
SUPPLEMENTARY MATERIAL

Effect of the enrichment of corn oil with *alpha*- or *gamma*-tocopherol on its *in vitro* digestion studied by ¹H NMR and SPME-GC/MS. Formation of hydroperoxy-, hydroxy-, keto-dienes and keto-*E*-epoxy-*E*-monoenes in the more *alpha*-tocopherol enriched samples

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Table S1. Composition and pH values of the juices employed in the *in vitro* digestion model employed in this study.

Components	Saliva	Gastric juice	Duodenal juice	Bile juice
KCl (mmol/L)	12.02	11.06	7.57	5.05
NaCl (mmol/L)	5.10	47.09	119.98	89.99
NaHCO ₃ (mmol/L)	20.17	-	40.33	68.86
NaH ₂ PO ₄ (mmol/L)	7.40	0.22	-	-
NH ₄ Cl (mmol/L)	-	5.72	-	-
KH ₂ PO ₄ (mmol/L)	-	-	0.59	-
Na ₂ SO ₄ (mmol/L)	4.79	-	-	-
KSCN (mmol/L)	2.06	-	-	-
MgCl ₂ (mmol/L)	-	-	0.53	-
CaCl ₂ *2H ₂ O (mmol/L)	-	2.72	1.36	1.51
HCl (37%) (mL/L)	-	6.50	0.18	0.15
Urea (mmol/L)	3.33	1.42	1.67	4.16
Glucose (mmol/L)	-	3.61	-	-
Glucuronic acid (mmol/L)	-	0.10	-	-
Uric acid (mmol/L)	0.09	-	-	-
Glucoseamine hydrochloride (mmol/L)	-	1.53	-	-
Bovine serum albumin (g/L)	-	1.00	1.00	1.80
Mucin (g/L)	0.025	3.00	-	-
<i>A. oryzae</i> α-amylase (g/L)	0.29	-	-	-
<i>A. niger</i> lipase (U/mL)	-	100	-	-
Pepsin (g/L)	-	2.50	-	-
Pancreatin (g/L)	-	-	9.00	-
Lipase type II from porcine pancreas (g/L)	-	-	1.50	-
Bovine bile extract (g/L)	-	-	-	18.75
pH	6.9±0.0	1.3±0.1	8.1±0.1	8.2±0.1

Table S2. Chemical shift assignments and multiplicities of the ^1H NMR signals in CDCl_3 of protons of glycerides. TG: triglycerides; DG: diglycerides; MG: monoglycerides. The signal letters agree with those given in Figure 1.

Signal	Chemical shift (ppm)	Multiplicity	Type of protons	Structures
Glycerides structure protons				
I	3.65	ddd	$\text{ROCH}_2\text{-CHOH-CH}_2\text{OH}$	glyceryl group in 1-MG
J	3.73	m^*	$\text{ROCH}_2\text{-CH(OR')-CH}_2\text{OH}$	glyceryl group in 1,2-DG
K	3.84	m^*	$\text{HOCH}_2\text{-CH(OR)-CH}_2\text{OH}$	glyceryl group in 2-MG
L	3.94	m	$\text{ROCH}_2\text{-CH}_2\text{OH-CH}_2\text{OH}$	glyceryl group in 1-MG
M	4.05–4.21	m	$\text{ROCH}_2\text{-CHOH-CH}_2\text{OR}'$	glyceryl group in 1,3-DG
N	4.18	ddd	$\text{ROCH}_2\text{-CHOH-CH}_2\text{OH}$	glyceryl group in 1-MG
O	4.22	dd,dd	$\text{ROCH}_2\text{-CH(OR')-CH}_2\text{OR}''$	glyceryl group in TG
P	4.28	ddd	$\text{ROCH}_2\text{-CH(OR')-CH}_2\text{OH}$	glyceryl group in 1,2-DG
Q	4.93	m	$\text{HOCH}_2\text{-CH(OR)-CH}_2\text{OH}$	glyceryl group in 2-MG
R	5.08	m	$\text{ROCH}_2\text{-CH(OR')-CH}_2\text{OH}$	glyceryl group in 1,2-DG

Abbreviations: d: doublet; m: multiplet.

