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Preservation of phytosterol and PUFA during ready-to-eat lettuce shelf-life in active bio-package

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ABSTRACT

Natural preservatives are used in food packages to improve the shelf life of perishable products. Carvacrol and thymol, the main components of oregano essential oil (OEO), are used in active packaging due to their antimicrobial and antioxidant properties. Here, the effect of a bioactive polylactic acid (PLA)/polybutylene succinate (PBS) package in the conservation of lettuce compounds with dietetic value is studied. Analytical pyrolysis (Py-GC/MS) was used to detect changes in dietary components such are phytosterols (PHSTs) and polyunsaturated fatty acids (PUFAs) after 1, 4 and 8 days of packaged in PLA/PBS (95:5%) films containing different OEO concentrations (2–10%). Lettuce PUFAs and PHSTs content decreased when packed in films without OEO. However, when packed in films containing 5 and 10% OEO, these bioactive components were preserved during the estimated lettuce shelf life, for up to 8 days of storage.

1. Introduction

The market of ready-to-eat leaf vegetables is rapidly growing at a global scale providing consumers with appealing products, rich in healthy and beneficial bioactive compounds. Among the most relevant nutritious components in leafy vegetables are phytosterols (Kim et al., 2015) and unsaturated/polyunsaturated fatty acids (Saini, Shang, Choi, Kim, & Keum, 2016). Lettuce is known to contain high quantities of dietary phytosterols (PHSTs) (Kaliora, Batzaki, Christe, & Kalogeropoulos, 2015) and of polyunsaturated fatty acids (PUFAs) (Saini, Shetty, & Giridhar, 2014).

Both, PHSTs and PUFAs are relevant bioactive components of vegetables known to have positive effects on health when included in the diets. Plant sterols exhibit cholesterol-lowering properties and are able to protect against cardiovascular diseases (Moreau, Whitaker, & Hicks, 2002; Weststrate & Meijer, 1998). Dietary PUFAs like α-linolenic acid (ALA) have also many beneficial effects in the control of chronic diseases i.e. inhibition of synthesis of vasoaggressive low-density lipoprotein (LDL) and acceleration of its elimination, reduction of blood pressure, prevention of cardiovascular disease and cancer (Abedi & Sahari, 2014). This has led to the development of functional foods enriched in such bioactive components like plant sterols and PUFAs (Lagarda, García-Llantas, & Farré, 2006; Volker, Weng, & Quaggiotto, 2005).

There is also interest in providing the industry with effective means for food preservation and of its nutritious beneficial properties. New trends are focusing in the development of active packaging, which can interact with the product or its environment and then improve food preservation. In general, active packaging containing essential oils (EOs) are developed to improve the shelf life of food and to avoid the undesirable flavours caused by direct addition of these substances (Gutiérrez, Sánchez, Battle, & Nerín, 2009). In this sense, oregano essential oil (OEO) is being included in these new food packaging materials due to its bioactive properties (Jouki, Mortazavi, Yazdi, & Koocheki, 2014; Wu et al., 2014) that are related to its content in bioactive monoterpenes, sesquiterpenes and phenolic compounds (Ortega-Nieblas et al., 2011) and because of their safety (Llana-Ruiz-Cabello, Pichardo et al., 2015; Llana-Ruiz-Cabello et al., 2017).

Therefore, different films containing OEO has been found useful in reducing the microbial counts of several microorganisms in food stuffs i.e. cooked salmon (Tammineni, Ünlü, & Min, 2013), cheese (Otero et al., 2014), chicken breast (Fernández-Pan, Carrión-Granda, & Maté, 2014), rainbow trout (Jouki, Yazdi, Mortazavi, Koocheki, & Khazaei, 2014) and also in lettuce (Llana-Ruiz-Cabello, Pichardo, Bermúdez

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et al., 2016). Moreover, OEO and films incorporated with this EO have shown different antioxidant activities related with the retardation of lipid peroxidation through their potent radical scavenging activity derived from their composition in carvacrol and thymol (Maisanaba et al., 2017). In fact, in a previous work we found that carvacrol, thymol, and their mixture (10:1) at low concentrations exert protective role against induced oxidative stress on Caco-2 cell lines system model (Llana-Ruiz-Cabello, Gutiérrez-Praena et al., 2015).

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Such antimicrobial and antioxidant properties of additives in bioplastics improve food preservation and consumer acceptance and this, desirably should include the preservation of the nutritional profile of the packed food. As far as we know there is no information available regarding the benefits of food packaged in active films in relation with the conservation of specific compounds with dietetic value i.e. PHSTs and PUFAs.

