LWT - Food Science and Technology 206 (2024) 116574

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Addition of olive by-product extracts to sunflower oil: Study by ^{1}H NMR on the antioxidant effect during potato deep-frying and further *in vitro* digestion

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ARTICLE INFO

Keywords:
Deep-frying
In vitro gastrointestinal digestion
Lipids
Olive extracts
Oxidation
Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy

ABSTRACT

Potatoes were fried in sunflower oil enriched or not with *Chetoui* olive by-product extracts (leaves and olive mill wastewater), and afterwards submitted to *in vitro* gastrointestinal digestion. Frying oils and fried potato lipids before and after digestion were analyzed using proton Nuclear Magnetic Resonance (^{1}H NMR) spectroscopy. Potential differences on lipid composition, oxidative status and after digestion also lipolysis degree, were studied. During deep-frying with oil replenishment, a higher generation of aldehydes was observed in non-enriched oils. During digestion, in the lipids of digested potatoes fried in non-enriched oil a higher degradation of linoleic chains and a higher generation of *cis,trans*-hydroperoxy- and *cis,trans*-hydroxy-octadecadienoates (primary oxidation compounds) and of alkanals was observed. A slightly higher lipolysis degree was reached in the lipids of potatoes fried in enriched oils. These findings suggest that the addition of both extracts could exert a potential antioxidant effect during frying and digestion, enhancing the quality, safety and nutritional value of fried food.

1. Introduction

Olea europaea L. is a crop of major agricultural importance in the Mediterranean basin. Despite the significant positive impact on the economy, society and culture of that area, olive industry leads to some environmental problems, particularly with regard to the large quantity of pollutant by-products generated that also pose an economic burden to manufacturers (Gullon et al., 2020). Among the several residues, olive mill wastewater (OMWW) and olive leaves outstand. The former is the main liquid effluent generated during olive oil production, which is characterized by a high organic load with a high chemical and biological oxygen demand; if directly discharged into soil or nearby river or sea, it causes not only oxygen depletion of water reservoirs, but also can exert toxic effects on plants and microorganisms (El-Abbassi, Kiai, & Hafidi, 2012). With regard to olive leaves, around 550−1000 kg/ha might be accumulated during pruning of the trees and the cleaning of olives (Contreras et al., 2019).

In order to achieve a more profitable and sustainable olive oil industry, the value-enhancement of olive by-products as sources of bioactive compounds for their potential application in the food and pharmaceutical industry has become a major focus of research (Athanasiadis et al., 2023; Ciont et al., 2023; Gullon et al., 2020; Ribeiro et al., 2021; Vidal, Moya, Alcalá, Romero, & Espinola, 2022). In fact, OMWW has a high content of phenolic compounds, being hydroxytyrosol and tyrosol the most abundant ones (Harzalli et al., 2022). Regarding olive leaves, it has been reported that oleuropein is quantitatively by far the major phenolic compound, regardless the variability observed in the phenolic profile due to variety, geographical zone, period of the year and climatic and soil conditions (Talhaoui, Taamalli, Gómez-Caravaca, Fernández-Gutiérrez, & Segura-Carretero, 2015).

All these compounds are known to be potent antioxidants and could be employed to increase food nutritional quality, as well as to extend the shelf life of food products (especially those rich in lipids), by preventing oxidation reactions that cause the deterioration of their sensory and nutritional quality and compromise their safety (Athanasiadis et al., 2023; Vidal et al., 2022). Several studies have reported that the

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https://doi.org/10.1016/j.lwt.2024.116574
Received 15 March 2024; Received in revised form 29 July 2024; Accepted 30 July 2024
Available online 2 August 2024

enrichment of different edible oils with phenolic extracts from olive by-products could be a cost-effective strategy to increase oil oxidative stability during storage (El Moudden et al., 2020; M'Rabet, Hosni, & Khwaldia, 2023) or to extend its usage during processing (Chiou, Kalogeropoulos, Salta, Efstathiou, & Andrikopoulos, 2009) Indeed, a previous study showed that enrichment of refined sunflower oil with OMWW extract can be a good mitigation strategy for polyunsaturated oil thermodegradation during frying of chicken and French fries, since the formation of certain oxidation products like volatile aldehydes and polymers was significantly reduced (Harzalli et al., 2022). Thus, the addition of olive by-products extracts to frying oils could lead to health benefits, as fried food with a lower content of potentially toxic compounds would be consumed.

Bearing in mind that bioactive compounds extracted from olive by-products, once added to enriched oils or foodstuffs, will be subsequently consumed, it is necessary to take a further step in the research and address the study of the potential benefits of these phenolic-rich extracts under conditions of gastrointestinal digestion. In the last decade, various studies have shown that under these particular conditions dietary lipids can undergo different chemical reactions, namely hydrolysis, oxidation, reduction, epoxidation, esterification and Maillard type reactions (Nieva-Echevarría, Goicoechea & Guillén, 2020ab). The extent of these reactions can be significantly affected by the simultaneous digestion with compounds showing antioxidant properties. To our knowledge and despite their possible health implications, the effect of the incorporation of olive by-product extracts to frying oils on lipid bioaccessibility, as well as on the nature and amount of oxidation products generated during gastrointestinal digestion, remains unknown. Recent works have been mainly focused on the study of the influence of digestion conditions on the content of main phenolic compounds from olive by-products extracts, either incorporated or not into a food product (Mercatante et al., 2022; Reboredo-Rodríguez et al., 2021; Ribeiro et al., 2021) and on the ways to increase their stability and further absorption, that is to say their bioaccessibility (Ciont et al., 2023; González et al., 2019). Only a few of them (Mercatante et al., 2022; Ribeiro et al., 2021) have also tackled potential changes on the antioxidant activity of assayed extracts due to digestion process, although *in vitro* chemical assays such as ABTS, ORAC, FRAP or DPPH, were employed. These methods have been extensively criticized because the information provided is limited and of doubtful significance (Schaich, Tian, & Xie, 2015).

In this scenario, the present study has two main objectives. Firstly, to investigate and compare the impact of oil enrichment with two phenolic-rich extracts obtained from olive leaves and OMWW corresponding to *Chetoui* variety on sunflower oil thermodegradation durying potato frying. Secondly, to study potential differences on lipid hydrolysis and oxidation during *in vitro* gastrointestinal digestion of potatoes fried in both enriched and non-enriched oils. Among Tunisian olive varieties, *Chetoui* stands out and accounts for approximately 30 % of the number of olive trees in the country (IOC, 2023). It must be noted that the use of olive industry by-products as sources of bioactive compounds can have a very positive environmental, economic and social impact. For this purpose, an advanced non-targeted technique such as Proton Nuclear Magnetic Resonance spectroscopy (^{1}H NMR) will be employed. This powerful tool not only provides a great deal of information in a simple, rapid and accurate way, but also overcomes many of the disadvantages of the techniques employed in the above-mentioned works. The study of lipid composition, lipid oxidation products and even lipolytic products can be made in one single run, which takes just some minutes. In contrast to chromatographic techniques, no chemical modification of the sample is required, neither the use of standard compounds for quantification purposes nor large amounts of polluting organic solvents, which is more environmentally friendly.

2. Materials and methods

2.1. Samples

Refined sunflower oil (Jadida brand, Medoil company, Tunisia) and potatoes of Spunta variety were purchased from a local supermarket.

2.2. Obtention of olive by-products extracts

The olive leaves and OMWW were collected from the "Bettaher Olive Oil" mill located in the Teboursouk region (Beja, Tunisia). The OMWW resulted from the three-phase crushing process of olives of the local *Chetoui* variety, benefiting from the Controlled Designation of Origin (AOC Teboursouk).

2.2.1. Obtention of olive leaves extract (L)

Leaves were randomly harvested from different parts of the trees and immediately transferred to the laboratory to be lyophilized after washing with distilled water. Then, they were ground to fine particles prior to extraction. A sample of 1 g of dry olive leaves was mixed with 20 mL of methanol. Hydrophilic compounds from olive leaves were extracted using an ultrasonic bath (40 kHz) (Ultrasons JP., Selecta, Barcelona, Spain) for 50 min at room temperature (25 °C). The mixture was then centrifuged for 15 min at 1693×*g* (Thermo Fisher Scientific Sorvall ST 16 Centrifuge). Afterwards, the methanolic phase was collected and filtered with a syring filter (nylon, pore size of 45 μm) for storage at −20 °C. This extraction method of *Chetoui* olive leaves is a slightly modified version of that described before by Mechi, Fernández, Baccouri, Abaza, and Martín-Vertedor (2022). This L extract was characterized as in a previous study and showed a total phenolic content of 18.20 mg/mL determined by Folin-Ciocalteau method (Harzalli et al., 2022).

