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ARTICLE

Gastric Emptying and Short-Term Digestibility of Thermally Oxidized Sunflower Oil Used for Frying in Fasted and Nonfasted Rats

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Four-hour *in vivo* digestibility of sunflower oil used in frying was tested in fasted and nonfasted rats. For three consecutive days, 12 male Wistar rats received 1 g of unused oil (controls, C), while 12 received 1 g of used oil (test group, T). On the night of day 3, 6 rats from each group were fasted (FC, FT) while the other 6 animals from each group had free access to food (NFC, NFT). On day 4, FC and NFC received 2 g of unused oil, while FT and NFT received 2 g of used oil. Luminal gastric and intestinal fats were studied by column and HPSE chromatography after endogenous corrections. Gastric emptying in FT was significantly slower than in NFT and FC. The luminal gastric fat profile differed from that of the oils administered, suggesting that nonoxidized triacylglycerols passed quickly into the intestines. All glyceridic compounds present in the luminal intestinal fat were affected by oil type (at least $P < 0.01$). Oil digestibility value order was FT < NFT < FC < NFC. FT and NFT presented lower ($P < 0.001$) triacylglycerol polymer and dimer digestibilities than NFC and FC. In conclusion, oil type determined luminal intestinal fat compounds and their digestibility more than nutritional status.

KEYWORDS: Digestibility; fasting; nonfasting; gastric emptying sunflower oil; frying; thermal oxidation

INTRODUCTION

Diets of North Americans and inhabitants of the European Union countries contain substantial quantities of unsaturated oils/fats that have been subjected to various degrees of processing and heat treatment (*1*−*3*). Food processed with thermally oxidized fat is generally thought to be digested less thoroughly than that prepared using oils with a low degree of alteration. This belief is based on scientific data regarding gastric emptying times (*4*) and the low absorption rate of thermally oxidized compounds (oligomers, dimers, and oxidized triacylglycerols) present in heated oils (*5*−*7*).

However, information related to digestibility and absorption of oils and fats used for frying is under debate. Digestibility of fats used in frying is commonly held to be low (*3*, *6*−*9*). According to Márquez-Ruiz et al. (*10*), the presence of large amounts of thermally oxidized compounds negatively affects hydrolysis of nonoxidized triacylglycerols. Our group has reported that true digestibility of the different alteration compounds from heated olive oil was 30−40%, whereas that of the unused olive oil was much higher (80%) (*11*). However, polar material and surfactants produced during frying may increase the hydrolytic effect of pancreatic lipase because the *in vitro* digestibility of nonoxidized triacylglycerols obtained from palm olein heated at 180 °C was lower when the oil was heated for 2 h than when heated for 4 h (*12*).

Moreover, most *in vitro* and *in vivo* studies on fat digestibility have been performed on fat compounds isolated from heated oils rather than from those used in frying and have not taken into consideration the influence of other dietary compounds (e.g., proteins, carbohydrates, fiber). Current concern regarding obesity and overweight has negatively affected dietary habits. A relatively large percentage of the population does not now eat breakfast (*13*), thus making lunch their first meal of the day. This meal pattern extends the fasting period for several hours and may lower pancreatic lipase action.

In the present paper we hypothesize that nutritional status conditions *in vivo* digestibility of sunflower oil used in frying. To date, few studies have considered the relationship between nutritional status (e.g., fasted or nonfasted condition) and the digestibility of compounds present in oils used for frying. The presence of food in the oil used for frying clearly makes its composition more complex than that of oil that is simply heated (*14*, *15*). Thus, during frying, food is submerged in oil that is heated in the presence of air. Moisture in the foodstuffs may induce hydrolytic alteration of the oil and lead to the production of hydrolytic compounds such as diacylglycerols (DG), monoacylglycerols (MG), and free fatty acids (FFA). Moreover, the moisture

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content of the foodstuffs being fried decreases the temperature of the frying oil. Furthermore, compounds that may migrate from the food into the oil during the frying process include phospholipids and other types of emulsifiers, lipid soluble vitamins, trace metals, cholesterol and cholesterol oxidation products, pigments, Maillard browning products, phenolic compounds, volatile compounds, etc.

While previous *in vivo* studies of fat digestibility (*6*, *16*) considered the entire gastrointestinal tract, the present study focused on the separate role of the stomach and small intestine. By combining an *in vivo* short-term experiment on fat digestibility in rats with high-performance size-exclusion chromatography (HPSEC), this study aimed to compare the effect on fasted and nonfasted rats of a 4 h exposure to unused sunflower oil and sunflower oil used in frying on (1) gastric emptying and (2) digestibility of both oils and their alteration compounds.

