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Chemical and biological evaluation of discarded frying palm oil from commercial restaurants

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A chemical and biological evaluation of two frying palm oil samples (A and B) discarded by commercial restaurants is reported. The degree of deterioration of the discarded palm oil was chemically estimated by 10 ordinary laboratory methods and five quick-test methods, and the biological effects of the oil were investigated by feeding rats with standard diet containing 20% discarded palm oil for 28 days. The chemical measurements indicated a severe deterioration during frying operations, particularly in sample A, which increased liver index and induced a nonsignificant decrease in platelet aggregation when compared with sample B. Both samples reduced spleen index, but had no effects on animal growth, heart and thymus indices, or aorta prostacyclin release.

INTRODUCTION

Fat or oil frying is one of the most commonly used methods for the preparation of foods. The fat or oil acts as a heat transfer medium and as an important ingredient of the fried food. However, repeated heating of edible oils, especially polyunsaturated fat, markedly modifies various parameters in the oil which are considered good indices of the degree of thermal oxidation (Fritsch, 1981; Giani *et al.*, 1985). The thermal degradation results in accumulation of decomposition products which not only affect the quality of fried foods, but are also of much concern for human health (Artman, 1969; Billek, 1979; Frankel *et al.*, 1984; Perrin, 1984; Sebedio *et al.*, 1988). Thermally oxidized oils are known to cause growth retardation, increase in liver and kidney weights, damage to the liver, thymus and testes (Alexander *et al.*, 1987), alteration of the production vascular eicosanoids (evaluation of platelets thromboxin formation) and decrease in vascular prostacyclin release (Giani *et al.*, 1985). Further, heating of oil leads to reduction of vitamin E, the latter preventing formation of lipid hydroperoxide free radicals (Duthie *et al.*, 1989) that are known to cause reduction of prostacyclin (and normally impair platelet aggregation) (Moncada *et al.*, 1976), tumour formation, toxic liver injury, neuromuscular disorders, arthritis, iron overload, gastrointestinal disorders (Halliwell and Grootveld, 1987) and damage to aortic endothelial cells (Yagi, 1987). However, in most of the published work on oil thermal degradation and its effects on various biological systems, the oil had been subjected to severe heating conditions in the laboratory which can be very different from those encountered in actual commercial practice.

In a previous study (Al-Kahtani, 1991), a survey of 62 commercial restaurants and fast-food outlets was conducted. The physical and chemical analysis of their discarded oils indicated particular deterioration in two of the 126 collected samples. The objective of this study was to investigate separately these two degraded frying palm oil samples more thoroughly by measuring the extent of chemical deterioration and studying the effects of feeding with a diet containing 20% discarded palm oil on aortic prostacyclin release, platelet aggregation, liver function, organ indices and growth in rats.

MATERIALS AND METHODS

Frying oil and frying techniques

The commercial frying pan oil (Al-Arabi, a product of the Saudi Vegetable Oil and Ghee Company, Jeddah,

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Table 1. Variables of commercial frying operations from which palm oil samples were obtained

Frying variables	Samples	
	A	B
Oil type	Commercial frying palm oil[a]	Commercial frying palm oil[a]
Frying vessel	Conventional steel frying pan (Fig. 1)	500 Pressure Fryer (Fig. 2) (Henny Penny Corp., Eaton, OH)
Oil quantity (litres)	4·5	27
Oil turnover rate	100% turnover (no replenishment)	100% turnover (no replenishment)
Temperature (°F)	340–360	350
Frying time per day	6	12
Days	3	4
Total frying time (h) (discarding point)	18	48
Food type	Various[b]	Various[c]
Amount of food fried (g per batch)	700–800	3600–4000
Batch time (min)	5	7–10
Frying frequency	Intermittent	Intermittent
Total amount of foods fried (kg)	18·5–22	260–300

[a] Frying palm oil is a product (Al-Arabi) of the Saudi Vegetable Oil and Ghee Company (Jeddah, Saudi Arabia).
[b] Fallafel (78%)—Middle Eastern vegetable patties; Sanbosak (22%)—Middle Eastern meat and vegetable mixture patties.
[c] Chicken (67%), fish (22%) and fresh potatoes (11%).

