JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2008,** *56,* 8475–8483 **8475**

Use of an in Vitro Digestion Model To Study the Bioaccessibility of 4-Hydroxy-2-nonenal and Related Aldehydes Present in Oxidized Oils Rich in Omega-6 Acyl Groups

ENCARNACIÓN GOICOECHEA,[†] KLAAS VAN TWILLERT,[§] MENNO DUITS,[§] ESTHER D. F. A. BRANDON,[§] PETER R. KOOTSTRA,[§] MARCO H. BLOKLAND,[§] AND MARÍA D. GUILLÉN*[,†]

Food Science and Technology Department, Faculty of Pharmacy, University of the Basque Country (UPV-EHU), Vitoria, Spain, and National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

Mixtures of either sunflower oil or thermodegraded sunflower oil and a standard meal were submitted to an in vitro digestion model. The same experiment was carried out with fluid deep-frying fat and thermodegraded fluid deep-frying fat. The thermodegradation of the oil and fat was provoked by submitting them to 190 °C with aeration in a convection oven, and the presence in the headspace of the thermodegraded oil and fat of oxygenated α,β-unsaturated aldehydes (OαβUAs), such as 4-hydroxy-2-nonenal (HNE), 4-oxo-2-nonenal (ONE), and 4,5-epoxy-2-decenal (EDE), was monitored by solid phase microextraction (SPME) followed by gas chromatography−mass spectrometry (GC-MS). The digestion products were separated by centrifugation in a lipidic phase, an aqueous phase, and a pellet phase. The headspace of these three phases was also studied by SPME/GC-MS to check if the toxic and very reactive OαβUAs above-mentioned remained unaltered after the in vitro digestion process or if they had reacted with the various compounds present in the digestion products, so disappearing from the samples. With the same aim the extract in ethyl acetate of the aqueous and pellet phases, and of the lipidic phase after dilution, were studied by GC-MS. All results obtained showed that a certain proportion of the toxic OαβUAs remains unaltered after digestion, dispersed in the three phases above-mentioned, and thus are bioaccessible in the gastrointestinal tract and so could reach the systemic circulation. Compounds that may originate in Maillard type reactions (2-pentylpyridine) are found among digestion products, proving that these reactions are possible in this process if adequate substrates are present. In addition, it has been shown that toxic metabolites from the synthetic antioxidant BHT, present in fat before digestion, remain unaltered after this process and could reach the systemic circulation.

KEYWORDS: Absorption from the diet; 4,5-epoxy-2-decenal (EDE); food; 4-hydroxy-2-nonenal (HNE); in vitro digestion model; oxygenated α,β-unsaturated aldehydes (OαβUAs); 4-oxo-2-nonenal (ONE)

INTRODUCTION

Oils and fats are constituents of many foods and play an important role as nutrients in the human diet, as well as having numerous technological functions in foodstuffs. Oxidation is the main cause of lipid deterioration, provoking the formation of several compounds, some of which give the typical rancid odor, whereas others can have harmful effects on human health.

In recent years a great deal of attention has been focused on a group of toxic compounds, called oxygenated α,β-unsaturated aldehydes (OαβUAs), which are generated in the oxidation of omega-6 and omega-3 polyunsaturated fatty acids and esters, both in biological systems (*1*) and in foodstuffs (*2*). They form a very broad family of compounds characterized by the presence in their structure of an aldehyde functional group, a double bond in the α,β position, and at least one other oxygenated functional group (possible groups include hydroperoxy, hydroxy, and oxo in position 4; or epoxy in position 4,5), which gives these compounds great reactivity and biological activity (*2*−*4*). When these compounds are generated endogenously in cells and tissues as a consequence of stress oxidative processes, they are

* Address correspondence to this author at Paseo de la Universidad 7, 01006 Vitoria, Spain (telephone 34-945-013081; fax 34-945-013014; e-mail mariadolores.guillen@ehu.es).

[†] University of the Basque Country.

[§] National Institute for Public Health and the Environment.

10.1021/jf801212k CCC: $40.75 © 2008 American Chemical Society
Published on Web 08/26/2008

Table 1. Composition of the Digestive Juices Used in the in Vitro Digestion Model under Fed Conditions[a]

	saliva	gastric juice	duodenal juice	bile
inorganic solution	896 mg of KCl 200 mg of KSCN 888 mg of NaH_2PO_4 570 mg of Na_2SO_4 298 mg of NaCl 1694 mg of $NaHCO_3$	2752 mg of NaCl 266 mg of NaH_2PO_4 824 mg of KCl 400 mg of $CaCl_2 \cdot 2H_2O$ 306 mg of NH_4Cl 6.5 mL of 37% HCl	7012 mg of NaCl 3388 mg of $NaHCO_3$ 80 mg of KH_2PO_4 564 mg of KCl 50 mg of $MgCl_2 \cdot 6H_2O$ 180 μL of HCl (37%)	5259 mg of NaCl 5785 mg of $NaHCO_3$ 376 mg of KCl 150 μL of HCl (37%)
organic solution	200 mg of urea	650 mg of glucose 20 mg of glucuronic acid 85 mg of urea 330 mg of glucosamine hydrochloride	100 mg of urea	250 mg of urea
add to mixture organic + inorganic solution	290 mg of amylase 15 mg of uric acid 25 mg of mucin	1 g of BSA 2.5 g of pepsin 3 g of mucin	200 mg of $CaCl_2 \cdot 2H_2O$ 1 g of BSA 9 g of pancreatin 1.5 g of lipase	222 mg of $CaCl_2 \cdot 2H_2O$ 1.8 g of BSA 30 g of bile
pH	6.8 ± 0.2	1.3 ± 0.1	8.1 ± 0.2	8.2 ± 0.2

[a] The inorganic and organic solutions are augmented to 500 mL with distilled water. After mixing of the inorganic and organic solutions, some further constituents are added and dissolved. If necessary, the pH of the juices is adjusted to the appropriate interval. Abbreviations: BSA, bovine serum albumin.

Table 2. Proportions of the Different Acyl Groups in Nondegraded Sunflower Oil (SO) and Fluid Deep-Frying Fat (DF) and in the Same Oil and Fat Thermodegraded (TSO and TDF) at 190°C for 3 h, Determined from ^{1}H NMR Data

	linolenic	linoleic	oleic	saturated
SO	0.0 ± 0.0	59.8 ± 2.1	30.0 ± 0.9	10.2 ± 0.5
TSO	0.0 ± 0.0	46.8 ± 1.9	33.3 ± 1.2	19.8 ± 0.7
DF	0.0 ± 0.0	38.1 ± 1.1	35.4 ± 0.9	26.5 ± 0.9
TDF	0.0 ± 0.0	25.7 ± 0.9	35.5 ± 1.3	38.8 ± 1.6

considered to be potential causal agents of several diseases, such as different types of cancer, chronic inflammation, adult respiratory distress syndrome, atherogenesis, diabetes, and neurodegenerative diseases such as Alzheimer's or Parkinson's disease, among others (*1, 5*). The most studied of the O$\alpha\beta$UAs is 4-hydroxy-*trans*-2-nonenal (HNE), produced by the oxidation of omega-6 polyunsaturated fatty acids. The absorption from the diet of toxic aldehydes formed in edible oil degradation has scarcely been studied (*6, 7*). However, some experimental studies with animals have proved that after its oral administration, 4-hydroxy-2-nonenal is absorbed and detected in rat tissues (*7, 8*); recently it has also been shown that 4-oxo-2-hexenal orally administered to mice forms adducts with esophageal, stomach, and intestinal DNA (*9*). Furthermore, a great deal of attention is being paid to lipid oxidation products from high-fat diets and their relation with the above-mentioned illnesses (*10*). It has been considered that the stomach acts as a "bioreactor" and the gastric fluid as a medium in which dietary lipid peroxidation and/or antioxidation can occur (*11, 12*); the bioreactor receives the masticated food in an aerobic environment because from time to time it is opened to the atmosphere, at least during meal time. In this context it has been evidenced that orally administered linoleic hydroperoxides decompose in the rat and pig stomach to hydroxides, epoxy ketones, and aldehydes (*13*).

