



Article Oxidative Stability of Long-Chain Fatty Acids with Different Unsaturation Degrees into Layered Double Hydroxides

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Abstract: Nowadays, there is increasing evidence that the intake of essential fatty acids (FAs) and oleic acid has high nutritional importance. However, the vulnerability of these FAs to oxidation deserves special attention. FA oxidation may be avoided or delayed by intercalation of its anionic form in inorganic matrices as layered double hydroxides (LDH). Thus, the aim of the study was to evaluate the protective effects of MgAl LDH towards oleate (O), linoleate (L) and α -linolenate (Ln) degradation. The incorporation and the loading of different FAs in anionic form in LDH was determined by X-ray diffraction and thermogravimetric analysis (TGA), respectively. In order to study the selectivity of LDH towards the FA, the inorganic solid was equilibrated with a mixture of O, L and Ln (1:1:1). TGA and gas chromatography showed that Ln was preferentially intercalated. Free FA (FFA) and intercalated FA (IFA) were heated at 40 °C in the dark and then analyzed weekly for a maximum of 42 days. Their oxidative stability was evaluated by monitoring the primary and secondary oxidative compounds. The volatile compounds were determined by solid-phase microextraction, coupled with gas chromatography-mass spectrometry. Peroxide values were higher in FFA samples than in IFA samples, such as hexanal and *trans,trans-2,4*-heptadienal % contents. The results showed the potential of LDH intercalation for FA preservation from oxidative modification.

Keywords: oleic acid; linoleic acid; α-linolenic acid; intercalation; layered double hydroxides; hydrotalcites; oxidative stability; SPME-HRGC-MS

1. Introduction

Epidemiologic studies provide important insight into the relationship between dietary fatty acid (FA) intake and the risk of disease development. The scientific community agrees on the importance of essential fatty acids (EFAs), linoleic and α -linolenic acids, and oleic acid in human nutrition and disease prevention [1]. In recent decades, researchers have focused their attention on n-3 polyunsaturated fatty acids (PUFAs), because of their health, therapeutic, and prophylactic properties [2]. It is known that for the maintenance of optimal health conditions, n-3 and n-6 PUFA must be consumed in a balanced proportion; the optimal n-6:n-3 PUFA ratio is 4:1 [1]. The minimum intake values to prevent deficiency symptoms are estimated to be 2.5% of total energy from linoleic acid (C18:2 n-6) plus 0.5% from α -linolenic acid (C18:3 n-3) [1]. Moreover, the scientific literature has attributed many interesting properties for human health to monounsaturated FAs (MUFAs), in particular, oleic acid (C18:1 n-9), [3]. For example, oleic acid content is responsible for a reduction in blood pressure, and it has been shown that a diet rich in high-oleic sunflower oil favorably alters some blood parameters [4]. In the daily diet, natural sources of these FAs are vegetable and animal foods [5,6]. However, sometimes, recourse to the use of supplements or functional foods enriched with particular FAs is necessary [7].



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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). It should not be overlooked that unsaturated FAs are particularly prone to undergo oxidation reactions. The primary products of autoxidation are hydroperoxides and peroxy radicals, unstable compounds that decompose into secondary oxidation products (e.g., unsaturated aldehydes, ketones and other reactive substances) [8]. The greater susceptibility of PUFA in respect to MUFA to oxidative and rancidity processes further decreases organoleptic properties, nutrition value and the shelf life of food. This condition makes the development of strategies and techniques to protect n-3 and n-6 PUFA from oxidation necessary; in fact, this topic is of great importance in the food field, and is still an open challenge for researchers [9].

In order to prevent/avoid the oxidative degradation of the fats during storage, technological, and household treatments, the most popular method is the addition of antioxidants, which are often molecules obtained by chemical synthesis [10]. Nowadays, the replacement of synthetic antioxidants with natural antioxidants in the food and pharmaceutical fields is of great interest, for greater safety and acceptance by the consumer [11].