*This signal shows different multiplicity if the spectrum, is acquired from the pure compound or taking part in the mixture.

**The intensity of some of these signals, also shown in Figure 1, together with signal F of Table S3, were used to estimate the molar percentages of different kinds of glyceryl structures using the equations [eq. S1–eq. S10].

***The assignment of the ^1H NMR signals of the protons was made as in previous studies (Guillén & Uriarte, 2012; Nieva-Echevarría et al., 2014).

Table S3. Chemical shift assignments and multiplicities of the ^1H NMR signals in CDCl_3 of protons of acyl groups and fatty acids. AG: acyl groups; FA: fatty acids. The signal letters agree with those given in Figure 1.

Signal	Chemical shift (ppm)	Multiplicity	Type of protons	Structures
Main acyl groups (AG) and fatty acids (FA)				
A	0.88	t	$-\underline{\text{CH}}_3$	saturated and monounsaturated ω -9 in AG and FA
	0.89	t	$-\underline{\text{CH}}_3$	linoleic in AG and FA
B	0.97	t	$-\underline{\text{CH}}_3$	linolenic in AG and FA
C	1.19–1.42	m^{**}	$-(\underline{\text{CH}}_2)_n-$	AG and FA
D	1.61	m	$-\text{OCO}-\text{CH}_2-\underline{\text{CH}}_2-$	AG in TG
	1.62	m	$-\text{OCO}-\text{CH}_2-\underline{\text{CH}}_2-$	AG in 1,2-DG
	1.63	m	$-\text{OCO}-\text{CH}_2-\underline{\text{CH}}_2-$, $\text{COOH}-\text{CH}_2-\underline{\text{CH}}_2-$	AG in 1,3-DG, 1-MG and FA
E	1.64	m	$-\text{OCO}-\text{CH}_2-\underline{\text{CH}}_2-$	AG in 2-MG
	1.92–2.15	m^{***}	$-\underline{\text{CH}}_2-\text{CH}=\text{CH}-$	AG and FA
F*	2.26–2.36	dt	$-\text{OCO}-\underline{\text{CH}}_2-$	AG in TG
	2.33	m	$-\text{OCO}-\underline{\text{CH}}_2-$	AG in 1,2-DG
	2.35	t	$-\text{OCO}-\underline{\text{CH}}_2-$, $\text{COOH}-\underline{\text{CH}}_2-$	AG in 1,3-DG, 1-MG and FA
	2.38	t	$-\text{OCO}-\underline{\text{CH}}_2-$	AG in 2-MG
G*	2.77	t	$=\text{HC}-\underline{\text{CH}}_2-\text{CH}=\text{CH}-$	linoleic in AG and FA

Abbreviations: d: doublet; t: triplet; m: multiplet.

*The intensity of these signals, also shown in Figure 1, was used to estimate the molar percentage of linoleic structures by using equation [eq. S11].

**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.

****The assignment of the ^1H NMR signals of the protons was made as in previous studies (Guillén & Ruiz, 2003; Nieva-Echevarría et al., 2014).

Table S4. Chemical shift assignments and multiplicities of the ^1H NMR signals in CDCl_3 of protons of some oxidation compounds detected in the digestates and formed during the *in vitro* digestion. The signal letters agree with those given in Figure 2.