In this context, the evaluation of the life of compounds of this nature from foods rich in lipids in the gastrointestinal tract can be considered of great interest and could be taken as an indicator of oral bioavailability, providing information about the possible ability of the ingested toxic compounds to reach the systemic circulation. It should be taken into account that the bioavailability could be lowered by reactions produced during the digestion process, in which these toxic compounds could react with proteins or other food components, forming adducts or other compounds (*2−4*).

Quantification of bioavailability of a compound from a certain matrix is difficult and is often hampered by the complex processes involved in human digestion. Oral bioavailability of a compound can be seen as the result of three processes: (1) release of the compound from its matrix into digestive juice in the gastrointestinal tract (bioaccessibility); (2) transport across the intestinal epithelium into the vena portae (intestinal transport); and (3) degradation of the compound in the liver and intestine (metabolism). Release of the compound from the ingested product in the gastrointestinal tract is a prerequisite for its uptake and bioavailability in the body. Afterward, the oral bioavailability of the compound can be then reduced by its partial transport across the intestinal epithelium or metabolism in the intestine or liver. Thus, determination of the bioaccessibility of a compound from its matrix can be seen as an indicator of its maximal oral bioavailability. An in vitro digestion model can be used to determine the bioaccessibility and simulates in a simplified manner the digestion processes in the mouth, stomach, and small intestine, in order to enable investigation of the bioaccessibility of compounds from their matrix during transit in the gastrointestinal tract (*14−17*).

Taking into account all of the above, the aim of this study was to evaluate if some oxygenated α,β-unsaturated aldehydes (O$\alpha\beta$UAs) present in oxidized food, such as 4-hydroxy-*trans*-2-nonenal (HNE), 4-oxo-*trans*-2-nonenal (ONE), and 4,5-epoxy-2-decenals (EDE), could reach the systemic circulation after ingestion or if these toxic compounds react with other components of food, enzymes, or compounds involved in the digestion, disappearing during this process. The results obtained can be considered as a measure of the bioaccessibility of these toxic compounds. To this aim an in vitro digestion model was used, which previously has been proved to be a simple, cheap, and reproducible tool for investigating the bioaccessibility of several compounds present in different matrices (*16−20*). The study was carried out on two edible oils rich in omega-6 acyl groups mixed with a standard meal. The edible oils had been submitted previously to thermodegradative conditions to provoke the formation of O$\alpha\beta$UAs, the presence of which in the oil and

Table 3. Main Compounds Detected by Means of SPME/GC-MS in the Headspace of Nondegraded (SO) and Thermodegraded Sunflower Oil (TSO) and in Their Digestion Products Obtained in the in Vitro Digestion Model, as well as Their Abundance Expressed as Base Peak (Bp) Area Counts Divided by 10^6

			digestion products (SO)				digestion products (TSO)		
compound[a]	Bp	SO	LiPh	AqPh	PePh	TSO	LiPh	AqPh	PePh
acids									
hexanoic acid *	60	0.58 ± 0.06		0.49 ± 0.12	1.01 ± 0.08	0.87 ± 0.12	1.18 ± 0.21		0.65 ± 0.13
heptanoic acid *	60			0.14 ± 0.03	0.13 ± 0.01	0.17 ± 0.04	0.88 ± 0.14		0.22 ± 0.01
octanoic acid *	60		0.03 ± 0.00	0.68 ± 0.07	0.37 ± 0.05	0.01 ± 0.00	5.00 ± 0.42	0.92 ± 0.09	1.68 ± 0.13
nonanoic acid *	60			0.30 ± 0.05	0.39 ± 0.08	0.19 ± 0.01	0.56 ± 0.09		0.20 ± 0.02
palmitoleic acid	55		0.25 ± 0.01				1.70 ± 0.19		
palmitic acid	256		24.40 ± 0.12	3.36 ± 0.21	2.01 ± 0.23		22.76 ± 0.85	3.13 ± 0.36	2.54 ± 0.16
linoleic acid	280		67.85 ± 1.05	0.24 ± 0.03	0.07 ± 0.01		19.44 ± 0.94	0.07 ± 0.00	0.21 ± 0.04
oleic acid	264		2.37 ± 0.26	0.44 ± 0.08	0.27 ± 0.06		11.53 ± 0.42	2.14 ± 0.67	0.35 ± 0.03
stearic acid	284		4.74 ± 0.31	1.84 ± 0.13	0.49 ± 0.07		12.90 ± 0.16	5.01 ± 0.29	0.67 ± 0.09
alcohols									
1-pentanol *	42					0.57 ± 0.13	0.06 ± 0.01	1.61 ± 0.13	
1-hexanol	56							0.92 ± 0.09	
2-hepten-1-ol	57							1.66 ± 0.15	
1-heptanol	70							2.58 ± 0.36	0.30 ± 0.05
1-octen-3-ol *	57					1.68 ± 0.15	0.60 ± 0.05	1.50 ± 0.13	0.50 ± 0.04
glycerol *	61		16.66 ± 0.42				42.09 ± 1.07		
2-octen-1-ol	57							1.05 ± 0.24	0.01 ± 0.00
1-octanol	56							0.86 ± 0.12	0.22 ± 0.03
(5-ethylcyclopent-1-enyl)methanol	97							0.67 ± 0.09	
furan derivatives									
2-pentylfuran *	81	0.07 ± 0.01				2.48 ± 0.95	0.92 ± 0.09	2.83 ± 0.46	0.67 ± 0.09
2-octylfuran	81					0.07 ± 0.02	0.01 ± 0.00	0.02 ± 0.00	0.08 ± 0.01
hydrocarbons									
octane *	43					0.07 ± 0.01	0.03 ± 0.00		
octene (or isomer)	55	0.39 ± 0.03				0.06 ± 0.02			
tetradecane	57					0.14 ± 0.02	0.02 ± 0.00	0.11 ± 0.03	
pentadecane	57					0.16 ± 0.04	0.06 ± 0.01	0.15 ± 0.02	
ketones									
3-hexen-2-one	83							0.28 ± 0.04	0.01 ± 0.00
2-octanone	58							1.60 ± 0.15	0.06 ± 0.01
3-octen-2-one *	55					0.16 ± 0.03	0.08 ± 0.02	0.65 ± 0.08	
2-nonanone *	58					0.78 ± 0.09			0.07 ± 0.01
3-nonen-2-one *	55					0.52 ± 0.07	0.09 ± 0.02	0.46 ± 0.07	
2-pentyl-2-cyclopenten-1-one	96							0.74 ± 0.09	0.22 ± 0.05
alkanals									
hexanal *	56	0.77 ± 0.08	0.02 ± 0.00			2.68 ± 0.36	0.73 ± 0.05	0.65 ± 0.05	
heptanal *	70					0.18 ± 0.04	0.10 ± 0.02	0.26 ± 0.03	
octanal *	43					0.13 ± 0.02	0.05 ± 0.01	0.12 ± 0.02	
nonanal *	57					2.71 ± 0.18	0.58 ± 0.08	1.10 ± 0.16	0.10 ± 0.01
decanal *	57					0.05 ± 0.01	0.05 ± 0.01	0.01 ± 0.00	
alkenals									
heptenal (or isomer)	83					0.74 ± 0.09	0.03 ± 0.00	0.16 ± 0.02	
trans-2-heptenal *	83	0.31 ± 0.04	0.08 ± 0.02		0.08 ± 0.01	6.74 ± 0.24	0.81 ± 0.06	2.87 ± 0.36	0.06 ± 0.01
octenal (or isomer)	70					0.06 ± 0.01		0.05 ± 0.00	
trans-2-octenal *	70					1.28 ± 0.08		2.27 ± 0.26	0.02 ± 0.00
nonenal (or isomer)	83					0.12 ± 0.01	0.32 ± 0.08	0.07 ± 0.01	
trans-2-nonenal *	55					0.52 ± 0.09	0.10 ± 0.04	1.01 ± 0.19	0.01 ± 0.00
decenal (or isomer)	70					0.15 ± 0.04			
trans-2-decenal *	70					3.38 ± 0.12	0.77 ± 0.12	0.52 ± 0.09	0.13 ± 0.01
undecenal (or isomer)	70					0.34 ± 0.07	0.06 ± 0.02		
trans-2-undecenal *	70					3.50 ± 0.13	1.45 ± 0.13	0.39 ± 0.07	0.27 ± 0.06
alkadi- and -trienals									
2,4-heptadienal	81					0.31 ± 0.16	0.05 ± 0.01	0.13 ± 0.05	
trans,trans-2,4-octadienal *	81					0.01 ± 0.00			
cis,trans-2,4-nonadienal *	81					0.46 ± 0.03	0.19 ± 0.04	0.35 ± 0.06	
trans,trans-2,4-nonadienal *	81					0.90 ± 0.12	0.80 ± 0.09	2.41 ± 0.19	0.05 ± 0.01
cis,trans-2,4-decadienal *	81					7.45 ± 0.36	3.79 ± 0.29	2.49 ± 0.024	0.21 ± 0.02
trans,trans-2,4-decadienal *	81					14.50 ± 1.15	8.08 ± 0.26	4.61 ± 0.37	1.42 ± 0.14
decatrienal (or isomer)	79					0.31 ± 0.08	0.03 ± 0.00	0.10 ± 0.02	
decatrienal (or isomer)	79					0.92 ± 0.12	0.15 ± 0.02	0.09 ± 0.02	
cis,trans-2,4-undecadienal	81					0.06 ± 0.01			
trans,trans-2,4-undecadienal	81					0.20 ± 0.04	0.06 ± 0.01	0.12 ± 0.01	0.07 ± 0.01
lactones									
5-ethyldihydro-2(3*H*)-furanone	85					0.27 ± 0.03	0.11 ± 0.02	0.13 ± 0.03	0.05 ± 0.01
5-butyl-2(5*H*)-furanone	84					0.11 ± 0.01	0.05 ± 0.01	0.03 ± 0.00	
5-butyldihydro-2(3*H*)-furanone	85					1.58 ± 0.24	0.82 ± 0.08	0.92 ± 0.08	0.40 ± 0.09
5-pentyl-2(5*H*)-furanone	84					1.97 ± 0.27	1.10 ± 0.09		
5-pentyldihydro-2(3*H*)-furanone	85								0.96 ± 0.13
oxygenated aldehydes									
4-oxo-*trans*-2-nonenal *	125					0.25 ± 0.04	0.05 ± 0.01		0.03 ± 0.01
4-oxononanal	43					2.67 ± 0.34			
2,3-epoxydecanal (or isomer)	71					0.63 ± 0.09	0.10 ± 0.01		
4-hydroxy-*trans*-2-nonenal *	57					2.42 ± 0.25	0.04 ± 0.00		
4,5-epoxy-2-decenal (isomer)	68					3.10 ± 0.37	1.42 ± 0.08	0.07 ± 0.01	0.12 ± 0.03
4,5-epoxy-2-decenal *	68					3.85 ± 0.39	1.83 ± 0.09	0.13 ± 0.01	0.51 ± 0.04
other compounds									
2-pentylpyridine	93						0.20 ± 0.06	0.15 ± 0.02	0.24 ± 0.03