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Analytical pyrolysis is a tool providing a direct fingerprinting and precise information about composition, quality and additives in foods and packages. The products of pyrolysis are amenable to chromatographic separation and when combined with a mass spectrometry detector (Py-GC–MS), yields molecular information about the structure of complex mixtures of natural and synthetic macromolecular substances (González-Pérez et al., 2007; González-Pérez, Jiménez-Morillo, de la Rosa, Almendros, & González-Vila, 2015). Other well-known advantages of the technique are the requirement of small sample sizes and little or no sample preparation. This technique has been used with success to detect EOs added to synthetic and bio-based polymers (Llana-Ruiz-Cabello, Pichardo, Jiménez-Morillo et al., 2016). Major plant lipid components such are sterols and fatty acids are also easily detected by direct analytical pyrolysis (Py-GC/MS) of biomass (González-Vila, Tinoco, Almendros, & Martín, 2001, 2009; Schnitzer, McArthur, Schulten, Kozak, & Huang, 2006).

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In previous works, a polylactic acid (PLA) & polybutylene succinate (PBS) (95:5) film containing OEO was developed (Llana-Ruiz-Cabello, Pichardo, Bermúdez et al., 2016), its antioxidant properties evaluated (Llana-Ruiz-Cabello, Gutiérrez-Praena et al., 2015) and the ability of this material to reduce microbial counts of yeasts and molds in ready-to-eat salad was confirmed (Llana-Ruiz-Cabello, Pichardo, Bermúdez et al., 2016).

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In this work we use a detailed Py-GC/MS analysis performed to detect changes in food composition of the relevant dietetic compounds mono, di and polyunsaturated fatty acids (PUFAs) and PHSTs, in iceberg lettuce (*Lactuca sativa*) after 1, 4 and 8 days packaged in PLA/PBS (95:5) films containing different quantities of OEO (0, 2, 5 and 10%). The concentrations of OEO were selected according to Llana-Ruiz-Cabello, Pichardo, Bermúdez et al. (2016).

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2. Materials and methods

2.1. Bio-polymer and additives

Plastic films were made of a mixture of polylactic acid (PLA) with polybutylene succinate (PBS) (950 g kg^{-1}:50 g kg^{-1}) and extruded with variable quantities of oregano essential oil (EO). The EO was obtained from El Jarpil® (Almería, Spain), PLA (2003D extr. grade) was purchased from Nature Works LLC (Minnetonka, MN, USA) and PBS (GS PlaTM FD92WD) from Mitshubishi Chemical Corporation (Tokyo, Japan). Chemicals for the different assays were purchased from Sigma-Aldrich (Spain) and VWR International Eurolab (Spain).

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The active PLA films were obtained by melt blending in a twin-screw extruder (DSE 20-40D; Brabender, Duisburg, Germany). Different concentrations (0, 2, 5 and 10% w/w) of OEO and were fed into the barrel at L/D 10. Barrel temperatures were set at 200–205 °C working at a screw speed of 70 min^{-1}. Final average thickness of the final films was 80 μm (315 Gauge).

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2.2. Packaging and storage

Developed lettuce packages containing 5 g of iceberg salad packed in PLA films (0, 2, 5 and 10% w/w OEO) as explained in Section 2.1.