Another strategy for preventing oxidative phenomena is the complexation of organic molecules (guest), such as FAs, in organic or inorganic structures with cavities (host), a process based on host–guest chemistry (e.g., molecular encapsulation, or nanoencapsulation). For example, the cyclodextrins (CD), natural cyclic oligosaccharides, containing six (α CD), seven (β CD), eight (γ CD), or more glucopyranose moieties, are widely used as host molecules [12]. The capacity of nanoencapsulation of the oleic acid and different volatile oils in CD and the thermal stability of linoleic acid encapsulated in α - and β -CD was investigated elsewhere [13].

Among inorganic structures, layered double hydroxides (LDH) are versatile layered hosts of molecular anions with biological activity. LDH have the following general formula, $[M(II)_{1-x}M(III)_x(OH)_2](A^{n-})_{x/n}$ nH₂O, where M(II) is Mg, Zn, Co, Cu, etc., M(III) is Al, Ga, or Fe, and A^{n-} is the anion, located into the interlayer region, which counterbalances the positive charge of the lamellae. In the last two decades, LDH have found wide advanced applications in the biomedical field because of their biocompatibility. For example, they are nanovehicles for drug delivery (i.e., anti-inflammatory, antimicrobial, antacid, anticancer), DNA segments, cosmeceuticals, as well as nanoparticles for diagnoses through fluorescent or magnetic analyses [14]. Some authors have studied the intercalation of FA: lauric, myristic and palmitic acids [15], caprylic, lauric, stearic, and behenic acids [16], oleic and stearic acids [17], elaidate, oleate, linoleate, and linolenate [18]. It was also reported that LDH could be used in water treatment, as adsorbents, as ion exchangers, and in other interesting agricultural and nutraceutical applications [19,20]. Recently, novel technological approaches for the protection of ϖ -3 PUFA against oxidation processes have been comprehensively reviewed [9].

The purpose of this work was to study the ability of LDH, constituted of Mg and Al, to intercalate anions deriving from oleic, linoleic, and linolenic acids. The selectivity of LDH towards the three different anions was investigated by means of thermogravimetric analysis and gas chromatography. Moreover, the thermal stability of mixtures of intercalated FA (IFA) was investigated and compared with that of free FA (FFA).

2. Materials and Methods

2.1. Chemicals

Oleic acid (\geq 99%), sodium chloride [NaCl (\geq 99.5%)], aluminum nitrate [Al(NO₃)₃· 9H₂O (98%)] and xylenol orange disodium salt were obtained from Fluka (Chemika, Buchs, Switzerland). Linoleic acid (\geq 99%), α -linolenic acid (\geq 99%), sodium sulfate (Na₂SO₄), and ethyl acetate (99.8%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (MeOH), ethanol absolute (EtOH) and n-hexane were purchased from Panreac (Barcelona, Spain). Sodium carbonate (Na₂CO₃) and hydrochloric acid 37-38% (HCl) were purchased from J.T. Baker, Mallinckrodt Baker B.V. (Denventer, Holland). Magnesium nitrate [Mg(NO₃)₂·6H₂O] (98%) and nitric acid (HNO₃) solution was from Carlo Erba reagenti Srl (Milan, Italy). Urea (98%) was purchased from Alfa Aesar (Ward Hill, MA, USA).

2.2. Preparation of LDH

MgAl LDH in carbonate form with the formula $[Mg_{0.63}Al_{0.37}(OH)_2](CO_3)_{0.185} \cdot 0.5H_2O$ (LDH-CO₃) was prepared according to the urea method [21]. The solid was first converted into the corresponding chloride form as follows: 1 g of LDH-CO₃ was dispersed in a 0.1 mol/dm³ NaCl solution and then titrated, at room temperature (r.t.), with a 0.1 mol/dm³ HCl solution by means of a radiometer automatic titrator operating in pH stat mode, and with a pH value of 5. The recovered solid of formula $[Mg_{0.63}Al_{0.37}(OH)_2](Cl)_{0.37} \cdot 0.6H_2O$ was suspended in a CO₂-free aqueous solution 0.5 mol/dm³ of NaNO₃ (1g/50 cm³ of solution; molar ratio NO₃⁻/Cl⁻ = 10) and gently stirred for 24 h, at r.t. [22]. The recovered solid was washed three times with CO₂-free deionized water and finally dried over, at r.t., with a saturated NaCl solution (75% of relative humidity). It had a formula of $[Mg_{0.63}Al_{0.37}(OH)_2](NO_3)_{0.37} \cdot 0.6H_2O$ (LDH-NO₃).

