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SUPPLEMENTARY MATERIAL

The key role of ovalbumin in lipid bioaccessibility and oxidation product profile during the *in vitro* digestion of slightly oxidized soybean oil

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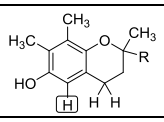
Some details of the in vitro digestion procedure

The digestion experiment started by adding 6 mL of saliva to each of the oil samples. After 5 min of incubation, 12 mL of gastric juice were added and the mixture was rotated head-over-heels at 40 rpm for 2 h at 37±2ºC. 1 hour after starting the gastric digestion, pH was set between 2 and 3 with HCl (37%), simulating the gradual acidification of the chyme occurring *in vivo*. After 2 h of gastric digestion, 2 mL of sodium bicarbonate solution (1 M), 12 mL of duodenal juice and 6 mL of bile juice were added. Subsequently, pH was set between 6 and 7, and the mixture was rotated again at 40 rpm and incubated at 37±2ºC for 4 h.

The enzymes used for the preparation of digestive juices, acquired from Sigma-Aldrich (St. Louis, MO, USA), were the following: α-amylase from *Aspergillus oryzae* (10065, ~30 U/mg); pepsin from porcine gastric mucosa (P7125, ≥400 U/mg protein); amano lipase A from *Aspergillus niger* (534781, ≥120,000 U/g); pancreatin from porcine pancreas (P1750) and lipase type II crude from porcine pancreas (L3126, 100-500 U/mg protein (using olive oil, 30 min incubation)).

Table S1. Chemical shift assignments and multiplicities of the ^{1}H NMR signals in $CDCl_3$ of the main protons of glycerides, fatty acids, some oxidation compounds and γ-tocopherol, present in the samples before and after *in vitro* digestion. TG: triglycerides; DG: diglycerides; MG: monoglycerides. The signal letters agree with those given in Figures 1 and S1.

Signal	Chemical shift (ppm)	Multiplicity	Functional group	
			Type of protons	Compound
Main acyl groups (AG) and fatty acids (FA)[a,b]				
A	0.88	t	–C**H**$_3$	saturated and monounsaturated **ω-9 AG** and **FA**
	0.89	t	–C**H**$_3$	linoleic **AG** and **FA**
B	0.97	t	–C**H**$_3$	linolenic **AG** and **FA**
C	1.19–1.42	m[*]	–(C**H**$_2$)$_n$–	**AG** and **FA**
D	1.61	m	–OCO–CH$_2$–C**H**$_2$–	**AG** in **TG**
	1.62	m	–OCO–CH$_2$–C**H**$_2$–	**AG** in **1,2-DG**
	1.63	m	–OCO–CH$_2$–C**H**$_2$–, COOH–CH$_2$–C**H**$_2$–	**AG** in **1,3-DG**, **1-MG** and **FA**
	1.64	m	–OCO–CH$_2$–C**H**$_2$–	**AG** in **2-MG**
E	1.92–2.15	m[**]	–C**H**$_2$–CH=CH–	**AG** and **FA**
F	2.26–2.36	dt	–OCO–C**H**$_2$–	**AG** in **TG**
	2.33	m	–OCO–C**H**$_2$–	**AG** in **1,2-DG**
	2.35	t	–OCO–C**H**$_2$–, COOH–C**H**$_2$–	**AG** in **1,3-DG**, **1-MG** and **FA**
	2.38	t	–OCO–C**H**$_2$–	**AG** in **2-MG**
G	2.77	t	=HC–C**H**$_2$–CH=	Linoleic **AG** and **FA**
H	2.80	t	=HC–C**H**$_2$–CH=	Linolenic **AG** and **FA**
I	3.65	ddd	ROCH$_2$–CHOH–C**H**$_2$OH	glyceryl group in **1-MG**
J	3.73	m[***]	ROCH$_2$–CH(O*R*')–C**H**$_2$OH	glyceryl group in **1,2-DG**
K	3.84	m[***]	HOC**H**$_2$–CH(O*R*)–C**H**$_2$OH	glyceryl group in **2-MG**
L	3.94	m	ROCH$_2$–C**H**OH–CH$_2$OH	glyceryl group in **1-MG**
M	4.05–4.21	m	ROC**H**$_2$–C**H**OH–C**H**$_2$OR'	glyceryl group in **1,3-DG**
N	4.18	ddd	ROC**H**$_2$–CHOH–CH$_2$OH	glyceryl group in **1-MG**
O	4.22	dd,dd	ROC**H**$_2$–CH(O*R*')–C**H**$_2$OR''	glyceryl group in **TG**
P	4.28	ddd	ROC**H**$_2$–CH(O*R*')–CH$_2$OH	glyceryl group in **1,2-DG**
Q	4.93	m	HOCH$_2$–C**H**(O*R*)–CH$_2$OH	glyceryl group in **2-MG**
R	5.08	m	ROCH$_2$–C**H**(O*R*')–CH$_2$OH	glyceryl group in **1,2-DG**
S	5.27	m	ROCH$_2$–C**H**(O*R*')–CH$_2$OR''	glyceryl group in **TG**
T	5.28–5.46	m	–C**H**=C**H**–	**AG** and **FA**
Oxidation compounds				
Conjugated dienic systems[c,d,e]				
–	5.44	ddd	–C**H**=C**H**–C**H**=C**H**–	(*Z*,*E*)-conjugated double bonds associated with hydroxy group (OH) in octadecadienoic **AG** and
-	5.66	dd		
-	5.97	t		