MATERIALS AND METHODS

Frying Performance. Forty domestic deep-fat frying operations of different foods were performed in a 4 wk period, at the rate of 10 procedures per week, using 2.5 L steel vessels (Solac, Vitoria, Spain). Refined sunflower oil (Koipesol, Andújar, Jaén, Spain) was employed for frying. The foodstuffs used in the experiment included frozen prefried potatoes and battered squid (Pryca hypermarkets, Spain), frozen croquettes (La Cocinera, Torrejón de Ardoz, Madrid, Spain), frozen tuna pastries (Findus-Nestlé España S.A., Esplugues de Llobregat, Barcelona, Spain), frozen spring rolls and frozen breaded veal fillets (Sánchez Romero, Jabugo, Huelva, Spain), frozen fish fingers (breaded hake) (Frudesa, l'Alcudia, Valencia, Spain), sausages (Gran Prix, Getafe, Madrid, Spain), fresh potatoes (Kennebec variety, Xinzo de Limia, Galicia, Spain) and battered anchovies, battered green peppers, battered sliced eggplant, and meatballs (ground beef plus wheat flour) prepared in the laboratory using standard culinary recipes. The following frying conditions were used: thermostat temperature 180 °C; heating time 9−12 min/frying; frying time 2−6 min/frying; and cooling time ∼4 h/frying. Frying oil was replenished with unused oil every ten frying operations to maintain, insofar as possible, a constant food/oil ratio. The amount of food selected for each frying procedure was based on commercial advertisements (e.g., one piece/person) and the criteria of the cook (standard quantities used at home for four persons). Details of food ratios have been published previously (*15*).

Animals and Treatments. Rats were obtained from the Animal Research Center, University of Alcalá (Madrid, Spain), homologated by the Spanish Ministerio de Agricultura, Reference 28005-22A, Real Decreto 233-88, and housed in an animal room under standard conditions of temperature (21 ± 2 °C) and humidity (55 ± 10%), with a 12 h light/12 h dark cycle. All experiments were performed in compliance with Directive 86/609/EEC of November 24, 1986, for the protection of scientific research animals. Twenty-four male Wistar rats with an average body weight of 200 g (190−210) were divided into four groups of six rats each. All animals had *ad libitum* access to water and chow diet (Panlab, Barcelona, Spain). For three consecutive days, the rats were administered 1 g of oil via esophageal probe at 9:00 a.m. Control rats received unused sunflower oil while test rats received sunflower oil that had been used to fry food 40 times. On the night of day 3, half of the rats were subjected to a 15 h fast (FC, FT) while the other half had *ad libitum* access to food and water (NFC, NFT). On day 4, beginning at 9 a.m., one animal at a time was taken at random from each of the four groups and administered additional sunflower oil. All control rats, fasted (FC) and nonfasted (NFC), received 2 g of unused oil by means of esophageal probe, while all test rats, fasted (FT) and nonfasted (NFT), were given 2 g of used oil. This oil amount was selected because after 4 h administration about the 40−50% of fat still remains in the gastrointestinal tract permitting one to test adequately the composition of remainder fat and thus to ascertain the digestibility (see below).

An additional six fasted and six nonfasted rats with an average body weight of 200 g, selected as negative controls for endogenous corrections, were given 2 mL of isotonic saline to determine endogenous luminal gastric and intestinal fats. After a 4 h exposure to the fat, the rats were anesthetized via intraperitoneal injection of sodium pentobarbital (60 mg kg^{-1} body weight) and euthanized by extracting blood from the descending aorta with a syringe. Slow perfusion of the stomach with 25 mL of isotonic saline solution enabled collection of the fat present in the gastric lumen. Fifty milliliters of isotonic saline solution were slowly perfused from the proximal duodenum to the distal ileum to collect the fat in the lumen of the small intestine. Samples were collected in chilled tubes and stored at −80 °C until analysis. Details of fat recovery has been previously reported and ranged between 93 and 101% (*11*).

Fat Extraction Procedure. After perfusion with saline solution, the fat remaining in the gastric and intestinal lumen was extracted with chloroform/methanol (*17*) and then purified using a chloroform/methanol/0.58% NaCl solution mix (vol:vol, 3/48/47) (*18*).

