









# In vitro digestion of potatoes fried in sunflower oil enriched with Olive by-product Extracts: impact on lipid bioaccesibility and oxidation

Zina Harzalli <sup>a,b</sup>, <u>Encarnacion Goicoechea</u><sup>b</sup>, Andrea Martínez-Yusta<sup>b</sup>, Barbara Nieva-Echevarria<sup>b</sup>, Wafa Medfai<sup>b</sup>, Imen Oueslati<sup>a</sup>

(a) Laboratoire de Biotechnologie de l'Olivier, Centre de Biotechnologie de Borj-Cedria, B.P. 901, 2050 Hammam-Lif, Tunisia. E-mail: <u>zina.harzalli@qmail.com</u> (b) Food Technology, Faculty of Pharmacy, Lascaray Research Center, University of the Basque Country (UPV/EHU), Vitoria-Gasteiz, Spain. E-mail: <u>encarnacion.goicoechea@ehu.eus</u>

## AIM OF THE WORK

The aim of this study was to investigate the potential antioxidant effect of olive by-product extracts during food *in vitro* gastrointestinal digestion. **Olive mill wastewater** and **olive leaves extracts** from the **Tunisian** *Chetoui* variety were selected, because they are considered as potential sources of novel bioactive compounds.

# MATERIALS AND METHODS

250 g of fresh potato were deep-fried for 10 min at 180 °C in 2.5 litres of either the control oil or the enriched oil with 100 ppm of olive leaves (**L**) or olive mill wastewater (**W**) extracts. Fried potato samples corresponding to 5<sup>th</sup> and 10<sup>th</sup> cycles of frying were submitted to *in vitro* gastrointestinal digestion (see Figure 1). Lipids of fried potatoes before (**P**) and after digestion (**DP**) were extracted and analyzed using Proton Nuclear Magnetic Resonance (<sup>1</sup>H NMR) (*1*).



# **RESULTS AND DISCUSSION**

#### Lipolysis extent

Before digestion, as expected, the main glyceryl structures present in all potato lipids were triglycerides (TG) (see **Table 1**). After digestion, a high level of lipolysis was observed. In agreement with the regiospecificity of the digestive lipases employed in the experiments, the most abundant glycerides in digested samples were glycerol (Gol), 2-monoglycerides (2-MG) and 1,2-diglycerides (1,2-DG). Remaining TG were only present in trace amounts.

Similar molar percentages of the different glycerides were observed in the different samples, evidencing that neither the enrichment of the oil nor the number of frying cycle affected the lipolysis extent reached during digestion.

**Table 1.** Lipolysis degree of lipids extracted from potatoes fried during 5 and 10 frying cycles in sunflower oil (P5, P10), sunflower oil enriched with olive leaves extract (PL5, PL10) and sunflower oil enriched with olive mill wastewater extract (PW5, PW10), together with those of the lipid extracts obtained after *in vitro* digestion of the corresponding potatoes (DP5, DP10, DPL5, DPL10, DPW5 and DPW10), expressed by the molar percentages of the different glyceryl structures present in the lipid samples. Different letters within each row of the two columns of the same kind of sample (before and after digestion) indicate a significant difference (p < 0.05).

	Glyceryl structures (molar %)							
Cycle 5	P5	DP5	PL5	DPL5	PW5	DPW5		
TG	98.27±0.02ª	$0.59{\pm}0.81^{\rm b}$	98.83±0.04ª	0.35±2.79 <sup>b</sup>	98.27±0.02ª	$0.35 \pm 0.70^{b}$		
1,2-DG	1.73±0.02 ª	$19.06 \pm 0.40^{b}$	$1.17{\pm}0.04^{a}$	$18.70 \pm 0.16^{b}$	1.44±0.04 <sup>a</sup>	$18.07 \pm 1.50^{b}$		
1, <b>3-DG</b>	-	$0.46{\pm}0.00^{b}$	-	$1.50{\pm}2.20^{b}$	-	$0.16{\pm}0.00^{a}$		
2-MG	-	$25.70 \pm 0.00^{b}$	-	30.30±0.63 <sup>b</sup>	-	$25.70{\pm}0.00^{b}$		
1-MG	-	$1.89{\pm}0.58^{a}$	-	2.37±0.63ª	-	$1.89{\pm}0.58^{a}$		
Gol	-	$32.07 \pm 0.72^{b}$	-	$35.13{\pm}0.97^{b}$	-	$32.07 \pm 0.72^{b}$		
Cycle 10	P10	DP10	PL10	DPL10	PW10	DPW10		
TG	98.81±0.02 <sup>a</sup>	$0.63 \pm 8.64^{b}$	98.68±0.14ª	$0.26{\pm}0.18^{b}$	98.68±0.14 <sup>a</sup>	0.26±0.18 <sup>b</sup>		
1,2-DG	$1.19{\pm}0.02^{a}$	$22.18 \pm 0.57^{b}$	1.32±0.14 <sup>a</sup>	$21.26 \pm 0.07^{b}$	1.28±0.05 <sup>a</sup>	$21.26 \pm 0.70^{b}$		
1, <b>3-DG</b>	-	$0.57 \pm 0.21^{b}$	-	$1.00{\pm}0.16^{b}$	-	$1.00{\pm}0.16^{b}$		
2-MG	-	$30.31 \pm 7.90^{b}$	-	$29.77 \pm 2.70^{b}$	-	$21.26 \pm 0.07^{b}$		
1-MG	-	$2.32{\pm}0.26^{b}$	-	$1.32{\pm}0.10^{b}$	-	$1.98{\pm}0.37^{b}$		
Gol	_	23 96+0 81 <sup>b</sup>	_	35 65+2 82b	_	40 15+1 48 <sup>b</sup>		

