ISSN 1330-9862
(FTB-1467)

review

Methodological Aspects and Relevance of the Study of Vegetable Oil, Fat and Lipoprotein Oxidation Using Pancreatic Lipase and Arylesterase

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Received: July 4, 2005
Revised version: November 23, 2005
Accepted: November 29, 2005

Summary

Fats and oils as major dietary components are involved in the development of chronic diseases. In this paper the physiological relevance and some methodological aspects related to the determination of two enzymes enrolled in metabolism of fat – pancreatic lipase and arylesterase – are discussed. Pancreatic lipase has been extensively used to study the triacylglycerol fatty acid composition and the *in vitro* digestion of oils and fats. The action of this enzyme may be coupled to analytical methods as GC, HPLC, HPSEC, TLC-FID, *etc.* as a useful tool for understanding the composition and digestion of thermal oxidized oils. Pancreatic lipase hydrolysis occurs in the water/oil interface, and it presents a behaviour that seems to be Michaelian, in which the apparent K_m and the apparent V_{max} of the enzymatic process depend more on the type of oil tested than on the degree of alteration. The kinetic behaviour of pancreatic lipase towards thermally oxidized oils also depends on the presence of natural tensioactive compounds present in the oil and surfactants formed during the frying. Arylesterase is an HDL binding enzyme that inhibits LDL oxidation. Low serum concentration of this enzyme has been related to increased cardiovascular disease risk. In this paper the most widely used methods for the determination of arylesterase activity are commented on. The importance of intrinsic factors (*e.g.* substrates, cofactors) participating in the enzyme reaction is also discussed. Moreover, several suggestions about further researches on the influence of extrinsic factors (*e.g.* diet, oxidative stress) upon the enzyme activity are proposed.

Key words: arylesterase, fat, lipoproteins, LDL, oils, pancreatic lipase, thermal oxidized fats

Introduction

Lipids are main components of food. They contain different molecules such as fatty acids, cholesterol, *etc.*, which play important physiological roles. Moreover, fats and oils used for cooking improve food taste and change food composition (*1*,*2*).

The excess of fat and saturated fatty acids in food has been related to some chronic diseases such as obe-

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sity and cardiovascular disease (*3*). Thus, the study of fat and oil composition has gained importance throughout the last decades. Moreover, fats are susceptible to oxidation and hydrolysis (*4*) and the origin of atherosclerosis has been related to low density lipoprotein (LDL) peroxidation (*5*). Several methods have been proposed for the study of fats, oils and plasma lipid carriers – the lipoproteins. Most of them are qualitative or semiquantitative, while quantitative methods are less available and common. Related to oxidation and thermal oxidation, many qualitative methods inform about the presence or absence of fats alteration and the susceptibility of LDL to oxidation, but there are less available quantitative methods.

This paper sums up some central aspects of fats and oils reviewing some major enzymes related to the study of the fat/oil metabolism. Among them, the pancreatic lipase deserves special mentioning. Some *in vivo* and *in vitro* related studies are reviewed. In addition, the arylesterase, a PON1 (paraoxonase 1) related enzyme, has demanded recent interest because it has been found bounded to high density lipoproteins (HDL) and seems to play a protective antioxidant (and thus, protective cardiovascular risk) effect. The relevance and some methodological aspects of this enzyme will also be reviewed.

Physiological Importance of Enzymes

Pancreatic lipase

Lipases (triacylglycerol acylhydrolases, E.C. 3.1.1.3) are ubiquitous enzymes of considerable physiological significance and industrial potential. In eukaryotes, lipases are involved in various stages of lipid metabolism including fat digestion, absorption and reconstitution, and also in lipoprotein metabolism. In plants, lipases are found in energy reserve tissues.

The major human lipases include the gastric, pancreatic and bile salt-stimulated lipases which aid in the digestion and assimilation of dietary fats, and the hepatic, lipoprotein and endothelial lipases that function in the metabolism of lipoproteins (*6*). The pancreatic, hepatic, lipoprotein and endothelial lipases are members of the lipase gene family. Pancreatic lipase is produced by the pancreatic acinar cells, and it is one of the exocrine enzymes of pancreatic juice that is essential for digestion of dietary fats in the intestinal lumen. The substrate of this enzyme is not a single molecule but a non-aqueous phase of aggregated lipids made up of aggregates of ester molecules, micelles or monolayers interfacing with an aqueous medium (*7,8*). It requires colipase as cofactor for its enzymatic activity. Colipase relieves phosphatidyl choline-mediated inhibition of the interfacial lipase-substrate complex, helps anchor the lipase to the surface and stabilizes it in the »open« active conformation (*9,10*).

In contrast to esterases, lipases are activated only when adsorbed to an oil–water interface (*11*) and do not hydrolyze dissolved substrates in the bulk fluid. A true lipase will split emulsified esters of glycerol and long-chain fatty acids such as triolein and tripalmitin. Lipases are serine hydrolases and display little activity in aqueous solutions containing soluble substrates. How lipases and lipids interact at the interface is still not entirely clear and is a subject of intense investigation (*12*).

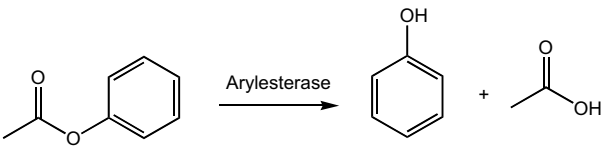
Due to their wide-ranging significance, lipases remain a subject of intensive study (*13,14*). Research on lipases is focussed particularly on structural characterization, elucidation of the mechanism of action, kinetics, sequencing and cloning of lipase genes, and general characterization of performance (*13,14*).

Lipases find promising applications in organic chemical processing, detergent formulations, synthesis of biosurfactants, the oleo chemical industry, the dairy industry, the agrochemical industry, paper manufacture, nutrition, cosmetics, and pharmaceutical processing. Development of lipase-based technologies for the synthesis of novel compounds is rapidly expanding the uses of these enzymes in these industries (*15*). Lower amounts of these enzymes are used in oleo chemical transformations (*14*). They can play an important role in the processing of γ-linolenic acid, a polyunsaturated fatty acid (PUFA); astaxanthine, a food colorant; methyl ketones, flavour molecules characteristic of blue cheese; 4-hydroxydecanoic acid, used as a precursor of the fruit flavour γ-decalactone; dicarboxylic acids, for use as prepolymers; cheaper glycerides, through interesterification to more valuable forms (*e.g.* cocoa butter replacements for use in chocolate manufacture) (*16*). Lipases can also be used to modify vegetable oils at position 2 of the triacylglycerol (TG), to obtain fats similar to human milk fat for use in baby feeds (*17*); to synthesize lipid esters (*18*), including isopropyl myristate, for use in cosmetics; and to produce monoglycerides to use as emulsifiers in food and pharmaceutical applications.

The increasing awareness of the importance of chirality in the context of biological activity has stimulated a growing demand for efficient methods for industrial synthesis of pure enantiomers, including chiral anti-inflammatory drugs such as naproxen (*19*) and ibuprofen (*20–24*); antihypertensive agents such as angiotensin-converting enzyme (ACE) inhibitors (*e.g.* captopril, enalapril, ceranopril, zofenapril, and lisinopril); and the calcium channel-blocking drugs such as diltiazem. Lipases are used in synthesis of these drugs (*25*).