Polar Material Assessment. Total polar material from the unused sunflower oil, the oil used in frying given to the rats, and that of the oils present in the gastric and intestinal lumen was determined by silica column chromatography (*19*).

High-Performance Size-Exclusion Liquid Chromatography (HPSEC) Assessment. To obtain further information about changes due to thermal oxidation during frying and *in vivo* hydrolysis and absorption of thermally oxidized oils, HPSEC analysis of the altered and nonaltered compounds present in the unused and used sunflower oils, and in the gastric and intestinal lumen of NFC, FC, NFT and FT and those of their negative control rats was performed following a slight modification of the AOCS method (*19*). A sample concentration (10−15 mg mL^{-1} tetrahydrofuran) was applied using a Waters 501 solvent-delivery pump (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 PLgel columns (Hewlett-Packard, Palo Alto, CA), connected in series, were operated at 40 °C. HPLC-grade tetrahydrofuran was used as the mobile phase with a flow of 1 mL min^{-1}. **Figure 1** shows a representative chromatogram of the polar material of oils, luminal gastric fat and luminal intestinal fat.

True Digestibility Ratios. True digestibility values of the whole oils and of the different hydrolytic and thermally oxidized compounds were calculated, after endogenous correction, taking into account the amount of oil or compounds administered and that present at the gastrointestinal lumen, according to the following equation:

$$100 \times [(\text{administered fat} - \text{luminal fat}) - \text{endogenous fat}]/\text{administered fat}$$

or similar formulas when calculating the digestibility values for individual compounds.

Statistical Study. Data from the different groups were compared by bifactorial analysis (oil type, the nutritional status and their interaction) using the general linear model followed by Bonferroni multiple comparison test. The NFC vs FC and NFT and the FT vs FC and NFT comparisons were considered when significant interaction for a given parameter was found. P values <0.05 were considered significant. The SAS 9.1.3 statistical packet was employed.

RESULTS

Forty discontinuous frying operations involving several types of fresh and frozen foods notably increased the polar and thermally oxidized compound content of sunflower oil while significantly decreasing the number of hydrolytic compounds present. As a consequence of repeated frying use, values of polymers (PTG) and dimers (DTG) of triacylglycerols in the oil rose from 0.0 to 78.0 mg/g oil and from 4.0 to 124.0 mg/g oil, respectively. Oxidized triacylglycerol monomers (OTG) increased from 15.0 to 74.0 mg/g oil. DG increased from 13.0 to 19.0 mg/g oil and FFA decreased from 4.0 to 3.0 mg/mg. The amounts of sunflower oil compounds administered to NFC, FC, NFT, and FT groups are presented in **Table 1**.

Although a relatively large amount of fat was administered, none of the rats tested in fasting or fed status presented diarrhea during the experiment. This fat dose was also used studying digestibility of very highly altered oils, (*11*, *16*) and no diarrhea was observed as well.