Saudi Arabia), is the most widely used frying oil in Saudi Arabia. The two discarded frying palm oil samples used in this study were chosen from 126 discarded frying oil samples collected during a survey of 62 commercial restaurants and fast-food outlets (Al-Kahtani, 1991). Restaurant operators were asked to follow their normal frying practices and save about a 1-litre oil sample in a clean glass bottle when they were about to discard the frying oils. Frying variables (the fryer design, fabrication material and capacity, and the frying conditions—temperature, frying frequency, total frying time and food type) of frying operations from which the two samples were obtained were reported during or after frying cycles (Table 1). Oil samples were then blanketed with nitrogen gas and stored at 20°C until required.

Chemical evaluation of used oils

Degree of deterioration of discarded oils was assessed by several methods, as follows.

Ordinary laboratory methods

The analytical procedures for quality evaluation of fresh and discarded oils include measurements of colour (Lovibond Tintometer, model E), viscosity (Brookfield Viscometer, 60 rpm at 38°C), refractive index (25°C), absorbance at 232 and 268 nm (Spectrophotometer, Beckmann model 35, USA), total polar materials (Billek *et al.*, 1978), iodine, *p*-anisidine and peroxide values, and free fatty acids (IUPAC, 1979; American Oil Chemists Society (AOCS), 1980). Fatty acid profile was determined by gas chromatography (Metcalf *et al.*, 1966) and the fatty acid methylesters were identified on a 5840 A gas chromatograph (Hewlett-Packard, PA, USA) with a flame ionization detector and 190 cm × 0·2 cm column packed with 70% DEGS on 100–120 high-performance Chromosorb W. The ratio of C 18:2/C16:0 was calculated from the fatty acid composition.

Quick-test methods

A food oil sensor (N1-21A, Northern Instrument Corp., Lino Lakes, MN) was used to measure the dielectric constant in discarded frying oils relative to fresh oils (Graziano, 1979). Four colorimetric diagnostic test kits (Fritest, Oxifrit or RAU-Test, Veri-Fry-FFA 500, and Veri-Fry-TAM 150) were used for visual evaluation of quality of discarded frying oil. Fritest and Oxifrit (products of E. Merck, Darmstadt) are sensitive to carbonyl and oxidized compounds, respectively (Croon *et al.*, 1986). Both kits have a colour scale consisting of four diagnostic colours—'good', 'still good', 'replace' and 'bad'—which were set to 1, 2, 3 and 4, respectively. The Veri-Fry-FFA 500 and Very-Fry-TAM 150 kits are products of Libra Laboratories, USA. The former is for the measurement of free fatty acids and the latter is for the estimation of total alkaline materials (soap concentration, in ppm) in discarded frying oils (Blumenthal *et al.*, 1985). The colour scale of these two kits consists of five diagnostic colours, each of which corresponds to a range of values for free fatty acids (%) or for total alkaline materials (ppm). The colour was read in steps of 0·5 over a range from 1 to 5.

Animals and dietary treatments

Male Sprague—Dawley rats weighing 200–250 g (Animal Care Centre of the College of Pharmacy, King Saud University) were divided into three groups. Each group was fed a standard ground diet containing 20% non-heated (fresh) oil, discarded frying oil of sample A or discarded frying oil of sample B for 28 days. Body weights were monitored each third day.

Preparation of platelet-rich plasma (PRP)

On Day 28, the animals were anaesthetized with ether and 5 ml of blood was withdrawn from each animal by cardiac puncture in a 10-ml plastic syringe containing sodium citrate. The blood and sodium tricitrate (3·6%) ratio was 9:1 (v/v). The citrated blood was then centrifuged at 400 *g* for 7 min and plasma-rich platelets was obtained for platelet aggregation (El-Tahir & Williams, 1980) and serum glutamic oxaloacetic transaminase (SGOT) determinations.

