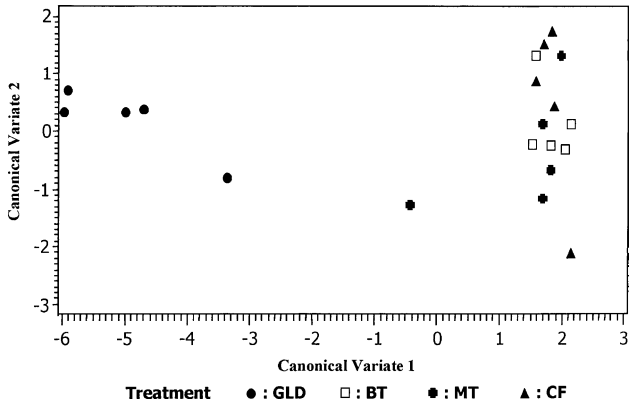

Fig. 3. Canonical discriminate analysis (CANDISC) plot for canonical variate 2 vs. canonical variate 1 values of PO samples adulterated with GLD, BT, MT, and CF. Abbreviations: see Figs. 1 and 2.

3.3. Multivariate analysis of HPLC data from PO adulterated with different animal fats

As mentioned previously, TAG peak changes in PO, due to different treatments (animal fat adulterations),

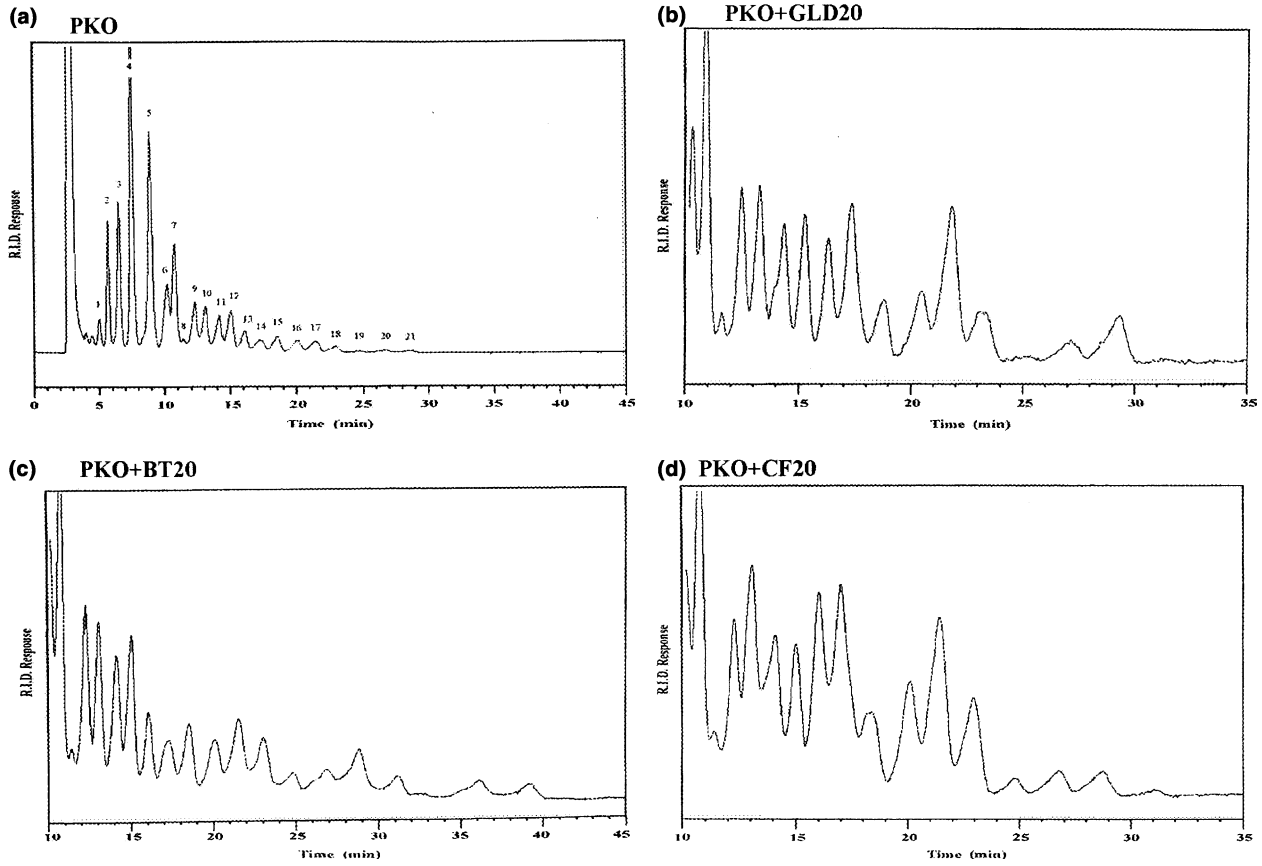

Fig. 4. HPLC chromatograms of: (a) palm kernel oil (PKO); (b) PKO adulterated with 20% GLD; (c) 20% BT; (d) 20% CF. Abbreviations: see Fig. 1.

could be designated into fourteen variables ranging from X_1 to X_{14}. ANOVA was performed on the means of all replicates of each treatment, in order to find any significant differences between the treatments and between the levels of adulterations. Multiple comparison test showed that X_1, X_2, X_4, X_5, X_6 and X_9 were the most suitable variables for the performance of CANDISC on different treatments (Table 4). The outcome of the CANDISC, when plotted for the first two canonical values, yielded a group separation, as shown in Fig. 3, where PO samples adulterated with other animal fats are tightly grouped while those adulterated with lard lie sufficiently far apart from them. Out of the GLD adulterated series, only one sample slightly deviated from the respective grouping. Likewise, among the sample series that are adulterated with other animal fats, only one sample showed slight deviation.

3.4. TAG analysis of adulterated samples of PKO by HPLC

