Short-Term in Vivo Digestibility of Triglyceride Polymers, Dimers, and Monomers of Thermoxidized Palm Olein Used in Deep-Frying

M. José González-Muñoz,† Sara Bastida,‡ and Francisco J. Sánchez-Muniz*,‡

Departamento de Nutrición y Bromatología, Facultad de Farmacia, Universidad de Alcalá de Henares, E-28871 Madrid, Spain, and Departamento de Nutrición y Bromatología I (Nutrición), Sección Lípidos, Facultad de Farmacia, Universidad Complutense de Madrid, E-28040 Madrid, Spain

Palm olein used to fry potatoes 40 and 90 times was tested in a short-term in vivo digestibility experiment. Total alteration, expressed as polar content, reached 26.4 g/100 g of olein, and the amount of triglyceride polymers, triglyceride dimers, and oxidized triglycerides increased severalfold, as a result of repeated frying use of the olein. True digestibility of used and unused whole palm olein and that of the nonoxidized triglycerides and different polar compounds was tested after 1 g of palm olein/100 g of body weight was administered to young adult Wistar rats by means of an esophageal probe. Luminal fat was obtained 4 h later, and its polar and nonpolar fractions were separated and quantified using column chromatography. Thermoxidized and hydrolytic compounds were quantified by high-performance size exclusion chromatography and compared to the compounds administered. The true digestibility coefficient of palm olein used in frying 90 times was 30% lower than that of unused palm olein. True digestibility of triglyceride polymers and dimers of unused palm olein was quite high (>50%). After 90 uses, digestibility of dimers was significantly lower ($p < 0.01$), being ~30%. Nonoxidized triglyceride hydrolysis was negatively affected by the presence of large amounts of thermoxidized compounds. The amount of monoglycerides and free fatty acids found in the luminal fat also decreased as a consequence of the arrest of pancreatic lipase activity by thermoxidized compounds formed through repeated frying.

Keywords: *Column chromatography; deep-frying; digestibility; HPSEC; in vivo; oxidized triglycerides; palm olein; polymers; rats*

INTRODUCTION

The present-day diet of the population of European Union countries and North America contains substantial quantities of fat subjected to different processing and heat treatments, for example, deep-fat frying. During frying, oxidative and thermal degradations take place in the unsaturated acyl groups of triglycerides, modifying their nutritional properties and leading to the formation of many different oxidized and polymerized compounds, most of which display higher polarity than the original triglycerides (Arroyo et al., 1992; Dobarganes et al., 1988; Garrido et al., 1994).

It has been suggested that diet is the source of oxidized lipoproteins found in the intestinal lymph of rodents (Aw et al., 1992; Stapräns et al., 1993a). Moreover, Stapräns et al. (1993a,b) observed a direct relationship between levels of oxidized lipids in the diet and the amount of oxidized lipids in mesenteric lymph chylomicrons of rats, suggesting that oxidized lipids in the diet are absorbed by the small intestine. After feeding rats a diet containing thermoxidized sunflower oil used in frying potatoes repeatedly 75 times, Sánchez-Muniz et al. (1998) found that hepatic thiobarbituric acid-reactive substances (TBARS) increased.

Digestibility is generally believed to decrease when frying fats are consumed, although some authors do not report important changes [for review, see Cuesta et al. (1988) and Márquez-Ruiz and Dobarganes (1996)]. According to Matsuchita (1975), Miyashita et al. (1990), and Perrin et al. (1985), triglycerides containing at least one oxygenated function are absorbed quite well. Moreover, it appears that triglyceride monohydroperoxides are hydrolyzed by pancreatic lipase to almost the same degree as the original triglycerides (Matsuchita, 1975; Miyashita et al., 1990). However, data concerning triglyceride oligomer digestibility are confusing (Bottino, 1962; Kajimoto and Mukai, 1970).