Abbreviations: TG, triglyceride; DG, diglyceride; MG, monoglyceride; Gol, glycerol.

REFERENCES

(1) Nieva-Echevarría B, Goicoechea E, Guillén MD (2019).. Food Research International (125), 108558. Acknowledgments: PID2021-1235210B-I00/ MCIN,AEI/ 10.13039/501100011033/ ERDF,UE and EJ-GV IT1490-22.

#### Degradation of linoleic chains

Linoleic chains (C18:2 $\omega$ 6) are the main acyl groups in fried potatoes, as sunflower oil was used for frying. As **Table 2** shows, after digestion a higher **degradation of linoleic** chains was observed in the lipid digests of potatoes fried in **non-enriched sunflower oil**, in comparison with those of lipid digests corresponding to potatoes fried in enriched oils (specially in the samples corresponding to the 5<sup>th</sup> frying cycle).

These results are in **agreement** with the differences observed regarding primary and secondary oxidation products, which are commented below.

**Table 2.** Main acyl groups of the lipids extracted from potatoes fried during frying cycles number 5 and 10 in sunflower oil (P5, P10), sunflower oil enriched with olive leaves extract (PL5, PL10) and sunflower oil enriched with olive mill wastewater extract (PW5, PW10), together with those of the lipid extracts obtained after *in vitro* digestion of the corresponding potatoes (DP5, DP10, DPL5, DP110, DPW5 and DPW10), expressed by the molar percentages of the several kinds of acyl groups plus fatty acids (AG+FA) present in the lipid sample. Different letters within each row of the two columns of the same kind of sample (before and after digestion) indicate a significant difference (p < 0.05).

Lipid composition (molar % of AG+FA)								
Cycle 5	P5	DP5	PL5	DPL5	PW5	DPW5		
Linoleic	57.70±0.13ª	54.65±0.22 <sup>b</sup>	57.92±0.07 ª	56.06±0.34ª	58.42±0.01ª	57.01±0.02ª		
Oleic	29.80±0.23ª	27.80±0.17 <sup>b</sup>	30.14±0.83ª	28.52±0.01b	29.59±0.29ª	28.58±0.05ª		
Total Unsaturated	87.50±0.12ª	82.46±0.40 <sup>b</sup>	88.06±0.04ª	84.58±0.32 <sup>b</sup>	88.01±0.01ª	84.59±0.021		
Saturated plus modified	12.50±0.21ª	17.53±0.40 <sup>b</sup>	11.93±0.04ª	15.41±0.33 <sup>b</sup>	11.98±0.02 <sup>a</sup>	15.40±0.03 <sup>k</sup>		
Cycle 10	P10	DP10	PL10	DPL10	PW10	DPW10		
Linoleic	57.58±0.10 ª	54.94±0.4 <sup>b</sup>	57.47±0.05ª	55.57±0.02 <sup>b</sup>	57.89±0.20ª	55.39±0.11 <sup>b</sup>		
Oleic	29.76±0.43ª	28.56±0.8ª	30.43±0.81ª	29.16±0.42ª	29.79±0.44ª	28.55±0.24ª		
Total Unsaturated	87.34±0.32ª	83.51±0.20 <sup>b</sup>	87.91±0.00ª	$84.74{\pm}0.50^{b}$	87.68±0.16ª	83.95±0.49 <sup>k</sup>		
Saturated plus modified	12.65±0.32 ª	16.49±0.20 <sup>b</sup>	12.08±0.00 <sup>a</sup>	15.25±0.50 <sup>b</sup>	12.31±0.16 ª	16.04±0.491		

#### Formation of primary and secondary oxidation products

As shown in **Table 3**, during digestion the generation of *cis,trans*hydroperoxy-octadecadienoates and of alkanals was higher in the lipid digests of potatoes fried in **non-enriched** sunflower oil, than in those fried in enriched oils (specially in the samples corresponding to the 10<sup>th</sup> frying cycle).

It is noteworthy that after digestion E,Z-2,4-alkadienals and 4-hydroxy-2alkenals were not detected in potato lipids. The former could have been isomerized to E,E,-2,4-alkadienals and the latter could have reacted with proteins.