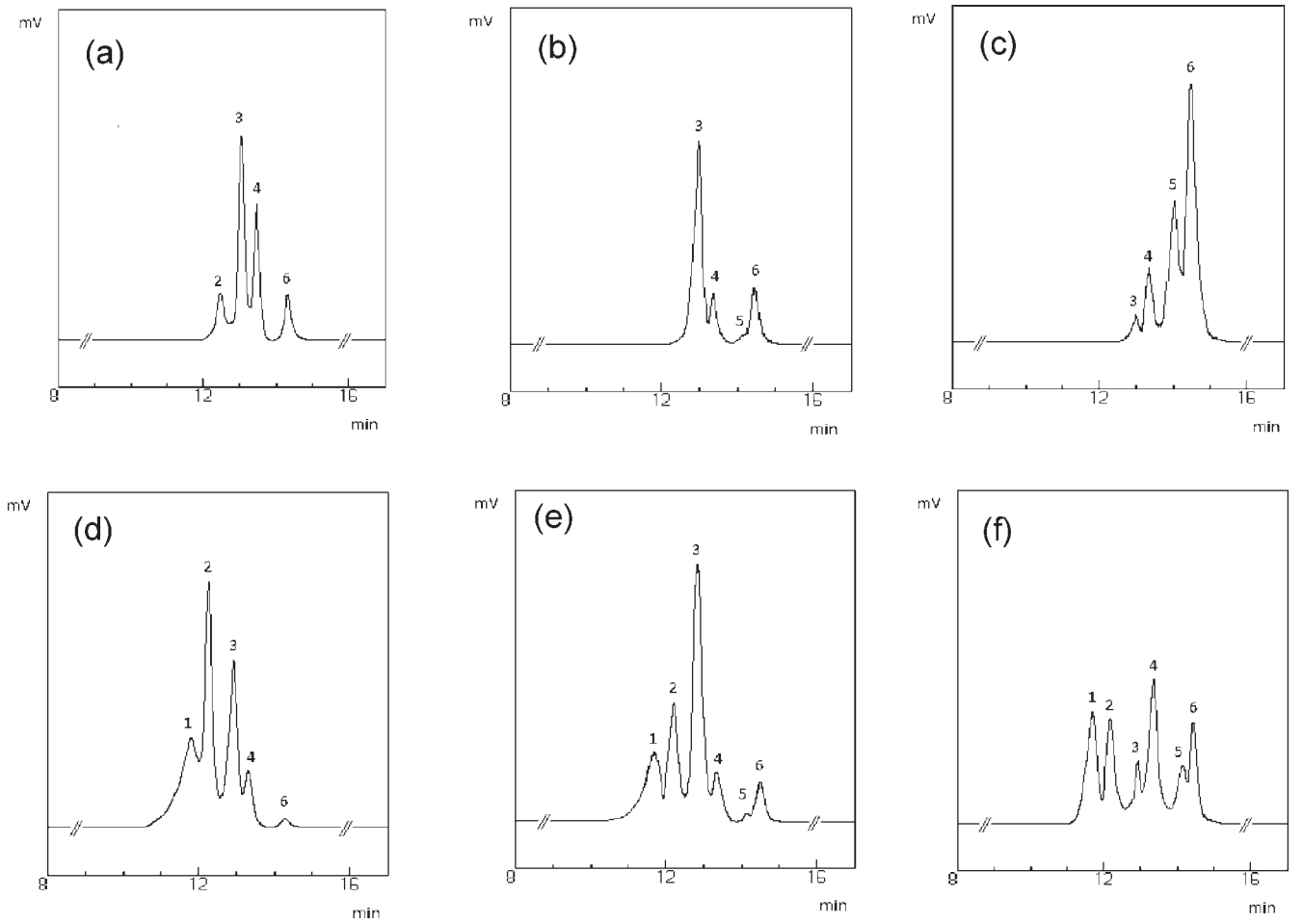

Figure 1. Representative HPSE chromatograms of polar fractions: (**a**) unused sunflower oil (control oil); (**b**) luminal gastric fat of animals administered the control oil; (**c**) luminal intestinal fat of animals administered the control oil; (**d**) sunflower oil used in frying (test oil); (**e**) luminal gastric fat of animals administered the test oil; (**f**) luminal intestinal fat of animals administered the test oil. 1, PTG; 2, DTG; 3, OTG; 4, DG; 5, MG; 6, FFA.

Table 1. Amount of Thermally Oxidized and Hydrolytic Compounds Present in Unused Sunflower Oil and Sunflower Oil Used in 40 Fryings Administered to 200 g Wistar Rats

	administered oil compounds (mg)	
	control sunflower oil	sunflower oil used in frying (test oil)
sunflower oil	2000.0	2000.0
PTG[a]	ND[b]	156.0
DTG	8.0	248.0
PTG + DTG	8.0	404.0
NOTG	1928.0	1368.0
OTG	30.0	148.0
DG	26.0	38.0
MG	ND	ND
FFA	8.0	6.0
hydrolytic compounds[c]	34.0	44.0

[a] PTG, triacylglycerol polymers; DTG, triacylglycerol dimers; NOTG, nonoxidized triacylglycerol monomers; OTG, oxidized triacylglycerol monomers; DG, diacylglycerols; MG, monoacylglycerols; FFA, free fatty acids. [b] ND, not detected. [c] Hydrolytic compounds: DG + MG + FFA.

Luminal Gastric and Intestinal Fat. The content and composition of both luminal gastric and intestinal fats were corrected by taking into account the endogenous fat obtained from saline administered rats. In the whole stomach small average amounts of NOTG (13.6 mg), OTG (0.37 mg), DG (0.21 mg), MG (0.0 mg) and FFA (0.13 mg) were found. In the whole small intestine amounts of NOTG (34.3 mg), OTG (2.6 mg), DG (10.3 mg), MG (12.9 mg) and FFA (25.7 mg). The amount of luminal gastric fat was significantly affected by the oil type−nutritional status interaction ($P < 0.01$), oil type ($P < 0.001$) and nutritional status ($P < 0.001$). Luminal gastric fat values were higher ($P < 0.001$) in FT animals than in their NFT and FC counterparts (**Table 2**).