Throughout recent decades in Spain, consumption of the traditional olive oil has declined in favor of that of other oils (Moreiras et al., 1990). The lower cost and high oxidative stability of palm olein (Gupta, 1993) explain the increased utilization of this oil for frying purposes and snack food preparation in Spain. Previous studies on in vitro hydrolysis of palm olein suggested that the kinetic parameters apparent Michaelis−Menten constant (K_M) and apparent maximum velocity of hydrolysis (V_M) were not related to the degree of alteration of the olein (Arroyo et al., 1996). Results obtained were related to a balance between the molecular weight of the triglyceride oligomers that negatively affect the enzymatic hydrolysis by the porcine lipase and the presence of polymers formed in fried oils that should have surfactant properties favoring the in vitro hydrolysis of the palm olein (Arroyo et al., 1996).

Studies on altered fat digestibility often use radiolabeled markers that do not always correspond with the same composition as that of the altered compounds

* Author to whom correspondence should be addressed (telephone 34-1-3941828; fax 34-1-3941732; e-mail frasan@eucmax.sim.ucm.es).

† Universidad de Alcalá de Henares.

‡ Universidad Complutense de Madrid.

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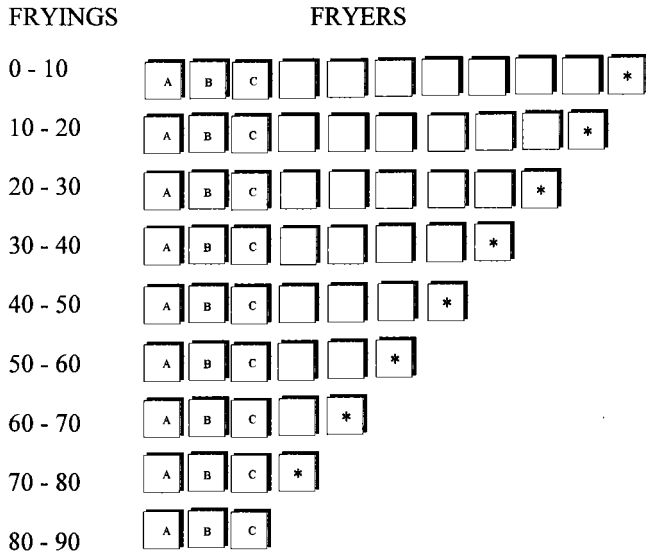

Figure 1. Performance of fryings with a null turnover of fresh oil: (A−C) samples of oils taken for analysis; (*) signifies fryer eliminated after each 10 fryings, its contents emptied to make up the volume of the other fryers to 3 L to keep the proportion of food to frying oil at 500 g/3 L.

present in used frying fats. Moreover, these markers are potentially harmful and rather expensive. In the present experiment a combination of the in vivo short-term fat digestibility, column chromatography, and high-performance size exclusion chromatography (HPSEC) techniques was employed to study the digestibility of palm olein used in frying, as well as of the different altered compounds (from thermal oxidative and hydrolytic alterations) formed during frying. The possible inhibitory role of some altered compounds, such as oligomers (polymers plus dimers of triglycerides), on fat digestibility was also tested by comparing the digestibility of palm oleins containing different amounts of altered compounds.

MATERIAL AND METHODS

Materials. Commercial palm olein was provided by AGRA, S.A. (Bilbao, Spain). The major fatty acid composition of unused palm olein is as follows: palmitic, 37.6%; stearic, 4.0%; oleic, 43.7%; and linoleic, 10.0%.

Methods. *Frying Performance.* Domestic deep-fat fryers with a 3 L aluminum vessel were used. After being heated for 20 min, the oil reached and maintained a temperature of 180 °C. Potatoes sliced ~2 mm thick were introduced into the oil at 180 °C and fried for 8 min. At the end of each frying operation, the oil was reheated for 10 min to 180 °C and more potatoes were fried. A total of 90 frying operations (18 sets of 5) were performed at the rate of 10 per day (one set in the morning and another in the afternoon). Between each set of five uses, the palm olein was allowed to cool to room temperature. The palm olein was heated for 20 h throughout the whole experiment. The food-to-oil ratio in the fryers was kept at 500 g/3 L without the addition of fresh oil by eliminating one fryer after 10 fryings and emptying its contents to make up the volume of the other fryers to 3 L. Figure 1 shows the scheme of the frying method used.