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes; SO, headspace components of nondegraded sunflower oil; TSO, headspace components of thermodegraded sunflower oil. The headspace components of the digestion products obtained from food based on SO or TSO: LiPh, lipidic phase; AqPh, aqueous phase; and PePh, pellet phase.

Table 4. Main Compounds Detected by Means of SPME/GC-MS in the Headspace of Nondegraded (DF) and Thermodegraded Fluid Deep-Frying Fat (TDF) and in Their Digestion Products Obtained in the Vitro Digestion Model, as well as Their Abundance Expressed as Base Peak (Bp) Area Counts Divided by 10^6

			digestion products (SO)				digestion products (TSO)		
compound[a]	Bp	DF	LiPh	AqPh	PePh	TDF	LiPh	AqPh	SePh
acids									
butanoic acid *	60			0.07 ± 0.02	0.10 ± 0.02				
pentanoic acid *	60		0.06 ± 0.01	0.11 ± 0.01	0.11 ± 0.02				
hexanoic acid *	60		0.54 ± 0.16	0.34 ± 0.06	1.14 ± 0.36	1.60 ± 0.13	0.64 ± 0.08	0.16 ± 0.04	0.20 ± 0.04
heptanoic acid *	60		0.07 ± 0.01	0.08 ± 0.02	0.22 ± 0.6	0.58 ± 0.07	0.47 ± 0.06	0.14 ± 0.03	0.12 ± 0.01
octanoic acid *	60		0.28 ± 0.06	0.49 ± 0.07	0.38 ± 0.05	0.56 ± 0.06	3.53 ± 0.34	1.78 ± 0.15	0.89 ± 0.21
nonanoic acid *	60		0.11 ± 0.02	0.19 ± 0.02	0.37 ± 0.07	1.81 ± 0.16	0.28 ± 0.04	0.10 ± 0.02	0.17 ± 0.03
decanoic acid *	60		0.08 ± 0.01	0.07 ± 0.01	0.10 ± 0.02	0.25 ± 0.04	0.41 ± 0.05		
dodecanoic acid	73		0.52 ± 0.06	0.03 ± 0.00	0.47 ± 0.06				
tetradecanoic acid	73		0.90 ± 0.12		0.61 ± 0.05				
palmitic acid	256		39.28 ± 1.93	3.34 ± 0.24	22.79 ± 0.48	9.49 ± 0.48	46.62 ± 2.06	3.96 ± 0.37	1.80 ± 0.24
linoleic acid	280		56.70 ± 2.06	0.10 ± 0.01	6.79 ± 0.28	0.93 ± 0.09	33.55 ± 1.86	0.06 ± 0.01	0.10 ± 0.02
oleic acid	264		21.24 ± 0.67	0.01 ± 0.00	5.58 ± 0.37		0.12 ± 0.02	0.01 ± 0.00	0.50 ± 0.03
stearic acid	284		5.77 ± 0.37	1.55 ± 0.16	3.31 ± 0.46	1.84 ± 0.24	20.88 ± 0.86	5.61 ± 0.42	0.21 ± 0.01
alcohols									
1-pentanol *	42					1.75 ± 0.18	0.16 ± 0.03	1.85 ± 0.24	0.10 ± 0.02
2-hexen-1-ol	57							0.21 ± 0.04	
1-hexanol	56							4.50 ± 0.59	1.70 ± 0.16
2-hepten-1-ol	57							2.02 ± 0.37	0.07 ± 0.01
1-heptanol	70							3.10 ± 0.28	1.39 ± 0.27
1-octen-3-ol *	57					1.92 ± 0.26	0.65 ± 0.15	3.36 ± 0.48	1.29 ± 0.18
glycerol *	61		18.95 ± 0.29				0.94 ± 0.12		
2-octen-1-ol	57							1.11 ± 0.09	0.05 ± 0.01
1-octanol	56							1.49 ± 0.16	0.45 ± 0.07
(5-ethylcyclopent-1-enyl)methanol	97							0.53 ± 0.06	
1-nonanol	56							0.83 ± 0.07	0.70 ± 0.09
furan derivatives									
2-pentylfuran *	81		0.17 ± 0.03	0.24 ± 0.02	0.07 ± 0.01	2.05 ± 0.37	1.38 ± 0.13	3.26 ± 0.31	1.33 ± 0.15
2-octylfuran	81					0.19 ± 0.04	0.07 ± 0.01	0.99 ± 0.12	0.15 ± 0.03
hydrocarbons									
octane *	43					0.20 ± 0.03	0.29 ± 0.03	0.11 ± 0.02	0.01 ± 0.00
decane *	57					0.04 ± 0.01			
dodecane *	57	1.76 ± 0.24		0.09 ± 0.01	0.01 ± 0.00	0.11 ± 0.02	0.01 ± 0.00	0.26 ± 0.03	0.01 ± 0.00
tetradecane	57					0.20 ± 0.03	0.05 ± 0.01	0.18 ± 0.02	0.04 ± 0.01
pentadecane	57					0.14 ± 0.02		0.07 ± 0.01	0.01 ± 0.00
ketones									
3-hexen-2-one	83			2.02 ± 0.15	0.17 ± 0.02			3.73 ± 0.23	0.20 ± 0.04
2-heptanone	43							1.04 ± 0.09	0.10 ± 0.02
2-octanone	58							1.21 ± 0.011	0.17 ± 0.04
3-octen-2-one *	55					0.18 ± 0.04	0.04 ± 0.01	0.75 ± 0.06	
2-nonanone *	58							1.05 ± 0.09	0.32 ± 0.07
3-nonen-2-one *	55					0.38 ± 0.08	0.07 ± 0.02	0.75 ± 0.08	
2-pentyl-2-cyclopenten-1-one	96							2.82 ± 0.16	0.40 ± 0.04