2.2.2. Obtention of olive mill waste water extract (W)

A liquid-liquid extraction of OMWW was performed following an acidification to pH 2 with hydrochloric acid (HCl 4.443 mol/L), and washed with hexane to remove the lipid fraction: 10 mL of OMWW was mixed with 15 mL of hexane. The mixture was vigorously shaken and centrifuged for 5 min at 1693×*g*. The phases were separated, and the washing was repeated successively two times. Extraction of phenolic compounds was then carried out with 10 mL of ethyl acetate. The phases were separated, and the extraction was repeated successively four times. The ethyl acetate was evaporated under vacuum, the dry residue was dissolved in methanol. This phenolic extract was named W. This extract was characterized in a previous study and showed a total phenolic content of 27.07 mg/mL determined by Folin-Ciocalteau method and using caffeic acid as standard (Harzalli et al., 2022).

2.3. Frying experiments

For the frying experiments, three types of sunflower oil were employed. The first one was the non-enriched sunflower oil, used as a control and named « O». The second one was sunflower oil enriched with 100 ppm of L extract, named « OL» and the third one was sunflower oil enriched with 100 ppm of W extract, named « OW». The enriched oils OL and OW were homogenized using an ultrasound bath for 45 min. Potatoes were peeled, cut into approximately uniform pieces (10x10 × 50 mm), washed and dried. For deep-frying, a domestic electric fryer (MaxiFry) was used, and the temperature was monitored using a digital thermometer (Ama-digit 14 CE th. Germany). In each frying cycle 250 g of fresh potatoes were fried for 10 min at 180 °C in 2.5 L of each kind of oil (O, OL, OW). The frying process was repeated 10 times (10 frying cycles), using new potatoes each time. The time between one frying cycle and another was 30 min, so the total duration of the potato deep-frying experiment was 6 h. This experiment was carried out in duplicate. During the frying process, the lid was kept closed. Each kind of oil was

added into the corresponding fryer (50 mL after each withdrawal), allowing a constant replenishment of the oil volume throughout the experiment. This replenishment strategy was implemented since it is a common practice during deep-frying of low-lipid porous foods, to ensure their total immersion in oil during successive frying cycles.

Used frying oils and fried potato samples selected for the subsequent studies were those corresponding to frying cycles number 5 (intermediate cycle) and 10 (final cycle). After cooling (approximately 30 min), they were stored at −20 °C until analysis (and further digestion of fried potatoes). Thus, the studied samples were the following: i) samples of non-enriched and enriched sunflower oils collected immediately after frying cycle number 5 (named O5, n = 2; OL5, n = 2 and OW5, n = 2) and frying cycle number 10 (named O10, n = 2; OL10, n = 2 and OW10, n = 2); ii) samples of potatoes fried in the corresponding non-enriched and enriched sunflower oils at frying cycle number 5 (named P5, n = 2; PL5, n = 2 and PW5, n = 2) and frying cycle number 10 (named P10, n = 2; PL10, n = 2 and PW10, n = 2).

2.4. In vitro gastrointestinal digestion experiments

The fried potato samples (4.5 g) described above, were submitted in duplicate to a static *in vitro* model that mimics digestion processes taking place in mouth, stomach and duodenum. The gastrointestinal model employed was initially developed by Versantvoort, Oomen, Van de Kamp, Rompelberg, and Sips (2005) in the National Institute for Public Health and the Environment of The Netherlands and slightly modified further to reach a lipolysis degree similar to that occurring *in vivo* (Nieva-Echevarría, Goicoechea, Manzanos, & Guillén, 2017). The full description of the model including a detailed composition of the digestive juices (saliva, gastric, duodenal and bile juices) and the references of the reagents employed for their preparation is provided in Supplementary Material (see Table S1 and Fig. S1). The digests obtained from potatoes fried during frying cycles number 5 and 10 in sunflower oil were named DP5 and DP10; those obtained from potatoes fried in sunflower oil enriched with olive leaves extract were named DPL5 and DPL10; and those obtained from potatoes fried in sunflower oil enriched with olive mill wastewater extract were named DPW5 and DPW10.

2.5. Lipid extraction of fried potatoes before and after digestion

Lipids from fried potato samples before and after digestion were extracted using dichloromethane as solvent (CH_2Cl_2, HPLC grade, Sigma-Aldrich, Burlington, MA, USA), as in previous studies (Nieva-Echevarría, Goicoechea, Manzanos, & Guillén, 2015, 2017). For undigested samples, the CH_2Cl_2 was used in a proportion of 1:2 (w/v) and assisted by an ultrasonic bath for 1 h. Afterwards, solvent was removed by means of a rotary evaporator under reduced pressure at room temperature, in order to avoid lipid oxidation. For the digested samples, a liquid-liquid extraction was done using the CH_2Cl_2 in a proportion of 2:3 (v/v). Afterwards, to ensure a complete protonation of fatty acids and/or the dissociation of the potential salts formed between fatty acids and cations, the remaining water phase was acidified to pH ≈ 2 with HCl (12.178 mol/L) and a second extraction was carried out. Both CH_2Cl_2 extracts of each sample were mixed and solvent was eliminated by means of a rotary evaporator under reduced pressure at room temperature, in order to avoid lipid oxidation. The lipid extracts obtained from fried potatoes before digestion were named as the above-mentioned potato samples (P5, PL5, PW5 and P10, PL10, PW10), and the lipid extracts obtained from the corresponding digestates were named DP5, DPL5, DPW5 and DP10, DPL10, DPW10, respectively.

2.6. Study by ^{1}H NMR of frying oils and lipid extracts of fried potatoes before and after digestion

2.6.1. ^{1}H NMR spectra acquisition

The frying oils and the lipid extracts from both undigested and digested fried potatoes were analyzed by ^{1}H NMR spectroscopy using a Bruker Avance 400 spectrometer operating at 400 MHz, as in previous studies (Guillén & Ruiz, 2003; Martínez-Yusta & Guillén, 2014; Nieva-Echevarría, Goicoechea, & Guillén, 2020b). To prepare the samples, 175 μL of each oil or lipid extract was mixed with 425 μL of deuterated chloroform ($CDCl_3$) in a 5 mm diameter ^{1}H NMR tube. The $CDCl_3$ contained a small amount (0.2 %) of non-deuterated chloroform and 0.03 % of tetramethylsilane (Eurisotop, Paris, France). Each sample was analyzed in duplicate. The relaxation delay and acquisition time were set to allow complete relaxation of the proton nuclei. This ensured that signal areas were directly proportional to the number of protons generating them. Consequently, these signal areas were used for quantitative analysis purposes. The spectra shown in Figs. 1, 2 and **3** were plotted at a fixed value of absolute intensity to be valid for comparative purposes using MNova program (Mestrelab Research, Santiago de Compostela, Spain). Assignment of the chemical shifts and multiplicities of the ^{1}H NMR signals in deuterated chloroform ($CDCl_3$) is provided in Supplementary Table S2.

2.6.2. Quantification from ^{1}H NMR spectral data

Several quantitative determinations were performed by using spectral data obtained from the recorded ^{1}H NMR spectra, because the area of each spectral signal is proportional to the number of protons that generate it, and the proportionality constant in the ^{1}H NMR spectrum is the same for all kinds of protons. Hence, it was possible to quantify different components in each sample: main lipid components (molar percentages of linoleic, oleic, and saturated plus modified chains), minor lipid component oleocanthal expressed as mmol/mol of acyl groups plus fatty acids (AG + FA), several oxidation compounds (expressed as mmol/mol AG + FA) and lipolytic species (molar percentages of triglycerides, diglycerides, monoglycerides and glycerol). The equations employed for all these determinations are the same as in previous studies (Nieva-Echevarria et al., 2015; 2017) and are provided in the Supplementary Materials Section. It must be noted that the presence of FA was negligible in frying oils and fried potato lipids before digestion.

2.7. Statistical analysis

The significance of the differences on the determinations regarding frying oils composition and lipolysis degree of the digestates were analyzed by one-way variance analysis (ANOVA) followed by Tukey's test at $p < 0.05$. The significance of the differences on the determinations regarding lipid composition and oxidative status of the lipid extracts of fried potatoes before and after digestion were analyzed by t-Student test at $p < 0.05$. In both cases SPSS Statistics 28 software was used (IBM, NY, USA).