Analytical Determinations. The polar fraction was evaluated using the column chromatographic method of Waltking and Wessels (1981), modifying the proportion of petroleum ether/diethyl ether used to fill the column and to elute the nonpolar fraction. A sample of ~1 g of palm olein was dissolved in 20 mL of petroleum ether/diethyl ether 87:13 (v/v) when unused oil was analyzed and 90:10 (v/v) when used oil was tested (Dobarganes et al., 1984). The sample was then transferred to a silica gel chromatographic column. A final elution of the column with chloroform/methanol 1:1 (v/v) was performed to improve recovery of the sample (Sánchez-Muniz et al., 1993). Three samples each of the unused palm olein and of that used in the 40th and 90th frying operations were analyzed. The separation of nonpolar fractions was checked by thin-layer chromatography on 0.5 mm thick 60 F250 silica gel plates (20 × 20 cm glass). Polar and nonpolar fractions were diluted 50 times (w/v) in hexane/diethyl ether 80:20 (v/v). Samples were applied as 10 μL spots using a 705 Hamilton microsyringe. Plates were developed with hexane/ethyl ether/acetic acid 80:20:1 (v/v/v) in a lined tank for ~25 min (~17 cm) and then removed for the solvent to evaporate. Spots were visualized by coating with iodine vapors.

HPSEC. Hydrolytic and/or thermoxidative alterations that occurred in the palm olein during frying were analyzed by HPSEC, following the method of Dobarganes et al. (1988). Isolated polar fractions were studied using a Waters 501 chromatograph (Milford, MA) with a 20 μL sample loop. A Waters 410 refractive index detector and two 300 mm × 7.5 mm i.d. (5 μm particle size) 0.01 and 0.05 μm pL gel (polystyrene−divinylbenzene) columns (Hewlett-Packard), connected in series, were operated at 40 °C. High-performance liquid chromatography (HPLC) grade tetrahydrofuran served as the mobile phase at a flow of 1 mL/min. The sample concentration was 10−15 mg/mL in tetrahydrofuran. All eluents and samples were precleaned by passage through a 2 μm filter. The quantity of each polar compound was calculated as previously indicated (Sánchez-Muniz et al., 1993).

Animals and Maintenance. The protocol used for this experiment was authorized by the Spanish Interministerial Commission of Science and Technology (Comisión Interministerial de Ciencia y Tecnología) and by an Internal Commission of the Pharmacy Faculty (Facultad de Farmacia) of the Universidad Complutense of Madrid (Spain).

Twenty-eight male Wistar rats [Instituto de Nutrición y Bromatología (CSIC), Facultad de Farmacia, Universidad Complutense de Madrid], weighing ~200 g each, were used. Groups of seven animals were housed together in plastic cages under controlled temperature conditions (22.3 ± 1.81 °C) and a 12 h light/dark cycle and were fed standard commercial rat pellets (Panlab S.L., Barcelona). Food and drinking water were supplied ad libitum. The rats were starved the night before the experiment took place but had free access to water until used. They were given a single dose (1 g/100 g of body weight) of raw palm olein or the same amount of used palm olein from the 40th or 90th frying operation by means of an esophageal probe. Control rats were administered isotonic saline solution at the rate of 1 mL/100 g of body weight. To avoid variations due to circadian cycles, experiments were started between 8:00 a.m. and 9:00 a.m. After a 4 h exposure to the fat, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg of body weight) and euthanized by blood extraction from the descending aorta using a syringe. Afterward, 50 mL of isotonic saline solution was slowly passed from the distal esophagus to the distal ileum to obtain the luminal fat. Because one rat of the group receiving the most altered palm olein showed a very different behavior throughout the test than the others (stress, aggressiveness, vomiting), only six animals from this group were studied.

Fat Extraction Procedure. Luminal fat was separated from the saline solution using a chloroform/methanol mix according to the method of Bligh and Dyer (1959) and then purified using the Folch et al. (1957) method.