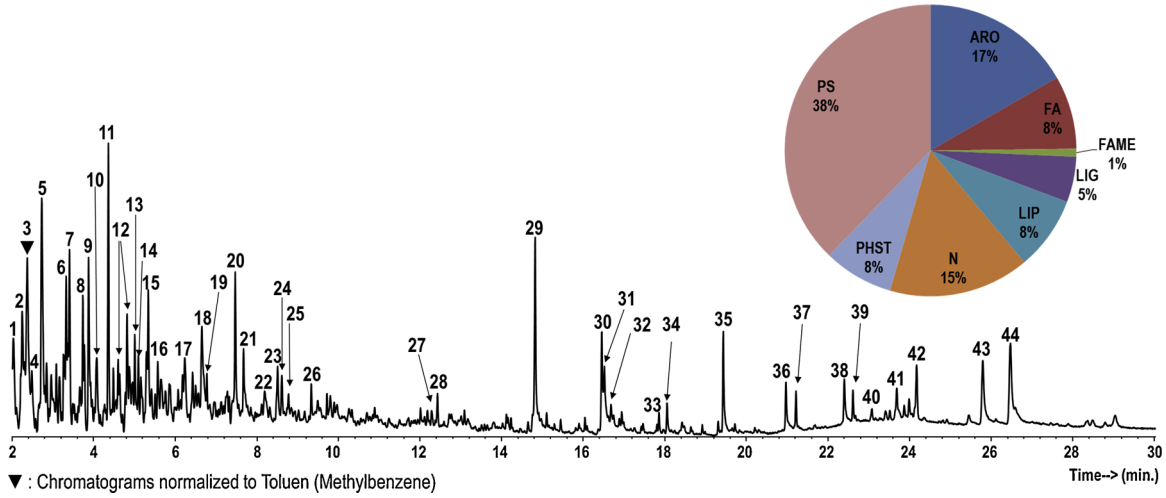

Fig. 1. Fresh lettuce fingerprinting (Py-GC/MS at 500 °C), with an indication of the relative contribution of the main compound families. Numbers on peaks corresponds to the major compounds identified and listed in Table 1. The insert units are in % of total chromatographic area.

were produced. Then, PLA bags were heat sealed with an initial modified atmosphere composed by 10% O_2, 10% CO_2 and 80% N_2. Sample bags of iceberg salad were stored at 4ºC for 8 days, simulating commercial conditions of production, transport and commercialisation.

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2.3. Analytical pyrolysis (Py-GC/MS)

Direct pyrolysis-gas chromatography–mass spectrometry (Py-GC/MS) of samples was performed using a double-shot pyrolyzer (Frontier Laboratories, model 2020i, Fukushima, Japan) attached to a GC system (Agilent Technologies, Palo Alto, CA. USA, model 6890N), 1, 4 and 8 days after packaged. Samples (0.3-0.4 mg dry lettuce biomass) were placed in crucible deactivated steel pyrolysis capsules and introduced into a preheated micro-furnace at (500 °C) for 1 min. The volatile pyrolysates were then directly injected into the GC/MS for analysis. The gas chromatograph was equipped with a low polar-fused silica (5%-phenyl-methylpolysiloxane) capillary column (Agilent J&W HP-5ms Ultra Inert, of 30 m × 250 μm × 0.25 μm film thickness. The oven temperature was held at 50 °C for 1 min and then increased to 100 °C at 30 °C min^{-1}, from 100 °C to 300 °C at 10 °C min-1, and stabilized at 300 °C for 10 min with a total analysis time of 32 min. The carrier gas was He at a controlled flow of 1 mL min^{-1}. The detector consisted of a mass selective detector (Agilent 5973 Technologies, Palo Alto, CA. USA, model 5973N) and mass spectra were acquired at 70 eV ionizing energy. Compound assignment was achieved by single-ion monitoring (SIM) for the major homologous series and by comparison with published data reported in the literature or stored in digital NIST 14 (Maryland, USA) and Wiley 7 (Weinheim, Germany) libraries.

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Table 1
Lettuce Py-GC/MS (500 °C) fingerprinting. Major compounds identified with indication of the retention time (RT: retention time in minutes), relative abundance (RA: % total chromatographic area), abundance (Ab μg/mg lettuce biomass dry weight pyrolyzed) and type (probable biogenic origin).