PKO is generally classified as a lauric oil. The non-adulterated sample seemed to consist of a complex mixture of TAG (Fig. 4(a)). The TAG peak identification of PKO in this study was based on retention times of TAG standards and a previous study of Tan and Che Man (2000). Accordingly, the peaks were identified as 1:UK (Y_1), 2:CCLa (Y_2), 3:CLaLa (Y_3), 4:LaLaLa (Y_4), 5:LaLaM (Y_5), 6:LaLaP (Y_6), 7:LaMO (Y_7), 8:LaPM (Y_8), 9:LaOO (Y_9), 10:LaPO (Y_{10}), 11:LaPP/MMO (Y_{11}), 12:MMP (Y_{12}), 13:MOO (Y_{13}), 14:MPO/POL (Y_{14}), 15:PPL (Y_{15}), 16:OOO (Y_{16}), 17:POO (Y_{17}), 18:PPO (Y_{18}), 19:PPP (Y_{19}), 20:SOO (Y_{20}), and 21:PSO (Y_{21}) (where UK stands for unknown, La for lauric, C for capric, and others as described previously).

As shown in the chromatogram (Fig. 4(a)), four major peaks can be found in the region starting from 5.0 to 10.0 min, out of which trilaurin and myristodilaurin are the only TAG species occurring in amounts greater than 10%. Most of the remaining peaks of the chromatogram represent either minor or trace level TAG species. By making a comparison between Fig. 4(a) and Fig. 1, it is clear that the TAG elution-range of PKO does not fall within the elution-range of animal fats. Thus, in PKO it is easy to see the changes caused by animal fat adulterants. The differences in the TAG profiles produced by different animal fat adulterants are shown in Fig. 4. In each case the 20% level of adulteration was used to illustrate the effects but the deviation from the pure sample of PKO could be recognized even at adulteration levels as low as 5%. Although the changes caused by MT on the composition of PKO have not been discussed in detail they were in fact very similar to those caused by BT adulteration.