Fat Digestibility Test. Luminal fat was corrected by taking into account the endogenous fat obtained from control rats. Purified samples of the luminal fat were transferred to a silica gel chromatographic column, polar and nonpolar fractions were separated, and purity was checked as indicated previously. Polar fractions were studied by means of HPSEC, as mentioned above. The amount of thermoxidized compounds (triglyceride polymers and dimers and oxidized triglycerides) and hydrolytic compounds (diglycerides, monoglycerides, and free fatty acids) was corrected by taking into account the amount

Table 1. Polar Content and Thermoxidized and Hydrolytic Compounds in Unused Palm Olein and in Palm Olein Used Repeatedly (40 and 90 Times) in Frying of Potatoes[a]

	ANOVA	unused	40 uses	90 uses
total polar content (mg/100 mg of oil)	$p < 0.001$	9.27 ± 0.10^a	14.81 ± 0.90^b	26.36 ± 0.30^c
triglyceride polymers (mg/100 mg of polar content)	$p < 0.001$	1.06 ± 0.09^a	5.90 ± 0.69^b	13.91 ± 0.03^c
triglyceride polymers (mg/100 mg of oil)	$p < 0.001$	0.10 ± 0.00^a	0.90 ± 0.10^b	3.70 ± 0.00^c
triglyceride dimers (mg/100 mg of polar content)	$p < 0.001$	11.17 ± 0.21^a	24.49 ± 1.19^b	30.06 ± 0.10^c
triglyceride dimers (mg/100 mg of oil)	$p < 0.001$	1.02 ± 0.30^a	3.63 ± 0.40^b	7.92 ± 0.10^c
oxidized triglycerides (mg/100 mg of polar content)	$p < 0.001$	12.33 ± 2.29^a	25.84 ± 0.47^b	31.73 ± 0.08^c
oxidized triglycerides (mg/100 mg of oil)	$p < 0.001$	1.12 ± 0.20^a	3.83 ± 0.30^b	8.32 ± 0.10^c
diglycerides (mg/100 mg of polar content)	$p < 0.001$	72.34 ± 2.18^a	42.30 ± 2.29^b	23.38 ± 0.21^c
diglycerides (mg/100 mg of oil)	$p < 0.05$	6.71 ± 0.20^a	6.28 ± 0.20^b	6.18 ± 0.10^b
monoglycerides (mg/100 mg of polar content)	$p < 0.05$	0.53 ± 0.08^a	0.23 ± 0.03^b	0.19 ± 0.02^b
monoglycerides (mg/100 mg of oil)	NS	0.04 ± 0.01^a	0.03 ± 0.00^a	0.04 ± 0.01^a
free fatty acids (mg/100 mg of polar content)	$p < 0.01$	2.66 ± 1.51^a	0.98 ± 0.03^b	0.74 ± 0.02^c
free fatty acids (mg/100 mg of oil)	NS	0.23 ± 0.10^a	0.16 ± 0.01^a	0.21 ± 0.01^a
thermoxidative alteration[b] (mg/100 mg of oil)	$p < 0.001$	2.25 ± 0.22^a	8.17 ± 0.89^b	19.93 ± 0.18^c
hydrolytic alteration[c]	$p < 0.05$	7.02 ± 0.20^a	6.48 ± 0.21^b	6.44 ± 0.07^b

[a] Results are mean of three samples ± SD. Values in the same row bearing a different letter are significantly different ($p < 0.01$). NS, not significant; ANOVA, analysis of variance. [b] Thermoxidative alteration = triglyceride polimers + triglyceride dimers + oxidized triglycerides. [c] Hydrolytic alteration = diglycerides + monoglycerides + free fatty acids.

Table 2. Thermoxidized and Hydrolytic Compounds in Polar Fractions Isolated from the Luminal Fat of Rats 4 h after Being Administered Unused Palm Olein and Palm Olein Used in Frying (40 and 90 Times)[a]

		luminal fat		
	ANOVA[b]	unused ($n = 7$)	40 uses ($n = 7$)	90 uses ($n = 6$)
nonpolar fraction (mg/100 mg of oil)	NS	43.90 ± 19.01^a	45.25 ± 14.30^a	60.18 ± 19.68^a
polar fraction (mg/100 mg of oil)	NS	56.10 ± 19.01^a	54.75 ± 19.01^a	39.82 ± 19.01^a
PTG (mg/100 mg of polar fraction)	$p < 0.0001$	0.19 ± 0.00^a	1.49 ± 0.45^b	7.23 ± 2.35^c
DTG (mg/100 mg of polar fraction)	$p < 0.0001$	1.34 ± 0.38^a	6.69 ± 1.49^b	15.37 ± 3.80^c
OTG (mg/100 mg of polar fraction)	$p < 0.0001$	3.43 ± 0.38^a	12.33 ± 5.67^b	19.53 ± 5.24^c
DG (mg/100 mg of polar fraction)	$p < 0.05$	47.90 ± 19.27^a	39.67 ± 14.12^a	23.51 ± 9.76^b
MG (mg/100 mg of polar fraction)	NS	19.84 ± 10.31^a	13.08 ± 5.20^a	15.73 ± 16.64^a
FFA (mg/100 mg of polar fraction)	BL ($p = 0.063$)	34.73 ± 13.93^a	26.75 ± 9.66^{ab}	18.63 ± 9.58^b