Arylesterase

Another group of enzymes related to fat and cholesterol metabolism are the esterases, which do not act at interfaces but rather in a homogeneous polar phase. One example of an esterase is arylesterase (E.C. 3.1.1.2), which is supposed to be one of the three activities that can show the PON1 (*26*). In recent studies, it has been hypothesized that arylesterase is a HDL-bound enzyme located at the same place as apolipoprotein (apo) A1 and apo J or chlusterin (*27,28*). Its native substrate is still unknown but it hydrolyses aromatic esters such as phenylacetate (PA) (Scheme 1):



Scheme 1. Scheme of phenylacetate hydrolysis by arylesterase. Phenol, which absorbs at 270 nm, and acetic acid are obtained

Arylesterase protects LDL and HDL-cholesterol from oxidation and also facilitates the cholesterol reverse transport (*29*,*30*). This protection is probably related to the ability of arylesterase to hydrolyze some oxidized phospholipids (*31*) and cholesteryl linoleate hydroperoxides (*30*), which are present in oxidized-LDL.

We must distinguish between two different actions of this enzyme: antioxidant and arylesterase. For its ability to protect LDL-cholesterol against oxidation, it needs the sulphydryl group on cysteine-284 to be free, as shown in the study of Aviram *et al.* (*32*), which used sulphydryl blocking agents like *p*-hydroxymercurybenzoate (PHMB). However, this cysteine-284 is not necessary for the arylesterase activity because the substitution of this amino acid by serine or alanine does not eliminate its arylesterase activity. On the other hand, calcium is required for its arylesterase activity but not for its ability to protect LDL-cholesterol against oxidation.

The arylesterase activity is rather variable among subjects. This variability has been related to mutations in the gene that codes for the production of PON1. The PON1 gene is located on the long arm of chromosome 7 (*33*). The major polymorphisms of PON1 include the replacement of glutamine (Q or A) by arginine (R or B) at position 192, and that of leucine (L) by methionine (M) at position 55. There is no discrimination between the allozymes for the phenylacetate substrate, but this gene polymorphism may affect the antioxidant action of PON1. Which allozyme is more protective against LDL-cholesterol oxidation is still under discussion.

Antioxidant and arylesterase activities are both affected by lifestyle and some living factors such as diet (*34*,*35*) and smoking (*36*). Furthermore, arylesterase activity is reduced in hypercholesterolemia, type 2 diabetes and cardiovascular diseases (*37*,*38*).

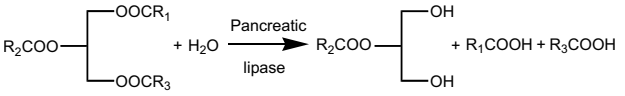
Arylesterase requires free calcium to work (*39*). Its activity is also enhanced when chloride is in the medium and inhibited when a high concentration of bicarbonate is available (*39*).

Methods for the Assessment of the Fat Composition and Quality

Plasma TG can be studied quantitatively and qualitatively. Most recent methods used in clinics and research measure total TG amount in plasma using sequential action of lipase, glicerol quinase, glicerol phosphate oxidase and peroxidase (*40*). The enzyme method is associated with the Trinder colour reaction (*41*). Measurements can be performed in a few minutes. Moderate hypertriglyceridemia is achieved for TG levels between 150–200 mg/dL but present recommendations suggest <110 mg/dL (1.24 mmol/L) for children and 130 mg/dL (1.46 mmol/L) for adults as desirable values.

TGs are usually molecules composed of glycerol esterified with long-chain fatty acids. The fatty acids differ in chain length (short, medium, large), ramification (branched and unbranched), unsaturation (saturated, monounsaturated, and polyunsaturated), position of double bounds (Δ for relative position of double bounds to the acid end; n or ω for the relative position to the methyl end), and geometric isomerization (*cis* or *trans*). The fatty acid composition of plasma TG gives information of the recent adherence to diet and the nutritional status (*e.g.* the trienic to tetraenic ratio (20:3/20:4) is a marker of malnutrition).

Differences in the distribution of fatty acids among the three possible positions of the glycerol moiety in TG from natural fats and oils were first demonstrated systematically using enzymatic hydrolysis (*42*). Pancreatic lipase specifically permits analysis of the fatty acids at position *sn*-2 (Scheme 2). More complex stereospecific hydrolysis procedures have been developed to determine fully the positional distributions of the fatty acids (*42*).



Scheme 2. Schematic reaction of pancreatic lipase hydrolysis on triacylglycerols

Lipases are among the most important enzymes used in the oil and fat industries. These enzymes catalyze the hydrolysis of TG and acyl and aryl esters (*42*,*43*) and many are also capable of catalyzing organic reactions in nonaqueous media (*44–46*).

Pancreatic lipase hydrolyzes or synthesizes TG with positional and fatty acid specificities, and is extensively used as gastrointestinal tract lipase in experimental systems since it is inexpensive and easily available. Human pancreatic lipase seems the »ideal« lipase to study the hydrolysis and digestion of fat with nutritional implications to human being. Nonetheless, the porcine pancreatic lipase displays 86 % homology with human pancreatic lipase (*47*), and therefore it is used instead of it.

During substrate hydrolysis by lipases, three stages can be defined: (*i*) interface enzyme adsorption, (*ii*) interfacial activation and (*iii*) catalysis. The enzyme, present in aqueous medium, is capable of penetrating into the interface between the lipophilic substrate and water. Only when both the substrate and the enzyme are at the interface, the enzyme-substrate complex occurs, and this leads to catalytic product formation and regeneration of the enzyme (*43*,*48*).

The pancreatic lipase hydrolyzes TG molecules that contain short-chain fatty acids more rapidly than molecules containing only long-chain fatty acids. Moreover, ester bonds of some very long polyunsaturated fatty acids such as docosahexaenoic acid and of some *trans* fatty acids such as *trans*-3-hexadecenoic, and the phytanic acid ester of glycerol are hydrolyzed more slowly, probably as a result of steric hindrance caused by the proximity of substituent groups to these ester bonds (*43*,*49*). TGs containing behenic acid are hydrolyzed with difficulty by pancreatic lipase, a fact of interest for food industry when obtaining food with less available energy (*50*).

The structural analysis of TG depends on certain important conditions: the use of calcium ions is essential, bile salts are necessary for the reaction, and TG must be well dispersed by previous vigorous shaking, as the enzyme attacks only the micellar form of fats. For this reason, methyl oleate (*51*) or hexane (*52*) are sometimes added as carriers to increase the solubility of saturated fats

with high melting points. Preincubation at 42 °C has also been recommended instead of the use of carriers (*53*). The use of isooctane and cyclohexane use has been recommended for microbial lipases (*54,55*). Concentrations of various cations, bile salts and the enzyme, the buffer pH and temperature should be adjusted to their optima so that an appreciable degree of hydrolysis (50–60 % is sufficient) occurs in a short time (*56*).