a	6.49	dd		**FA**
-	5.47	ddm	–C**H**=C**H**–C**H**=C**H**–	(*E*,*E*)-conjugated double bonds associated with hydroperoxy group (OOH) in octadecadienoic **AG** and **FA**
-	5.76	dtm		
-	6.06	ddtd		
b	6.27	ddm		
-	5.51	dtm	–C**H**=C**H**–C**H**=C**H**–	(*Z*,*E*)-conjugated double bonds associated with hydroperoxy group (OOH) in octadecadienoic **AG** and **FA**
-	5.56	ddm		
-	6.00	ddtd		
c	6.58	dddd		
-	5.58	dd	–C**H**=C**H**–C**H**=C**H**–	(*E*,*E*)-conjugated double bonds associated with hydroxy group (OH) in octadecadienoic **AG** and **FA**
-	5.71	dd		
-	6.03	dd		
d	6.18	dd		
Epoxides				
Epoxy-derivatives				
e1	2.88[f]	m	-C**H**O**H**C-	(*Z*)-9,10-epoxystearate
e2	2.9[g]		-C**H**O**H**C-	monoepoxy-octadecenoate groups
e3	2.94[****]	m	-C**H**O**H**C-	(*Z*)-12,13-epoxy-9(*Z*),15(*Z*)-octadecadienoic acid
Epoxy-hydroxy-derivatives				
e4	2.93[h]	dt	-C**H**OHC-CHOH-CH=CH-	*threo*-11-hydroxy-(*E*)-12,13-epoxy-(Z)-9-octadecenoate
f1	3.09[i]/3.097[j]	dd	-CHO**H**C-CH=CH-CHOH-	9-hydroxy-(*E*)-12,13-epoxy-(*E*)-10-octadecenoate
Epoxy-hydroperoxy-derivatives				
f2	3.11[i]	dd	-CHO**H**C-CH=CH-CHOOH-	9-hydroperoxy-(*E*)-12,13-epoxy-(*E*)-10-octadecenoate[k]
Aldehydes				
g	9.75[l]	t	-C**H**O	n-alkanals
			γ-Tocopherol[m]	
h	6.36[****]	s		

Abbreviations: t: triplet; m: multiplet; d: doublet. *Overlapping of multiplets of methylenic protons in the different acyl groups either in β-position, or further, in relation to double bonds, or in γ-position, or further, in relation to the carbonyl group; **Overlapping of multiplets of the α-methylenic protons in relation to a single double bond of the different unsaturated acyl groups; ***This signal shows different multiplicity if the spectrum is acquired from the pure compound or taking part in the mixture; ****Assignment made with the aid of standard compounds.
[a]Assignments of AG in TG taken from M. D. Guillén and A. Ruiz, *J. Sci. Food Agric.*, 2003, **83**, 338-346.