Isolation of thoracic aorta and various organs

The animals were killed and the abdomen and thorax were opened. The thoracic aorta was cleared from adhering tissues and placed on ice-cold Kreb's solution (pH 8) for prostacyclin (PG12) production estimation. The thymus, heart, liver and spleen were then removed and weighed.

Determination of PG12 release from thoracic aorta

The tissues were blotted dry and 20 mg of thoracic aorta was placed in a small plastic cuvette containing 0·2 ml of Kreb's solution (pH 8). The tissues were incubated for 30 min at 37°C, then the aorta was chopped into small pieces and incubated in an aggregometer chamber for 3 min. A siliconized stainless stirrer was used during the 3-min incubation. The cuvette was then removed and placed in an ice bath. The PG12 content of the incubation medium was estimated against authentic PG12 (Sigma Chemical Co., St Louis, MO, USA) using platelet antiaggregatory assay (El-Tahir & Williams, 1980).

Platelet aggregation

To study platelet aggretability, the minimum dose of adenosine diphosphate (ADP, Sigma Chemical Co.), which induces irreversible aggregation lasting at least 3 min was used. The results was expressed as per cent of control.

Liver function test

SGOT was estimated by a colorimetric method (Reitman & Frankel, 1957) using a commercial kit (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany).

Statistical analysis

Data were statistically analysed using the analysis of variance (Steel & Torrie, 1980) and SAS programs (Statistical Analysis Systems Institute, 1982).

RESULTS AND DISCUSSION

Frying techniques

Sample A was collected from a primitive frying technique using an open conventional gas-fried steel pan (Fig. 1), which is simply a household appliance type of oil fryer and does not meet the frying operation requirements. In contrast, the frying operation from which sample B was obtained utilized a well-designed stainless steel electric broaster (Fig. 2; 500 Pressure Fryer, Eaton, OH, USA) in which frying conditions can be easily controlled.

Chemical evaluation of used oils

Ordinary laboratory methods

The extents of palm oil degradation during frying are depicted in Table 2. The colour of frying oils changed from light yellow of fresh oil to amber and reddish brown, particularly in sample B, as a result of the diffusion of pigments into the oil during frying. The change in colour may be due to oxidation (Peled *et al.*, 1975; Fritsch, 1981; Yoon *et al.*, 1987). Rossell and Phil (1986) mentioned that palm oil is known to darken more quickly than other oils, but this does not necessarily mean a reduction in quality.

The increase in viscosities of discarded oils (Table 2) was due to polymerization which resulted in formation of higher molecular weight compounds (carbon to carbon and/or carbon to oxygen—to carbon bridges between fatty acids (Artman, 1969; Landers & Rathmann, 1981). Many workers (Rock, 1964; Defouw *et al.*, 1981; Bessler and Orthoefer, 1983) have reported increases in oil viscosities during heating or frying. Refractive indices of discarded oils were higher than that of fresh oil (Table 2), and this is in agreement with the finding of Yoon *et al.* (1987).

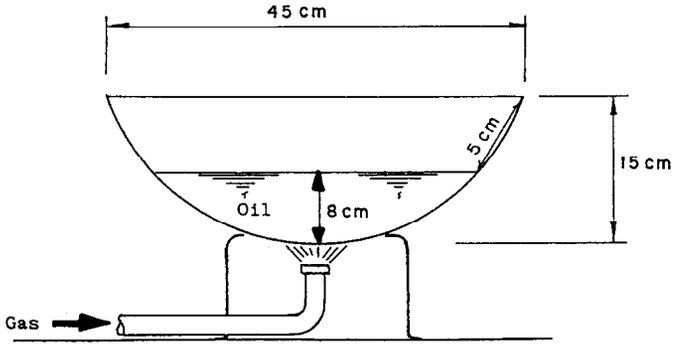

Fig. 1. Conventional gas-fired steel pan from which sample A was collected.