The semi-micro method developed by Luddy *et al.* (*57*) has been recommended as the best practical procedure for the structural analysis of TG (*58*). Facts of this method are commented below:

Tris(hydroxymethyl)methylamine (Tris) buffer (1 M, pH=8, 1 mL), calcium chloride solution (2.2 %, 0.1 mL) and a solution of bile salts (0.05 %, 0.25 mL) are added to the TG (up to 5 mg) in a stoppered test tube, and the whole is allowed to equilibrate at 40 °C in a water-bath for 1 min before the pancreatic lipase preparation (1 mg) is added. The mixture is shaken vigorously at this temperature by means of a mechanical shaker for 2–4 min until the desired degree of hydrolysis is attained, when the reaction is stopped by the addition of ethanol (1 mL) followed by 6 M hydrochloric acid (1 mL). The solution is extracted three times with diethyl ether (10-mL portions), with centrifugation if necessary to break any emulsions, the solvent layer is washed twice with distilled water (5-mL portions) and dried over anhydrous sodium sulphate. On removal of the solvent, the products obtained after the hydrolysis can be studied by different methods such as thin layer chromatography (TLC), or high performance liquid chromatography (HPLC). For its determination by GC after the removal of the solvent, it is necessary to resuspend the hydrolysis products with *N*-methyl-*N*-trimethylsilylheptafluorbutyramide (MSHFBA) to convert the free fatty acids into the corresponding sylil ethers (*59*).

The 2-monoacyl-*sn*-glycerols formed during the procedure are generally isolated by TLC and then transesterified to fatty acid methyl esters prior to gas chromatographic analysis. However, the presence in altered oils of many different compounds with repartion factor (R_f) values similar to those of the 2-monoacyl-*sn*-glycerols makes the TLC separation rather inadvisable, so HPLC should be preferred.

A commonly and rapidly used procedure for investigating esterase and lipase activity employs *p*-nitrophenyl esters with aliphatic acyl chains of various lengts: short chains (acetate or butyrate) to measure esterases activity, and long chains (palmitate or oleate) to measure lipases activity. The release of *p*-nitrophenol is measured spectrophotometrically at 410 nm. But this method has many limitations, as it must be performed at neutral or alkaline pH where some lipases cannot work (*60*).

No lipolytic enzyme has yet been isolated that is capable of distinguishing between the positions 1 and 3 of a triacyl-*sn*-glycerol. In order to determine the compositions of positions *sn*-1, *sn*-2 and *sn*-3 a number of ingenious stereospecific analytic procedures have been developed and reviewed (*42,61*).

Regioisomers (reverse isomers) of 1,2-diacyl-*sn*-glycerols with various pairs of saturated and unsaturated acyl groups, which were prepared by partial Grignard degradation of the corresponding TG and derivatized into 3,5-dinitrophenylurethanes (DNPU), 3,5-dinitrobenzoates, nicotinates, 1- and 2-anthrylurethanes, have successfully been identified by electrospray ionization mass spectrometry (ESI-MS), chiral-phase HPLC and GC (*62,63*).

According to Christie (*42*), by using small variations of the classical method of Brockerhoff (*64*), it is possible to obtain phospholipid derivatives (*e.g.* phosphatidylcholines) from diacylglycerols after pancreatic lipase hydrolysis. These compounds can be further hydrolyzed by phospholipases. Analysis of the resulting diacylglycerols can determine the structure of the original TG.

Enzymatic Methods to Study Oxidized Oils and Lipid Peroxidation

Fats and oils are susceptible to structural changes due to hydrolysis and autooxidation. These changes are increased at high temperatures, producing a higher amount of thermal oxidation compounds (*2*). Unsaturation increases the fat oxidation susceptibility (*e.g.* linoleic acid is 40 times more oxidizing than oleic acid) (*65*). Thermally oxidized oils, such as those produced by repeated frying, contain a complex mixture of products such as oxidized TG monomers, TG dimers, and TG polymers. These products are mainly associated with changes in the physicochemical properties of fats (*66–68*). Consequently, the oil/water interface could vary and the lipolytic activity of the lipase could be altered. Moreover, no studies on the hydrolysis of oxidized or polymerized oils have been carried out with lipases other than pancreatic lipase. Two groups of studies related to oxidized oils, *in vitro* and *in vivo* techniques are reviewed in this section.

In vitro techniques

As is mentioned before, pancreatic lipase displays greater reactivity towards TGs, which contain more hydrophilic fatty acids, such as C10 and C12, than towards those containing C16 and C18 (*69*). Linoleic and linolenic acids are normal constituents of fats and oils. When prooxidant conditions are present they are converted to their respective hydroperoxydes. Studies of oxidized oils employing pancreatic lipase have suggested that altered oils are less hydrolyzed than unaltered oils (*4*). However, some »paradoxical« results have been published and will also be commented on. The preferential hydrolysis of hydroperoxy linoleoyl and linolenoyl groups by pancreatic lipase may therefore be due to the presence of hydrophilic hydroperoxy groups that are more susceptible to lipase hydrolysis. Miyashita *et al.* (*70*) suggested that hydroperoxides do not inhibit lipase activity, although lipid peroxidation products react with enzyme protein, reducing its biological activity. The loss of enzyme activity could be due to structural changes in the proteins, caused mainly by protein-centered free radical intermediates (*71–73*).

Yoshida and Alexander (*74*) studied the enzymatic hydrolysis of acylglycerol products obtained from thermally oxidized corn, sunflower and soybean oils. These oils were heated at 180 °C for 50, 70 and 100 h, with aeration, and then three fractions were eluted from the oils: the non-polar fraction contained monomeric compounds,

the slightly polar fraction contained dimeric compounds and the polar fraction included polymeric compounds. The monomers in the non-polar fraction were hydrolyzed by pancreatic lipase as rapidly as those in the corresponding unheated oils. The dimers in the slightly polar fraction were hydrolyzed much more slowly, and the polymers in the polar fraction were barely hydrolyzed at all. The source of the oil had no effect: pancreatic lipase hydrolyzed heated corn, sunflower and soybean oils to a similar degree. Taking into account the studies of Ohfuji *et al.* (*75*) with thermally oxidized dimers, Yoshida and Alexander (*74*) explain the lower hydrolysis of dimers in relation to monomers. This is further discussed in the *in vivo* techniques section.

Márquez-Ruiz *et al*. (*76*) tested the *in vitro* action of pancreatic lipase on complex glycerides from thermally oxidized oils. Pure commercial olive oil was heated at 180 °C for 150 h. Samples of non-heated olive oil, heated olive oil, a 1:1 mix of both and the polar fraction isolated from olive oil heated for 150 h were subjected to enzymatic hydrolysis with pancreatic lipase under standardized conditions for 2 min and for 30 min. As expected, the percentage of the released free fatty acids increased substantially with hydrolysis time but decreased in proportion to the degree of oil alteration. A much higher degree of hydrolysis by pancreatic lipase was found for dimers of TG than for polymers in the olive oil heated for 150 h. Similar results were obtained when the polar fraction of the olive oil heated for 150 h was hydrolyzed. Hydrolysis produced no further significant triacylglycerol polymer loss and fatty acid release after a 15-minute reaction period, demonstrating the difficulty involved in the hydrolysis of these high molecular mass products.