Concentrations of nonoxidized triacylglycerol monomers (NOTG) in gastric luminal fat were significantly affected by the oil type−nutritional status interaction ($P < 0.05$), the nutritional status ($P < 0.001$) and the oil type ($P < 0.01$) (**Table 2**). FT rats presented significantly higher NOTG values (both $P < 0.01$) in gastric luminal fat than their NFT and FC counterparts.

All thermally oxidized compounds in the gastric luminal fat were significantly affected by the interaction between the oil type and nutritional status (all, $P < 0.001$). The amount of PTG and DTG present in the luminal gastric fat was affected by oil type and nutritional status (both $P < 0.001$). Thus, luminal gastric PTG and DTG values were significantly higher in the NFT and FT groups (at least $P < 0.01$) than in the NFC and FC groups, respectively, and significantly higher in the FT rats than in the NFT animals ($P < 0.001$) (**Table 2**). Oil type and the interaction between oil type and nutritional status significantly affected ($P < 0.05$) concentrations of OTG in the luminal gastric fat. These OTG values of FT rats were significantly higher than those of NTF ($P < 0.01$) and FC ($P < 0.05$) animals (**Table 2**).

DG levels in luminal gastric fat were affected by oil type ($P < 0.001$) and nutritional status ($P < 0.01$). FFA values were significantly affected by nutritional status ($P < 0.05$). Hydrolytic

Table 2. Amount (mg) of Different Acylglycerol Compounds Present in the Gastric Lumen after 4 h Administration of Unused Sunflower Oil and Sunflower Oil Used in 40 Fryings[a]

	nonabsorbed oil (mg)						
	stomach				ANOVA		
	NFC[b]	FC	NFT	FT	oil effect	nutritional status	interaction
total	65.3 ± 35.0	79.9 ± 34.8	83.4 ± 34.1	201.3 ± 27.8 c***	<0.001	0.001	<0.01
PTG[c]	ND	ND	9.4 ± 3.4 c	20.9 ± 5.9 c***	<0.001	<0.001	<0.001
DTG	ND	ND	13.9 ± 5.7 b	26.8 ± 4.9 c***	<0.001	<0.001	<0.001
PTG + DTG	ND	ND	23.3 ± 8.9 c	47.7 ± 9.3 c***	<0.001	<0.001	<0.001
NOTG	18.1 ± 19.1	37.7 ± 27.4	27.8 ± 23.3	93.2 ± 15.9 b**	<0.01	<0.001	<0.05
OTG	19.1 ± 10.7	14.6 ± 7.8	19.2 ± 8.3	39.7 ± 15.4 b*	<0.05	NS	<0.05
DG	3.3 ± 1.7	6.5 ± 1.7	8.6 ± 3.4	12.1 ± 3.2	<0.001	<0.01	NS
MG	0.3 ± 0.8	0.0 ± 0.0	0.3 ± 0.6	0.3 ± 0.6	NS	NS	NS
FFA	6.5 ± 3.2	7.3 ± 2.1	4.2 ± 0.7	8.3 ± 3.7	NS	<0.05	NS
hydrolytic compounds[d]	10.1 ± 4.7	13.8 ± 3.7	13.1 ± 4.2	20.7 ± 6.3	<0.05	<0.05	NS

[a] Data are mean ± SD of 6 animals per group. NFT and FT data bearing a letter (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$; multiple comparison test when significant factor interaction exists) were significantly different from NFC and FC respectively, and FC and FT data bearing asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; multiple comparison test when significant factor interaction exists) were significantly different from NFC and NFT, respectively; ND, not detected; NS, not significantly. [b] FC, fasted rats receiving the control oil; FT, fasted rats receiving the test oil; NFC, non-fasted rats receiving the control oil; NFT, non-fasted rats receiving the test oil. [c] PTG, triacylglycerol polymers; DTG, triacylglycerol dimers; NOTG, nonoxidized triacylglycerol monomers; OTG, oxidized triacylglycerol monomers; DG, diacylglycerols; MG, monoacylglycerols; FFA, free fatty acids. [d] Hydrolytic compounds: DG + MG + FFA.