Signal	Chemical shift (ppm)	Multiplicity	Type of protons	Structures
Oxidation Compounds (OC)				
Conjugated dienic systems associated with hydroperoxy groups				
-	5.51	dtm	$-\underline{\text{CH}}=\text{CH}-\text{CH}=\text{CH}-$	<i>(Z,E)</i> -conjugated double bonds associated with hydroperoxy group (OOH) in octadecadienoic AG and FA HPO-c(Z,E)dEs
-	5.56	ddm		
-	6.00	ddtd		
b	<u>6.58</u>	dddd		
Conjugated dienic systems associated with hydroxy groups				
-	5.44	ddd	$-\underline{\text{CH}}=\text{CH}-\text{CH}=\text{CH}-$	<i>(Z,E)</i> -conjugated double bonds associated with hydroxy group (OH) in octadecadienoic AG and FA HO-c(Z,E)dEs
-	5.66	dd		
-	5.97	t		
a	<u>6.49</u>	dd		
-	5.58	dd	$-\underline{\text{CH}}=\text{CH}-\text{CH}=\text{CH}-$	<i>(E,E)</i> -conjugated double bonds associated with hydroxy group (OH) in octadecadienoic AG and FA HO-c(E,E)dEs
-	5.71	dd		
-	6.03	dd		
c	<u>6.18</u>	dd		
Conjugated keto-dienes (KO-c-dEs)				
d	<u>7.49</u>	ddd	$-\text{CH}=\underline{\text{CH}}-$ (C-11)	<i>(Z,E)</i> - conjugated double bonds associated with ketodiene of octadecadienoic AG and FA acyl groups KO-c(Z,E)dEs
	6.16	d	$-\underline{\text{CH}}=\text{CH}-$ (C-10)	
	6.12	m	$-\underline{\text{CH}}=\text{CH}-$ (C-12)	
	5.91	dt	$-\underline{\text{CH}}=\text{CH}-$ (C-13)	
	2.54	t	$-\text{CH}_2-\text{CO}$	
Keto-epoxy-monoenes (KO-EPO-mEs)				
	6.52	dd	$-\text{CH}=\text{CH}-$	13-KO-9,10- <i>E</i> -EPO-11- <i>E</i> -ene 9-KO-12,13- <i>E</i> -EPO-10- <i>E</i> -ene KO-E-EPO-E-mEs
	6.38	d	$-\text{CH}=\text{CH}-$	
e	<u>3.22</u>	dd	$-\text{HCO}\underline{\text{CH}}-$	
	2.91	td	$-\text{HCO}\text{CH}-$	
	2.53	t	$-\text{CH}_2-$	
Aldehydes				
f	<u>9.75</u>	t	$-\underline{\text{CHO}}$	n-alkanals
	2.40	dt	$-\text{CH}_2-$	

Abbreviations: d: doublet; t: triplet; m: multiplet.

*The intensities of the signals indicated in bold, together with signal F of Table S3, were used to estimate the concentration (mmol/molAG+FA) using the equation [eq. S12].

**The assignment of the ^1H NMR signals of the protons was made with the aid of standard compounds and with the data taken from literature (Guillén & Ruiz, 2005; Dufour & Loonis, 2005; Guillén & Goicoechea, 2007; Lin et al., 2007; Ramsden et al., 2017).

Table S5. Chemical shift assignments and multiplicities of the ^1H NMR signals in CDCl_3 of protons of some tocopherols detected in the samples before and after *in vitro* digestion. The signal letters agree with those given in Figure 2.

Signal	Chemical shift (ppm)	Multiplicity	Type of protons	Compounds
Tocopherols				
αT	2.16 ^{**}	s	- <u>CH</u> ₃ (C-7)	<i>alpha</i> -Tocopherol
γT	6.36 ^{**}	s	- <u>CH</u> (C-5)	<i>gamma</i> -Tocopherol

Abbreviation: s: singlet.

*The intensity of these signals, together with signal F of Table S3, were used to estimate the concentration (mmol/molAG+FA) using the equation [eq. S12].

**Assignment was made with the aid of standard compounds and with the data taken from the literature (Baker & Mayers, 1991)