The same group, Márquez-Ruiz *et al.* (*77*), has studied two aspects of the *in vitro* hydrolysis of abused oils using pancreatic lipase: (*i*) the susceptibility to hydrolysis of altered compounds present in the used frying oils, (*ii*) the influence of the degradation level of used frying fats on the hydrolysis of non-oxidized TG. Frying oils ranging from 3.1 to 61.4 % in polar compound content were studied using a combination of adsorption chromatography, high-performance size exclusion chromatography (HPSEC), TLC-FID and *in vitro* hydrolysis for 2 min and for 20 min with pancreatic lipase. Three types of substrates were subjected to enzymatic hydrolysis: (a) polar fractions isolated from trilinolein samples subjected to heating at 180 °C, (b) polar fractions isolated from thermally oxidized frying oils, and (c) oils used in frying but without any preliminary separation of the non-oxidized TG fractions.

Hydrolysis of polar fractions produced similar results in terms of the relative hydrolysis rate. Oxidized TG monomers were the type of altered compounds most extensively hydrolyzed (80–89 %) after 20 min. Polymers displayed low hydrolysis values (11–42 %), while dimers gave intermediate values. Hydrolysis of oils used in frying revealed that the most degraded oils produce a significantly reduced level of total hydrolytic products (diacylglycerols, monoacylglycerols and fatty acid monomers). An increase in the amount of polymers and dimers affects the hydrolysis rate of non-oxidized TG to a remarkable degree: their percentage of hydrolysis in slightly degraded oils is about 95 %, while this percentage falls to as low as 52 % in degraded oils, containing 47.6 % of dimers plus polymers.

The position of the oxygenated group in the TG could modulate its enzymatic hydrolysis, which has physiological importance. The classical method of Brockerhoff (*64*) would be useful to differentiate between oxidized acyls in position 1 and 3 in the triacyl-*sn*-glycerol.

In vitro hydrolysis of thermally oxidized TG by pancreatic lipase has also been investigated (*78–80*). Two different aspects have been studied: total hydrolysis of thermally oxidized oils (final point technique), and enzymatic hydrolysis kinetics of altered oils (dinamic hydrolysis technique).

Final point technique

The final point technique includes the study and quantitation of the compounds obtained after hydrolyzing altered oils for a given time. Our group has used this method extensively for the study of unused and used oils. Thus, the enzymatic hydrolysis of palm olein that had been used 60 or 90 times to fry potatoes was compared to that of unused palm olein. Porcine pancreatic lipase was used for the hydrolysis reaction. Five hundred-gram sets of potato slices were fried in 3 L of olein without any replenishment with unused oil. The food to oil ratio in the fryers was kept at 500 g per 3 L by emptying the content of one fryer into the others after each 10 uses (*78–80*).

This successive frying of potatoes significantly increased the total polar content in the palm olein from (9.3±0.1) mg/100 mg of oil to (26.4±0.3) mg/100 mg of oil after 90 uses (Table 1). Using a combination of col-

Table 1. Composition of palm olein and sunflower oil before and after potato frying

	Number of fryings	*w*(total polar content)	*w*(TG polymers)	*w*(TG dimers)	*w*(oxidized TG)	*w*(DG)	*w*(non-oxidized TG)
		mg/100 mg of oil					
Palm olein	0	9.3±0.1	0.1±0.0	1.0±0.3	1.1±0.2	6.7±0.2	93.7±0.1
	60	18.2±0.2	1.6±0.0	5.0±0.1	5.3±0.1	6.1±0.1	82.8±0.5
	90	26.4±0.3	3.7±0.0	7.9±0.1	8.3±0.1	6.2±0.1	73.6±0.3
Sunflower oil	0	4.0±0.2	0.1±0.0	0.5±0.0	1.9±0.1	1.0±0.1	96.0±0.8
	30	18.9±0.3	2.2±0.1	7.8±0.2	7.0±0.2	1.4±0.1	91.1±0.3
	60	27.7±0.3	5.6±0.2	11.0±0.2	8.9±0.2	1.7±0.1	72.3±0.3

Data are the mean of three samples±s.d., TG: triacylglycerols, DG: diacylglycerols

umn chromatography and HPSEC (*81*), it was found that polymer, dimer, and oxidized triacylglycerol contents in palm olein also increased several times (Table 1).

Unused palm olein and palm oleins from the 60th and 90th frying, as well as polar fractions from the unused and used palm oleins separated by the column chromatographic method of Waltking and Wessels (*82*) were hydrolyzed by porcine pancreatic lipase (E.C. 3.1.1.3) at 37 °C for 20 min following a slight variation of the Luddy *et al.* method (*57*).

The degree of hydrolysis of the unused olein was similar to that of the olein used 40 times for frying potatoes (Table 2). However, hydrolytic compounds from palm olein used 90 times were significantly lower.

These results could be explained by the presence of non-oxidized TG in the samples, which are the natural substrates for pancreatic lipase. The palm olein that had been used 90 times contained a lower amount of unaltered TG than the unused olein or that used 40 times (Table 1).

The net hydrolysis of TG was much higher in unused palm olein (83.8 %), and in palm olein from the 40th frying (87.1 %) than in palm olein from the 90th frying (65.8 %). This means that pancreatic lipase hydrolysis of TG is maintained or increased when palm olein is moderately altered, but that it decreases when a high degree of alteration exists.

In a previous study (*83*), the existence of a balance between factors that improve or impair the pancreatic lipase hydrolysis was suggested. This balance will be discussed in the next section. Although non-oxidized and oxidized TG were not tested separately, it also seems possible that the increased proportion of oligomers in samples might decrease hydrolysis of intact TG, as Márquez-Ruiz *et al.* (*77*) have demonstrated. All these data clearly indicate that the lipolytic hydrolysis of palm olein is greater when the alteration of the substrate is lower.

The amount of polymers hydrolyzed from palm oleins and from their corresponding polar fractions is shown in Table 3. The percentage of polymers remaining non-hydrolyzed was much higher in the polar fractions than in their corresponding oleins, suggesting that some compounds in the oils must play a role in the hydrolytic action of pancreatic lipase.

Dynamic hydrolysis technique

The titrimetric assay was used to determine lipolytic activity (*80*). The acid released during the hydrolysis was continuously titrated at 37 °C with NaOH solutions at pH=8.3 with the aid of pH stat and pH meter. The reaction time was 10 min. In all cases, lipase activity was measured as initial reaction rates in order to avoid the possible inhibition that might take place due to the appearance of reaction products. Specific lipase activity was defined as the μmol of free fatty acids released per min and per mg of crude enzyme.

Substrate concentration in the reactor was determined taking into account its molecular mass, which was calculated by HPSEC (*84*), extrapolating oil sample R_f in the calibration curves performed using acylglycerol standards and polyethylene glycol in different states of matter (*83*).