Table 3. Amount (mg) of Different Acylglycerol Compounds Present in the Intestinal Lumen after 4 h Administration of Unused Sunflower Oil and Sunflower Oil Used in 40 Fryings[a]

	nonabsorbed oil (mg)						
	intestine				ANOVA		
	NFC	FC	NFT	FT	oil effect	nutritional status	interaction
total	509.6 ± 133.1	576.7 ± 164.5	668.3 ± 60.2	761.1 ± 256.0	<0.05	NS	NS
PTG	ND	ND	56.9 ± 13.7	61.3 ± 17.3	<0.001	NS	NS
DTG	ND	ND	59.3 ± 7.2	48.6 ± 7.4	<0.001	NS	NS
PTG + DTG	ND	ND	116.2 ± 10.5	109.9 ± 12.3	<0.001	NS	NS
NOTG	293.4 ± 78.3	222.1 ± 107.6	389.1 ± 51.0	455.9 ± 180.3	<0.01	NS	NS
OTG	0.7 ± 2.2	5.2 ± 3.1	29.9 ± 5.2 c	25.7 ± 7.3 c	<0.001	NS	<0.05
DG	24.2 ± 10.4	36.0 ± 25.5	73.7 ± 18.1	82.1 ± 22.5	<0.001	NS	NS
MG	56.1 ± 25.7	94.0 ± 11.8	27.1 ± 5.3	35.0 ± 17.9	<0.001	<0.01	NS
FFA	135.2 ± 60.0	219.4 ± 27.6 *	32.3 ± 4.6 c	52.5 ± 26.9 c	<0.001	<0.01	<0.05
hydrolytic compounds	215.5 ± 78.3	349.4 ± 63.9	133.1 ± 19.7	169.6 ± 63.9	<0.001	NS	NS

[a] For abbreviations see **Table 2**. Data are mean ± SD of 6 animals per group. NFT and FT data bearing a letter (a, $P < 0.05$; b, $P < 0.01$; c, $P < 0.001$; multiple comparison test when significant factor interaction exists) were significantly different from NFC and FC, respectively, and FC and FT data bearing asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; multiple comparison test when significant factor interaction exists) were significantly different from NFC and NFT, respectively; NS, not significantly.

compounds (DG + MG + FFA) were significantly affected by oil type and nutritional status (both $P < 0.05$) (**Table 2**).

Oil type affected luminal intestinal fat amount ($P < 0.05$) (**Table 3**). Of the thermally oxidized compounds present in the luminal intestinal fat, only OTG were affected ($P < 0.05$) by oil type−nutritional status interaction. FFA were the hydrolytic compounds significantly affected by this interaction ($P < 0.05$) (**Table 3**). Nutritional status did not significantly affect ($P > 0.05$) the concentration of thermally oxidized compounds in luminal intestinal fat but did significantly influence that of hydrolytic compounds (MG and FFA, both $P < 0.01$). Oil type significantly affected (at least, $P < 0.01$) all compounds. FT and NFT rats presented higher OTG in luminal intestine than FC and NFC rats ($P < 0.001$) while FC contains more OTG than NFC ($P < 0.05$). FT and NFT rats presented less ($P < 0.001$) FFA than FC and NFC rats (**Table 3**). Alteration compounds in the total luminal—gastric plus intestinal—fat were not affected by the interaction between oil type and nutritional status (data not shown). However, nutritional status did not significantly affect ($P > 0.05$) the concentration of thermally oxidized compounds in luminal intestinal fat but did significantly affect that of hydrolytic compounds (MG and FFA) (both $P < 0.01$). Oil type significantly affected ($P < 0.001$) all thermally oxidized and hydrolytic compounds. FT and NFT rats presented higher (at least $P < 0.01$) PTG, DTG, OTG, and DG values, but lower (at least $P < 0.05$) MG and FFA levels in luminal intestinal fat than their FC and NFC counterparts (data not shown).

Whether or not NOTG values are considered, fat composition profiles of the oils administered differ greatly from those of the luminal gastric and intestinal fat (Supplemental data 1 and 2 in the Supporting Information).

Digestibility Ratios of Unused Sunflower Oil and Sunflower Oil Used in Frying. Table 4 shows the digestibility of whole control and test oils in fasted and nonfasted animals. Not significant oil type−nutritional status interaction was observed for any compounds studied. The type of oil affected the digestibility of all compounds studied (at least, $P < 0.01$).