alkanals									
hexanal *	56	0.40 ± 0.07	0.05 ± 0.01		0.10 ± 0.01	1.84 ± 0.24	0.59 ± 0.05	1.18 ± 0.13	0.01 ± 0.01
heptanal *	70					0.25 ± 0.06	0.15 ± 0.03	0.25 ± 0.05	
octanal *	43					0.36 ± 0.07	0.07 ± 0.02		
nonanal *	57					2.93 ± 0.34	0.86 ± 0.07	2.94 ± 0.34	0.22 ± 0.03
decanal *	57					0.16 ± 0.02	0.04 ± 0.03	0.17 ± 0.03	0.06 ± 0.01
alkenals									
heptenal (or isomer)	83					0.18 ± 0.02		0.06 ± 0.02	
trans-2-heptenal *	83		0.06 ± 0.02	0.10 ± 0.03	0.02 ± 0.00	3.35 ± 0.27	0.75 ± 0.07	2.31 ± 0.34	0.07 ± 0.01
octenal (or isomer)	70					0.10 ± 0.02			
trans-2-octenal *	70					1.74 ± 0.14	0.50 ± 0.06	1.45 ± 0.16	0.03 ± 0.01
nonenal (or isomer)	83					0.24 ± 0.04			
trans-2-nonenal *	55					0.53 ± 0.07	0.19 ± 0.03	0.70 ± 0.09	0.06 ± 0.02
decenal (or isomer)	70					0.51 ± 0.08	0.02 ± 0.00	0.06	tr
trans-decenal *	70					3.24 ± 0.29	1.43 ± 0.16	1.44 ± 0.13	0.41 ± 0.06
undecenal (or isomer)	70					0.05 ± 0.01	0.02 ± 0.01		
trans-2-undecenal *	70					4.25 ± 0.35	1.87 ± 0.16	1.24 ± 0.13	0.70 ± 0.08
alkadi- and -trienals									
2,4-heptadienal	81					1.30 ± 0.12	0.09 ± 0.02	0.20 ± 0.03	
cis,trans-2,4-nonadienal *	81					0.33 ± 0.04	0.14 ± 0.03	0.06 ± 0.01	0.02 ± 0.00
trans,trans-2,4-nonadienal *	81					1.33 ± 0.06	0.96 ± 0.09	0.91 ± 0.09	0.03 ± 0.01
cis,trans-2,4-decadienal *	81					5.05 ± 0.38	2.83 ± 0.24	1.99 ± 0.19	0.34 ± 0.06
trans,trans-2,4-decadienal *	81					11.02 ± 0.49	5.96 ± 0.37	3.99 ± 0.42	2.17 ± 0.37
decatrienal (or isomer)	79					0.01 ± 0.00	tr	0.04 ± 0.01	
decatrienal (or isomer)	79					0.32 ± 0.05	tr	0.08 ± 0.01	
cis,trans-2,4-undecadienal	81					0.05 ± 0.02			
trans,trans-2,4-undecadienal	81					0.55 ± 0.03	0.13 ± 0.02	tr	0.16 ± 0.02
lactones									
5-ethyldihydro-2(3*H*)-furanone	85					0.63 ± 0.05	0.07 ± 0.02	0.32 ± 0.05	0.06 ± 0.03
5-butyl-2(3*H*)-furanone	98					0.29 ± 0.04			
5-butyl-2(5*H*)-furanone	84					0.08 ± 0.02	tr	tr	
5-butyldihydro-2(3*H*)-furanone	85					1.89 ± 0.18	0.74 ± 0.08	1.47 ± 0.34	0.61 ± 0.07
5-pentyl-2(5*H*)-furanone	84					2.32 ± 0.28	0.99 ± 0.11		
5-pentyldihydro-2(3*H*)-furanone	85					1.14 ± 0.19	0.49 ± 0.06	0.69 ± 0.08	1.63 ± 0.18
oxygenated aldehydes									
4-oxo-*trans*-2-nonenal *	125					0.08 ± 0.02	tr		tr
4-oxononanal	43					1.95 ± 0.23			

Table 4. Continued

compound[a]	Bp	DF	digestion products (SO)			TDF	digestion products (TSO)		
			LiPh	AqPh	PePh		LiPh	AqPh	SePh
4-hydroxy-*trans*-2-octenal (or isomer)	57					0.02 ± 0.01			
2,3-epoxydecanal (or isomer)	71					0.50 ± 0.05			
4-hydroxy-*trans*-2-nonenal *	57					2.26 ± 0.11	0.03 ± 0.01		
4,5-epoxy-2-decenal (isomer)	68					2.78 ± 0.16	0.42 ± 0.03	0.12 ± 0.02	0.02 ± 0.01
4,5-epoxy-2-decenal *	68					3.14 ± 0.42	1.58 ± 0.24	0.04 ± 0.01	0.03 ± 0.01
other compounds									
2-pentylpyridine	93						0.18 ± 0.04	0.34 ± 0.06	0.29 ± 0.05
benzaldehyde *	106					0.32 ± 0.06	tr	0.74 ± 0.09	
BHT quinone methide	161	0.36	2.50	0.24	0.21	tr			
butylated hydroxytoluene (BHT)	205	40.29	29.12	28.39	33.14	0.93 ± 0.09	0.24 ± 0.04	0.05 ± 0.02	0.34 ± 0.6

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes; DF, headspace components of nondegraded fluid deep fat; TDF, headspace components of thermodegraded fluid deep fat. The headspace components of the digestion products obtained from food based on DF or TDF: LiPh, lipidic phase; AqPh, aqueous phase; PePh, pellets phase. tr, traces.