The kinetics of the enzymatic hydrolysis of vegetable oils that had been used in frying and that presented different degrees of alteration were also investigated using porcine pancreatic lipase (*83*). The aim of the study

Table 2. Concentration of diacylglycerols, monoacylglycerols and free fatty acids in unused palm olein and in palm olein used 40 and 90 times for frying potatoes after a 20-minute enzymatic hydrolysis by pancreatic lipase

Hydrolyzed oil	Number of fryings	*w*(MG)	*w*(DG)	*w*(FFA)
		mg/100 mg of sample		
Palm olein	0	24.82±1.10	24.95±0.20	34.99±1.50
Palm olein	60	25.37±1.70	24.33±1.60	36.95±1.50
Palm olein	90	13.41±0.10	26.49±0.30	23.70±0.30

Data are the mean of three samples±s.d., MG: monoacylglycerols, DG: diacylglycerols, FFA: free fatty acids

Table 3. *In vitro* enzymatic hydrolysis of polymers from palm olein and from the polar fraction of unused palm olein and that used 40 and 90 times for frying potatoes

	w(polymers)		
	Initial	After hydrolysis	Hydrolyzed
	mg/100 mg of sample		%
Palm olein (40th frying)	0.70±0.02	0.30±0.02	57.10
Polar fraction from palm olein (40th frying)	5.92±0.50	4.67±0.20	21.10
Palm olein (90th frying)	4.12±0.02	2.65±0.10	35.70
Polar fraction from palm olein (90th frying)	13.94±0.00	13.93±0.10	00.00

Data are the mean of three samples±s.d.

was to understand the effect of the thermally oxidized products that appear during frying on *in vitro* lipolysis by pancreatic lipase. To better understand lipase hydrolysis of thermally oxidized oils, the effect of thermally oxidized acylglycerol models upon pancreatic lipase activity was also studied (*79*).

Successive uses in potato frying, with no replenishment of unused oil, significantly increased total polar compound content of palm olein to 26 mg/100 mg of oil and that of sunflower oil to 28 mg/100 mg of oil after 90 and 60 frying operations, respectively. TG polymers, TG dimers, and oxidized TG increased 37.0, 7.9, and 7.5 times respectively in palm olein, and 56.0, 22.0, and 4.7 times in sunflower oil (Table 1).

The activity of pancreatic lipase can be modulated not only by the presence of complex compounds (*e.g.* dimers and polymers of TG) in the substrate but also by altered compounds arising during the hydrolysis step (*e.g.* altered fatty acids). With this objective in mind, velocity *vs* substrate concentration relationships were measured and the corresponding kinetic parameters were calculated: v refers to the initial velocity in μmol/min·mg of enzyme; and S to the substrate concentration in mM.

Fig. 1 shows the activity of pancreatic porcine lipase toward palm olein and sunflower oil, both with approximately 18 mg of polar compounds/100 mg of oil. Both oils were hydrolyzed by pancreatic lipase following Michaelis–Menten saturation kinetic behaviour. Each set of data was fitted to the Michaelis-Menten equation by non-linear regression, and the corresponding apparent V_{max} and apparent K_m were estimated.

Comparison of the activity of pancreatic lipase on palm olein and sunflower oil, both with approximately 18 mg polar content/100 mg of oil (Table 1, Fig. 1), clearly suggests that the hydrolytic efficiency of pancreatic lipase is greater in palm olein than in sunflower oil.

As can be seen from Table 4, kinetic parameters for palm olein and sunflower oil did not indicate a clear relationship with the number of frying operations, and therefore with the content of altered compounds in the oils. However, looking at the apparent K_m or V_{max} of palm olein or sunflower oil samples (unused or used in frying), it can be concluded that palm olein is more easily hydrolyzed by pancreatic lipase than sunflower oil. The difference in the kinetic behaviour of the enzyme with unused and used oils must be related to their different fatty acid composition and degree of alteration. Palm olein has a higher content of palmitic and oleic acids but a lower content of linoleic acid than sunflower oil; oleic acid is known to be released by pancreatic lipase with the highest relative speed (*85*). Comparison of both oils, whose polar compound content was similar, shows that sunflower oil has a higher amount of polymers and dimers, and a lower amount of diacylglycerols than palm olein (Table 1). Another aspect to be taken into account is the presence of minor compounds that

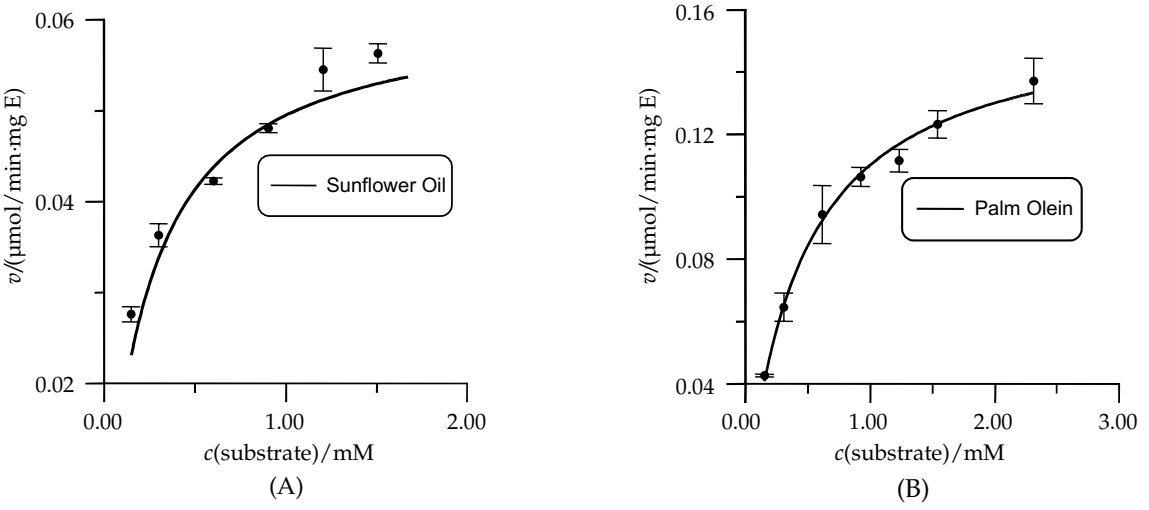


Fig. 1. Activity of pancreatic porcine lipase toward (A) palm olein and (B) sunflower oil (both with approximately 18 % in polar content). Experiment conditions in the text. Solid line corresponds to computer fitting of the data to the Michaelis-Menten equation, and error bars refer to 95 % confidence limits using the Student's t test

Table 4. Composition of non polar triacylglycerols of palm olein (NPTPO) before and after thermal oxidation at 180 °C

	Heated time	*w*(total polar content)	*w*(TG dimers)	*w*(TG polymers)	*w*(oxidized TG)
	h	mg/100 mg of sample			
NPTPO	0	0	0	0	0
	1	16.28	5.16±0.02	1.30±0.01	9.82±0.04
	4	60.48	14.80±0.03	16.60±0.12	29.10±0.10

Data are the mean of three samples±s.d., TG: triacylglycerol

can affect the enzyme reaction. Palm olein has a high amount of tocotrienols, while sunflower oil is rich in tocopherols. The amount of some antioxidants (*e.g.* polyphenols) and tensioactive substances is also quite different in both oils (*86,87*), explaining the highest hydrolysis of palm olein. All these facts affect the enzyme activity and thus the reaction rate, which will be discussed below.