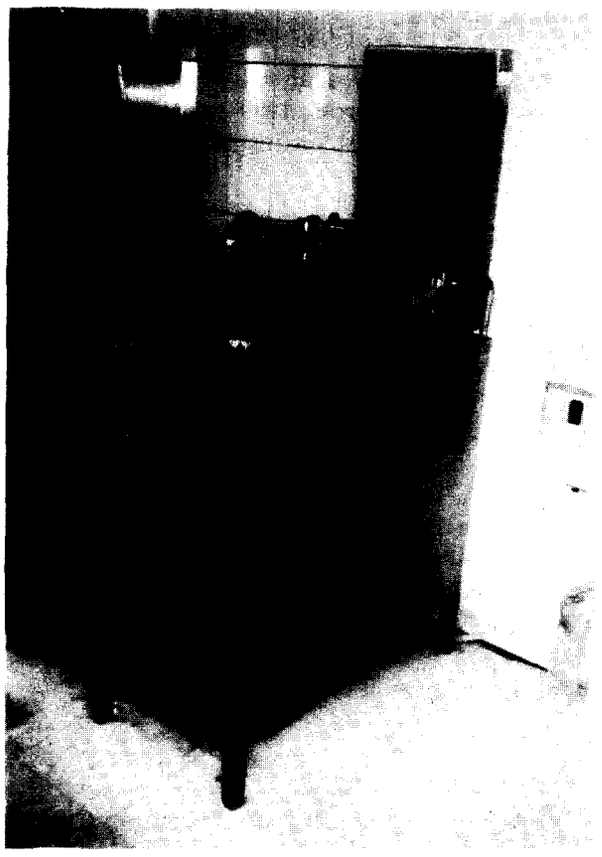

Fig. 2. Electric broaster (500 Pressure Fryer, Henny Penny Corp, Eaton, OH) from which sample B was collected.

Extinctions at both 232 and 268 nm were higher in discarded oils, particularly sample A, than in fresh oils (Table 2). This indicates the formation of conjugated compounds (dienes and trienes) in frying oils. According to Peled *et al.* (1975) and Yoon *et al.* (1987), these compounds could form polymers and an equilibrium might occur during frying.

The contents of total polar materials (TPM) in discarded oils are shown in Table 2. The polar fraction of discarded oil of sample A was much higher than that of sample B, and this indicates the degree of deterioration, as TPM represents all oxidation and decomposition products formed during frying (Billek, 1979). The increase of TPM has been reported by many researchers (Paradis & Nawar, 1981; Frankel *et al.*, 1984; Defiellietaz-Goethart *et al.*, 1985; Augustin *et al.*, 1987). It has been suggested that TPM content is the most reliable measure of fat deterioration during frying (Fritsch, 1981), and that 25—30% is the rejection point for frying oil (Billek *et al.*, 1978; Billek, 1979). However, these values were adopted for polyunsaturated oils and might not be applicable to more saturated oils (palm oil), as these oils are less susceptible to oxidation during frying (Difielliettaz-Goethart *et al.*, 1985).

Peroxide, iodine and *p*-anisidine values, are the oil oxidation parameters, but the last is the most reliable, as it measures the secondary stage of oxidation rather than unstable peroxides, which are formed during primary oxidation (Augustin *et al.*, 1987; Sebedio *et al.*, 1988). The rapid decomposition of these peroxides makes the peroxide value a less reliable method for monitoring thermal deterioration of palm oil (Lee, 1962; Okiy & Oke, 1986; Sebedio *et al.*, 1988). Oxidation at the reactive double bonds in a fatty acid chain decreased the total number of double bonds, and, consequently, a reduction in iodine value in sample A was obtained. The fried food ingredients that leached out into the frying medium affected the stability and decomposition of the frying oil. Chlorophyll and pheophytin from fallafel are known as pro-oxidants in fats and oils (Usuki *et al.*, 1984). Many investigators (Augustin *et al.*, 1987; Stevenson *et al.*, 1984; Yoon *et al.*, 1987) have reported a decrease in iodine value in palm oil products during heating and frying. However, sample B was slightly higher in iodine value, possibly because the fat that leached out from the foods (fish and chicken) altered the iodine value of the frying oil. Table 2 shows an increase in palmitic acid and a decrease in linoleic acid (lower C18:2/C16:0 ratio) in discarded oils. Peers and Swoboda (1982) found this ratio to be a reliable indicator of fat deterioration during frying, whereas others (Augustin *et al.*, 1987; Yoon *et al.*, 1987) reported a higher correlation between this ratio and iodine value, TPM and dielectric constant.