3. Results and discussion

3.1. Effect of the addition of olive by-products extracts on sunflower oil thermodegradation during frying

Food frying is a very complex process in which several reactions take place simultaneously, not only in foods, but also in the oils used to fry them. It is well known that when submitted to repeated frying episodes, the original oil composition changes due to the migration of lipids from food (if present), and also due to the thermal degradation of both oil and

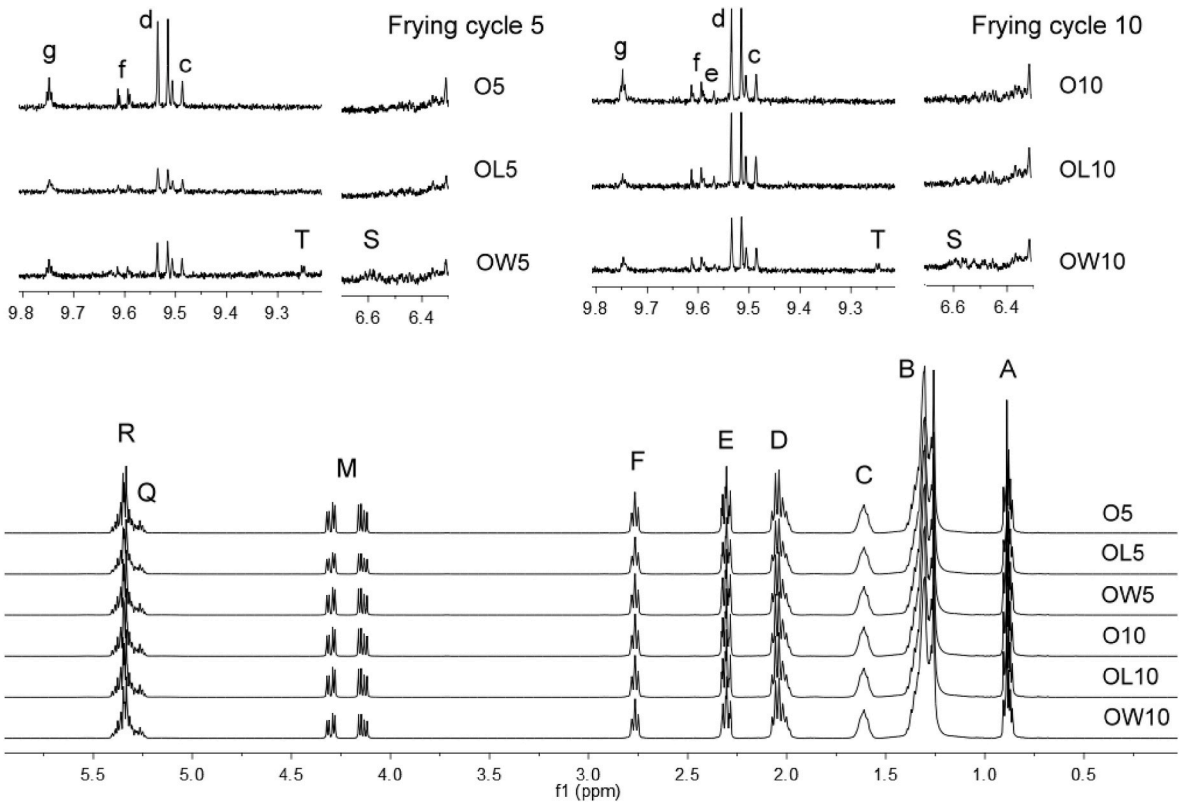

Fig. 1. [1]H NMR spectra of sunflower oil samples obtained after having been used for frying potatoes for 5 and 10 cycles, either non-enriched (O5, O10) or enriched with olive leaves extract (OL5, OL10) or with olive mill wastewater extract (OW5, OW10). Some spectral regions have been properly enlarged and the signal letters agree with those in Table S2.

migrated food lipids (Martínez-Yusta & Guillén, 2014). In this study, as selected foods are potatoes, which have a very low lipid content (below 0.5 %), no migration of potato lipids to the frying oil was expected, being oil absorption favored. Thus, thermal degradation will be the main cause of the changes occurring in frying oils composition. It should be noted that several degradation reactions (thermo-oxidation, polymerisation, hydrolysis, double bond isomerization to *trans* configuration, cyclisation, etc.), not only can affect oil major components, i.e. its triglycerides supporting acyl groups, but also its minor components of interest such as lipophilic vitamins, phytosterols, phenolics, among others (Choe & Min, 2007; Mitrea et al., 2022). Thus, in addition to the generation of potential cytotoxic compounds, bioactive molecules can be significantly lost, jeopardyzing the nutritional quality of both cooking oils and foods fried in them (Falade, Oboh, & Okoh, 2017).

3.1.1. *Changes in the frying oil profile of main acyl groups*

Fig. 1 shows the [1]H NMR spectral regions from 0 to 6 ppm of enriched (OL, OW) and non-enriched (O) sunflower oils corresponding to the frying cycles number 5 and 10. These spectra contain the typical main proton signals related to this kind of vegetable oil, and their assignment is provided in Table S2. Without further enlargement, the main proton signals present in the spectra in enriched (OL, OW) and non-enriched (O) sunflower oils corresponding to frying cycles 5 and 10, seemed quite similar. Table 1 shows the composition in main acyl groups of enriched (OL, OW) and non-enriched (O) sunflower oils before heating and after being submitted to frying cycles number 5 and 10. After repeated frying episodes of potatoes over a total period of 6 h, in all samples only a very slight degradation of linoleic chains was observed (up to 0.8 %), which is the main unsaturated acyl group in this type of oil. These results are in agreement with previous studies in which a decrease of 0.63 % of linoleic chains was observed in sunflower oil after being used for 7.5 h at 190 °C to fry dough for Spanish doughnut every 2.5 h, which is another carbohydrate-rich food with low lipid content, like potato (Martínez-Yusta & Guillén, 2016). Moreover, Aşkın and Kaya (2020) reported a decrease of 0.95 % of linoleic chains during repetitive potato deep-frying in sunflower oil submitted to 180 °C for 7 h. On the other hand, it is worth mentioning that when sunflower oil samples of a smaller volume were submitted to heating experimental conditions in the absence of food a higher degradation of linoleic groups was observed. In this sense, a decrease of 1.63 % of linoleic groups was reported by Mitrea et al. (2022) when 20 mL of oil were heated at 180 °C for 30 min, and a decrease of almost 3 % by Giuffrè, Capocasale, Zappia, and Poiana (2017) when 100 g of oil were heated at 180 °C for 120 min.

As for the potential influence of the addition of olive phenolic-rich extracts, no significant differences on linoleic degradation were observed among enriched (OL, OW) and non-enriched oils (O), regardless the number of frying cycles carried out (5 or 10). Nevertheless, it should be noted that, if any, the effect would hardly be observed considering that this essential fatty acid was scarcely degraded during the frying experiment performed. In this line, a longer experiment would have been suitable to elucidate the influence of both extracts on the changes occurring in the frying oil fatty acid profile.

Table 1
Composition of the unheated sunflower oils and of those obtained after frying cycles number 5 and 10, either non-enriched (O, O5, O10) or enriched with olive leaves extract (OL, OL5, OL10) or with olive mill wastewater extract (OW, OW5, OW10). Data regarding main acyl groups (AG) are expressed as molar percentages of total AG, and data regarding minor lipid oxidation compounds and minor components of interest are expressed as millimol of compound per mol of AG. Different letters within each row of the three columns of the same kind of sample (unheated, cycles 5 and 10) indicate a significant difference ($p < 0.05$).