Table 3. Estimated concentrations of lipid oxidation compounds detected the lipid extracts of potatoes fried during frying cycles number 5 and 10 in sunflower oil (P5, P10), sunflower oil enriched with olive leaves extract (PL5, PL10) and sunflower oil enriched with olive mill wastewater extract (PW5, PW10), together with those of the lipid extracts obtained after *in vitro* digestion of the corresponding potatoes (DP5, DP10, DPL5, DPL10, DPW5 and DPW10), expressed as millimol of compound per mol of acyl groups plus fatty acids (AG+FA) present in the lipid semple. Different letters within each row of the two columns of the same kind of sample (before and after digestion) indicate a significant difference (p < 0.05).

		,	•	e e	,			
Lipid oxidation compounds (mmol/ mol AG+FA)								
Cycle 5	P5	DP5	PL5	DPL5	PW5	DPW5		
Z,E-CD-OOH	0.04±0.15 <sup>a</sup>	$0.47 \pm 0.02^{b}$	0.16±0.00 <sup>a</sup>	$0.26 \pm 0.02^{a}$	-	0.34±0.06 <sup>b</sup>		
Z,E-CD-OH	$0.20{\pm}0.02^{a}$	$0.80{\pm}0.04^{\rm b}$	0.26±0.60 <sup>a</sup>	$0.45{\pm}~0.02^{\rm b}$	-	$0.36 \pm 0.03^{b}$		
Alkanals	$0.16{\pm}0.00^{a}$	$0.48 \pm 0.10^{b}$	$0.14 \pm 0.00^{a}$	$0.29 \pm 0.04^{b}$	$0.14{\pm}0.00^{a}$	$0.31 \pm 0.00^{b}$		
E,Z-2,4-alkadienals	-	-	0.08±0.00 <sup>b</sup>	-	0.06±0.01 <sup>b</sup>	-		
4-hydroxy-E-2-alkenals	-	-	-	-	-	-		
E,E-2,4-alkadienals	$0.24{\pm}0.00^{b}$	$0.21{\pm}0.03^{\rm b}$	$0.24 \pm 0.00^{b}$	$0.21{\pm}\ 0.03^{b}$	$0.22{\pm}~0.01^{a}$	$0.14 \pm 0.07^{b}$		
E-2-alkenals	$0.10{\pm}~0.01^{\rm b}$	$0.08{\pm}0.05^{\rm b}$	0.13± 0.00 <sup>a</sup>	$0.03{\pm}\;0.02^{\rm b}$	$0.13{\pm}~0.00^{b}$	$0.10\pm\!\!0.02^b$		
Cycle 10	P10	DP10	PL10	DPL10	PW10	DPW10		
Z,E-CD-OOH	0.21±0.05ª	$1.29{\pm}0.00^{\rm b}$	0.16±0.12 <sup>a</sup>	$0.44{\pm}~0.10^{\rm b}$	0.32±0.02ª	0.50±0.13 <sup>b</sup>		
Z,E-CD-OH	$0.42{\pm}0.09^{a}$	$0.80{\pm}0.04^{\rm b}$	0.35±0.14ª	$0.65\pm0.20^{b}$	$0.40{\pm}0.00^{a}$	$0.50 \pm 0.17^{b}$		
Alkanals	$0.15{\pm}0.00^{a}$	$0.46{\pm}0.02^{b}$	$0.14 \pm 0.00^{a}$	$0.29{\pm}0.04^{b}$	$0.17 \pm 0.00^{a}$	$0.43{\pm}0.00^{b}$		
E,Z-2,4-alkadienals	$0.12{\pm}0.01^{b}$	-	0.11±0.01 <sup>b</sup>	-	$0.08 {\pm} 0.00^{\rm b}$	-		
4-hydroxy-E-2-alkenals	$0.05{\pm}0.00^{\rm b}$	-	0.03±0.01b	-	$0.02{\pm}0.00^{b}$	-		
E,E-2,4-alkadienals	$0.50{\pm}0.00^{\rm a}$	$0.39{\pm}0.03^{\rm b}$	0.42± 0.01 <sup>a</sup>	$0.26 {\pm}~ 0.07^{\ b}$	$0.34{\pm}~0.00^{\rm a}$	$0.29{\pm}0.00^{b}$		
E-2-alkenals	$0.20{\pm}0.01^a$	$0.07{\pm}0.02^{\rm b}$	0.19± 0.01 <sup>a</sup>	$0.05{\pm}0.05^{\:b}$	$0.17{\pm}~0.01^{\text{ a}}$	$0.10 \pm 0.02$ <sup>a</sup>		

Abbreviations: Z,E-CD-OUH, cis,trans-conjugated double bonds associated with hydroperoxy group in octadecadienoic acyl groups and fatty acids; Z,E-CD-OH: cis,trans- conjugated double bonds associated with hydroxy group in octadecadienoic acyl groups and

### CONCLUSIONS

In short, in this study it was observed that during *in vitro* digestion of potatoes fried in non-enriched sunflower oil a higher degradation of linoleic chains and a higher generation of certain oxidation products occurred, in comparison to potatoes fried in oils enriched with olive by-product extracts.

These findings suggest that the addition of these extracts from *Chetoui* olive leaves and olive mill wastewater to frying oils could potentially have an **antioxidant effect** on food lipids during digestion. Further *in vivo* digestion studies would be needed to confirm these results.