Ref	RT	RA	AB	Compound	Type	Ref	RT	RA	AB	Compound	Type
1	2.01	3.29	9.66	2,5-Dimethylfuran	PS	**23**	8.49	1.44	4.24	2-Pentylcyclopentanone	LIP
2	2.23	5.35	15.74	Cyclopentene, 1-methyl-	PS	**24**	8.59	0.95	2.78	1H-Indole, 3-methyl-	N
3	2.35	7.06	20.78	Toluene	ARO	**25**	8.76	0.74	2.19	Vanillin	LIG
4	2.47	2.88	8.48	Cyclopentane-1,2-diol	PS	**26**	9.32	0.45	1.33	Phenol, 2-methoxy-5-(1-propenyl)-, (E)-	LIG
5	2.71	7.38	21.71	1H-Pyrrole, 1-methyl-	N	**27**	12.27	0.32	0.93	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	LIP
6	3.31	2.82	8.28	2(5 H)-Furanone	PS	**28**	12.41	0.51	1.49	Furfural phenylhydrazone	N
7	3.39	3.82	11.22	2-Hydroxy-2-cyclopenten-1-one	PS	**29**	14.81	3.81	11.20	n-Hexadecanoic acid	FA
8	3.72	2.59	7.60	5 Methyl furfural	PS	**30**	16.44	1.98	5.81	9,12-Octadecadienoic acid (Z,Z)-	FA
9	3.85	4.40	12.94	Phenol	ARO	**31**	16.50	1.79	5.26	9,12,15-Octadecatrienoic acid (Z,Z,Z)-	FA
10	4.05	2.77	8.14	Benzofuran	PS	**32**	16.67	0.53	1.56	n-Octedecanoic acid	FA
11	4.34	4.98	14.64	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	PS	**33**	17.85	0.31	0.93	Alk	LIP
12	4.58	1.46	4.30	Phenol, 2-methyl-	ARO	**34**	18.04	0.57	1.66	Alk	LIP
12	4.80	2.39	7.03	Phenol, 4-methyl-	ARO	**35**	19.42	1.86	5.46	Alk	LIP
13	4.99	1.81	5.32	Phenol, 2-methoxy-	LIG	**36**	20.96	1.09	3.21	Alk	LIP
14	5.08	1.50	4.42	Ethanol, 2-butoxy-	LIP	**37**	21.20	0.51	1.51	Tetracosanoic acid, methyl ester	FAME
15	5.32	2.86	8.41	2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	PS	**38**	22.38	1.08	3.17	Alk	LIP
16	5.55	1.72	5.06	Benzyl nitrile	N	**39**	22.60	0.38	1.13	Hexacosanoic acid, methyl ester	FAME
17	6.22	2.40	7.07	5-Hydroxymethyldihydrofuran-2-one	PS	**40**	23.06	0.23	0.66	Sitosterol acetate	PHST
18	6.63	4.02	11.83	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	PS	**41**	23.68	1.06	3.11	Stigmasterol acetate	PHST
19	6.76	1.07	3.15	Benzenepropanenitrile	N	**42**	24.16	1.34	3.93	Stigmastan-3,5-diene	PHST
20	7.46	3.96	11.66	Indole	N	**43**	25.79	2.29	6.73	Stigmasterol	PHST
21	7.66	2.03	5.96	2-Methoxy-4-vinylphenol	LIG	**44**	26.46	2.84	8.34	Sitosterol	PHST
22	8.18	1.38	4.05	3,5-Dihydroxytoluene	ARO						

ARO: aromatics unspecific; FA: fatty acid; FAME: fatty acid methyl ester; LIG: methoxyphenol from lignin; LIP: lipid; N: Nitrogen compound; PHST: phytosterol.

Laboratories, model 2020i, Fukushima, Japan) attached to a GC system (Agilent Technologies, Palo Alto, CA. USA, model 6890N), 1, 4 and 8 days after packaged. Samples (0.3-0.4 mg dry lettuce biomass) were placed in crucible deactivated steel pyrolysis capsules and introduced into a preheated micro-furnace at (500 °C) for 1 min. The volatile pyrolysates were then directly injected into the GC/MS for analysis. The gas chromatograph was equipped with a low polar-fused silica (5%-phenyl-methylpolysiloxane) capillary column (Agilent J&W HP-5ms Ultra Inert, of 30 m × 250 μm × 0.25 μm film thickness. The oven temperature was held at 50 °C for 1 min and then increased to 100 °C at 30 °C min^{-1}, from 100 °C to 300 °C at 10 °C min-1, and stabilized at 300 °C for 10 min with a total analysis time of 32 min. The carrier gas was He at a controlled flow of 1 mL min^{-1}. The detector consisted of a mass selective detector (Agilent 5973 Technologies, Palo Alto, CA. USA, model 5973N) and mass spectra were acquired at 70 eV ionizing energy. Compound assignment was achieved by single-ion monitoring (SIM) for the major homologous series and by comparison with published data reported in the literature or stored in digital NIST 14 (Maryland, USA) and Wiley 7 (Weinheim, Germany) libraries.

3. Results and discussion

3.1. Lettuce Py-GC/MS fingerprint

The analytical pyrolysis of lettuce produced typical biomass chromatograms. A detailed pyrolysis fingerprint of lettuce is depicted in Fig. 1 and the identified compounds in Table 1. A complete list of the pyrolysis results obtained for all samples can be found in Supl. Table 1.