Table 5. Distribution Percentages of Some O$\alpha\beta$UAs Found in the Three Phases Obtained after the Digestion of the Foods Based on both Thermodegraded Sunflower Oil (TSO) and Fluid Deep-Frying Fat (TDF)

O$\alpha\beta$UA[a]	TSO			TDF		
	LiPh (%)	AqPh (%)	PePh (%)	LiPh (%)	AqPh (%)	PePh (%)
4-oxo-*trans*-2-nonenal *	33.14 ± 1.11	66.19 ± 2.53	0.68 ± 0.22	81.93 ± 3.03	16.74 ± 1.12	1.32 ± 0.33
4-hydroxy-*trans*-2-nonenal *	59.06 ± 2.41	38.66 ± 1.41	2.28 ± 0.31	70.91 ± 2.92	26.52 ± 1.61	2.57 ± 0.41
4,5-epoxy-2-decenal (isomer)	80.00 ± 3.12	19.41 ± 1.01	0.58 ± 0.18	94.28 ± 3.25	5.53 ± 0.63	0.19 ± 0.04
4,5-epoxy-2-decenal *	79.39 ± 3.02	20.06 ± 0.98	0.55 ± 0.17	94.79 ± 3.21	4.91 ± 0.57	0.30 ± 0.05

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes. LiPh, lipidic phase; AqPh, aqueous phase; PePh, pellet phase.

digestion products was monitored by means of solid-phase microextraction/gas chromatography−mass spectrometry (SPME/GC-MS). Recently, some authors have used SPME/GC-MS to study the interactions between proteins and aldehydes by measuring the content of aldehydes in the gas phase (*21, 22*). The presence of these toxic compounds in the fluid or semifluid phases obtained after digestion was also studied by GC-MS.

MATERIALS AND METHODS

Edible Oil Samples and Characterization. Fluid deep-frying fat, named "DF", recommended for deep-frying, and sunflower oil, named "SO", were acquired in two different local supermarkets. Of each oil three bottles of the same batch were acquired for the study. The DF label indicates that it was made of vegetable oils and fats of unspecified origin, containing the synthetic antioxidant *tert*-butylhydroxytoluene (BHT; E321) and one antifoaming agent (E900). Both oils were characterized by proton nuclear magnetic resonance (^{1}H NMR) and by study of their headspace composition using SPME/GC-MS.

^{1}H Nuclear Magnetic Resonance. The ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz, as in previous studies (*23*). Each oil sample, weighing 0.2 g, was mixed with 400 μL of deuterated chloroform and a small proportion of tetramethylsilane (TMS, 0.03%) as internal reference; this mixture was introduced into a 5 mm diameter tube. The acquisition parameters were as follows: spectral width, 5000 Hz; relaxation delay, 3 s; number of scans, 64; acquisition time, 3.744 s; pulse width, 90°; total acquisition time, 12 min and 54 s. The experiment was carried out at 25 °C. The deuterated chloroform and the TMS were acquired from Cortec (Paris, France). The main signals of the region between 0 and 5.5 ppm were integrated to determine the proportions in acyl groups, as in previous studies (*23*).

SPME. Extraction of the volatile and semivolatile components of the headspace of the oil and fat samples (5 g in a 20 mL vial) was accomplished automatically using a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland). The fiber used was coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS; 50/30 μm film thickness, 1 cm long), acquired from Supelco, which was inserted into the headspace of the sample and was maintained for 60 min (50 °C, 250 rpm). Selection of the fiber type and extraction conditions was based on previous studies carried out in our laboratory.

GC-MS. The extracted compounds retained on the SPME fiber were desorbed, separated, identified, and semiquantified in a gas chromatograph. To this aim the fiber with the adsorbed compounds was injected into a Hewlett-Packard gas chromatograph model HP 6890A equipped with a Jeol GCmate II benchtop double-focusing magnetic sector mass spectrometer operating in electron ionization (EI) mode with TSS-2000 software (Shrader Analytical Laboratories, Detroit, MI). The column used was a Varian Factor Four capillary column (VF-1 ms, 60 m long, 0.25 mm i.d., 0.25 μm film thickness, coated with a nonpolar stationary phase of 100% dimethylpolysiloxane). The operation conditions were the following: the oven temperature was set initially at 50 °C (5 min hold), increased to 290 °C at 4 °C/min (30 min hold); helium was used as carrier gas; splitless mode was used for injection with a purge time of 5 min, and the fiber was maintained in the injection port for 10 min (250 °C). To avoid carry-over problems, after each sample, the fiber was submitted to a 15 min bakeout at 250 °C in the fiber cleaning and conditioning station of the CombiPAL autosampler.

Many components were identified by using standards. Asterisked compounds in the several tables were acquired commercially and used as standards for identification. Many other components were tentatively identified. In the latter case, retention times, together with mass spectra, and matching with mass spectra of a commercial library higher than 85%, were taken as identification criteria (Wiley 275.L, Mass Spectral Database, Rev. D.01.00, June 2000) as in previous studies (*24*). The semiquantification of the components was based on the area counts of the base peak of the mass spectrum of each compound divided by 10^6. The base peak is the most intense peak (intensity 100%) in the mass spectrum of a compound. Although the chromatographic response factor of each compound is different, the area counts thus determined are useful for the purpose of comparing each compound in different samples. The results were obtained as average values of three determinations.

Compounds such as 4-oxo-*trans*-2-nonenal (CAS Registry No. 103560-62-9), 4-hydroxy-*trans*-2-nonenal (CAS Registry No. 75899-68-2), and 4,5-epoxy-*trans*-2-decenal (CAS Registry No. 134454-31-2), acquired from Cayman Chemical (Ann Arbor, MI), and the other compounds asterisked in the different tables acquired from Aldrich (Milwaukee, WI), were used as standard compounds for identification purposes.

Thermodegradation of Edible Oil Samples. The original oils were submitted to thermodegradative conditions: 10 g of oil was kept in

open crystal 100 mL beakers in a *Selecta* convection oven, with circulating air, the temperature of which was maintained at 190 °C with a stability of ±0.5% for 3 h. These degradative conditions were chosen to obtain an oxidative level similar to that of oil used repeatedly for frying. The oxidation level of these samples was monitored by the study of their headspace by means of SPME/GC-MS, and their proportions in acyl groups were determined from ^{1}H NMR data, as above.

Preparation of Food Samples for Digestion. Samples for digestion were made of 5 g of oil, either non-thermodegraded or thermodegraded, and 4.5 g of a standard meal to simulate a high-fat diet. The standard meal selected was a specific infant formula acquired in a local supermarket, considered as representative of the mean food intake of adults in The Netherlands for a cooked meal with regard to macronutrient and caloric composition (Third Dutch National Food Consumption Survey from 1998 in The Netherlands), after the addition of 2 mL of sunflower oil per 100 g of infant formula. According to the producer's label, this infant formula was made of 39.5% vegetables (15% small peas, 10% leek, 10% mushrooms, 4.5% tomatoes), 22.5% water, 22% potatoes, 10% ham, 4% wheat flour, 1.5% corn oil, 0.5% corn starch, salt, and basil. The food samples submitted to digestion contained proteins, 0.18 g; carbohydrates, 0.37 g; lipids, 5.10 g; fiber, 0.09 g; and sodium, 0.004 g.

In Vitro Digestion. The in vitro digestion model used was described by Versantvoort et al. (*19*). This model implied a three-step procedure simulating digestive processes in the mouth, stomach, and small intestine. The large intestinal tract was not taken into account, because in vivo food digestion and absorption of compounds mainly take place in the small intestine.