Since the objective of this study was to distinguish lard adulteration in PKO, the TAG profiles of the adulterated samples may be required to supplement further details. By closely examining the chromatographic profiles of the adulterated samples, it can be seen that, with the increasing concentration of animal fats, all peaks, from

Table 2
TAG compositional changes in PKO after adulteration with different concentrations (%) of GLD[a]

Peak No.	Adulteration level					
	0	2	5	10	15	20
1 (UK)	2.09 ± 0.02	2.0 ± 0.04	1.90 ± 0.00	1.88 ± 0.03	1.73 ± 0.04	1.83 ± 0.05
2 (CCLa)	6.63 ± 0.09	6.20 ± 0.11	6.12 ± 0.01	5.74 ± 0.06	5.39 ± 0.04	5.29 ± 0.02
3 (CLaLa)	9.57 ± 0.05	8.92 ± 0.09	8.82 ± 0.02	8.29 ± 0.07	7.72 ± 0.21	7.64 ± 0.06
4 (LaLaLa)	20.7 ± 0.08	20.0 ± 0.07	19.8 ± 0.04	18.4 ± 0.15	17.6 ± 0.22	16.9 ± 0.13
5 (LaLaM)	17.0 ± 0.10	15.8 ± 0.06	15.4 ± 0.12	14.5 ± 0.14	14.0 ± 0.08	13.5 ± 0.06
6 (LaLaP)	6.00 ± 0.11	5.65 ± 0.03	5.55 ± 0.00	5.22 ± 0.07	4.91 ± 0.04	4.89 ± 0.03
7 (LaMO)	8.50 ± 0.05	7.98 ± 0.02	7.88 ± 0.04	7.31 ± 0.03	7.01 ± 0.10	6.77 ± 0.08
8 (LaPM)	1.11 ± 0.02	1.08 ± 0.03	1.12 ± 0.03	1.16 ± 0.02	1.07 ± 0.01	1.16 ± 0.03
9 (LaOO)	4.98 ± 0.05	4.78 ± 0.05	4.69 ± 0.01	4.33 ± 0.04	4.07 ± 0.02	3.88 ± 0.05
10 (LaPO)	4.30 ± 0.00	4.34 ± 0.03	4.38 ± 0.04	4.35 ± 0.02	4.40 ± 0.01	4.39 ± 0.00
11 (LaPP/MMO)	3.70 ± 0.03	3.75 ± 0.01	3.79 ± 0.02	3.68 ± 0.00	3.74 ± 0.00	4.05 ± 0.04
12 (MMP)	4.29 ± 0.00	4.23 ± 0.00	4.15 ± 0.05	4.06 ± 0.03	3.95 ± 0.01	3.72 ± 0.00
13 (MOO)	2.30 ± 0.04	2.66 ± 0.01	2.72 ± 0.02	3.03 ± 0.02	3.23 ± 0.05	3.41 ± 0.06
14 (MPO/POL)	1.84 ± 0.05	2.53 ± 0.04	2.83 ± 0.06	3.77 ± 0.02	4.59 ± 0.05	5.25 ± 0.09
15 (PPL)	1.89 ± 0.07	2.20 ± 0.00	2.20 ± 0.03	2.45 ± 0.03	2.55 ± 0.02	2.34 ± 0.03
16 (OOO)	1.67 ± 0.03	2.19 ± 0.00	2.20 ± 0.01	2.42 ± 0.06	2.69 ± 0.02	2.71 ± 0.01
17 (POO)	1.54 ± 0.14	2.81 ± 0.10	3.08 ± 0.06	4.39 ± 0.02	5.41 ± 0.05	6.25 ± 0.03
18 (PPO)	0.79 ± 0.03	1.47 ± 0.02	1.60 ± 0.00	2.31 ± 0.08	2.50 ± 0.04	2.57 ± 0.02
19 (PPP)	0.29 ± 0.01	0.21 ± 0.02	0.10 ± 0.00	–	–	–
20 (SOO)	0.50 ± 0.05	0.84 ± 0.01	0.88 ± 0.03	1.31 ± 0.04	1.20 ± 0.07	1.16 ± 0.03
21 (PSO)	0.33 ± 0.00	0.35 ± 0.00	0.82 ± 0.04	1.44 ± 0.04	2.29 ± 0.07	2.32 ± 0.06

[a] Each value in the table represents the mean ± SD of triplicate analyses. Abbreviations: PKO, palm kernel oil; UK, unknown; C, capric; La, lauric. For other abbreviations see Table 1.

peak 1 to peak 9, except peak 8 tended to decrease while peak 13 and above tended to increase (Table 2, data for BT and CF not shown). Peak 8 remained unaffected by the addition of any of the animal fats. In the cases of peak 10 and peak 11, both GLD and CF adulteration tended to increase the peak while BT tended to decrease the peak. Peak 12 also decreased upon addition of all three types of adulterants.