Sample B was higher in free fatty acids (%) than sample A (Table 2). The acidity was mainly formed by hydrolysis of triglycerides, which was promoted by the presence of food moisture, and by oxidation (Fritsch, 1981) or by the reaction of oil with moisture formed during other deterioration reactions (Peled *et al.*, 1975; Augustin & Berry, 1984; Okiy & Oke, 1984; Peers & Swoboda, 1982; Usuki *et al.*, 1984; Carlson & Tabacchi, 1986; Yoon *et al.*, 1987). However, the measurement of free fatty acids (%) cannot determine suitability of frying oils for further use (Berger 1984).

Quick-test methods

The change in dielectric constant of discarded frying oils, which resulted from the accumulation of polar molecules during oil breakdown, was measured by a food-oil sensor after calibration with corresponding fresh oil (Fritsch *et al.*, 1979). Sample A appeared to be heat damaged to a greater extent than sample B (Table 3). According to Paradis and Nawar (1981), a food-oil sensor reading of 3·7 (27% polar components value) or higher has been suggested to represent low-quality frying corn oil. Food-oil sensor values of 3·1 and 3·5 corresponded to polar component values of 27% and 29%, respectively, in high-stability frying oils such as palm and hydrogenated oils (Croon *et al.*, 1986). A study by Augustin *et al.* (1987) on refined, bleached, deodorized (RBD) olein showed that 27% polar components gave a dielectric constant reading of 3·7 on a food-oil sensor.

The food-oil sensor is accurate, quick and considered to be an alternative method to TPM (Graziano, 1979; Fritsch, 1981). However, readings of the instrument become less accurate at later stages of frying (Fritsch *et al.*, 1979; Stevenson *et al.*, 1984), as a result of the pres-

Table 2. Quality assessment[a] of discarded frying palm oils, by ordinary methods

Characteristic	Fresh oil	Discarded frying oil	
		Sample A	Sample B
Colour			
Red	3·05 ± 0·80	8·00 ± 1·00	45·50 ± 6·40
Yellow	28·00 ± 2·60	47·00 ± 2·50	54·30 ± 5·10
Blue	—	—	5·50 ± 2·10
Viscosity (cP)	48·60 ± 1·60	107 ± 10·70	92·60 ± 9·35
Refractive index	1·4666 ± 0·00	1·4686 ± 0·00	1·4683 ± 0·00
Absorbance			
232 nm	0·44 ± 0·07	2·51 ± 0·23	1·16 ± 0·19
268 nm	0·02 ± 0·01	0·52 ± 0·09	0·37 ± 0·03
Total polar materials (%)	3·50 ± 0·45	26·7 ± 1·31	16·5 ± 0·75
Iodine value	55·9 ± 1·30	50·9 ± 0·30	57·6 ± 0·68
p-Ansidine value	3·95 ± 0·49	98·2 ± 5·80	68·6 ± 6·90
Peroxide value (mequiv kg^{-1})	1·85 ± 0·49	15·5 ± 1·90	10·7 ± 0·70
Free fatty acids (%)	0·19 ± 0·20	1·40 ± 0·18	2·58 ± 0·30
Fatty acids (%)			
14:0	1·93 ± 0·09	3·60 ± 0·06	5·40 ± 0·32
16:0	34·6 ± 0·14	39·2 ± 0·63	36·2 ± 0·78
18:0	5·15 ± 0·07	5·60 ± 0·06	5·20 ± 0·11
18:1	41·4 ± 0·42	39·9 ± 0·27	40·0 ± 0·80
18:2	11·7 ± 0·21	7·00 ± 0·64	10·3 ± 0·92
20:0	0·63 ± 0·02	0·40 ± 0·05	0·32 ± 0·01
Others	4·85 ± 0·35	4·70 ± 0·29	3·09 ± 0·52
18:2/16:0	0·34 ± 0·01	0·18 ± 0·02	0·27 ± 0·01

[a] Mean of three determinations.

ence of water or fat extracted from the fried food in the frying oils.