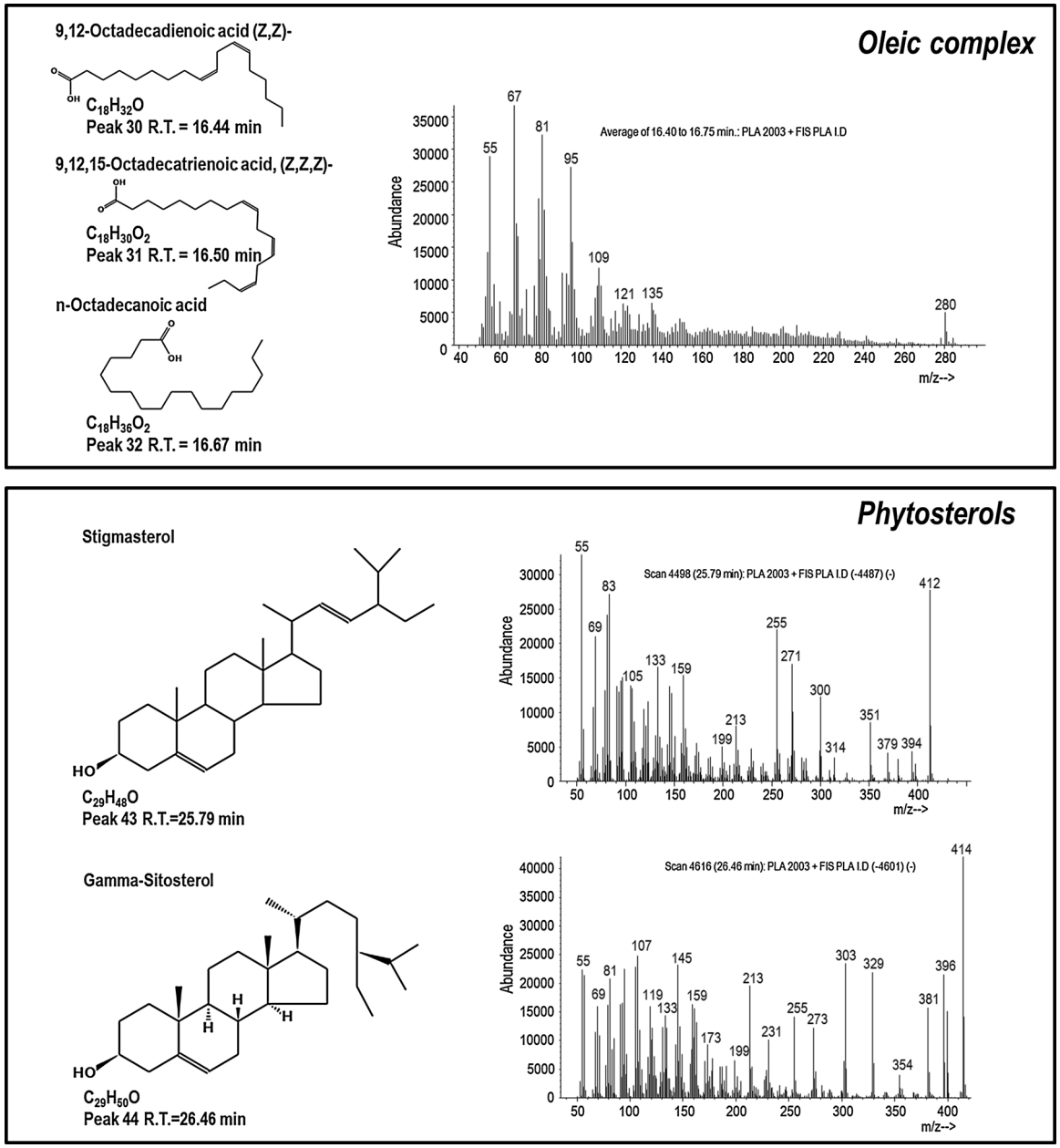

Fig. 2. Chemical structure and mass spectra of the main compounds with dietetic value detected by direct analytical pyrolysis of lettuce (Py-GC/MS at 500 °C). The mass spectra correspond to those obtained in our instrument.

The first part of the chromatogram (min 2 to 14) is dominated by pyrolysis products from lignocellulose that represent c. 43% of the total chromatographic area and included products from the major polysaccharide component (38%) i.e. furan [1,6,8,10,17,18] and cyclopentane [2,4,7,11,15] derivatives and methoxyphenols [13,21,25,26] from the polyphenolic lignin domain (5%). Most long chain lipids, mainly alkane/alkene doublets [33–36, 38] and fatty acids (FA) [29–32] are eluted in the central section of the chromatogram (min 14 to 23), with a major prominent peak of palmitic acid [29] (c. min 14.8) and an oleic complex cluster that include the polyunsaturated (PUFAs) linoleic [30] and linolenic [31] acids, the monounsaturated oleic [co-eluted] and saturated FA stearic [32] acids. The last section of the chromatogram (min 23 to 28) is dominated by triterpenes, plant sterols known as PHSTs. Other compounds identified in the pyrograms from iceberg lettuce included: aromatic structures of unknown origin (ARO), alkyl benzenes [3,22], phenol [9] and methyl phenols [12]; compounds with nitrogen (N) probably derived from the protein/polypeptide domain, nitriles [16, 19], hydrazones [28] and the heterocyclic pyrroles [5] and indoles [20,24]. Also a small proportion (1%) of methylated FAs (FAME) were identified [37, 39] probably derived from the pyrolysis of epicuticular waxes.