The chromatographic profile beyond peak 13 is the most important region, where characteristic features, corresponding to each animal fat adulterant, seem to emerge. Particularly, differences could clearly be seen between GLD and CF in the ways that they influenced peak 14, peak 17 and peak 21. These were the peaks which greatly helped in determining the lard-adulterated samples of PKO since they were strongly influenced by the three dominant TAG peaks of lard, namely LPO, OPO and SPO.

Differentiation between GLD-adulterated series and BT-adulterated series was relatively easy since the influences of GLD and BT on the original profile of PKO differed considerably. This was because the lard TAG profile was distinctly different from that of beef tallow, as noted in the foregoing discussion and, hence, there were considerable differences in the influences of BT adulteration on the TAG peaks, such as peak 14, peak 17 and peak 21. In addition to this, a few smaller peaks also tended to emerge beyond peak 21 as the adulteration gradually increased.

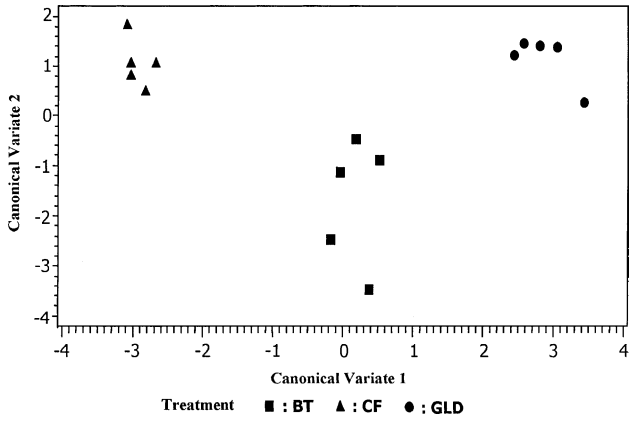

Fig. 5. CANDISC plot for canonical variate 2 vs. canonical variate 1 values of PKO samples adulterated with GLD, BT, and CF. Abbreviations: see Figs. 1, 3 and 4.

3.5. Multivariate analysis of HPLC data from PKO adulterated with different animal fats

Multivariate analysis of HPLC data using CANDISC could also offer an alternative way of detecting lard