Visual estimation of the used frying oils indicated the degree of deterioration during frying (Table 3). The Oxifrit (RAU-Test) colour readings 'bad' showed that the oils of both samples were degraded, and the Fritest reading 'replace' indicated the right time of oil change. However, because of clarity it was easier to compare the colour of the reaction mixtures to the colour scale of the RAU-Test than that of Fritest (Croon *et al.*, 1986).

The observations of the reaction mixture colours for free fatty acid (Veri-Fry FFA-500 kit) showed green–blue or a reading of 1 (0–0·2% FFA) for fresh oil, and green—yellow or a reading of 4 (2·1–3·5% FFA) for sample A and yellow or a reading of 5 (3·6–5% FFA) for sample B (Table 3). The higher acidity in sample B is qualitatively consistent with the results obtained by the ordinary laboratory method of free fatty acid measurement (Table 2).

Table 3. Quality evaluation of discarded frying oils by quick-test methods

Methods	Fresh oil	Discarded frying oil	
		Sample A	Sample B
Food-oil sensor	1·97 ± 0·06	7·17 ± 1·18	4·83 ± 0·55
Fritest	Good	Replace	Replace
RAU-Test (Oxifrit-Test)	Good	Bad	Bad
Veri-Fri-FFA-500 (% FFA)	0·0–0·2	2·1–3·5	3·5–5·0
Veri-Fri-TAM-150 (Soap concentration, ppm)	0·0–20	86–110	86–110

The colours of reaction mixtures for total alkaline materials (Veri-Fry TAM-150 kit) were lemon yellow or a reading of 1 (0–20 ppm soap concentration) for fresh oil and blue–green or a reading of 4 (86–110 ppm soap concentration) for both samples A and B (Table 3). The accumulation of these materials in discarded frying oils was a result of the interaction of food materials with oil degradation products.

Al-Kahtani (1991) found a highly significant ($P < 0·001$) correlation (CV = 0·75–0·9) between the standard method (column chromatographic determination of polar materials) and each of the quick-test methods food-oil sensor (dielectric constant), Oxifrit (RAU-Test), Fritest and Veri-Fry-FFA-500, but a lower correlation (CV = 0·43) between the standard method and Veri-Fry TAM-150. Other correlation coefficients between ordinary methods have been reported by Al-Kahtani and by other workers (Peled *et al.*, 1975; Croon *et al.*, 1986).

Biological studies

Feeding the animals with either sample for 28 days had no significant effect on body weight (Fig. 3). Discarded oil sample A significantly increased liver weight (Table 4) when compared with either sample B or fresh palm oil. The increase in liver weight is associated with a

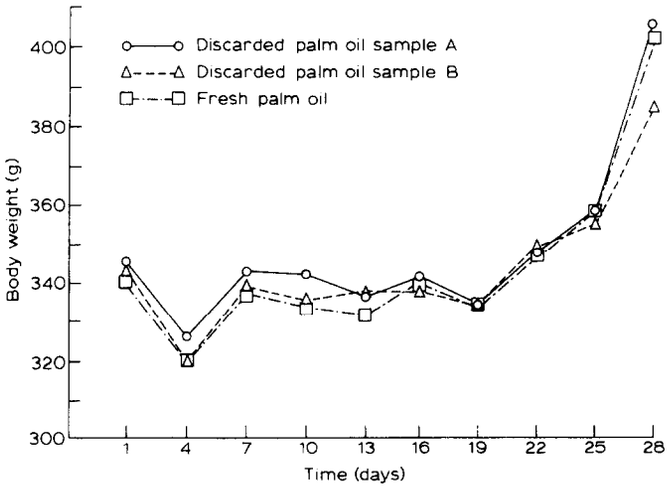

Fig. 3. Body weight of rats during feeding with a diet containing 20% discarded palm oil.