Oil samples	Unheated oils			Frying cycle 5			Frying cycle 10		
	O	OL	OW	O5	OL5	OW5	O10	OL10	OW10
Main acyl groups (molar %)									
Linoleic	58.47 ± 0.21^{a}	58.71 ± 0.18^{a}	58.61 ± 0.20^{a}	57.86 ± 0.05^{a}	58.41 ± 0.20^{a}	57.91 ± 0.31^{a}	57.79 ± 0.01^{a}	57.76 ± 0.16^{a}	57.72 ± 0.04^{a}
Oleic	29.73 ± 0.18^{a}	29.55 ± 0.20^{a}	29.64 ± 0.19^{a}	29.94 ± 0.07^{a}	29.61 ± 0.09^{a}	29.74 ± 0.22^{a}	30.23 ± 0.25^{a}	29.70 ± 0.04^{a}	29.79 ± 0.21^{a}
Saturated + modified[a]	11.80 ± 0.06^{a}	11.75 ± 0.01^{a}	11.75 ± 0.01^{a}	12.20 ± 0.02^{a}	11.98 ± 0.11^{a}	12.35 ± 0.54^{a}	11.98 ± 0.24^{a}	12.54 ± 0.20^{a}	12.49 ± 0.17^{a}
Main phytosterols (mmol/mol AG)									
Δ7-avenasterol	0.94 ± 0.03^{a}	0.94 ± 0.03^{a}	0.94 ± 0.03^{a}	0.91 ± 0.03^{a}	0.91 ± 0.03^{a}	0.92 ± 0.01^{a}	0.79 ± 0.01^{a}	0.79 ± 0.01^{a}	0.83 ± 0.03^{a}
β-sitosterol + Δ5-campesterol + Δ5-avenasterol	1.87 ± 0.01^{a}	1.87 ± 0.02^{a}	1.89 ± 0.01^{a}	1.87 ± 0.03^{a}	1.88 ± 0.01^{a}	1.89 ± 0.02^{a}	1.77 ± 0.01^{a}	1.77 ± 0.00^{a}	1.77 ± 0.01^{a}
Phenolic components (mmol/mol AG)									
Oleocanthal	–	nd	0.11 ± 0.00	–	nd	0.08 ± 0.01	–	nd	0.05 ± 0.01
Lipid oxidation compounds (mmol/mol AG)									
***E,E*-2,4-alkadienals**	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.09 ± 0.02^{a}	0.51 ± 0.00^{a}	0.20 ± 0.02^{c}	0.25 ± 0.02^{b}	0.67 ± 0.04^{a}	0.48 ± 0.00^{b}	0.47 ± 0.02^{b}
***E*-2-alkenals**	0.07 ± 0.02^{a}	0.07 ± 0.01^{a}	0.06 ± 0.01^{a}	0.20 ± 0.02^{a}	0.12 ± 0.02^{a}	0.16 ± 0.04^{a}	0.24 ± 0.04^{a}	0.24 ± 0.00^{a}	0.23 ± 0.02^{a}
Alkanals	0.09 ± 0.01^{a}	0.10 ± 0.01^{a}	0.10 ± 0.01^{a}	0.16 ± 0.02^{a}	0.11 ± 0.00^{b}	0.13 ± 0.01^{b}	0.23 ± 0.00^{a}	0.09 ± 0.02^{b}	0.11 ± 0.02^{b}
***E,Z*-2,4-alkadienals**	–	–	–	0.11 ± 0.00^{a}	0.05 ± 0.00^{c}	0.08 ± 0.00^{b}	0.15 ± 0.02^{a}	0.12 ± 0.02^{a}	0.11 ± 0.04^{a}
4-hydroxy-*E*-2-alkenals	–	–	–	-	–	–	0.06 ± 0.01^{a}	0.06 ± 0.00^{a}	0.05 ± 0.00^{a}

Abbreviations: nd: not detected.
[a] Modified AG are those oxygenated and/or truncated AG generated as a result of the reactions occurring during frying.

3.1.2. Formation of thermo-oxidation products in frying oils

Due to the thermo-oxidation of linoleic acyl groups during frying, new compounds were generated. Fig. 1 also shows two enlarged ^{1}H NMR spectral regions of enriched (OL, OW) and non-enriched (O) sunflower oils corresponding to the frying cycles number 5 and 10: the region at 6.3–6.7 ppm, where the signals due to protons of conjugated dienes supported in primary oxidation compounds appear, and the region at 9.2–9.8 ppm, where the signals related to aldehydic protons can be observed. The corresponding signal assignments are shown in Table S2.

As for primary oxidation compounds, i.e. hydroperoxides (or hydroxides) supporting also *Z,E*- and *E,E*-conjugated dienic systems, no signals were observed. These results are in agreement with previous studies carried out on oils submitted to food frying (Martínez-Yusta & Guillén, 2014, 2016; Nieva-Echevarría, Goicoechea, Manzanos, & Guillén, 2016). At frying temperatures, these compounds are very unstable and, if formed, they immediately evolve to give the so-called secondary or further oxidation compounds (aldehydes, ketones, alcohols, acids, hydrocarbons, furan, etc), among which aldehydes are the predominant ones (Frankel, 2005).

On the contrary, in the spectra of all enriched and non-enriched oils corresponding to frying cycles 5 and 10 signals due to the aldehydic proton of *E*-2-alkenals (signal **c**), *E,E*-2,4-alkadienals (signal **d**), *E,Z*-2,4-alkadienals (signal **f**) and alkanals (signal **g**) were observed (see 9.2–9.8 ppm in Fig. 1). In addition, in the samples corresponding to frying cycle 10 a very small doublet due to the aldehydic proton of 4-hydroxy-*E*-2-alkenals was also noticed (signal **e**). These aldehydic protons can be supported either on small molecules or on modified acyl chains. These results are in agreement with those of Petersen, Jahreis, Busch--Stockfisch, and Fritsche (2013), who studied the formation of volatile compounds during deep-frying of French fries in several oils (at 170 °C for up to 32 h), and reported the detection of these aldehydes in sunflower oil: alkanals from 6 to 9 carbon atoms, *E*-2-alkenals of 7, 8 and 10 carbon atoms, and among *E,E*-2,4-alkadienals specially high proportions of those of 10 carbon atoms, followed by those of 7. Furthermore, other authors have also described the occurrence of 4-hydroxy-*E*-2-alkenals in sunflower oil submitted to heating at high temperatures (Guillén & Uriarte, 2012; Yuan, Shoeman, & Csallany, 2018) and to deep-frying of carbohydrate-rich food with low lipid content (Spanish doughnut), like potato (Martínez-Yusta & Guillén, 2016). As for oil samples of frying cycle 5, it was observed that in the spectra of both enriched oils (OL5, OW5) signals due to aldehydes showed lower intensities than in the spectrum of the corresponding non-enriched oil (O5), which evidenced a lower generation of aldehydes in the formers than in the later. This lower formation is especially noteworthy in the case of *E,E*-2,4-alkadienals (signal **d**), which are one of the most characteristic aldehydes generated during the frying process using sunflower oil (Petersen et al., 2013). Table 1 shows the estimated concentrations of aldehydes in the oil samples subject of study. In O5 the concentration of *E,E*-2,4-alkadienals was twice as high as in enriched OL5 and OW5. The other three kinds of aldehydes follow the same trend, showing higher concentrations in non-enriched (O5) than in enriched oils (OL5, OW5). Regarding oil samples of frying cycle 10, non-enriched O10 contained higher concentrations of *E,E*-2,4-alkadienals and of alkanals than in enriched OL10 and OW10, being the concentrations of *E*-2-alkenals and of *E,Z*-2,4-alkadienals similar in the three kinds of oils. Considering all the above, it can be stated that the addition of both kinds of *Chetoui* olive extracts to sunflower oil exerted a protective effect towards oil thermodegradation during potatoes frying. These results are in agreement with previous studies carried out by other techniques on the antioxidant activity of these extracts (Harzalli et al., 2022; Mechi et al., 2022). The protective effect observed was more pronounced at the first frying cycles, which could be related to the extent of thermal degradation undergone by olive polyphenols, as reported by other authors (Brenes, García, Dobarganes, Velasco, & Romero, 2002).

3.1.3. Degradation of bioactive compounds of interest, either naturally present or added to sunflower oil

During potato deep-frying it was also possible to follow the evolution of some phytosterols naturally present in sunflower oil, this is, Δ7-avenasterol, and β-sitosterol, Δ5-campesterol and Δ5-avenasterol (see signal assignment in Table S2). As can be observed in Table 1, regarding samples of each frying cycle no significant differences were observed among enriched and non-enriched oils. However, although in frying cycle 5 the sterols content remained unchanged, after 10 frying cycles a slight but significant degradation took place in all samples ($p < 0.05$, not shown in Table 1). These results are in agreement with those of Chiou et al. (2009), who reported that the content of phytosterols in sunflower oil enriched or not with olive leaf extracts decreased after being used to pan-fry potatoes, but that the supplementation of the oil with the extracts did not affect in a detectable degree the phytosterols oxidative deterioration.

On the other hand, as consequence of oil enrichment with phenolic-rich extracts, some signals due to protons of olive minor components of interest could be expected in the spectra of enriched sunflower oil samples. Nevertheless, they were only detected in the spectra of those enriched with the W extract, and not in those enriched with the L extract. This is probably due to the fact that in the latter samples olive minor components of interest were in very low concentrations, not detectable by ^{1}H NMR (it must be noted that, as indicated in the Materials and Methods section, the total phenolic content of W extract was almost 2-fold higher than that of L extract).