Changes in the apparent K_m and apparent V_{max} were not related to the degree of alteration of the oils. Thus, enzymatic hydrolysis catalyzed by porcine pancreatic lipase is not deeply affected by compounds of high molecular mass and high polarity. However, these data must be evaluated in light of the process itself, because dynamic hydrolysis data account for what occurs in the oil-water interface. Thus, it should be indicated that there exist factors that interfere with (*e.g.* complexity of sample, losses of antioxidants) and factors that enhance the enzymatic reaction (interface substrates, sample polarity, surfactant concentration, *etc.*). Regardless of the complexity of the sample, the preferential interface substrates for pancreatic lipase are always the same: TG and diacylglycerols, which are in much higher proportion than oligomers. It is also known (*88,89*) that the concentration of tocopherols decreases significantly as a consequence of heating.

Yoshida and Alexander, and Ohfuji *et al.* (*74,75*) reported that the lower the degree of enzymatic hydrolysis, the higher the molecular mass of the acylglycerol samples. As previously mentioned, Miyashita *et al.* (*70*) demonstrated that porcine pancreatic lipase is not inactivated in the presence of monohydroperoxides and the hydrolysis preferentially takes place in molecules with esterified monohydroperoxide fatty acids rather than in those containing non-oxidized fatty acids. Henderson *et al.* (*90*) concluded that the high molecular mass polymers of TG present in oxidized fish oils can be hydrolyzed by pancreatic lipase *in vitro*. The polymers formed in oils during frying display surfactant properties (*91*). Emulsions with higher surfactant content are more stable and their interface surface is larger, suggesting that the presence of surfactants enhances the enzymatic hydrolysis.

The efficiency of pancreatic lipase in hydrolyzing palm olein (expressed by the V_{max}) tends to increase with the alteration level of the substrate (Table 4). However, the enzymatic affinity for this substrate (expressed by the apparent K_m) does not change. The kinetic parameter apparent V_{max} / apparent K_m in unused oils shows a tendency to increase in oils with about 18 % in polar content, and to decrease in oils containing about 27 % of polar compounds. The hydrolytic behaviour of non-polar TG of palm oleins (NPTPO) subjected to 180 °C for 1 to 4 h also suggests that this balance between factors exists (Table 2) (*79*). Comparison between kinetic behaviour of unheated NPTPO and unused palm olein clearly indicates than the latter is a better substrate for pancreatic lipase than NPTPO. Both samples differ in major and minor compounds because NPTPO contains only isolated non-oxidized TG, while unused palm olein contains, in addition to those compounds, other molecules with higher polarity (oligomers, oxidized TG, diacylglycerols and free fatty acids). These differences can affect the hydrolytic behaviour of pancreatic lipase. A decrease in the apparent K_m and apparent V_{max} after 2 h can be related to the disappearance of natural emulsifier in NPTPO, while increased production of polar surfactants would explain the increase in the K_m after 4 h, in relation to the NPTPO samples heated for 2 h.

In vivo studies

Digestion of TG implies complicated physicochemical reactions and numerous interactions between lipolytic products, phospholipids, bile salts, proteins, and carbohydrates. The process can be summarized as follows: alimentary canal enzymes, mostly pancreatic lipase, prepare fatty acids for absorption and transport through the enterocyte membrane by converting water-insoluble TG into more hydrophilic molecules, diacylglycerols, monoacylglycerols and free fatty acids (*92*).

Few systematic studies investigating the major steps in fat digestion and absorption of thermally oxidized and polymerized oils (*e.g.* micelle formation, micelle-cell membrane transport) have been carried out. Although there is a consensus in that heated fats present reduced digestion and absorption (*93*), other authors (*94–96*) did not find that frying had any significant effect on the digestion of different oils.

The main reason reported (*97*) for the lower digestion of diets that included linseed oil heated to 275 °C was the presence of dimers of fatty acids in this polymerized fat. Deuel (*98*) considered that the extent to which frying fats were polymerized was one of the principal factors affecting their digestibility. Potteau *et al.* (*99,100*) reported that as polymerization of oil increases, its digestive use declines, as part of the polymers are eliminated in the faeces.

On the other hand, polar dimers and polymers of TG (*101*) appear to be better hydrolyzed and their products absorbed than non-polar dimers in lymph cannulation studies. Márquez-Ruiz *et al.* (*102*) found high digestibility values for oxidized dimers and polymers. Bottino *et al.* (*49*) reported apparent digestibility of dimers between 30–70 %, but Kajimoto and Mukai (*103*) questioned such high values. Ohfuji *et al.* (*75*) found that dimeric compounds from thermally oxidized oils are absorbed by rats. However, 2 out of 3 of the ester bonds in TG monomers are readily hydrolyzed whereas in dimers, which are larger, due to a number of different chemical entities and C-C linkages, internal ester groups are not available for hydrolysis (*104*). According to Paulose and Chang (*105*), as a result of heating, much of the dimeric structure is complex because intramolecular and intermolecular linkages can coexist. This would interfere with the hydrolysis of dimers and polymers even more, resulting in lower hydrolysis for heated oils. Ohfuji and Kaneda (*104*) obtained no appreciable enzymatic hydrolysis of polymeric compounds in oxidized oils.

Studies on altered fat digestion often use radiolabelled markers, which do not always correspond to the same composition as that of the altered compounds present in heated or used frying fats. In addition, these markers are potentially harmful and rather expensive. During the last few years the *in vivo* digestibility and absorption coefficients of some oils have been studied by our group without using radiolabelled materials (*106,107*). True digest-

ibility of unheated olive oil was tested 2, 4, 6 and 7 h after administrating 1 g of olive oil/100 g of body mass to young adult Wistar rats by means of esophageal probes. Control rats were administered isotonic saline solution at the rate of 1 mL/100 g of body mass. Afterwards, 50 mL of isotonic saline solution were slowly passed from the distal esophagus to the distal ileum in order to obtain the luminal fat. Remaining gastrointestinal luminal fat showed a linear but inverse relationship ($r=-0.99$; $p<0.001$) with the duration of the experiment. A 4-hour test was found to be adequate, because after this period half of the oil administered remained in the lumen, making it possible to accurately determine the different non-digested and/or non-absorbed thermally oxidized compounds (*108*).

The *in vivo* digestibility and absorption coefficients of palm oleins submitted to potato frying were related to their thermal oxidation level (*106*). The palm oleins studied were the same ones that were discussed in the *in vitro* studies section (Table 1). When these used palm oleins were administered by esophageal probe at the rate of 1 g/100 g of body mass to young adult Wistar rats which had fasted overnight, a significant decrease was noted in the true digestibility and true absorption coefficients after the 4-hour experiment. However, no changes in the intracellular gastrointestinal fat content were found. Modifications in fat digestibility and absorption were highly correlated ($p<0.001$) with total alteration and the presence of TG polymers, TG dimers, and oxidized TG.

These results concur with those of Carey *et al.* (*109*) and suggest that the most critical events under the experimental conditions applied take place in the intestinal lumen, because clearance of the intracellular gastrointestinal fat does not change significantly. True digestibility and absorption coefficients were positive and significantly correlated with hydrolytic alteration products administered. Results suggest that palm olein with a high level of thermally oxidized products was digested less efficiently than unused palm oleins but the level of thermally oxidized products did not affect the clearance of intracellular gastrointestinal fat.