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Compounds with a particular dietetic interest found in the lettuce chromatograms were the bioactive PUFAs included in the oleic domain (c. min 16.5) that represented c. 4% of total chromatographic area and the PHSTs, eluted at the end of the chromatogram that represents c. 8% of total chromatographic area. The chemical structures of these compounds as well as their mass spectra are in Fig. 2. The preservation of these compounds during the shelf life of the packed lettuce was considered as the main target for this study.

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3.2. Lettuce decay with time

The evolution of lettuce pyrolysis fingerprint packed in PLA/PBS bioplastic without OEO is shown in Fig. 3. A conspicuous disappearance of peaks of particular dietetic interest: oleic and PUFAs complex as well as of PHSTs, can be observed at a first sight in the chromatogram from days 4 and 8. This confirms that iceberg lettuce rapidly and progressively lost relevant dietetic compounds during conservation time when packed in our bioplastic (PLA/PBS) without any OAO additive.

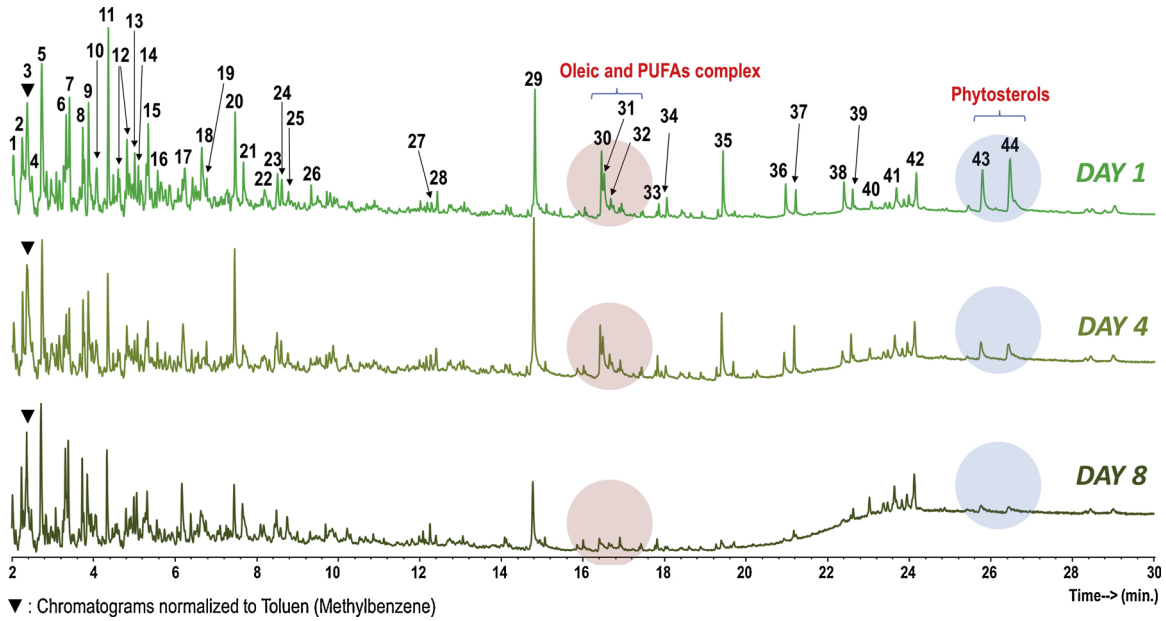

Fig. 3. Evolution of lettuce fingerprinting (Py-GC/MS at 500 °C) with time (1, 4 and 8 days) packed in PLA/PBS bioplastic (95:5) film without OEO. Numbers on peaks corresponds to the major compounds identified and listed in Table 1.