Thus, in OW, OW5 and OW10 spectra at 9.22 ppm, signal **T,** is tentatively attributed to the aldehydic proton of oleocanthal (dialdehydic form of decarboxymethyl ligstroside aglycone, *p*-HPEA-EDA), which is the elenolic acid ester of tyrosol. As this signal does not overlap with any other one, the estimated concentration of oleocanthal was given in Table 1. As can be observed, a 2-fold decrease of this phenolic alcohol was observed after 6 h of frying (cycle 10). Furthermore, at 6.60 ppm, some overlapped signals (signal **S**) due to protons of hydroxytyrosol and its derivatives plus tyrosol derivatives were detected (Ruiz-Aracama, Goicoechea, & Guillén, 2017; Tsiafoulis, Liaggou, Garoufis, Magiatis, & Roussis, 2023). Although quantification was not possible, it must be noted that the intensity of signal S was lower in OW10 than in OW5 (see Fig. 1) and much lower than in OW (not shown), indicating that these phenolics degraded at high temperatures, as occurring with oleocanthal. These results are in total agreement with previous studies, in which virgin olive oils were submitted to heating at 180 °C and it was reported that tyrosol, hydroxytyrosol and their derivatives degraded faster than other olive oil phenolics (Brenes et al., 2002; Carrasco-Pancorbo et al., 2007). This is of special interest since these phenolics have received a great deal of attention in the last decades, not only for their antioxidant activity, but also for other health properties attributed to them (El-Abbassi et al., 2012; Gullon et al., 2020).

3.2. Effect of the addition of olive by-products extracts to frying oils during in vitro digestion of potatoes fried in them

3.2.1. Differences observed on the occurrence of lipid oxidation

The oxidative status of the lipids extracted from potatoes fried during cycles number 5 and 10 in enriched and non-enriched sunflower oils, before and after being submitted to *in vitro* gastrointestinal digestion, were studied from two different approaches: on the one hand, through the evolution of the different kinds of unsaturated acyl groups (AG), and after digestion also fatty acids (FA); and on the other hand, through the formation of oxidation compounds, either primary or secondary.

3.2.1.1. Degradation extent of unsaturated acyl groups and fatty acids during digestion. The ^{1}H NMR spectra of the lipid extracts of fried potatoes before digestion (not shown) contained the same main signals related to AG as those present in the corresponding sunflower oils used to fry the potatoes (see O10, OL10 and OW10 spectra in Fig. 1). This can be explained by the well-known phenomenon of oil absorption by the potato during frying (Martínez-Yusta & Guillén, 2014; Nieva-Echevarria et al., 2016). These qualitative similarities observed in the spectra were confirmed by the quantitative data reported on Table 2, on the molar percentages of the main AG present in potato lipids before digestion (see linoleic, oleic and saturated plus modified acyl groups % in P5-10, PL5-10 and PW5-10). These values are very similar to those of the corresponding sunflower oils used to fry the potatoes (see O5-10, OL5-10 and OW5-10 in Table 1).

During *in vitro* digestion of fried potatoes, a loss of total unsaturated AG and FA occurred, especially in the case of the most unsaturated ones, this is, linoleic. As shown in Table 2, regarding samples of frying cycle 5, this decrease of linoleic chains ranged from 3.05 % DP5 to 1.41 % in DPW5, and regarding oleic chains this decrease ranged from 1.98 % in DP5 to 1.01 % in DPW5. It must be noted that this decrease was only statistically significant in DP5. The fact that the highest losses of unsaturated chains occurred in DP5, suggests that in DPL5 and DPW5 the presence of components of olive by-product extracts coming from the absorbed oils used to fry, exerted an antioxidant effect during digestion.

Table 2 also shows the molar percentages of the main AG and FA present in the lipids of digested potatoes of frying cycle 10. The decrease in linoleic chains during digestion ranged from 2.64 % in DP10 to 1.9 % in DPL10. As for the decrease in oleic chains during digestion it was quite similar in the three kinds of samples (1.20–1.27 %). The decrease in linoleic chains during digestion was statistically significant in all the samples of frying cycle 10. This could be explained by the fact that after the 10 frying cycles carried out, the antioxidant compounds initially present in the enriched sunflower oils were degraded, and that the remaining ones, if any, were not absorbed by the potatoes during frying in enough concentrations to exert any protection during subsequent digestion. In this sense, it must be noted that before digestion the

Table 2
Main components 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, DPL10, DPW5 and DPW10). Data are expressed as the molar percentages of the several kinds of acyl groups plus fatty acids (AG + FA) 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 ($p < 0.05$).

Potatoes Lipid composition (molar % of AG + FA)						
Frying cycle 5	P5	DP5	PL5	DPL5	PW5	DPW5
Linoleic	57.70 ± 0.13^a	54.65 ± 0.22^b	57.92 ± 0.07^a	56.06 ± 0.34^a	58.42 ± 0.01^a	57.01 ± 0.02^a
Oleic	29.79 ± 0.23^a	27.81 ± 0.17^b	30.14 ± 0.83^a	28.52 ± 0.01^b	29.59 ± 0.29^a	28.58 ± 0.05^a
Saturated plus modified[a]	12.50 ± 0.21^a	17.53 ± 0.40^b	11.93 ± 0.04^a	15.41 ± 0.33^b	11.98 ± 0.02^a	15.40 ± 0.03^b
Frying cycle 10	**P10**	**DP10**	**PL10**	**DPL10**	**PW10**	**DPW10**
Linoleic	57.58 ± 0.10^a	54.94 ± 0.11^b	57.47 ± 0.05^a	55.57 ± 0.08^b	57.89 ± 0.20^a	55.39 ± 0.11^b
Oleic	29.76 ± 0.43^a	28.56 ± 0.08^a	30.43 ± 0.81^a	29.16 ± 0.42^a	29.79 ± 0.44^a	28.55 ± 0.24^a
Saturated plus modified[a]	12.65 ± 0.32^a	16.49 ± 0.20^b	12.08 ± 0.02^a	15.25 ± 0.50^b	12.31 ± 0.16^a	16.04 ± 0.49^b

[a] Modified AG or FA are those oxygenated and/or truncated chains generated. The presence of FA before digestion was negligible.