A 4-hour experiment was also designed to investigate how thermally oxidized and polymerized compounds present in palm oleins used repeatedly for frying of potatoes were hydrolyzed *in vivo* (*110*). A combination of *in vivo* short-term fat digestion, column chromatography and HPSEC techniques was employed. The possible inhibitory role of some altered compounds, such as oligomers, on fat digestibility of non-thermally oxidized TG was also tested by comparing the hydrolysis of palm oleins with a different composition in altered compounds. After the administration of palm olein used in frying, the percentage of oligomers and oxidized TG in the polar fraction of the luminal fat markedly increased, while the percentage of diacylglycerols and free fatty acids decreased. This is in part due to the high content of thermally oxidized compounds in these oils and in part to the reduced hydrolytic activity of pancreatic lipase for such compounds.

True digestibility of non-oxidized TG was greater than that of oligomers. However, as the number of uses, and thus the content of polar compounds of the palm olein increased, the digestibility ratio decreased (12 and 37 % lower after the 40th and 90th frying operation, respectively). These results suggest that under the experimental conditions applied, hydrolysis of non-oxidized TG by pancreatic lipase is inhibited, or at least retarded, by the presence of thermally oxidized compounds. Henderson *et al.* (*90*) reported that in the case of oils containing low amounts (less than 4 %) of polymers as substrates both TG and polymers were almost completely hydrolyzed by pancreatic lipase after 1 hour *in vitro*, but when highly oxidized oils (containing 20 or 30 % of TG polymers) were used, some TG remained intact.

Data related to oxidized TG digestibility coefficient are difficult to be explained as it depends on a complex balance between their formation from polymers and dimers and their disappearance by hydrolysis (Fig. 2). Relatively low digestibility of these compounds contrasts with the data of other authors (*70,111*) who suggest that oxidized TG are well-absorbed and appear to be adequately hydrolyzed by pancreatic lipase because of the higher polarity and similar molecular mass of oxidized TG and non-altered TG. According to Carey *et al.* (*109*), during the first stage of fat digestion, absorption depends on lipolytic enzyme activity. Afterwards, molecular polarity greatly influences the entry of lipidic products into the micellar phase and finally, molecular mass further limits luminal uptake of fat.

As the number of uses of the palm olein for frying increased, luminal fat tended to present a lower percentage and amount of monoacylglycerols and free fatty acids. This could be attributed to lower pancreatic lipase activity. However, monoacylglycerols and free fatty acids from highly altered palm oleins are more polar than those from less altered palm olein and thus may undergo increased absorption, contributing to the lower percentages found in the remaining luminal fat.

Similar results were found when studying short-term digestion of olive oil and sunflower oil both heated at 180 °C for 50 h (*107,108*). Heating increased significantly the polar material of both oils. After 4 h, the remaining lumen fat (non-hydrolyzed and/or non-absorbed) was higher in the heated samples, due to the lower hydroly-

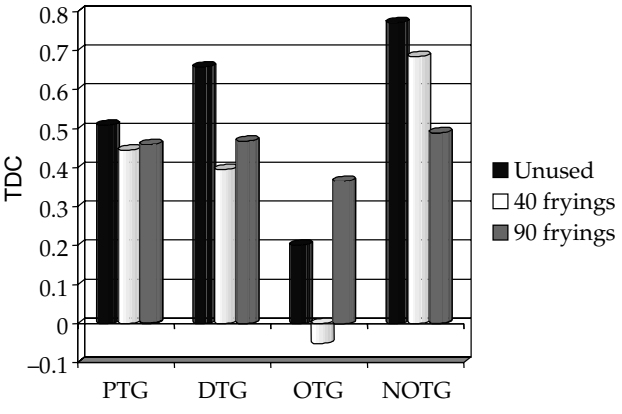


Fig. 2. True digestibility coefficient (TDC) of triacylglycerol polyers (PTG), triacylglycerol dimers (DTG), oxidized triacylglycerols (OTG) and non-oxidized triacylglycerols (NOTG) from unused palm olein and from palm olein used 40 and 90 times for frying of potatoes after 4-hour *in vivo* experiment

sis and absorption of the polymers. In conclusion, after a 4-hour experiment, true digestibility coefficients of altered oils, measured by fat disappearance from intestinal lumen, were significantly lower than those of the unused oils. True digestibility of polymers and dimers was quite high but decreased as the alteration of the oil increased. Non-oxidized TG hydrolysis was negatively affected by the presence of large amounts of thermally oxidized compounds.

Studies with Arylesterase

The other group of enzymes studied in this paper is the esterases, arylesterase particularly. The interest in arylesterase has grown much in the last few decades, as we have previously commented on. Nonetheless, the available information about the uses of this enzyme is much reduced in comparison with that of pancreatic lipase. This enzyme is used to predict cardiovascular risk and gives information about the anti-oxygen-free radical action of HDL (*29,30*). Arylesterase has just been purified and added to the Protein Data Bank (*112*), but systems and spectrophotometric methods to determine its enzymatic activity had already been developed (*89,113*).

Eckerson first proposed a method based on the spectrophotometric measure of the appearance rate of phenol as the product of the reaction catalyzed by the arylesterase (Scheme 1). The initial rates of hydrolysis were determined at 270 nm. The assay mixture included 1.0 mM phenyl acetate and 0.9 mM $CaCl_2$ in 20 mM Tris/HCl, pH=8.0 at 25 °C (*89*). The E_{270} for the reaction is 1295 M^{-1} cm^{-1} and one unit of arylesterase activity is equal to 1 mol of PA hydrolyzed per L per minute. This seems to be the elective method for many authors, although modifications such the addition of NaCl as an effective stimulator for the reaction have been made to the buffer (*114*).

Due to the low reproducibility and sensitivity of the Eckerson *et al.* method (*89*), our group has developed a new method using simulated body fluid (SBF) as buffer instead of Tris/HCl, at 37 °C and pH=7.34–7.4. Fewer errors and higher precision were achieved using Nus *et al.* method (*39*) rather than the Eckerson *et al.* (*89*).

Experiments using the method by Eckerson *et al.* as well as that by Nus *et al.* (*39*) show that arylesterase exhibits Michaelis-Menten kinetics (Fig. 3), with the apparent K_m and V_{max} values shown in Table 5. As this enzyme has not been commercialized, the results are given per μL of serum diluted in ratio 1:40 used for the essay.

Another method has recently been proposed by Lorentz *et al.* (*113*). It is a mechanized essay based on the diminution of the hexacyanoferrate-III as a consequence of the coupled reaction of the tiophenyl acetate hydrolysis catalysed by the arylesterase.

Many studies have established the relationship between arylesterase and HDL and LDL. In this way, the group of La Du has postulated the role of arylesterase in the prevention of LDL and HDL oxidation (*30*), and in further studies they demonstrate the inhibition of this enzyme by the oxidized LDL (*115*).

Many factors such as diet can affect arylesterase activity. Sarandol et al. (*34*) studied the effect of red wine consumption on this activity and concluded that taking 0.375 g alcohol/kg of body mass diminishes the arylesterase activity because of the positive antioxidant status produced by the moderate consumption of red wine. Wallace *et al.* (*35*) studied the effect of a diet rich in olive oil on a population of women with type 2 diabetes, and demonstrated the antiatherogenic role of oleic acid by increasing arylesterase activity.