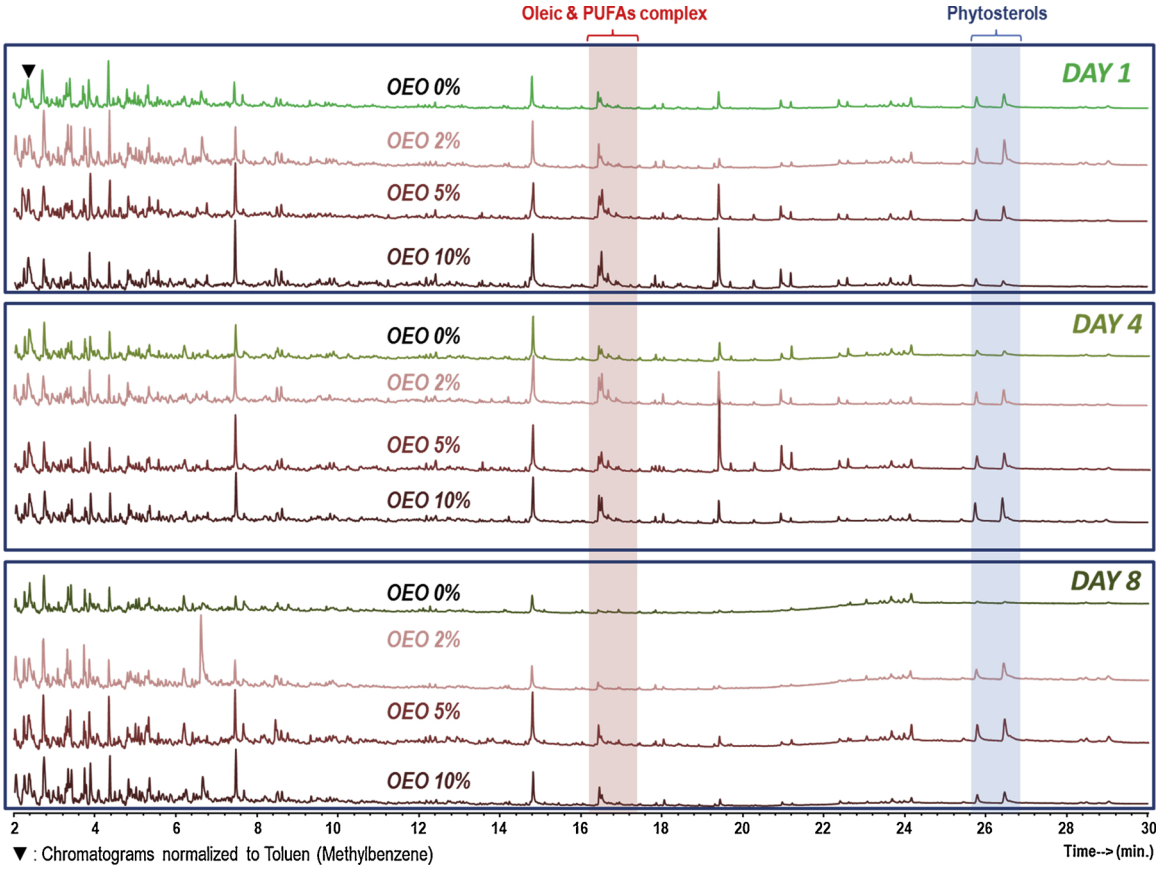

Fig. 4. Evolution of lettuce fingerprinting (Py-GC/MS at 500 °C) with time (1, 4 and 8 days) packed in PLA/PBS bioplastic (95:5) film casted with variable concentrations of OEO (0, 2, 5, 10% w/w).

The evolution of lettuce pyrolysis fingerprint packed in PLA/PBS bioplastic without OEO is shown in Fig. 3. A conspicuous disappearance of peaks of particular dietetic interest: oleic and PUFAs complex as well as of PHSTs, can be observed at a first sight in the chromatogram from days 4 and 8. This confirms that iceberg lettuce rapidly and progressively lost relevant dietetic compounds during conservation time when packed in our bioplastic (PLA/PBS) without any OAO additive.

When analysing the evolution of the lettuce pyrogram fingerprint with time and packed in the PLA/PBS bioplastic without (OEO 0%) or with variable concentrations of the OEO additive (2, 5, 10%), the preservation of the peaks corresponding to compounds of particular dietetic interest is also evident (Fig. 4). In this regard, even when the lettuce is packed in bioplastic with the minimum OEO content (OEO 2%) the relative content of PHST is preserved along the storage time and when packaged in 5 and 10% OEO containing films, both PUFAs and PHST relative contents are preserved even after 8 days of storage.