[b]Assignments of AG in partial glycerides (DG and MG) and of FA taken from B. Nieva-Echevarría, E. Goicoechea, M. J. Manzanos and M. D. Guillén, *Food Res. Int.*, 2014, **66**, 379-387.

[c]Data taken from E. Goicoechea and M. D. Guillén, *J. Agric. Food Chem.*, 2010, **58**, 6234-6245 (conjugated (*Z*,*E*)- and (*E*,*E*)-hydroperoxy-dienes).

[d]Data taken from M. Dong, Y. Oda and M. Hirota, *Biosci., Biotech. Biochem.*, 2000, **64**, 882-886 (conjugated (*Z*,*E*)-hydroxy-dienes).

[e]Data taken from P. Tassignon, P. De Waard, T. De Rijk, H. Tournois, D. de Wit and L. De Buyck, *Chem. Phys. Lipids*, 1994, **71**, 187-196 (conjugated (*E*,*E*)-hydroxy-dienes).

[f]Data taken from G. Du, A. Tekin, E. G. Hammond and L. K. Woo, *J. Am. Oil Chem. Soc.*, 2004, **81**, 477-480.

[g]Data taken from H. A. J. Aerts and P. A. Jacobs, *J. Am. Oil Chem. Soc.*, 2004, **81**, 841-846.

[h]Data taken from G. J. Garssen, G. A. Veldink, J. F. Vliegenthart and J. Boldingh, *FEBS J.*, 1976, **62**, 33-36.

[i]Data taken from H. W. Gardner, D. Weisleder and R. Kleiman, *Lipids*, 1978, **13**, 246-252.

[j]Data taken from P. A. Van Os Cornelis, J. F. G. Vliegenthart, C. G. Crawford and H. W. Gardner, *Biochim. Biophys. Acta*, 1982, **713**, 173-176.

[k]δ-Ketols (hydroxy-keto-derivatives) could also contribute to this signal (H. W. Gardner, R. Kleiman and D. Weisleder, *Lipids*, 1974, **9**, 696-706).

[l]Data taken from M. D. Guillén and A. Ruiz. *Eur. J. Lipid Sci. Technol.*, 2004, **106**, 680-687.

[m]Assignment taken from J. K. Baker and C. W. Myers. *Pharm. Res.*, 1991, **8**, 763-770.

Quantification from ^{1}H NMR spectral data of several compounds present in the starting oil samples and/or in the lipid extracts of the digestates, and of Lipid Bioaccessibility

A. Lipolytic products and Lipid Bioaccessibility

The number of moles (N) of all the glycerides and fatty acids present in the lipid samples were expressed as follows:

$N_{2\text{-}MG}=Pc*A_K/4$ [eq. S1]
$N_{1\text{-}MG}=Pc*A_L$ [eq. S2]
$N_{1,2\text{-}DG}=Pc*(A_{I+J}-2A_L)/2$ [eq. S3]
$N_{TG}=Pc*(2A_{4.26\text{-}4.38}-A_{I+J}+2A_L)/4$ [eq. S4]
$N_{1,3\text{-}DG}=Pc*(A_{4.04\text{-}4.38}-2A_{4.26\text{-}4.38}-2A_L)/5$ [eq. S5]
$N_{FA}=Pc*(A_F-6N_{TG}-4N_{1,2\text{-}DG}-4N_{1,3\text{-}DG}-2N_{1\text{-}MG}-2N_{2\text{-}MG})/2$ [eq. S6]
$N_{Gol}=(N_{FA}-N_{1,2\text{-}DG}-N_{1,3\text{-}DG}-2N_{2\text{-}MG}-2N_{1\text{-}MG})/3$ [eq. S7]

where Pc is the proportionality existing between the area of the ^{1}H NMR signals and the number of protons that generate them, A_K, A_L, A_{I+J} and A_F are the areas of the corresponding signals indicated in Table S1, and $A_{4.26\text{-}4.38}$ and $A_{4.04\text{-}4.38}$ represent the areas of the signals between 4.26 and 4.38 ppm, and between 4.04 and 4.38 ppm, respectively (see Figure S1). Gol: glycerol.