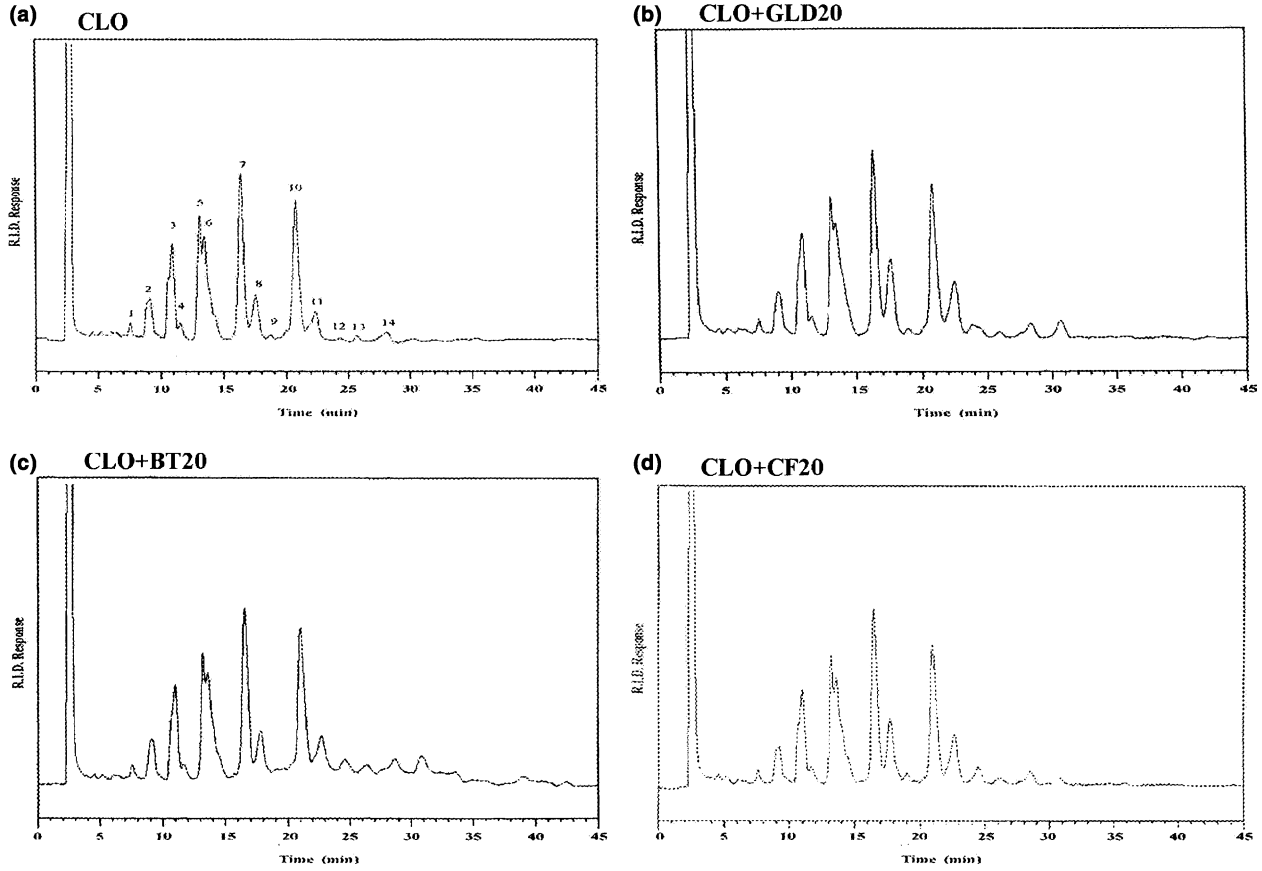

Fig. 6. HPLC chromatograms of: (a) canola oil (CLO); (b) CLO adulterated with 20% GLD; (c) 20% BT; (d) 20% CF. Abbreviations: see Fig. 1.

adulteration in PKO. As mentioned earlier, there were 21 TAG peaks in the HPLC profile of the unadulterated sample of PKO. By taking each peak as a variable, a minimum of twenty one variables (ranging from $Y_1, Y_2, \ldots, Y_{21}$) can be determined for each adulterated sample (Table 2). Based on the multiple comparison test, Y_1, Y_6, Y_8, Y_{10}, Y_{12}, Y_{13}, Y_{14}, Y_{17} and Y_{21} were the variables found suitable for performing CANDISC on different treatments (Table 4).

By plotting the first and second canonical values associated with each sample, a two-dimensional representation of the grouping by characteristic types could be obtained (Fig. 5). This clearly showed that adulterated samples belonging to each different animal fat type lie in a particular spatial region and, hence, there is adequate discrimination among them. Therefore, this could serve as a model to predict samples of PKO adulterated with GLD.

3.6. TAG analysis of adulterated samples of CLO by HPLC

CLO is a highly unsaturated, oleic acid-rich oil in which, monounsaturated TAG molecules are present predominantly. The TAG profile of the unadulterated sample, as shown in Fig. 6(a), was found to have fourteen TAG peaks. The TAG peak identification of CLO was based on retention times of TAG standards and a previous study of Tan and Che Man (2000). Accordingly, TAG peaks of CLO were: 1:LnLnL (Z_1), 2:LLLn (Z_2), 3:LLL (Z_3), 4:PLLn (Z_4), 5:OLLn (Z_5), 6:OOLn (Z_6) 7:OOL (Z_7), 8:POL/SLL (Z_8), 9:PSLn (Z_9), 10:OOO (Z_{10}), 11:POO/SOL (Z_{11}), 12:PPO (Z_{12}), 13:OOGa (Z_{13}), and 14:SOO (Z_{14}) (where Ln stands for linolenic, Ga for gadoleic, and others are as described previously).