In short, digestive juices were prepared artificially accordingly to **Table 1** and were heated to 37 ± 2 °C before use. The digestion was started by adding 6 mL of saliva to the sample and was incubated for 5 min. Then 12 mL of gastric juice was added, and the pH was set between pH 2 and 3. Then the mixture was rotated head-over-heels for 2 h (55 rpm at 37 ± 2 °C). Finally, 12 mL of duodenal juice, 6 mL of bile, and 2 mL of sodium bicarbonate solution (1 M) were added simultaneously, and the pH of the chyme was set between 6 and 7. Then the mixture was rotated for another 2 h. Each sample was digested in triplicate.

Evaluation of the Occurrence of Oα*β*UAs in the Digestion Products. At the end of the in vitro digestion process, the digestion tubes were centrifuged for 15 min at 2750*g*, yielding three phases: the lipidic phase (about 5 g), named "LiPh"; the aqueous phase (about 38 mL), named "AqPh"; and the pellet phase (about 4−5 g), named "PePh". The evaluation of the occurrence of Oα*β*UAs in the three digestion phases was carried out by study of their headspace composition using SPME/GC-MS (experimental conditions described above). In addition, the presence of these toxic compounds in the digestion products was also confirmed and studied by the extraction from AqPh and PePh with an organic solvent, ethyl acetate, or by dilution in the case of LiPh, and subsequent analysis by GC-MS (*25*). To this aim 1 g of AqPh or PePh was extracted by liquid−liquid extraction with 1 and 2 mL of ethyl acetate, respectively, and the extract was studied by GC-MS. In the case of LiPh, 0.2 g of this was diluted with 1 mL of ethyl acetate, and the mixture was also studied by GC-MS. The results were obtained as average values of at least three determinations.

RESULTS AND DISCUSSION

Characterization of the Food Samples Based on Sunflower Oil and of Their Digestion Products. The composition in acyl groups of the non-thermodegraded sunflower oil (SO) determined from ^{1}H NMR data is given in **Table 2** and, as expected, linoleic is the main one, in double proportion to oleic acyl groups. The study of its headspace revealed, as **Table 3** shows, that it contains just a few volatile compounds, among which are hexanoic acid, 2-pentylfuran, hexanal, and *trans*-2-heptenal.

Table 2 shows that the thermodegraded sunflower oil (TSO) has a lower proportion of linoleic acyl groups than the original SO due to its degradation. As can be seen in **Table 3**, TSO headspace contains a great number of volatile components generated in this degradation process. Among the latter, there are some acids, a reduced number of alcohols, furan derivatives, certain ketones, and a very high number of aldehydes. Many of these compounds are well-known as secondary oxidation products and have been found in oxidized sunflower oil at intermediate temperatures (70 °C with aeration) (*24*). It is also worth noting the presence in TSO headspace of some oxygenated Oα*β*UAs, such as 4-hydroxy-*trans*-2-nonenal (HNE), 4-oxo-*trans*-2-nonenal (ONE), and 4,5-epoxy-2-decenals (EDE), also previously detected in oxidized oils (*2, 24, 25*) and the toxic properties of which have been widely studied (*1, 3−5*). The area counts of the base peak of their mass spectra, given in **Table 3**, provide information about the abundance of these volatile compounds in the thermodegraded sunflower oil TSO.

As commented on above, both kinds of oils SO and TSO were mixed with a standard meal in proportions in weight of 4.5 g of a standard meal/5 g of oil to simulate a high-fat diet. After the in vitro digestion process and centrifugation, three different phases were collected: the lipidic phase (about 5 g; LiPh), the aqueous phase (about 38 mL; AqPh), and the pellet phase (about 4−5 g; PePh).

Table 3 shows the main components of the headspace of the three phases resulting from the digestion of the food based on sunflower oil SO, named LiPh(SO), AqPh(SO), and PePh(SO); it can be observed that they contain only certain proportions of free fatty acids, which come from the lipolysis provoked by the enzyme lipase present in the duodenal juice. These free fatty acids are distributed in the three phases, and their abundance in their headspace depends on their concentration in the correspondent phase, on their volatility, on their solubility in the matrix and in definitive, on the interactions established between each of these compounds and the phase matrix. Among these three phases only the lipidic LiPh(SO) could be considered comparable with that of the sunflower oil SO. However, it should be mentioned that despite both being lipophilic, the polarity of the LiPh(SO) generated in the in vitro digestion process is higher than that of the initial oil SO, due to the presence of high proportions of free fatty acids in comparison to the oil, the main components of which are triglycerides. As expected, glycerol was also released in lipolysis during digestion (see **Table 3**).

As for the headspace composition of the three phases obtained in the digestion of the food based on thermodegraded sunflower oil TSO, named LiPh(TSO), AqPh(TSO), and PePh(TSO), **Table 3** reveals that their headspace contains, in addition to the fatty acids and glycerol, many other compounds that were present in the thermodegraded oil (TSO); among these are some alkylfurans and hydrocarbons, certain ketones and furanones, and a great number of aldehydes. Despite the great reactivity of Oα*β*UAs, they are also distributed in the three phases, being present in a higher proportion in the headspace of the lipidic phase, LiPh(TSO), than in the other two. The detection of all these compounds in the headspace of these three phases shows that they persist after digestion and that a certain amount of these toxic aldehydes is bioaccessible in the gastrointestinal tract and could reach the systemic circulation.

The presence of 2-pentylpyridine in the headspace of the three phases obtained from the food based on thermodegraded sunflower oil TSO is also noteworthy. This compound is known to be formed through Maillard type reactions between 2,4-decadienal and amino acids (*26*). Its presence among the

digestion products proves that reactions of this type take place in the digestion process. It should be noted that although there have been a great many studies on the digestibility of Maillard reaction products (*27*), to the best of our knowledge the possibility of this reaction taking place during the digestion process has never been shown previously.

As has been commented on above with free fatty acids, the abundance of the toxic O$\alpha\beta$UAs found in the headspace of these three phases is a function of their solubility, and their escape from these phases to the headspace depends on their concentration, on their volatility and solubility in each phase, and, in short, on the established interactions between each compound and the phase matrix. Therefore, these abundances cannot be considered as representative of the concentration of these toxic compounds in each of these phases, and for this reason, abundance data can be taken as only indicative but not valid to determine precise data of bioavailability.

To ascertain the distribution of the O$\alpha\beta$UAs in these three phases in a more accurate way, they were extracted from AqPh(TSO) and PePh(TSO) with ethyl acetate, a solvent used in previous studies to extract 4-oxo-*trans*-2-hexenal from several food products (*25*), and afterward the extract was studied by GC-MS in scan mode. The same was done with LiPh(TSO) after dilution with ethyl acetate. The results obtained, summarized in **Table 5**, show that the four toxic O$\alpha\beta$UAs studied were present in the three phases, in higher concentrations in the lipidic and aqueous phases.

From these results it is proved that although O$\alpha\beta$UAs can react with proteins, aminophospholipids, carbohydrates, and DNA (*2*−*4*), these toxic compounds do not disappear during digestion, but rather persist and are distributed in the three phases obtained, LiPh(TSO), AqPh(TSO), and PePh(TSO), with higher concentrations in the first two. They are bioaccessible in the gastrointestinal tract and so are susceptible to being absorbed by the intestinal cells and thus able to reach the systemic circulation.