higher level of polar compounds in the used oil, such as that of sample A, which contained higher concentrations of polar compounds than sample B. Billek (1979) reported that higher levels of polar compounds of heated sunflower oil produced an increase in the liver weight index, but there is no significant change in SGOT (Table 4). A longer duration of feeding with used oil might be needed to produce significant liver damage (Billek, 1979). Discarded oil of either sample significantly reduced spleen index when compared with fresh palm oil (Table 4). The reduction in spleen index may be a result of spleen lymphocyte loss (Patricia *et al.*, 1982). The loss of spleen lymphocytes may lead to reduction in immune function, as lymphocytes represent an important part of the immune system (Michael & James, 1987). Neither sample altered the thymus or heart weights (Table 4).

Platelet aggregation were not significantly altered by either sample (Table 4), and there is a nonsignificant decrease in platelet aggregation in animals treated with sample A. This might be due to a high level of polar compounds, as the oxygen radical, which may be present in polar compounds, decreases ADP-induced platelet aggregation (Norman *et al.*, 1981).

Aortic prostacyclin contents were not altered by either sample (Table 4). However, it has been reported that feeding with polyunsaturated fatty acid reduces prostacyclin level, presumably through enhanced generation of peroxidation products in tissues (Okuma *et al.*, 1980; Karpen *et al.*, 1981) and an increase in mixed function oxidase (Morio *et al.*, 1990).

Table 4. Effects of feeding diet containing 20% discarded frying palm oil on various biological systems

	Fresh oil	Discarded	
		Sample A	Sample B
Liver weight index (g)	2·95 ± 0·08	3·52 ± 0·12[a]	3·09 ± 0·21
Liver function (SGOT[b] UI^{-1})	29·3 ± 3·35	27·2 ± 0·76	28·1 ± 3·36
Spleen index (g)	0·23 ± 0·04[a]	0·19 ± 0·02	0·19 ± 0·02
Heart weight index (g)	0·27 ± 0·01	0·28 ± 0·01	0·28 ± 0·01
Thymus weight index (g)	0·13 ± 0·01	0·14 ± 0·01	0·12 ± 0·01
Prostacyclin production (ng mg^{-1} tissue)	9·50 ± 0·50	13·0 ± 2·20	12·4 ± 2·40
Platelet aggregation (% of control)	61·00 ± 16·00	40·00 ± 5·00	62·0 ± 0·15

[a] Significantly different from other oils; $P < 0.05$ (by Newman–Keul's range test), mean ± SE.
[b] SGOT—Serum glutamin oxaloacetic transaminase.

The lack of effect of discarded frying palm oil on prostacylin production is not surprising, as it is saturated oil which is less susceptible to oxidation (Defiellettaz-Goethart *et al.*, 1985). It is suggested that the peroxides generated from thermal oxidation of unsaturated fat are unstable and may not be responsible for reduction of prostacyclin production (Giani *et al.*, 1985).

In conclusion, discarded oil sample A, which was more degraded (in particular, it contained more polar components), produced more detrimental effects in the rats than sample B. However, the significant biological effects produced were limited to an increased liver weight and decreased spleen index. This might be due to (1) a lower susceptibility of palm oil to oxidation during frying, compared with polyunsaturated oils, whose thermal oxidation products were reported in the literature to have more detrimental biological effects, or (2) the duration of feeding, might have been too short to produce significant effects. However, this study has highlighted the extent of deterioration of palm oil under commercial frying conditions when it is not discarded or replaced at the right time, and the biological consequences. The history of the two samples represented actual commercial practice, without the exaggeration in thermal conditions (temperature and time) that sometimes occur in laboratory oil-heating or frying experiments.

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