DISCUSSION

Polar material and thermally oxidized compound content of fresh sunflower oil coincides with that of other good quality oils (*15*). As expected, discontinuous frying increased polar material and thermally oxidized compound content (*7*, *15*). Furthermore, after 40 discontinuous frying operations the polar

Table 4. Short-Term Digestibility (%) of Unused Sunflower Oil and Sunflower Oil Used in 40 Frying Oils and That of Polymer, Dimers and Monomers of Triacylglycerol Compounds Administered to Wistar Rats[a]

					ANOVA		
	NFC	FC	NFT	FT	oil effect	nutritional status	interaction
oil	72.0 ± 8.8	67.7 ± 8.6	62.6 ± 4.9	51.5 ± 13.7	<0.01	NS	NS
PTG	ND	ND	57.8 ± 9.8	47.3 ± 12.2	<0.001	NS	NS
DTG	100.0 ± 0.0	100.0 ± 0.0	81.2 ± 4.8	77.6 ± 3. 5	<0.001	NS	NS
NOTG	83.8 ± 4.8	86.5 ± 5.9	69.8 ± 4.4	59.9 ± 13.3	<0.001	NS	NS
OTG	33.9 ± 35.4	33.9 ± 24.6	72.1 ± 6.8	64.5 ± 11.4	<0.01	NS	NS

[a] For abbreviations see **Table 2**. Data are mean ± SD of 6 animals per group. NS, not significantly.

material and polymeric compound values (PTG + DTG) exceeded the cutoff points recommended for oil discarding (*20*, *21*). These findings, consistent with those of other studies (*7*, *22*), indicate that repeated frying use increases the amount of thermally oxidized compounds in the oil.

Large amounts of fat in the stomach lengthen gastric emptying time. Moreover, digestion time increases when a large quantity of altered oil is consumed. Thus, Benini et al. (*23*) studied the effect of heat-treated fats on gastric emptying in asymptomatic volunteers given two meals that were identical except for the oils (unused and used in frying) employed in food preparation. Total gastric emptying time, measured using ultrasonography, and the sensation of satiety following the meal were significantly greater after the meal prepared with oil previously used in frying. According to the present data, less fat was present in gastric lumen in NFT than in FT rats. As the amount of endogenous luminal gastric fat was very low, the presence of food in the stomach may have somewhat counteracted the expected negative effect of altered fat on the gastric emptying rate (*4*). When eaten alone, carbohydrates exit the stomach more rapidly than when they are consumed with other nutrients and more rapidly than proteins, fats, and fibrous foods. The presence of fat and protein in the small intestine stimulates secretion of the hormone cholecystokinin, which in turn reduces the rate of gastric emptying (*24*). McHugh and Moran (*25*) observed that isocaloric loads of carbohydrate, protein, and fat given to rhesus monkeys produced similar gastric emptying rates, suggesting that gastric emptying is regulated according to the caloric density of the meal, regardless of its nutrient composition. Adaptation to a high fat diet also accelerated the rate of gastric fat emptying in humans (*26*). Kaplan et al. (*27*) observed in rats that doubling the amount of orally infused oil increased gastric emptying time 2-fold, while Harkins et al. (*28*) observed that gastric retention of dietary fat rose as the levels of dietary fat administered increased. Kunz et al. (*29*) showed that gastric emptying of fat was influenced by the order of ingestion of the different meal components. These authors found that gastric fat emptying time was shorter when the fat component was ingested at the beginning of the meal than when it was taken toward its end.

Assuming that gastric lipase activity in rats is rather low and that gastric lipase has little effect on large fatty acid triglycerides (*30*), present data suggest that gastric emptying time of each alteration compound differs, as luminal gastric fat contains a much lower amount of NOTG than the original oils. However, when NOTG were not taken into account the relative percentage of polymerization and hydrolytic compounds in the luminal gastric fat did not greatly differ between NFC and FC, and between NFT and FT rats (Supplemental data 2 in the Supporting Information).

The presence of other dietary compounds (e.g., carbohydrates, proteins) thus appears to accelerate gastric emptying of NOTG. However, a detailed study of the data indicates that there were slight differences between the absolute amounts of FFA and DG, suggesting that a certain level of gastric lipase activity occurred under our experimental conditions (**Figure 1**; Supplemental data 1 and 2 in the Supporting Information).