Using these equations, the molar percentages of the different kinds of glycerides in relation to the total number of moles of glyceryl structures present (NT_{GS}) were determined as follows:

$NT_{GS}=N_{TG}+N_{1,2\text{-}DG}+N_{1,3\text{-}DG}+N_{2\text{-}MG}+N_{1\text{-}MG}+N_{Gol}$ [eq. S8]
$G\%=100N_G/NT_{GS}$ [eq. S9]

where G is each kind of glyceride (TG, 1,2-DG, 1,3-DG, 2-MG and 1-MG) and N_G the respective number of moles.

$Gol\%=100N_{Gol}/NT_{GS}$ [eq. S10]

Likewise, the Lipid Bioaccessibility parameter was calculated as follows:

$L_{BA}\%=100(N_{1\text{-}MG}+N_{2\text{-}MG}+N_{FA})/NT_{AG+FA}$ [eq. S11]
$NT_{AG+FA}=Pc*A_F/2$ [eq. S12]

where NT_{AG+FA} is the total number of moles of AG plus FA present.

B. Polyunsaturated acyl groups and fatty acids

The concentrations of linolenic (Ln) and linoleic (L) AG and FA, expressed as millimoles per mole of the sum of AG+FA present in either the starting oils or the lipid extracts of the digested samples were estimated by using the following equations:

$$[Ln] = [(A_H/4)/(A_F/2)]*1000 \quad \text{[eq. S13]}$$

$$[L] = [(A_G/2)/(A_F/2)]*1000 \quad \text{[eq. S14]}$$

where A_H and A_G are the areas of signals H and G indicated in Table S1. It must be noted that due to partial overlapping of signals H and G, a previous correction of both areas must be carried out to properly assess the area corresponding to each one of them. For this purpose, trilinolenin and trilinolein were used as references.

Finally, it should be pointed out that signal F is due to methylenic protons bonded to carbon atoms in *alpha* position in relation to carbonyl/carboxyl groups of AG and FA, modified or not, as well as to carbonyl groups of other compounds formed during oxidation such as aldehydes. However, as the oxidation level of both the non-digested and the *in vitro* digested samples is very low, the inclusion in this signal of methylenic protons in *alpha* position in relation to carbonyl groups different from those of AG and FA does not affect the calculations in which A_F is included, because the concentration of aldehydes is negligible in relation with that of AG+FA.

C. Oxidation compounds and γ-tocopherol

The concentration of the several kinds of oxidation compounds, as well as that of γ-tocopherol, expressed as millimoles per mol of the sum of AG+FA present, was estimated by using the following equations:

$$[OP] = [(A_{OP}/n)/(A_F/2)]*1000 \quad \text{[eq. S15]}$$

$$[\gamma\text{-T}] = [(A_{\gamma T}/n)/(A_F/2)]*1000 \quad \text{[eq. S16]}$$

where A_{OP} and $A_{\gamma T}$ are the areas of the signals selected for the quantification of each oxidation product (OP) and of γ-T, shown in Table S1, and n the number of protons that generate each signal. In the case of the epoxides giving signal at approximately 2.9 ppm (signals "e1-e4" in Table S1), the overlapped area due to the side band of *bis*-allylic protons signals G and H must be subtracted. Although the epoxy-compounds included in signal "e" can contribute with two ("e1-e3") or one ("e4") protons, it has been assumed that all contribute with two protons. This type of epoxides has been quantified together with those giving signal at approximately 3.1 ppm (see signals "f1+f2" in Table S1).