[a] Results are mean ± standard deviation. Number of animals in parentheses. PTG, triglyceride polymers; DTG, triglyceride dimers; OTG, oxidized triglycerides; DG, diglycerides; MG, monoglycerides; FFA, free fatty acids. [b] ANOVA, analysis of variance of luminal fat content. Values in the same row bearing a different letter are significantly different ($p < 0.05$). NS, nonsignificant; BL, borderline for statistical significance.

present in control rats and compared with the amount of polar compounds present in the oil administered to the rats. Control rats display the following values: total gastrointestinal lumen fat, 0.016 ± 0.003 g; triglyceride polymers, 0.000 ± 0.000 g; triglyceride dimers, 0.000 ± 0.000 g; total triglycerides, 0.003 ± 0.001 g; nonoxidized triglycerides, 0.003 ± 0.001 g; diglycerides, 0.004 ± 0.001 g; monoglycerides, 0.001 ± 0.001 g; and free fatty acids, 0.008 ± 0.002 g. As cholesterol and bile acids are present in the intestinal tract and display molecular weights, and thus HPSEC retention times, which are similar to those of free fatty acids, the influence of these compounds in HPSEC determinations was tested. Huge amounts of cholic acid resulted in only minor chromatographic peaks, for which reason the influence of bile acids can be considered inconsequential. However, the addition of cholesterol to the samples increases the area related to free fatty acids. Cholesterol content in the luminal fat samples was calculated using Boehringer Mannheim's (Mannheim, Germany) enzymatic colorimetric methods, and the HPSEC free fatty acid data were corrected.

Statistical Analysis. Data from the different palm oleins were compared by one-way analysis of variance (ANOVA) of repeated measures. When comparisons by ANOVA were significant, the Bonferroni test was performed to study differences between specific groups.

RESULTS

The polar fraction and all of the compounds caused by thermoxidative alteration (triglyceride polymers, triglyceride dimers, and oxidized triglycerides) increased significantly ($p < 0.001$) in absolute terms (mg/100 mg of oil) in the palm olein used for frying. Compounds formed by hydrolytic alteration (diglycerides, monoglycerides, and free fatty acids) decreased ($p < 0.05$), although only diglycerides were significantly affected (Table 1). Results on milligrams per 100 mg of polar content indicate significant changes (at least $p < 0.05$) for all altered compounds.

Table 2 indicates how the percentage of the polar fraction in the luminal fat after the 4 h long experiment tended to decrease as the number of uses of the palm olein increased. Diglycerides and free fatty acids were the major compounds in the polar fractions of the luminal fat after unused palm olein was administered, and their contribution decreased ($p < 0.05$ and 0.063, respectively) as the number of uses of the olein increased. However, as the number of uses of the palm olein in frying increased, luminal fat displayed significantly higher percentages of triglyceride dimers and oxidized triglycerides (both $p < 0.0001$).

Table 3 presents detailed information about the absolute amount (g) of fat administered and that which was recovered, in addition to that of different altered components. The data seem to indicate some similar trends for administered and luminal fat. Thus, the amount of polymers, dimers, and oxidized triglycerides presented in luminal fat significantly increased (at least $p < 0.01$) as the number of frying uses of palm olein increased. However, the levels of nonoxidized triglycerides and diglycerides showed a very different trend.