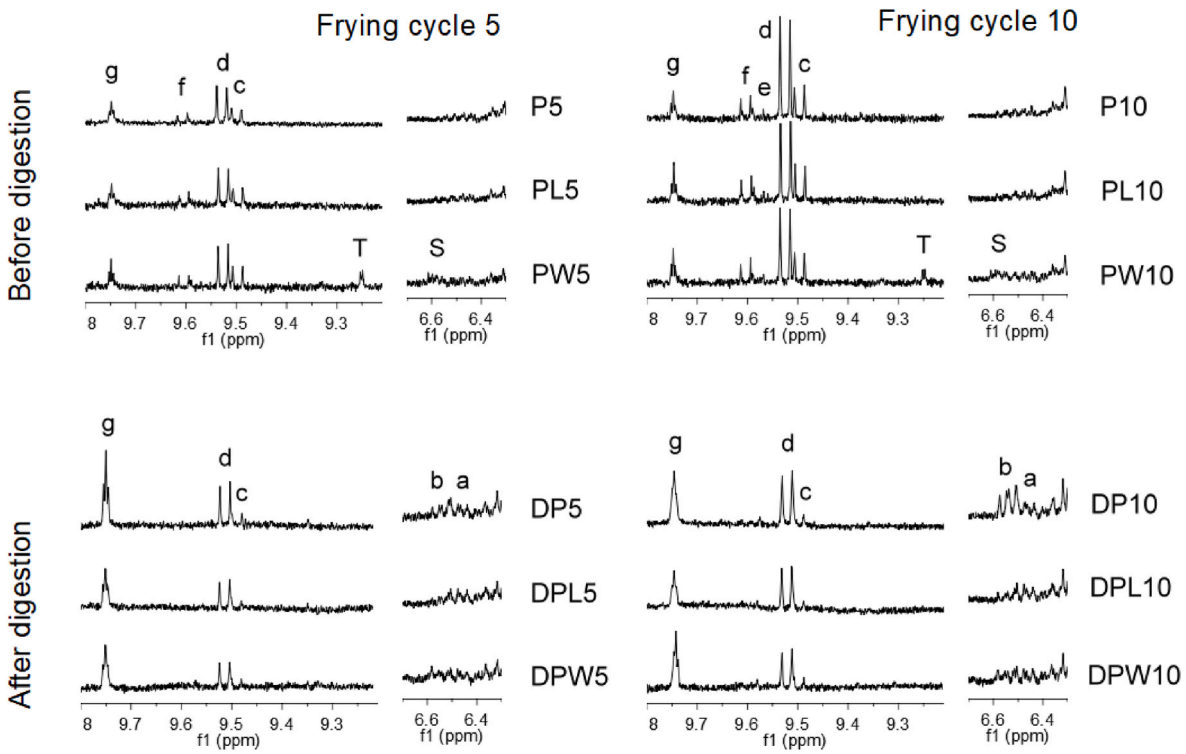

Fig. 2. ^{1}H NMR spectra of lipids extracted from potatoes fried during frying cycles number 5 and 10 in sunflower oil (P5,P10), sunflower oil enriched with olive leaves extract (PL5,PL10) and sunflower oil enriched with olive mill wastewater extract (PW5,PW10), together with those of the lipid extracts obtained after *in vitro* digestion of the corresponding potatoes (DP5, DP10, DPL5, DPL10, DPW5 and DPW10). Some spectral regions have been properly enlarged to show the signals related to main primary and secondary oxidation compounds. Signal letters agree with those given in Table S2.

estimated concentrations of oleocanthal in potato lipids PW5 and PW10 were very similar as those described above for the corresponding frying oils (see OW5 and OW10 in Table 1).

Anyway, it must be highlighted that the decrease of linoleic fatty chains observed during the *in vitro* digestion of fried potatoes is in agreement with the results reported in other studies on the *in vitro* digestion of sunflower and walnut oils (Calvo, Lozano, Espinosa-Mansilla, & González-Gómez, 2012; Nieva-Echevarria et al., 2017).

3.2.1.2. Generation of lipid oxidation products during digestion. As a result of the degradation of unsaturated lipids during digestion, oxidation products were formed. Fig. 2 shows two enlarged ^{1}H NMR spectral regions of the lipids extracted from potatoes fried during cycles 5 and 10 in enriched and non-enriched oils, before and after *in vitro* digestion of the potatoes. Regarding the spectrum of each sample, two regions are shown: at 6.3–6.7 ppm, where the signals due to protons of conjugated dienes supported in primary oxidation compounds can be observed, and at 9.2–9.8 ppm, where the signals related to aldehydic protons of secondary oxidation compounds appear. Table 3 shows the estimated concentrations of the several oxidation products detected in fried potatoes lipids before and after digestion. It can be observed that before digestion, the spectra of the lipids of all potatoes fried in enriched and non-enriched oils during frying cycles 5 and 10, are very similar to those of the corresponding frying oils (see Fig. 1). As commented above, during frying process, an oil-uptake by low-lipid content foods like potatoes takes place (Martínez-Yusta & Guillén, 2014). Thus, at 6.3–6.7 ppm no signals due to protons of conjugated dienes supported in primary oxidation compounds were detected. Moreover, in the region at 9.2–9.8 ppm in all the samples signals related to aldehydes of the same nature of those detected in the corresponding oils used to fry the potatoes were observed, this is: *E*-2-alkenals (signal **c**), *E,E*-2,4-alkadienals (signal **d**), *E,Z*-2,4-alkadienals (signal **f**) and alkanals (signal **g**). In addition, in potato lipids corresponding to frying cycle 10 also very small doublet due to 4-hydroxy-*E*-2-alkenals was observed (signal **e**).

As shown in Table 3, the estimated concentrations of these aldehydes present in potato lipids before digestion follow the same trend as in the corresponding frying oils, being *E,E*-2,4-alkadienals the aldehydes present in the highest concentrations, followed by *E*-2-alkenals, alkanals and *E,Z*-2,4-alkadienals, in this order. In addition, in P10, PL10 and PW10 4-hydroxy-*E*-2-alkenals were present in much lower concentrations. It must be noted that these concentration values of aldehydes present in potato lipids before digestion were not exactly the same as those of the corresponding frying oils (see Table 1). This can be explained by the fact that when the food absorbs frying oil, aldehydes coming from the oil once in the food can undergo transformation reactions or react with other food components, such as carbohydrates or proteins.

After submitting the potatoes to *in vitro* digestion, it can be observed in the spectra of their lipids that incipient signals due to protons located in *cis,trans*-conjugated dienes supported on octadecadienoic AG or FA having also hydroxy (*Z,E*-CD-OH, signal **a**) or hydroperoxy (*Z,E*-CD-OOH, signal **b**) groups are observed, especially in those samples corresponding to potatoes fried in non-enriched oil (DP5, DP10). The formation of these primary oxidation compounds during digestion is in agreement with previous studies that reported their generation during *in vitro* gastrointestinal digestion of sunflower oil (Nieva-Echevarria et al., 2017) or *in vitro* gastric digestion of turkey muscle tissue (Gorelik et al., 2005). The estimated concentrations of these primary oxidation compounds ranged from traces of *Z,E*-CD-OOH in DPW5 to 1.29 mmol/mol of AG + FA in DP10 (see Table 3). Regarding samples of each frying cycle, during digestion these primary oxidation compounds were generated in higher concentrations in the lipids of potatoes fried in non-enriched oils, than in those fried in enriched oils (compare DP5 with DPL5 and DPW5, and compare DP10 with DPL10 and DPW10). These results confirm the above-described antioxidant effect of olive by-product extracts.

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). Data are expressed as millimol of compound per mol of acyl groups plus fatty acids (AG + FA) present in the potato 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 ($p < 0.05$).

Potatoes Lipid oxidation compounds (mmol/mol AG + FA)						
Frying cycle 5	P5	DP5	PL5	DPL5	PW5	DPW5
Z,E-CD-OOH	–	0.47 ± 0.02	–	0.26 ± 0.02	–	tr
Z,E-CD-OH	–	0.51 ± 0.02	–	0.45 ± 0.02	–	0.36 ± 0.03
E,E-2,4-alkadienals	0.24 ± 0.08^{a}	0.21 ± 0.03^{a}	0.24 ± 0.06^{a}	0.13 ± 0.02^{b}	0.23 ± 0.02^{a}	0.19 ± 0.02^{a}
E-2-alkenals	0.10 ± 0.01^{a}	0.08 ± 0.05^{a}	0.13 ± 0.03^{a}	0.03 ± 0.02^{b}	0.13 ± 0.03^{a}	0.10 ± 0.02^{a}
Alkanals	0.16 ± 0.03^{a}	0.48 ± 0.10^{b}	0.14 ± 0.03^{a}	0.29 ± 0.04^{b}	0.14 ± 0.04^{a}	0.31 ± 0.09^{b}
E,Z-2,4-alkadienals	0.07 ± 0.01	–	0.08 ± 0.01	–	0.06 ± 0.01	–
4-hydroxy-*E*-2-alkenals	–	–	–	–	–	–
Frying cycle 10	**P10**	**DP10**	**PL10**	**DPL10**	**PW10**	**DPW10**
Z,E-CD-OOH	–	1.29 ± 0.05	–	0.34 ± 0.10	–	0.46 ± 0.13
Z,E-CD-OH	–	0.56 ± 0.04	–	0.45 ± 0.09	–	0.47 ± 0.17
E,E-2,4-alkadienals	0.51 ± 0.04^{a}	0.34 ± 0.03^{b}	0.42 ± 0.10^{a}	0.26 ± 0.07^{b}	0.34 ± 0.06^{a}	0.29 ± 0.04^{a}
E-2-alkenals	0.20 ± 0.01^{a}	0.07 ± 0.02^{b}	0.19 ± 0.01^{a}	0.05 ± 0.01^{b}	0.17 ± 0.01 ^{a}	0.08 ± 0.02^{b}
Alkanals	0.15 ± 0.03^{a}	0.46 ± 0.02^{b}	0.17 ± 0.04^{a}	0.29 ± 0.04^{b}	0.17 ± 0.05^{a}	0.43 ± 0.07^{b}
E,Z-2,4-alkadienals	0.12 ± 0.02	–	0.12 ± 0.01	–	0.08 ± 0.01	–
4-hydroxy-*E*-2-alkenals	0.05 ± 0.01	–	0.04 ± 0.01	–	0.02 ± 0.00	–

Abbreviations: Z,E-CD-OOH: *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 fatty acids, tr: traces.