Digestion of TG is primarily due to pancreatic lipase hydrolysis. Responding to stimulation by the hormone cholecystokinin, pancreatic lipase is released by the pancreatic tissue, enters the duodenum, binds to the surface of TG using colipase as mediator to prevent its expulsion by bile acids into the water phase, and subsequently digests the TG (*31*, *32*). The degradation process is regiospecific and ideally results in the formation of MG and FFA (*33*).

Fasting conditions affected the presence of hydrolytic compounds in the intestinal lumen in the case of the control oil but not in that of the test oil, suggesting that in fasting conditions less pancreatic lipase was available during the 4 h *in vivo* study. However, the effect of the gastric emptying slowing-down on these results should be not ruled out. The oil alteration affects the concentrations of all polymerization and hydrolytic compounds and that of NOTG in the luminal intestinal fat. According to the literature, polymers (*6*, *11*, *34*) and OTG exert significant effects on digestibility of altered and nonaltered fat compounds (*10*−*12*).

Reduced digestibility and absorption are observed in all cases in which heated oils and oils used in frying have been studied (*7*, *33*, *34*). In the present study, FT, NFT, FC, and NFC rats displayed oil digestibility values of 51.5%, 62.6%, 67.7%, and 72.0%, respectively, suggesting that true digestibility of sunflower oil used in frying was lower than that of unused sunflower oil, coinciding with previous data from other authors (*8*, *9*). However, research conducted by Lanteaume et al. (*35*), Le Floch et al. (*36*), and Varela et al. (*37*) did not conclude that digestibility of different oils used for frying varied to any great degree in relation to unused oils. Some years ago, Crampton et al. (*38*) reported that the low nutritive value of diets that included linseed oil heated to 275 °C was principally due to the presence of one or more fatty acid radicals in this polymerized fat. Deuel (*39*) considered that the extent to which frying fats were polymerized was one of the principal factors behind their diminished digestibility, results that concur with those found in the present paper.

Present data also indicate that OTG digestibility values in NFT and FT rats were higher than those of NFC and FC animals, respectively, suggesting that OTG digestibility improves when the amount of NOTG present is reduced. This trend was observed in a previous study of palm olein used in frying (*16*) but not in the case of highly altered, thermally oxidized sunflower oil (*7*). NOTG and OTG digestibility values in FT rats tended to decrease, suggesting that fasting limited the amount of pancreatic lipase available during the 4 h study. In fact, after administration of sunflower oil used in frying, the total amount of MG and FFA in the luminal fat decreased. According to control oil data, the NOTG digestibility was higher than that of DTG and OTG. These results agree in general with the literature. However, in the case of the test oil, NOTG show lower digestibility than OTG and DTG. We have not a clear explanation for these figures; nonetheless, according to

the literature a large amount of OTG induces decreases in the digestibility of NOTG (*10*). Moreover, pancreatic lipase activity is modulated by both the complexity and the polarity of the acylglycerol substrate. In altered oils OTG are more polar than NOTG increasing the K_m of the pancreatic lipase (*11*) explaining the higher digestibility of OTG. Nonetheless, when acylglycerol structures are very complex (*12*) the pancreatic lipase action seems to be decreased. In previous studies using monounsaturated oils (olive oil) heated at 180 °C for 50 h NOTG digestibility was higher than that of the other polar compounds (*11*), suggesting that DTG and PTG were rather complex and the action of pancreatic lipase in the acylglycerol molecule was decreased even though those compounds had higher polarity.

Taking into account both nutritional status and oil type it can be concluded that the fasting condition affects gastric emptying when altered oils are ingested but only slightly affects the presence of different alteration compounds in luminal intestinal fat. However, the factor which most influenced fat digestibility in the present study was oil type (unused sunflower oil or that used in 40 frying operations) rather than nutritional status (fasting or nonfasting conditions).

ABBREVIATIONS USED

DG, diacylglycerols; DTG, dimers of triacylglycerols; FC, fasted rats receiving the control oil; FT, fasted rats receiving the test oil; FFA, free fatty acids; HPSEC, high-performance size-exclusion chromatography; MG, monoacylglycerols; NFC, nonfasted rats receiving the control oil; NFT, nonfasted rats receiving the test oil; NOTG, nonoxidized triacylglycerol monomers; OTG, oxidized triacylglycerol monomers; PTG, polymers of triacylglycerols.

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Supporting Information Available: Acylglycerol compound profiles of administered oil, luminal gastric fat and luminal intestinal fat in fasted rats. This material is available free of charge via the Internet at http://pubs.acs.org.

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