The differences in the TAG profiles produced by different animal fat adulterants are shown in Fig. 6; in each case the 20% level of adulteration was used to illustrate the effects. Under similar operating-conditions, TAG peak elution-ranges of both CLO and GLD are similar. Therefore, most of the changes due to lard adulteration took place on the existing TAG peaks while a few minor peaks tended to emerge in the latter part of the chromatogram with increasing concentration of adulterant.

On a comparison basis, it may be possible to discriminate lard adulterated samples from those adulterated with BT, since BT adulteration in CLO caused decreases of peaks 1–10 while corresponding increases were noticed for peaks 11–14. Apart from these changes, peak15 emerged as an additional peak, even at adulteration levels as low as 2% while peak 16, peak 17 and peak 18 emerged as additional peaks at adulteration levels 10% and above (data not shown). However, most of the TAG compositional changes due to CF adulteration were seemingly comparable to those due to lard adulteration. Owing to this apparent similarity between the lard-and chicken fat-adulterated series, determination of lard adulteration in CLO may not be feasible through a superficial examination of HPLC data. Therefore, in order to resolve this issue, TAG compositional variations, due to different animal fat adulterations, were subjected to multivariate data analysis by taking each TAG peak as a variable.

3.7. Multivariate analysis of HPLC data from CLO adulterated with different animal fats

Based on the results presented in Table 3, a minimum of thirteen variables could be selected for multivariate

Table 3
TAG compositional changes in CLO after adulteration with different concentrations (%) of GLD[a]

Peak No.	Adulteration level					
	0	2	5	10	15	20
1 (LnLnL)	1.43 ± 0.00	1.50 ± 0.02	1.40 ± 0.01	1.45 ± 0.00	1.30 ± 0.03	1.30 ± 0.02
2 (LLLn)	6.14 ± 0.08	5.55 ± 0.05	5.50 ± 0.03	5.25 ± 0.06	5.00 ± 0.01	4.95 ± 0.00
3 (LLL)	12.0 ± 0.07	11.7 ± 0.10	11.2 ± 0.06	11.0 ± 0.04	10.9 ± 0.07	10.2 ± 0.12
4 (PLLn)	1.85 ± 0.02	1.80 ± 0.01	1.80 ± 0.00	1.65 ± 0.04	1.45 ± 0.05	1.70 ± 0.07
5 (OLLn)	9.58 ± 0.03	9.80 ± 0.05	9.35 ± 0.08	9.15 ± 0.08	9.40 ± 0.14	8.90 ± 0.11
6 (OOLn)	15.8 ± 0.04	15.2 ± 0.06	15.0 ± 0.04	14.8 ± 0.03	14.7 ± 0.00	14.3 ± 0.16
7 (OOL)	19.0 ± 0.16	18.5 ± 0.10	17.9 ± 0.09	17.3 ± 0.07	17.4 ± 0.04	16.9 ± 0.07
8 (POL/SLL)	6.48 ± 0.03	6.40 ± 0.08	6.80 ± 0.15	7.30 ± 0.13	8.10 ± 0.12	8.70 ± 0.09
9 (PSLn)	0.71 ± 0.00	0.70 ± 0.01	0.80 ± 0.00	0.80 ± 0.03	0.70 ± 0.00	0.95 ± 0.02
10 (OOO)	19.0 ± 0.05	19.8 ± 0.08	19.2 ± 0.11	18.3 ± 0.17	18.4 ± 0.13	17.0 ± 0.08
11 (POO/SOL)	5.43 ± 0.07	5.65 ± 0.04	6.20 ± 0.12	7.00 ± 0.08	7.45 ± 0.07	8.00 ± 0.05
12 (PPO)	0.28 ± 0.06	0.70 ± 0.09	0.90 ± 0.13	1.40 ± 0.14	1.80 ± 0.11	2.30 ± 0.18
13 (OOGa)	0.52 ± 0.00	0.70 ± 0.03	1.00 ± 0.02	0.95 ± 0.03	0.75 ± 0.00	0.65 ± 0.02
14 (SOO)	1.68 ± 0.02	1.70 ± 0.04	2.35 ± 0.07	2.20 ± 0.04	1.65 ± 0.06	2.00 ± 0.05
15 (UK)	–	0.40 ± 0.07	0.95 ± 0.05	1.50 ± 0.08	1.20 ± 0.07	2.10 ± 0.02

[a] Each value in the table represents the mean ± SD of triplicate analyses. Abbreviations: CLO, canola oil; Ln, linolenic; Ga, Gadoleic. For other abbreviations see Table 1.