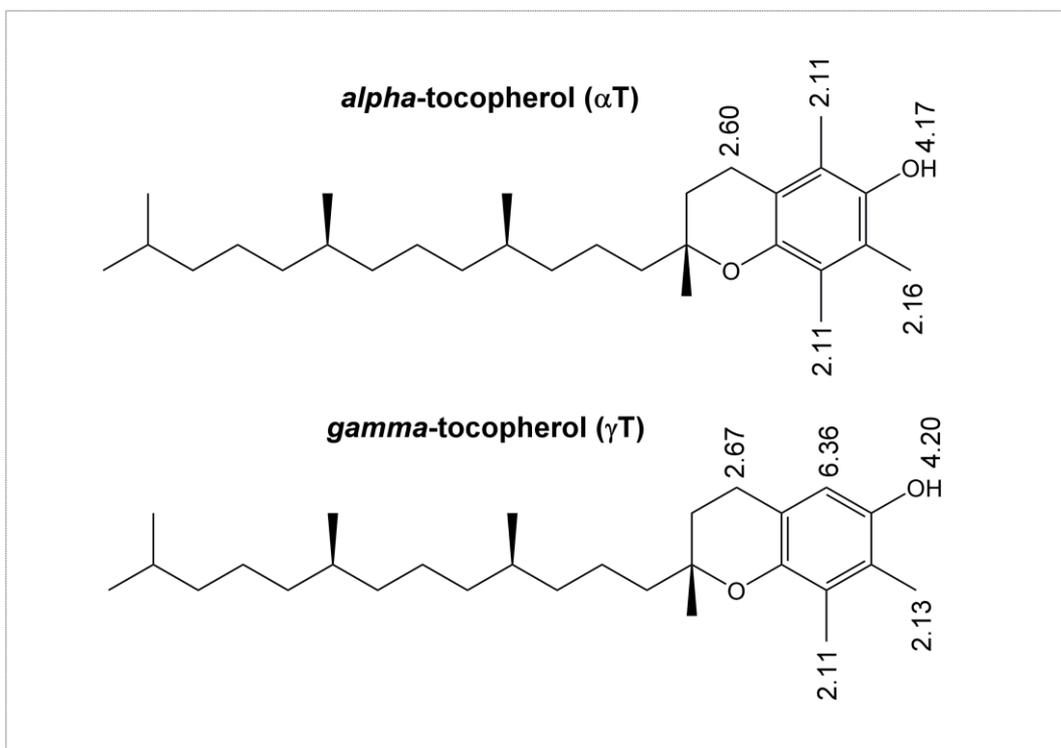


Figure S1. Chemical structures of tocopherols involved in this of study, together with some chemical shifts (ppm) of some of their hydrogen atoms.

Quantification from ¹H NMR spectral data of several compounds present in the starting samples and/or in the lipid extracts of the digestates

Bearing in mind that the area of each ¹H NMR spectral signal is proportional to the number of protons that generate it, and that the proportionality constant is the same for all kinds of protons, the area of some spectral signals can be employed to quantify a wide variety of compounds, as detailed below.

A. Equations used to estimate the molar percentage (%) of the several glyceride structures present in the lipid extract of digestates and the glycerol.

In these equations the number of moles (N) of fatty acids and all the glycerides were expressed as follows:

$$N_{2-MG} = Pc * A_K / 4 \quad [\text{eq. S1}]$$

$$N_{1-MG} = Pc * A_L \quad [\text{eq. S2}]$$

$$N_{1,2-DG} = Pc * (A_{I+J} - 2A_L) / 2 \quad [\text{eq. S3}]$$

$$N_{TG} = Pc * (2A_{4.26-4.38} - A_{I+J} + 2A_L) / 4 \quad [\text{eq. S4}]$$

$$N_{1,3-DG} = Pc * (A_{4.04-4.38} - 2A_{4.26-4.38} - 2A_L) / 5 \quad [\text{eq. S5}]$$

$$N_{FA} = (Pc * A_F - 6N_{TG} - 4N_{1,2-DG} - 4N_{1,3-DG} - 2N_{1-MG} - 2N_{2-MG}) / 2 \quad [\text{eq. S6}]$$

$$N_{Gol} = (N_{FA} - N_{1,2-DG} - N_{1,3-DG} - 2N_{2-MG} - 2N_{1-MG}) / 3 \quad [\text{eq. S7}]$$

where Pc is the proportionality constant existing between the area of the ¹H 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 S2 and Figure 1, and A_{4.26-4.38} and A_{4.04-4.38} represent the areas of the signals between 4.26 and 4.38 ppm, and between 4.04 and 4.38 ppm, respectively.