Comparing the abundance of the oleic complex (peaks 30–32) and of the major PHSTs (stigmasterol and sitosterol, peaks 43 and 44 respectively) allow us to compare the evolution of compounds with dietetic interest with time and with the different concentrations of OEO added to the package bioplastic. The evolution of dietetic relevant compounds is shown in Fig. 5A, B. Also, an attempt was made to detect possible degradation or cracking of long chain fatty acids with storage time. For this, we compared the chromatographic areas of the most abundant long chain FA (palmitic acid) with that of acetic acid. Acetic acid is found in relative high abundance in biomass pyrolyates and is usually consider a degradation product of fatty acids (Fig. 5C).

Therefore, lettuce packaged in films with high concentrations of OEO (5% and 10%) maintained the values of oleic complex nutrients at levels similar to those observed for the first day of storage (Fig. 5A) that may be attributed to an effective antioxidant activity exerted by the additive in the film. In addition, values of PHSTs experimented an increased in lettuce packaged in films containing OEO (Fig. 5B), this may reflect the occurrence of a selective preservation of PHSTs with time. Although not conclusive, the results also point to a positive effect of OEO in films by diminishing long chain palmitic acid degradation to short chain acids (Fig. 5C).

4. Conclusions

Analytical pyrolysis was found useful in characterizing lettuce composition and particularly in tracing the evolution with time of dietetic relevant components like the bioactive PUFAs and PHSTs. Using this technique, we were able to evaluate the effect of an active film bio-package (PLA/PBS) containing variable quantities of OEO in the conservation of these specific dietetic compounds in packed food. The use of active bio-packages containing OEO, allowed appropriate preservation of both PHSTs and PUFAs relative contents during the shelf life (8 days) of the packed food.

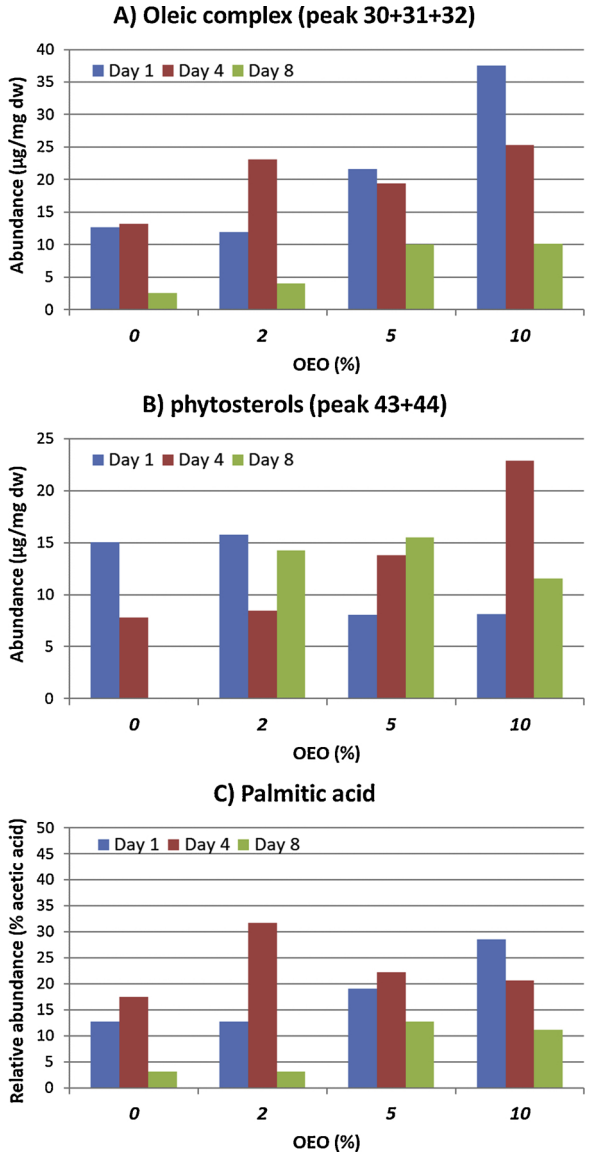

Fig. 5. (A) and (B); evolution of the abundance (µg/mg lettuce biomass dry weight) of the main compounds with dietetic value and (C); evolution of the relative abundance of palmitic acid vs acetic acid (% chromatographic area) in lettuce with conservation time (1, 4 and 8 days) packed in PLA/PBS (95:5) film containing OEO (0, 2, 5 and 10% w/w).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.fpsl.2019.100410.

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