Furthermore, after *in vitro* digestion, signals due to aldehydic protons were also observed in all the spectra of potato lipids (see Fig. 2). However, only those signals due to *E*-2-alkenals (signal **c**), *E,E*-2,4-alkadienals (signal **d**) and alkanals (signal **g**) were detected. Signals related to the aldehydic proton of *E,Z*-2,4-alkadienals (signal **f**) and in samples corresponding to frying cycle 10 also of 4-hydroxy-*E*-2-alkenals (signal **e**), were not observed after digestion. As for *E,E*-2,4-alkadienals and *E*-2-alkenals, in some cases their concentrations remained very similar, but in most of the samples it significantly decreased after digestion (see Table 3). This suggests that they remained unaltered after the *in vitro* digestion process or they reacted with the various components of the digestive juices, so disappearing from the samples. On the contrary, in all the samples the concentration of alkanals significantly increased after digestion. The formation of alkanals was higher during the digestion of potatoes fried in non-enriched oils than in that of potatoes fried in enriched oils, as can be observed in Table 3 and Fig. 2. This result is in agreement with the above-described higher formation of primary oxidation compounds during the digestion of potatoes fried in non-enriched oils, than in that of potatoes fried in enriched oils. To our knowledge only two previous studies tackled the influence of olive oil phenolic-rich fractions on the advance of lipid oxidation during *in vitro* gastric and gastrointestinal digestion of cooked turkey meat (Martini, Cavalchi, Conte, & Tagliazucchi, 2018; Tirosh, Shpaizer, & Kanner, 2015). However, controversial results were reported, this is, none (Tirosh et al., 2015), and either antioxidant or prooxidant effects depending on the assayed dosage (Martini et al., 2018). This could be due to the selected markers and unspecific methodologies for monitoring lipid oxidation progress: concentration of hydroperoxides measured by FOX-2 assay and concentration of Thiobarbituric Acid Reactive Substances (TBARS) determined by TBARS test.

3.2.1.3. Decrease of olive phenolic components and evolution of phytosterols during digestion. It is noteworthy that before digestion in the spectra of the lipids of potatoes fried in the sunflower oils enriched with the olive mill wastewater extract (PW5, PW10) signal **S** due to certain dienic protons of hydroxytyrosol and its derivatives plus tyrosol derivatives, and signal **T** due to the aldehydic proton of oleocanthal were observed (see Fig. 2). This evidences that these olive-derived components were also absorbed by the potatoes during frying. In this context, several authors have pointed out that these absorbed oil minor components can have positive effects not only on fried food quality, but also on human health (Chiou, Kalogeropoulos, Boskou, & Salta, 2012).

Nevertheless, after digestion the above-mentioned signals **S** and **T** were not observed in the spectra of DPW5 and DPW10. This fact is probably related to the different polarity of these minor components and that of the solvent used for the extraction of digested lipids, and maybe also to their potential oxidation and/or aggregation with proteins present in digestive juices (Nieva-Echevarria et al., 2020b).

Finally, it must be noted that regarding the bioaccesibility of phytosterols after digestion, the signal of Δ7-avenasterol remained

Table 4
Molar percentages of the different glyceryl structures present in the lipid extracts of the digestates corresponding to potatoes fried during frying cycles number 5 and 10 in sunflower oil, either non-enriched (DP5, DP10) or enriched with olive leaves extract (DPL5, DPL10) and olive mill wastewater extract (DPW5, DPW10). Different letters within each row indicate a significant difference ($p < 0.05$).

Glyceryl structures (molar %)	DP5	DPL5	DPW5	DP10	DPL10	DPW10
TG	18.1 ± 2.2^{b}	12.9 ± 1.0^{ab}	13.4 ± 2.6^{ab}	20.0 ± 1.2^{b}	11.4 ± 4.1^{ab}	8.6 ± 0.3^{a}
1,2-DG	17.0 ± 2.2^{a}	18.7 ± 2.1^{a}	18.1 ± 0.2^{a}	19.0 ± 0.7^{a}	21.3 ± 1.4^{a}	19.3 ± 0.0^{a}
1,3-DG	0.3 ± 0.5^{a}	0.6 ± 0.0^{a}	0.9 ± 1.0^{a}	1.2 ± 0.0^{a}	0.6 ± 0.1^{a}	0.6 ± 0.2^{a}
2-MG	30.5 ± 0.5^{a}	30.3 ± 3.2^{a}	26.8 ± 3.1^{a}	25.7 ± 0.0^{a}	29.8 ± 3.1^{a}	29.3 ± 3.9^{a}
1-MG	2.2 ± 1.5^{a}	2.4 ± 0.5^{a}	3.0 ± 0.5^{a}	1.9 ± 0.8^{a}	1.3 ± 0.4^{a}	2.0 ± 0.5^{a}
Gol	31.9 ± 2.6^{a}	35.1 ± 0.5^{a}	37.9 ± 1.1^{a}	32.1 ± 1.1^{a}	35.7 ± 2.1^{a}	40.1 ± 4.0^{a}
LB (%)	70.3 ± 1.0 ^{ab}	74.2 ± 2.5^{ab}	74.0 ± 3.4^{ab}	66.5 ± 1.7^{a}	74.0 ± 5.1^{ab}	78.1 ± 0.4^{b}

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

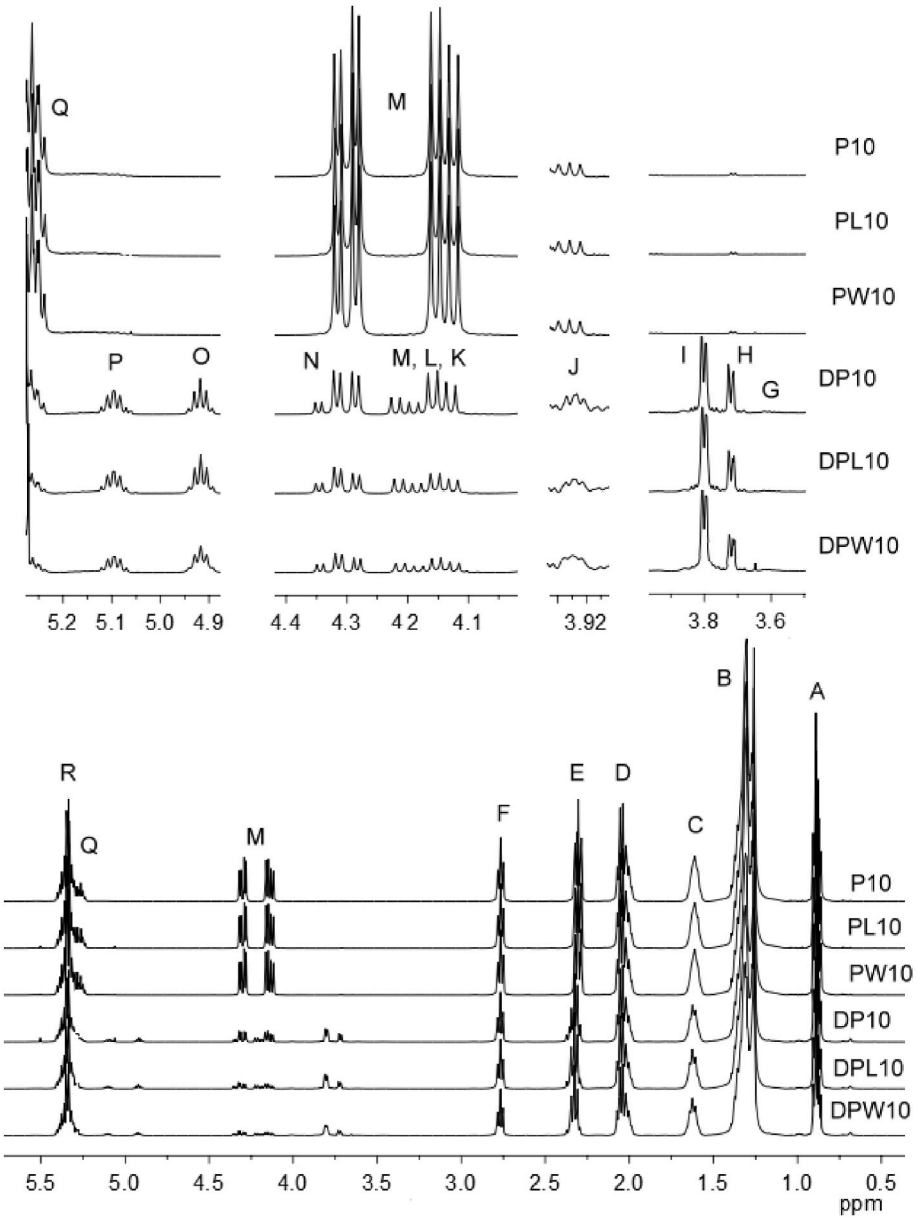

Fig. 3. ^{1}H NMR spectra of lipids extracted from potatoes fried during frying cycle number 10 in sunflower oil (P10), sunflower oil enriched with olive leaves extract (PL10) and sunflower oil enriched with olive mill wastewater extract (PW10), together with those of the lipid extracts obtained after *in vitro* digestion of the corresponding potatoes (DP10, DPL10 and DPW10). Some spectral regions have been properly enlarged and the signal letters agree with those in Table S2.

unchanged, as in previous studies (Alberdi-Cedeño, Ibargoitia, & Guillén, 2020). The evolution of β-sitosterol, Δ5-campesterol and Δ5-avenasterol could not be studied, because their signal overlapped with that of cholesterol present in in digestive bile juice.