Our group is now studying the effect of some dietary compounds on the arylesterase activity. With this aim, a restructured beef meat containing 20 % of nut mass was given to a population of men and women with at least two cardiovascular disease risk factors. The design of the study was cross-over controlled by placebo. The study had two different 5-week experimental periods (intervention and control) and a washout period of 1 month and a half between each of them. During the intervention period, volunteers were given restructured beef steak containing nuts 4 times a week and 2 restructured beef

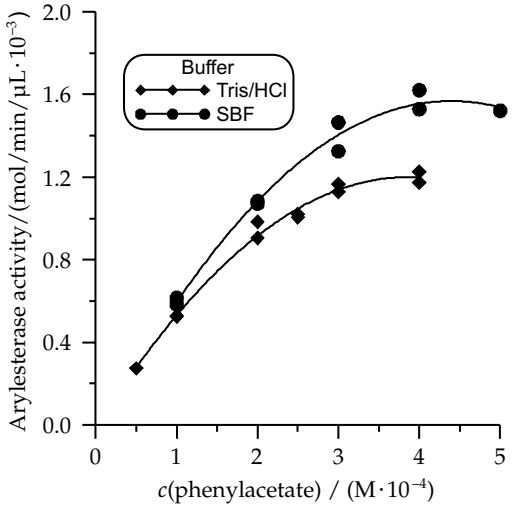


Fig. 3. Michaelis-Menten kinetic adjustment of human serum arylesterase using simulated body fluid (SBF) and Tris/HCl. Curves were obtained changing phenylacetate (PA) concentration ($1 \cdot 10^{-4}$ to $5 \cdot 10^{-4}$ M) and maintaining a fixed serum volume (50 μL)

Table 5. Apparent kinetic parameters of arylesterase obtained using Tris/HCl according to Eckerson *et al.* (*89*) and using simulated body fluid (SBF) buffer according to the Nus *et al.* (*39*)

Buffer	V_{max} / U	V_{max} 95 % CL / U	K_m / M	K_m 95 % CL / M
Tris/HCl	$5.299 \cdot 10^{-5}$	$4.18 \cdot 10^{-5} - 6.42 \cdot 10^{-5}$	$2.765 \cdot 10^{-4}$	$1.62 \cdot 10^{-4} - 3.91 \cdot 10^{-4}$
SBF	$6.305 \cdot 10^{-5}$	$4.62 \cdot 10^{-5} - 7.99 \cdot 10^{-5}$	$2.643 \cdot 10^{-4}$	$1.13 \cdot 10^{-4} - 4.16 \cdot 10^{-4}$

CL: confidence limits

Table 6. Arylesterase activity (U/L) of 18 patients consuming restructured beef meat containing nuts (intervention period) and without nuts (control period)

	Intervention period			
	0 week	3 weeks	5 weeks	7 weeks
Men	63.98	59.88	58.89	63.63
Women	71.65	76.81	73.75	77.39
Total	67.27	67.14	65.26	69.53
	Control period			
Men	79.86	78.88	60.07	66.81
Women	67.59	61.21	72.14	66.53
Total	75.26	72.26	64.60	66.70

sausages containing nuts once a week. After the washout period, volunteers started the control period, where they were given restructured beef steaks without nuts 4 times a week and 2 restructured beef sausages without nuts once a week. No other meat products were allowed to be eaten during the intervention and control periods.

Table 6 shows preliminary results of 18 patients at weeks 0, 3, 5 and 7 of both intervention and control periods. Arylesterase activities were calculated by the extrapolation of the increasing absorbance measured with the Nus *et al*. method (*39*) of each sample, from a calibration curve, using a constant concentration of the substrate phenylacetate and different volumes of enzyme. As this enzyme arylesterase has still not been commercialized, the results are expressed per L of enzyme instead of mg of protein.

These results show that the inclusion of nuts in the diet decreased arylesterase activity to a lesser extent due to the high antioxidant agents present in this food. Nevertheless, when nuts were not in the diet, the arylesterase activity increased in women, while it decreased in men, suggesting marked gender differences (*116*).

Conclusions and Future Studies

Pancreatic lipase has been extensively employed to study oils and fats. Its use can be extended to know which fatty acids, oxidized or not, are located at 1,3 positions of glycerol. Moreover, this enzyme can be used for studying thermal oxidized fats. Future studies must be conducted to investigate the kinetic behaviour of isolated altered compounds (such as polymers, dimers, *etc.*), and to understand the possible influence of minor compounds (*e.g.* sterols, polyphenols) present in some oils on the hydrolytic behaviour of the digestive enzyme towards oils and specific altered compounds. The possible *in vitro* balance between factors that improve and impair lipase hydrolysis has to be tested extensively. Finally, efforts must be addressed to find relationships between *in vitro* and *in vivo* studies to better comprehend the major stages of the digestion and absorption of thermally oxidized oils, and to assess the oil alteration level when the enzymatic capacity of lipase is completely inhibited.

Arylesterase is a fairly unknown enzyme that seems to play a very important role against LDL oxidation. Its mechanism of action has not been described, so further studies are necessary to be done to better understand the process and how to inhibit LDL oxidation. The modulation of its activity by prooxidant/antioxidant compounds and/or other substances *in vitro* as well as *in vivo* should be investigated.

Acknowledgements

The study was supported by the project AGL2001-2398-C03-03 from the Ministry of Education and Science of Spain. We also thank the Complutense University of Madrid for the predoctoral fellowship to Meritxell Nus.

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Metode i važnost ispitivanja oksidacije biljnih ulja, masti i lipoproteina pomoću lipaze pankreasa i arilesteraze

Sažetak

Masti i ulja, kao glavni sastojci prehrane, uzrok su razvoja nekih kroničnih bolesti. U radu su ispitivani fiziološka važnost i neke metode određivanja dvaju enzima koji sudjeluju u metabolizmu masti, i to lipaze pankreasa i arilesteraze. Lipaza pankreasa vrlo se često koristila u ispitivanju sastava masnih kiselina u triacilglicerolima i u *in vitro* digestiji ulja i masti. Djelovanje tog enzima može se povezati s analitičkim metodama kao što su GC, HPLC, HPSEC i TLC-FID kako bi se utvrdio sastav i digestija toplinski oksidiranih ulja. Hidrolitsko djelovanje lipaze pankreasa provodi se na granici faza voda/ulje, te se čini da se pokorava Michaelis-Mentenovoj kinetici, u kojoj prividne K_m i V_{max} vrijednosti enzimskog procesa više ovise o vrsti ispitivanog ulja nego o stupnju hidrolize. Kinetika lipaze pankreasa prema toplinski oksidiranim uljima ovisi također o prisutnosti prirodnih tenzioaktivnih spojeva u ulju i o površinski aktivnim spojevima nastalim tijekom prženja. Arilesteraza je enzim koji veže HDL i time inhibira oksidaciju LDL. Mala koncentracija tog enzima u serumu povezana je s povećanim rizikom kardiovaskularnih bolesti. U radu su prikazane najčešće primijenjeni postupci za određivanje aktivnosti arilesteraze. Ujedno je razmotrena važnost unutarnjih faktora (npr. supstrata, kofaktora) koji sudjeluju u enzimskoj reakciji. Nadalje, predložena su daljnja istraživanja o utjecaju vanjskih faktora (npr. dijeta, oksidativni stres) na aktivnost enzima.