Table 4 indicates the true digestibility coefficient of the whole olein and of its various thermoxidized com-

Table 3. Thermoxidized and Hydrolytic Compounds of Unused Palm Olein and Palm Olein Used in Frying (40 and 90 Times) Administered to Rats and Present in the Luminal Fat after 4 h Experiment[a]

	administered fat				luminal fat			
	ANOVA[b]	unused ($n = 7$)	40 uses ($n = 7$)	90 uses ($n = 6$)	ANOVA[b]	unused ($n = 7$)	40 uses ($n = 7$)	90 uses ($n = 6$)
oil (g)	NS	2.031± 0.273[a]	2.056 ± 0.137[a]	2.046 ± 0.166[a]	$p < 0.05$	0.968 ± 0.285[a]	1.231± 0.206[ab]	1.296 ± 0.194[b]
PTG (g)	$p < 0.001$	0.002 ± 0.000[a]	0.018 ± 0.001[b]	0.075 ± 0.006[c]	$p < 0.001$	0.001 ± 0.000[a]	0.010 ± 0.003[b]	0.040 ± 0.013[c]
DTG (g)	$p < 0.001$	0.021 ± 0.003[a]	0.075 ± 0.005[b]	0.162 ± 0.013[c]	$p < 0.001$	0.007 ± 0.002[a]	0.045 ± 0.010[b]	0.085 ± 0.021[c]
NOTG (g)	$p < 0.01$	1.840 ± 0.248[a]	1.751 ± 0.116[a]	1.506 ± 0.124[b]	$p < 0.05$	0.425 ± 0.184[a]	0.557± 0.176[ab]	0.780 ± 0.255[b]
OTG (g)	$p < 0.001$	0.023 ± 0.003[a]	0.079 ± 0.005[b]	0.171 ± 0.014[c]	$p < 0.01$	0.018 ± 0.010[a]	0.083 ± 0.036[b]	0.108 ± 0.029[b]
DG (g)	NS	0.136 ± 0.019[a]	0.129 ± 0.008[a]	0.126 ± 0.011[a]	$p < 0.05$	0.251 ± 0.101[a]	0.267 ± 0.121[a]	0.130 ± 0.054[b]
MG (g)	NS	0.001 ± 0.000[a]	0.001 ± 0.000[a]	0.001 ± 0.000[a]	NS	0.104 ± 0.054[a]	0.086 ± 0.035[a]	0.087 ± 0.092[a]
FFA (g)	$p < 0.001$	0.005 ± 0.001[a]	0.003 ± 0.000[b]	0.004 ± 0.000[c]	BL	0.182 ± 0.073[a]	0.180 ± 0.065[a]	0.103 ± 0.053[a]

[a] Results are mean ± standard deviation. Number of animals in parentheses. PTG, triglyceride polymers; DTG, triglyceride dimers; NOTG, nonoxidized triglycerides; OTG, oxidized triglycerides; DG, diglycerides; MG, monoglycerides; FFA, free fatty acids. [b] ANOVA, analysis of variance. Values in the same row bearing a different letter are significantly different ($p < 0.05$). NS, nonsignificant; BL, borderline for statistical significance.

Table 4. True Digestibility of Unused Palm Olein and Palm Olein Used in Frying and That of Polymers, Dimers, and Monomers of Triglycerides after 4 h Experiment[a]

	ANOVA[b]	unused ($n = 7$)	40 uses ($n = 7$)	90 uses ($n = 6$)
oil (g/g)	$p < 0.01$	0.527 ± 0.085[a]	0.403 ± 0.081[b]	0.368 ± 0.059[b]
PTG (g/g)	NS	0.508 ± 0.112[a]	0.444 ± 0.138[a]	0.458 ± 0.197[a]
DTG (g/g)	$p < 0.01$	0.657 ± 0.064[a]	0.394 ± 0.114[b]	0.467 ± 0.159[b]
oligomers (PTG + DTG) (g/g)	$p < 0.01$	0.636 ± 0.065[a]	0.404 ± 0.116[b]	0.464 ± 0.171[ab]
total TG (NOTG + OTG) (g/g)	$p < 0.001$	0.768 ± 0.083[a]	0.652 ± 0.093[a]	0.480 ± 0.117[b]
NOTG (g/g)	$p < 0.001$	0.772 ± 0.080[a]	0.684 ± 0.098[a]	0.489 ± 0.121[ab]
OTG (g/g)	NS	0.200 ± 0.398[a]	−0.052± 0.454[a]	0.365 ± 0.167[a]

[a] Results are mean ± standard deviation. Number of animals in parentheses. PTG, triglyceride polymers; DTG, triglyceride dimers; NOTG, nonoxidized triglycerides; OTG, oxidized triglycerides. [b] ANOVA, analysis of variance. Values in the same row bearing a different letter are significantly different ($p < 0.05$). NS, nonsignificant.