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

$$N_{TGS} = N_{TG} + N_{1,2-DG} + N_{1,3-DG} + N_{2-MG} + N_{1-MG} + N_{Gol} \quad [\text{eq. S8}]$$

$$G\% = 100N_G / N_{TGS} \quad [\text{eq. S9}]$$

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.

$$Gol\% = 100N_{Gol} / N_{TGS} \quad [\text{eq. S10}]$$

B. Estimation of the molar percentage of linoleic FA plus AG (FA+AG). The molar percentage of linoleic (L%), AG plus FA, in relation to the total number of moles of AG plus FA (N_{TAG+FA}) present in the starting oils and in the lipid extracts of the corresponding digestates was estimated as follows:

$$L\% = 100 * A_G / A_F \quad [\text{eq. S11}]$$

where A_G and A_F are the areas of signals G and F indicated in Table S3 and Figure 1.

C. Estimation of the concentration of specific compounds in oil samples and in the lipids extract from digestates

The concentration of the several kinds of specific compounds (SC), expressed as millimoles per mole of the sum of AG+FA, was estimated by using the following equations:

$$[\text{SC}] = [(A_{SC}/n)/(A_F/2)] * 1000 \quad [\text{eq. S12}]$$

where A_{SC} , is the area of the signal selected for the quantification of each specific compound present in the sample, n the number of protons that generate the signals given in Tables S4 , S5 and Figure 2 and A_F is the area of the signal F in Table S3 and in Figure 1.

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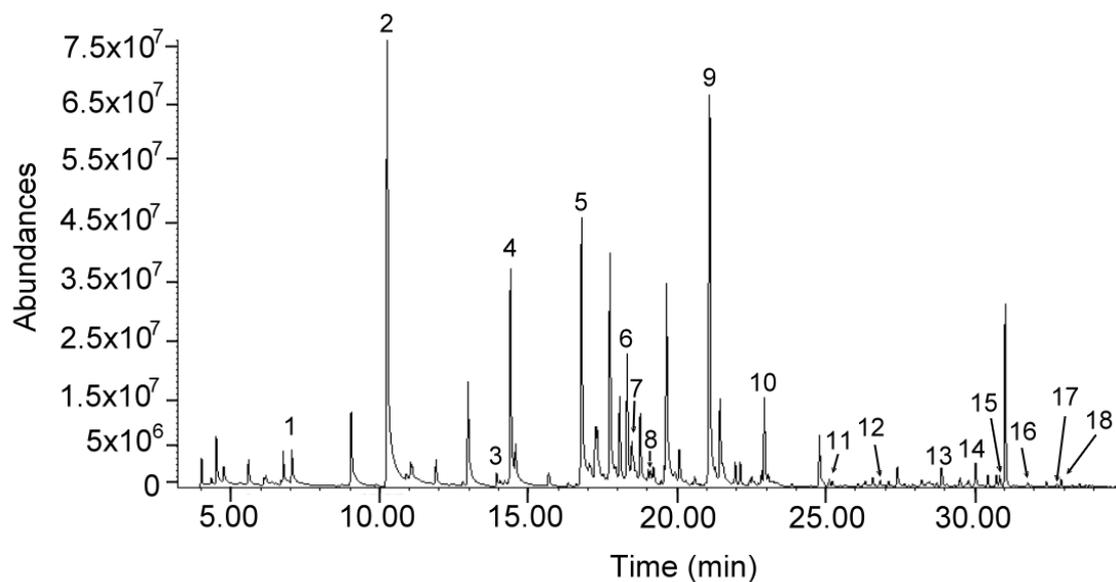


Figure S2. Region between 4-34 min of the total ion chromatogram obtained by SPME-GC/MS of the digestate of corn oil sample enriched in *alpha*-tocopherol DC5 α T. Peaks identified: **(1)** pentanal; **(2)** hexanal; **(3)** furan, 2-butyl; **(4)** heptanal; **(5)** (*E*)-2-heptenal; **(6)** furan, 2-pentyl; **(7)** (*Z,E*)-2,4-heptadienal; **(8)** (*E,E*)-2,4-heptadienal; **(9)** (*E*)-2-octenal; **(10)** nonanal; **(11)** (*E*)-2-nonenal; **(12)** decanal; **(13)** (*E*)-2-decenal; **(14)** (*Z,E*)-2,4-decadienal; **(15)** (*E,E*)-2,4-decadienal; **(16)** 5-pentyl-2(5H)-furanone; **(17)** 4,5-epoxy-2-decenal (isomer); **(18)** 4,5-epoxy-(*E*)-2-decenal.