Table 4
Summary of the statistical analysis for variable selection in each oil[a]

PO		PKO		CLO	
Variable	Treatmentwise CL	Variable	Treatmentwise CL	Variable	Treatmentwise CL
X_1	*	Y_1	*	Z_1	ns
X_2	*	Y_2	ns	Z_2	ns
X_3	ns	Y_3	ns	Z_3	ns
X_4	*	Y_4	ns	Z_4	*
X_5	*	Y_5	ns	Z_5	**
X_6	**	Y_6	*	Z_6	ns
X_7	ns	Y_7	ns	Z_7	ns
X_8	ns	Y_8	*	Z_8	*
X_9	*	Y_9	ns	Z_9	*
X_{10}	ns	Y_{10}	*	Z_{10}	ns
X_{11}	ns	Y_{11}	ns	Z_{11}	*
X_{12}	ns	Y_{12}	*	Z_{12}	**
X_{13}	ns	Y_{13}	**	Z_{13}	*
X_{14}	ns	Y_{14}	**	–	–
–	–	Y_{15}	ns	–	–
–	–	Y_{l6}	ns	–	–
–	–	Y_{17}	**	–	–
–	–	Y_{18}	ns	–	–
–	–	Y_{19}	ns	–	–
–	–	Y_{20}	ns	–	–
–	–	Y_{21}	*	–	–

[a] Abbreviations: CL, confidence level; ns, not significant; for other abbreviations see Tables 1–3.
* $P < 0.05$.
** $P < 0.001$.

data analysis. The multiple comparison test indicated that, out of the thirteen variables, Z_4, Z_5, Z_8, Z_9, Z_{11}, Z_{12} and Z_{13} were found to show significant differences with respect to different treatments (Table 4). Therefore, these seven variables were subsequently used to perform CANDISC analysis. Fig. 7 shows a two-dimensional representation of the groupings resulting from the first and second canonical values associated with each sample. This shows that adequate discrimination could be achieved using CANDICS analysis for the detection of lard-adulteration in CLO.

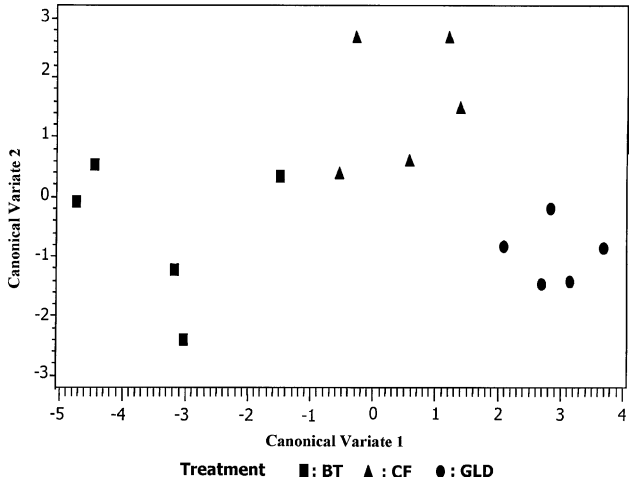

Fig. 7. CANDISC plot for canonical variate 2 vs. canonical variate 1 values of CLO samples adulterated with GLD, BT, and CF. Abbreviations: see Figs. 1, 3 and 6.

4. Conclusion

It is possible to distinguish lard-contaminated samples of vegetable oils by subjecting liquid chromatographic data to CANDISC analysis. The potential of the method is evident, as oil samples that are contaminated with as little as 2% lard could easily be distinguished. Based on this preliminary investigation, the usefulness of this approach could be tested for other oils in the future.

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