Characterization of the Food Samples Based on Fluid Deep-Frying Fat and of Their Digestion Products. The composition in acyl groups of the original DF was determined by ^{1}H NMR, and as can be seen in **Table 2** it has similar proportions of linoleic and oleic acyl groups and higher proportions of saturated groups than sunflower oil SO. The study of DF headspace revealed, as **Table 4** shows, that it contains just a few volatile compounds, among which are dodecane and hexanal. As indicated by the producer label, DF also contains butylated hydroxytoluene (BHT, E321), a synthetic phenolic antioxidant widely used as a food additive. However, it is noteworthy that the DF headspace also contains a toxic compound derived from BHT, called BHT quinone methide (2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone), the tumor promoting activity of which has been proved (*28, 29*).

After being submitted to thermodegradative conditions, the headspace of fluid deep-frying fat (TDF) is greater in both number and concentrations of compounds resulting from fat degradation (see **Table 4**). In general, they are the same families as the ones detected in TSO headspace (see **Table 3**), that is, acids, alcohols, furan derivatives, hydrocarbons, ketones, lactones, and saturated and unsaturated aldehydes. It is also remarkable that in TDF headspace some oxygenated α,β-unsaturated aldehydes (O$\alpha\beta$UAs) can also be detected, such as 4-hydroxy-*trans*-2-nonenal (HNE), 4-oxo-*trans*-2-nonenal (ONE), and 4,5-epoxy-2-decenals (EDE); however, they are in lower proportions than in TSO headspace, which is in agreement with the higher proportion of linoleic acyl groups in sunflower oil than in fluid deep fat (see **Table 2**). It should be remembered that these O$\alpha\beta$UAs are formed mainly in the degradation of linoleic acyl groups.

As in the parallel study of sunflower oil, both kinds of fats DF and TDF were mixed with a standard meal in proportions by weight of 4.5 g of a standard meal/5 g of fat to simulate a high-fat diet. After the in vitro digestion process and centrifugation, three phases were also collected: the lipidic phase (about 5 g; LiPh), the aqueous phase (about 38 mL; AqPh), and the pellet phase (about 4−5 g; PePh).

As can be seen in **Table 4**, the headspaces of the three phases from the digestion of the food based on fluid deep fat DF [LiPh(DF), AqPh(DF), and PePh(DF)] contain, among other compounds, certain proportions of glycerol and free fatty acids, which come from the lipolysis provoked by the enzyme lipase present in the duodenal juice. As explained before, these free fatty acids show a different distribution in the headspace of each of the three phases, depending on several factors. It is also noteworthy that the toxic antioxidant BHT metabolite called BHT quinone methide (2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadienone) (*28*) remains unaltered in the headspace of the three phases, which means that after digestion it is bioaccessible in the gastrointestinal tract and susceptible to being absorbed and exerting toxic effects.

The headspace composition of the three phases obtained in the digestion of the food based on thermodegraded fluid deep-frying fat TDF [LiPh(TDF), AqPh(TDF), and PePh(TDF)] (see **Table 4**) contain many other compounds apart from the fatty acids and glycerol, most of which were present in the thermodegraded fat (TDF) and remain unaltered after the digestion process; among the latter there are alcohols, acids, furan derivatives, hydrocarbons, furanones, ketones, and aldehydes. Also in this case the O$\alpha\beta$UAs persist after digestion, and they are distributed in the three phases, present in higher proportions in the headspace of the lipidic phase, LiPh(TDF), than in the other two. These results are in agreement with the parallel study of the food based on thermodegraded sunflower oil TSO and confirm that, after digestion, a certain amount of these toxic aldehydes is bioaccessible in the gastrointestinal tract and could reach the systemic circulation.

As **Table 3** shows, 2-pentylpyridine, which is known to be formed in the reaction of 2,4-decadienal with amino acids, as previously mentioned (*26*), is also present in the headspace of the three phases obtained after digestion of the food made of TDF, which reinforces the results obtained with thermodegraded sunflower oil TSO.

As mentioned before, the abundance of O$\alpha\beta$UAs in the headspace of these three phases, LiPh(TDF), AqPh(TDF), and PePh(TDF), does not reflect their concentration in the phases due to several influential factors already mentioned. For this reason, again to more accurately establish the distribution of O$\alpha\beta$UAs in these three phases, extraction with ethyl acetate of aqueous and pellets phases was carried out, as was dilution of the lipidic phase in the same solvent, and afterward the three of them were studied by GC-MS. As can be seen in **Table 5**, the results obtained showed that the four toxic O$\alpha\beta$UAs subject of the study were present in the three phases, in higher concentrations in the lipidic phase LiPh(TDF). In general, **Table 5** shows that for both oil and fat the higher proportion of these toxic compounds is found in the lipidic phases, followed by the aqueous phases, the differences in proportions between these two phases being higher in those from the food based on thermodegraded fluid deep fat TDF than in those from the food based on thermodegraded

sunflower oil TSO. These four toxic compounds are also present in the pellets from the two foods but in lower proportions than in the other phases.

In conclusion, despite the great reactivity of O$\alpha\beta$UAs, the study of the headspace and of the fluid matrix of the three phases obtained after the in vitro digestion of the foods based on thermodegraded sunflower oil TSO and thermodegraded fluid deep fat TDF confirmed that these compounds, 4-hydroxy-*trans*-2-nonenal (HNE), 4-oxo-*trans*-2-nonenal (ONE), and 4,5-epoxy-2-decenals (EDE), persist after digestion, are susceptible to absorption in the gastrointestinal tract, and so could reach the systemic circulation. This is a matter of great importance because as mentioned above they have been considered to be potential causal agents of several diseases, such as different types of cancer, chronic inflammation, adult respiratory distress syndrome, atherogenesis, diabetes, and neurodegenerative diseases such as Alzheimer's or Parkinson's disease, among others (*1, 5*). As there is evidence that these toxic compounds could be absorbed through the diet (*7, 8*), special attention should be paid both to oils that are repeatedly heated in households and restaurants and to foods enriched with omega-3 and omega-6 polyunsaturated fatty acids, in which these compounds can be generated, too (*30*). Further studies should be carried out to confirm these results with in vivo digestion experiments.

Moreover, although it was not the main aim of this study to show the formation of 2-pentylpyridine, the origin oif which is attributed to Maillard type reactions between 2,4-decadienal and amino acids, it may be concluded that these types of reactions take place during digestion if adequate substrates are present.

Finally, this study has also shown that the antioxidant BHT, when added to foods, can evolve to give toxic metabolites that are bioaccessible after digestion. This fact is of great importance and requires further attention to safeguard human health.

ACKNOWLEDGMENT

Our thanks go to Maya Kartasasmita and Leen van Ginkel for their kind assistance.