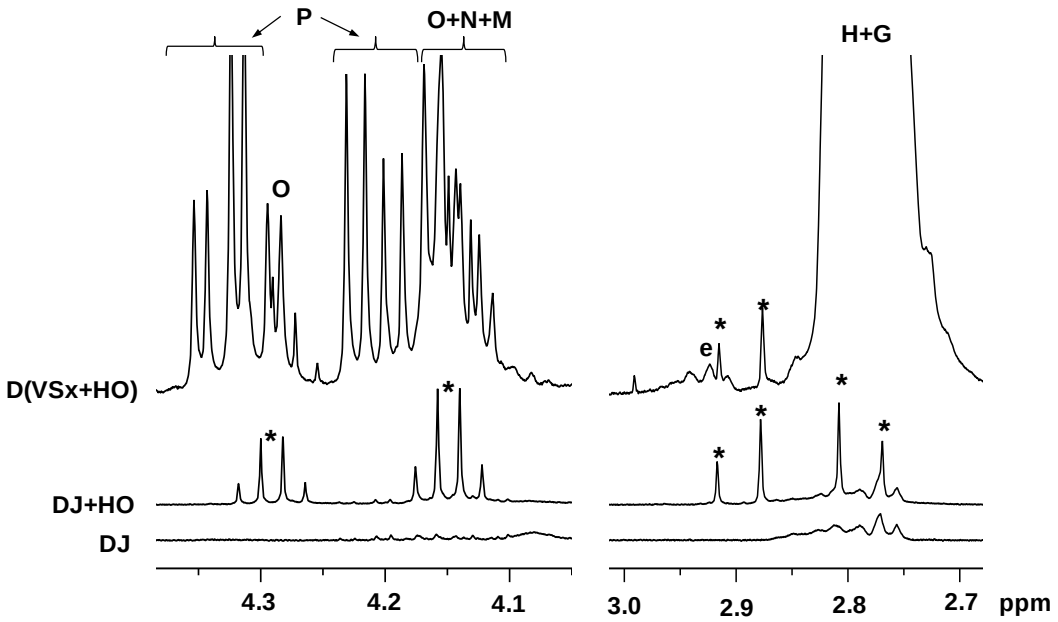


Figure S1. Enlargement of some spectral regions of the ^{1}H NMR spectra of the lipid extracts of: the digestive juices subjected to digestion conditions (DJ); the digestive juices subjected to digestion conditions mixed with ovalbumin at the high proportion tested (DJ+HO); and the slightly oxidized virgin soybean oil digested in presence of the high ovalbumin proportion (D(VSx+HO)). The signal letters agree with those in Table S1, considering that signal "e" includes signals "e1 to e4". Signals marked with an asterisk are considered to come from the ovalbumin sample used.

Levels of some minor components in the oxidized oils studied, determined by Direct Immersion Solid Phase Microextraction followed by Gas Chromatography/Mass Spectrometry (DI SPME-GC/MS), according to the methodology described by J. Alberdi-Cedeño, M. L. Ibargoitia, G. Cristillo, P. Sopelana and M. D. Guillén, Food Chem., 2017, 221, 1135-1144.

Table S2. Abundances, expressed as arbitrary area units of the mass spectrum base peak (BP) of each compound, extracted from the total ion chromatograms obtained by DI SPME-GC/MS, divided by 10^6, of the main minor components of the studied soybean oils, together with their respective molecular weights (MW).

Compounds (MW)	**BP**	**VSx**	**RSx**
Free fatty acids			
Total free fatty acids†	55	136.8 ± 19.9	-
Tocopherols			
δ-Tocopherol (402)‡	402	11.0 ± 0.4	15.8 ± 1.3
β-Tocopherol (416)‡	416	0.5 ± 0.1	1.2 ± 0.1
γ-Tocopherol (416)‡	416	8.4 ± 1.1	34.7 ± 4.7
α-Tocopherol (430)‡	165	0.3 ± 0.1	0.5 ± 0.1
Hydrocarbons			
Squalene (410)‡	69	22.9 ± 1.7	31.5 ± 0.1

-: not detected

†This total includes linoleic, oleic and linolenic acids, whose mass spectra base peaks are 67, 55 and 79, respectively. However, given that all of them overlap, ion 55, common to all these fatty acids, has been used to quantify them altogether.

‡Standard compounds were acquired commercially and used for identification purposes.