Table 5. Luminal Fat to Administered Fat Ratio of Hydrolytic Compounds of Unused Palm Olein and Palm Olein Used in Frying (40 and 90 Times) after 4 h Experiment[a]

	ANOVA[b]	unused ($n = 7$)	40 uses ($n = 7$)	90 uses ($n = 6$)
DG (g/g)	$p < 0.05$	1.83 ± 0.59[a]	2.06 ± 0.90[a]	1.05 ± 0.47[b]
MG (g/g)	NS	129.75 ± 75.55[a]	143.87 ± 54.67[a]	101.88 ± 100.74[a]
FFA (g/g)	$p < 0.05$	35.43 ± 13.32[ab]	54.55 ± 19.92[a]	25.22 ± 13.85[a]
MG + FFA (g/g)	NS	48.06 ± 20.84[a]	62.45 ± 21.42[a]	36.03 ± 15.87[a]

[a] Results are mean ± standard deviation. Number of animals in parentheses. DG, diglycerides; MG, monoglycerides; FFA, free fatty acids. [b] ANOVA, analysis of the variance. Values in the same row bearing a different letter are significantly different ($p < 0.05$). NS, nonsignificant.

pounds. Digestibility of the whole palm olein, dimers ($p < 0.01$), nonoxidized triglycerides ($p < 0.001$), and total triglycerides ($p < 0.001$) was significantly reduced ($p < 0.01$) in used palm oleins.

Table 5 shows that in the case of hydrolytic compounds (diglycerides and free fatty acids), the luminal fat/administered fat ratio decreased significantly ($p < 0.05$) when palm oleins from the 40th and 90th frying operations were compared.

DISCUSSION

The fact that the polar fraction of the palm olein increased along with the increase in the number of frying uses is in line with data published previously by our research group (Cuesta et al., 1993; Romero et al., 1995; Sánchez-Muniz et al., 1993) and other authors (Sebedio et al., 1990; Pérez-Camino et al., 1991). However, this study confirms the high stability of palm olein used in frying, as it withstood 90 uses, without the addition of fresh oil, before reaching the critical level of 25% in altered triglycerides, above which no oil can legally be used in food preparation (Firestone, 1996; Presidencia del Gobierno, 1989). This figure compares very favorably with that of a sunflower oil, analyzed in a previous study (Arroyo et al., 1996), which had to be discarded after only 60 frying uses. Moreover, >6% of the polar fraction in both used oleins were diglycerides, which have never been associated with oil toxicity and must not differ much from the original diglycerides present in unused palm olein.

According to previous studies (Cuesta et al., 1993; Romero et al., 1995; Sánchez-Muniz et al., 1993), HPSEC data in palm olein show that after repeated frying uses, the amount of thermoxidized compounds clearly increases. Thus, absolute amount (mg/100 mg of oil) of triglyceride polymers increases ∼30-fold, whereas that of dimers and oxidized triglycerides increase >7-fold. This increase, together with the tendency of the number of products of hydrolytic alteration to decrease, confirms the fact that in fat used for deep-frying potatoes, thermoxidative reactions are more prevalent than hydrolytic ones.

After the administration of palm olein used in frying, the percentage of oligomers and oxidized triglycerides in the polar fraction of the luminal fat markedly increased, whereas the percentage of diglycerides and free fatty acids decreased. These results are first attributable to the great content of thermoxidized compounds in these oils and second to the reduction of the hydrolytic action of pancreatic lipase over these compounds. As a result of these two factors, the nonpolar fraction/polar fraction ratio also tended to

increase along with the number of uses of the palm olein administered.