3.2.2. *Differences observed on lipid bioaccesibility*

The lipids of potatoes fried in the three types of sunflower oil at the frying cycles 5 and 10 were almost exclusively composed by triglycerides (TG), which accounted for 98.7 ± 0.2 %. Together with them, 1,2-diglycerides (1,2-DG) were also present, although in very small amount (1.3 ± 0.1 %). However, these glycerides were subjected to the hydrolytic action of lipases during *in vitro* gastrointestinal digestion process, yielding as consequence new glyceryl species like monoglycerides (MG) and glycerol (Gol). In order to examine potential differences on the advance of lipolysis among fried potato samples subject of study, the profile of the hydrolytic products present in each type of digests was investigated by ^{1}H NMR, following the same methodology as in previous studies (Nieva-Echevarría et al., 2015). Quantitative data corresponding to the estimated molar percentages of the different glyceryl structures present in fried potato digests are detailed in Table 4, together with the lipid bioaccessibility value (LB %). This latter provides a more physiological point of view, and considers that for the complete absorption of a TG, only its conversion into MG and two FA is required (although further lipolysis could be possible). As an example, Fig. 3 shows the ^{1}H NMR spectra of the lipids extracted from potatoes fried during cycle 10 in P10, PL10, PW10, together with the lipids of their corresponding *in vitro* digests (DP10, DPL10, DPW10).

As shown in Table 4, the molar percentage of TG substantially decreased to values ranging from ≈9 to 20 %. In all the samples, glycerol was the main glyceryl structure formed, accounting for ≈32–40 % of total glyceryl structures, followed by 2-MG and 1,2-DG, which is in agreement with the regiospecificity of the digestive lipases employed (Desnuelle & Savary, 1963). Thus, lipid bioaccessibility of fried potatoes varied from 70 to 80 %. The overall advance of hydrolysis obtained is

much higher than that reported in a previous study tackling the *in vitro* digestion of fried food (Liu et al., 2020). In this latter, the authors observed that the percentage of TG decreased only from 87.0 to 83.4 after submitting fried clams to a standardized gastrointestinal digestion protocol.

Changes provoked by digestion process could be also easily inferred from the observation of Fig. 3. As for the non-digested samples (P10, PL10, PW10), signals **M** and **Q** due to protons of the glyceryl backbone of TG were the only proton signals easily detectable in the spectral region ranging from approximately 3.55 to 5.25 ppm whereas their intensity greatly decreased in the case of the digestates (DP10, DPL10, DPW10). Instead, in the spectra of these latter samples, proton signals of certain partial glycerides arose showing high intensity, that is to say, signals **H**, **N** and **O** due protons of the glycerol backbone of 1,2-DG, and signals **I** and **P** due to the same kind of protons but in 2-MG. Although detected, signals due to 1-MG (signals **G**, **J** an **L**) and to 1,3-DG (signal **K**) were hardly visible without further enlargement. The presence of negligeable amounts of these partial glycerides (see quantitative data in Table 4) can be explained by the occurrence isomerization reactions of 2-MG and 1,2-DG, respectively, during intestinal step. Previous authors have indicated that in aqueous media, this reaction might be favored by neutral or alkaline pH and moderate temperatures (Miettinen & Siurala, 1971).

Besides common features of the lipids of digestates, it should be noticed that lipid bioaccessibility was higher in digested potatoes fried in enriched oils, than in those fried in non-enriched oils, being this difference statistically significant ($p < 0.05$) in the case of samples showing a higher advanced oxidation level, that is to say digestates from frying cycle 10 (compare LB % in DP10 with that of DPW10). The differences observed on lipid bioaccessibility can be mostly explained by the differences on the molar percentages of TG and of Gol among the samples. Indeed, as Table 4 shows, TG % was 2-fold higher in DP10 than in DPL10 and DPW10; in relation to samples from the frying cycle 5, although not significant, this difference was of 1.5-fold order. This can also be deduced from the observation of Fig. 3 when comparing the intensity of signals **M** and **Q** due to TG in DP10 spectrum with that in DPL10 and DPW10 spectra. On the contrary, Gol % was lower ($p > 0.05$) in DP5 and DP10 than in the rest of the samples. Likewise, lipolysis advance was slightly lower in DP10 than in DP5 (see LB % in Table 4, 66.5 *vs* 70.3 %).

The results obtained clearly highlight that digestive lipases were slightly less active during digestion of potatoes fried in non-enriched oils than during that of potatoes fried in enriched oils, which may be due to the higher oxidation level of the formers than the latters. As commented above, DP5 and DP10 showed higher concentrations of aldehydes than DPL5 and DPW5 and that DPL10 and DPW10, respectively. Because of the high reactivity of their aldehydic functional groups, these oxidation products could react with proteins, among which digestive lipases can be cited, impairing their activity (Márquez-Ruiz, Guevel, & Dobarganes, 1998). Previous studies also reported a lower hydrolysis level during *in vitro* digestion of heated oils as the initial oxidation status of the starting oil increased (David, Sánchez-Muniz, Bastida, Benedi, & González-Muñoz, 2010; Nieva-Echevarria et al., 2017).

4. Conclusions

In conclusion, the enrichment of sunflower oil with both *Chetoui* olive by-product extracts provoked a decrease in the extent of oxidation reactions, not only during potato frying, but also during the *in vitro* digestion of fried potatoes, being higher as higher the total phenolic content of the extract is. During frying process, this effect was evidenced by the lower generation of aldehydes in enriched than in non-enriched oils, especially during the first frying cycles. During *in vitro* digestion, this effect was evidenced by the lower degradation of linoleic chains and the lower generation of *cis,trans*-hydroperoxy- and *cis,trans*-hydroxy-octadecadienoates (primary oxidation compounds) and of alkanals in the lipids of digested potatoes fried in enriched oils, in comparison with those of potatoes fried in non-enriched oils. Moreover, a higher lipid bioaccessibility was reached in the digestion of potatoes fried in enriched oils than in non-enriched oil. Thus, the addition of both extracts to frying oils could be a suitable strategy for improving the quality, safety and nutritional value of fried foods. Considering the potential antioxidant activity of both extracts, it would be considered of great interest to further optimize the extraction process of both extracts on a larger scale, as well as to get further knowledge on their effect under *in vivo* digestion conditions. It must be emphasized that this interest on potential antioxidant compounds can be considered even greater if they are obtained from by-products of the agri-food industry, in this case olive industry, because this reuse of olive by-products as source of high-added value compounds would provide benefits not only from an environmental point of view, but also from an economic and social point of view.

Finally, it must be noted that ^{1}H NMR is a very useful technique for the evaluation of the effectiveness of antioxidant compounds, because it provides a global view of the changes occurring in several lipidic main and minor components, either initially present or generated during oxidation or lipolysis. All this qualitative and quantitative information is obtained in a single run which takes just some minutes.

CRediT authorship contribution statement

Zina Harzalli: Writing – original draft, Investigation, Formal analysis. **Barbara Nieva-Echevarria:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Andrea Martinez-Yusta:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Imen Oueslati:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Wafa Medfai:** Writing – review & editing. **Ridha Mhamdi:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Encarnacion Goicoechea-Oses:** Writing – review & editing, Writing – original draft, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work has been supported by the research project PID 2021-123521OB-I00 (funded by the Spanish Ministry of Science, Innovation and Universities MCIU/AEI/10.13039/501100011033 and by “ERDF A way of making Europe”), as well as by the grant IT1490-22 of the Basque Government (EJ-GV). This work also was supported by the Ministry of High Education and Scientific Research of Tunisia. Open Access funding provided by UPV/EHU.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116574.

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