LITERATURE CITED

(1) Esterbauer, H.; Schaur, R. J.; Zollner, H. Chemistry and biochemistry of 4-hydroxy-2-nonenal, malonaldehyde and related aldehydes. *Free Radical Biol. Med.* **1991**, *1*, 81–128.
(2) Guillén, M. D.; Goicoechea, E. Toxic oxygenated α,β-unsaturated aldehydes and their study in foods. A review. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 119–136.
(3) Lee, S. H.; Oe, T.; Blair, I. A. 4,5-Epoxy-2(*E*)-decenal induced formation of 1,*N*-6-etheno-2′-deoxyadenosine and 1,*N*-2-etheno-2′-deoxyguanosine adducts. *Chem. Res. Toxicol.* **2002**, *15*, 300–304.
(4) Sayre, L. M.; Lin, D.; Yuan, Q.; Zhu, X.; Tang, X. Protein adducts generated from products of lipid oxidation: focus on HNE and ONE. *Drug Metab. Rev* **2006**, *38*, 651–675.
(5) Zarkovic, N. 4-Hydroxynonenal as a bioactive marker of pathophysiological processes. *Mol. Aspects Med.* **2003**, *24*, 281–291.
(6) Grootveld, M.; Atherton, M. D.; Sheerin, A. N.; Hawkes, J.; Blake, D.; Richens, T. E.; Silwood, C. J. L.; Lynch, E.; Claxson, A. W. D. In vivo absorption, metabolism, and urinary excretion of α,β-unsaturated aldehydes in experimental animals. *J. Clin. Invest.* **1998**, *101*, 1210–1218.
(7) Kanazawa, K.; Ashida, H. Dietary hydroperoxides of linoleic acid decompose to aldehydes in stomach before being absorbed into the body. *Biochim. Biophys. Acta Lipids Lipid Metab.* **1998**, *1393*, 349–361.
(8) Oarada, M.; Miyazawa, T.; Kaneda, T. Distribution of ^{14}C after oral administration of [U-^{14}C]labeled methyl linoleate hydroperoxides and their secondary oxidation products in rats. *Lipids* **1986**, *21*, 150–154.
(9) Kasai, H.; Maekawa, M.; Kawai, K.; Hachisuka, K.; Takahashi, Y.; Nakamura, H.; Sawa, R.; Matsui, S. O.; Matsuda, T. 4-Oxo-2-hexenal, a mutagen formed by omega-3 fat peroxidation, causes DNA adduct formation in mouse organs. *Ind. Health* **2005**, *43*, 699–701.
(10) Kanner, J. Dietary advanced lipid oxidation endproducts are risk factors to human health. *Mol. Nutr. Food Res.* **2007**, *51*, 1094–1101.
(11) Gorelik, S.; Ligumsky, M.; Kohen, R.; Kanner, J. A novel function of red wine polyphenols in humans: prevention of absorption of cytotoxic lipid peroxidation products. *FASEB J.* **2008**, *22*, 41–46.
(12) Gorelik, S.; Ligumsky, M.; Kohen, R.; Kanner, J. The stomach as a "bioreactor": when red meat meets red wine. *J. Agric. Food Chem.* **2008**, *56*, 5002–5007.
(13) Suomela, J. P.; Ahotupa, M.; Kallio, H. Triacylglycerol oxidation in pig lipoproteins after a diet rich in oxidized sunflower seed oil. *Lipids* **2005**, *40*, 437–444.
(14) Glahn, R. P.; Wien, E. M.; Van Campen, D. R.; Miller, D. D. Caco-2 cell iron uptake from meat and casein digests parallels in vivo studies: use of a novel in vitro method for rapid estimation of iron bioavailability. *J. Nutr.* **1996**, *126*, 332–339.
(15) Ruby, M. V.; Schoof, R.; Brattin, W.; Goldade, M.; Post, G.; Harnois, M.; Mosby, D. E.; Casteel, S. W.; Berti, W.; Carpenter, M.; Edwards, D.; Cragin, D.; Chappell, W. Advances in evaluating the oral bioavailability of inorganics in soil for use in human health risk assessment. *Environ. Sci. Technol.* **1999**, *33*, 3697–3705.
(16) Oomen, A. G.; Hack, A.; Minekus, M.; Zeijdner, E.; Cornelis, C.; Schoeters, G.; Verstraete, W.; Wiele, T. Vd.; Wragg, J.; Rompelberg, C. J. M.; Sips, A. J. A. M.; Wijnen, J. V. Comparison of five in vitro digestion models to study the bioaccessibility of soil contaminants. *Environ. Sci. Technol.* **2002**, *36*, 3326–3334.
(17) Oomen, A. G.; Rompelberg, C. J. M.; Bruil, M. A.; Dobbe, C. J. G.; Pereboom, D. P. K. H.; Sips, A. J. A. M. Development of an *in vitro* digestion model for estimation of bioaccessibility of soil contaminants. *Arch. Environ. Contam. Toxicol.* **2003**, *44*, 281–287.
(18) Versantvoort, C. H. M.; Van de Kamp, E.; Rompelberg, C. J.M. Development of an *in vitro* digestion model to determine the bioaccessibility of contaminants from food. Report 320102002, 2004National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands; available from http:/www.rivm.nl/en.
(19) Versantvoort, C. H. M.; Oomen, A. G.; Van de Kamp, E.; Rompelberg, C. J. M.; Sips, A. J. A. M. Applicability of an *in vitro* digestion model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* **2005**, *43*, 31–40.
(20) Brandon, E. D. F. A.; Oomen, A. G.; Rompelberg, C. J. M.; Versantvoort, C. H. M.; van Engelen, J. G. M.; Sips, A. J. A. M Consumer product *in vitro* digestion model: bioaccessibility of contaminants and its application in risk assessment. *Regul. Toxicol. Pharmacol.* **2006**, *44*, 161–171.
(21) Chopin, C.; Kone, M.; Serot, T. Study of the interaction of fish myosin with the products of lipid oxidation: the case of aldehydes. *Food Chem.* **2007**, *105*, 126–132.
(22) Perez-Juan, M.; Flores, M.; Toldra, F. Binding of aroma compounds by isolated myofibrillar proteins: effect of protein concentration and conformation. *Food Chem.* **2007**, *105*, 932–939.
(23) Guillen, M. D.; Ruiz, A. Rapid simultaneous determination by proton NMR of unsaturation and composition of acyl groups in vegetable oils. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 688–696.
(24) Guillén, M. D.; Cabo, N.; Ibargoitia, M. L.; Ruiz, A. Study of both sunflower oil and its headspace throughout the oxidation process. Ocurrence in the headspace of toxic oxygenated aldehydes. *J. Agric. Food Chem.* **2005**, *53*, 1093–1101.
(25) Kawai, K.; Matsuno, K.; Kasai, H. Detection of 4-oxo-2-hexenal, a novel mutagenic product of lipid peroxidation, in human diet and cooking vapor. *Mutat. Res.* **2006**, *603*, 186–192.

(26) Kim, Y.-S.; Hartman, T. G.; Ho, C.-T. Formation of 2-pentylpyridine from the thermal interaction of amino acids and 2,4-decadienal. *J. Agric. Food Chem.* **1996**, *44*, 3906–3908.

(27) De Zorzi, M.; Curioni, A.; Simonato, B.; Giannattasio, M.; Pasini, G. Effect of pasta drying temperature on gastrointestinal digestibility and allergenicity of durum wheat proteins. *Food Chem.* **2007**, *104*, 353–363.

(28) Kahl, R. Butylated hydroxytoluene toxicity. In *Lipid-Soluble Antioxidants: Biochemistry and Clinical Applications*; Ong A. S. H., Packer, L., Eds.; Birkhäuser: Basel, Switzerland, 1992; pp 590–605.

(29) Meier, B. W.; Gomez, J. D.; Zhou, A.; Thompson, J. A. Immunochemical and proteomic analysis of covalent adducts formed by quinone methide tumor promoters in mouse lung epithelial cell lines. *Chem. Res. Toxicol.* **2005**, *18*, 1575–1585.

(30) Surh, J.; Lee, S. O.; Kwon, H. 4-Hydroxy-2-alkenals in polyunsaturated fatty acids-fortified infant formulas and other commercial food products. *Food Addit. Contam.* **2007**, *24*, 1209–1218.

Received for review April 17, 2008. Revised manuscript received July 23, 2008. Accepted July 23, 2008. We thank the Laboratory for Food and Residue Analyses and Centre for Substances and Integrated Risk Assessment at the National Institute for Public Health and the Environment (RIVM, Bilthoven, The Netherlands) for funding the analyses and welcoming E.G. to the institute to carry out the in vitro digestion work. This work has also been supported by the Spanish Ministry of Science and Technology (MCYT, AGL2006-01381) and the Basque government (EJ-GV, IT-403-07). E.G. thanks the EJ-GV for a predoctoral fellowship/contract and for funding the travel costs.

JF801212K