As previously stated, reduced digestibility and absorption are observed in all cases in which heated fats have been studied [for review, see Cuesta et al. (1988) and Márquez-Ruiz and Dobarganes (1996)]. The significant decrease in the true digestibility coefficient of whole palm olein seen in this study concurs with previous results of other authors (Alexander, 1966; Nolen, 1973). However, research conducted by Lanteaume et al. (1966), Le Floch et al. (1968), and Varela et al. (1986) did not find important effects on the fat digestibility of different oils used for frying.

Some years ago, Crampton et al. (1953) reported that the principal reason for the lower nutritive value of diets that included linseed oil heated to 275 °C was the presence of one or more dimer fatty acid radicals in this polymerized fat. Deuel (1955) considered that the extent to which frying fats were polymerized was one of the principal factors behind their diminished digestibility.

Some time later, Potteau et al. (1970, 1977) likewise reported that as polymerization of oil increased, its digestive utilization declined, as part of the polymers were eliminated in the feces. The lower digestibility of thermoxidized oils, as compared to that of unused oils, has been attributed to the presence of the nondistillable urea−nonadductable fraction.

According to Combe et al. (1981), polar dimers and polymers appear to be comparatively better absorbed than nonpolar dimers in lymph cannulation studies. Márquez-Ruiz et al. (1992) found high digestibility values for oxidized dimers and polymers. Bottino (1962) reported apparent digestibility of dimers of between 30 and 70%, but Kajimoto and Mukai (1970) questioned such high values. Although the present data for both triglyceride polymers and dimers are in line with the former results, digestibility of polymers tended to decrease ∼10% as the number of frying operations increased, whereas that of dimers decreased by at least 29%. Márquez-Ruiz et al. (1992) indicate that a high percentage of triglyceride polymers remains after pancreatic lipase action in vitro and that no further significant changes in triacylglyceride polymer hydrolysis occur after the first 15 min.

The present results with regard to nonaltered triglycerides are interesting. According to the unused palm olein data, true digestibility of these products was greater than that of oligomers. However, as the number of uses, and thus the polar content, of the palm olein increased, the digestibility ratio decreased (12 and 37% lower) after the 40th and 90th frying operations, respectively. These results suggest that in our experimental conditions, hydrolysis of nonoxidized triglycerides by pancreatic lipase is inhibited, or at least retarded, by the presence of altered compounds. Henderson et al. (1993) reported that in the case of oils containing low amounts (<4%) of triglyceride polymers as substrates, both triglycerides and polymers of triglycerides were almost completely hydrolyzed by pancreatic lipase after 1 h in vitro, but when highly oxidized oils, containing 20 or 30% triglyceride polymers, were used, some triglycerides remained intact.

Data on oxidized triglycerides (OTG) from the present study are difficult to explain and may result from the complex balance between their formation from polymers and dimers and their disappearance due to hydrolysis by pancreatic lipase. In unused oil, the amount of OTG decreased after 4 h, with a net digestion of 20%. The absolute amount of OTG in the luminal fat after the administration of palm olein used for frying 40 times increased by 5.2%. When palm olein from the 90th frying was used, true digestibility was 36.5%. These results contrast with those of other authors (Matsuchita, 1975; Miyashita et al., 1990), who suggest that OTG are well-absorbed and appear to be adequately hydrolyzed by pancreatic lipase because of the higher polarity and similar molecular weights of OTG and nonaltered triglycerides. According to Carey et al. (1983), during the first stage of fat digestion, absorption depends on lipolytic enzyme activity. Afterward, molecular polarity greatly influences the entry of lipidic products into the micellar phase and, finally, molecular weight further limits luminal uptake.

As the number of frying uses of the palm olein administered increased, luminal fat tended to present a lower percentage and amount of monoglycerides and free fatty acids. This could be attributed to lower pancreatic lipase activity. The possibility of increased absorption of these compounds should be discarded because the actual amount of luminal fat increased.

In conclusion, palm olein used repeatedly for frying potatoes undergoes thermoxidation, and its content in polymeric triglycerides and oxidized triglycerides increases with use. After a 4 h experiment, true digestibility coefficients of palm oleins used in frying were significantly lower than those of the unused palm olein. True digestibility of polymers and dimers was quite high but decreased as the alteration of the oil increased. Nonoxidized triglyceride hydrolysis was negatively affected by the presence of large amounts of thermoxidized compounds.

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