2.3. Intercalation of O, L and Ln

The intercalation compounds were obtained by ion exchange reactions achieved by equilibrating LDH-NO₃ with solutions containing the species of interest in anionic form. The compound containing oleate (O) was prepared by equilibrating the LDH-NO₃ with an aqueous solution of 0.1 M sodium oleate (0.2 g/6.4 cm³ of solution; molar ratio O/NO₃ = 1), for 24 h at r.t. in the dark. The same experimental conditions were used for linoleate (L), linolenate (Ln) and for the mixture of the three anions (molar ratio O:L:Ln = 1:1:1). Intercalation compounds with O, L and Ln were also prepared by using a mixture of H₂O/EtOH (1:1, *v/v*) as solvent. Subsequently, the intercalation compounds will be referred to as MgAl-O, MgAl-L, MgAl-Ln, and MgAl-OLLn (IFA).

2.4. Sample Characterization

The samples were characterized by chemical analysis, X-ray powder diffraction (XRPD), and thermal analysis. XRPD patterns were taken with a Philips X'PERT PRO MPD diffractometer operating at 40 kV and 40 mA, with a step size 0.0170 2 θ degrees, and step scan 20 s, using Cu K α radiation and an X'Celerator detector.

Thermogravimetric analysis (TGA) and differential thermal analysis (DTA) were performed with Netzsch STA 449C apparatus, in air flow and with a heating rate of 10 °C/min. Metal analyses were performed with Varian 700-ES series inductively coupled plasma-optical emission spectrometers (ICP-OES), using solutions prepared by dissolving the samples in concentrated HNO₃ and properly diluted.

2.5. Heating of FFA and IFA

Seven 2 mL amber glass vials, capped with a PTFE (polytetrafluoroethylene) septum, containing a mixture of O, L, Ln in equimolar ratios (FFA), were placed in an oven maintained at 40 °C for 42 days. Another seven vials, containing IFA, were subjected to the same treatment. Every seven days, a vial containing FFA and a vial containing IFA were taken out and subjected to analyses reported in the following paragraphs (IFA samples were extracted as reported in Section 2.6. Then all samples were subjected to the analyses described in the following Sections 2.7–2.9.

2.6. Procedure of Extraction of FA from IFA

The LDH functionalized with anions deriving from FA were suspended in water and maintained under magnetic stirring at r.t. for 10 min; then, Na₂CO₃ was added. The samples were stirred for another 40 min. HCl was added until pH 3–4. Ethyl acetate was used for the extraction. The collected organic phases were washed with saturated NaCl solution, dried over anhydrous Na₂SO₄, and totally dried under nitrogen flow.

2.7. Peroxide Value Determination

The peroxide value (PV) was determined by FOX (ferrous oxidation–xylenol orange) assay and expressed as meq peroxide/kg sample [23].

2.8. Analysis of Volatiles

Volatile compounds were analyzed by solid-phase microextraction (SPME), coupled with high-resolution gas chromatography–mass spectrometry (HRGC-MS), as reported in a previous paper [6]. SPME was performed using a 50/30 μ m Divinylbenzene/Carboxen/Polydimethylsiloxane StableFlex (DVB/Carboxen/PDMS) fiber mounted in an SPME manual holder gray assembly (Supelco, Bellefonte, PA, USA). Compounds were identified by comparing their mass spectra with those reported in the Wiley138 mass spectral library and using the linear retention indexes from the literature [24].

2.9. Analysis of FA Percentage Composition by HRGC

FA percentage composition was determined after the derivatization of FA to methyl esters with methanol, as reported by Maurelli et al. (2009) [25], and successive HRGC analysis with a flame ionization detector was carried out, as reported in a previous paper [26].

2.10. Statistical Analysis

The reported results were expressed as the mean and standard deviation of duplicate samples. Microsoft Excel 2007 (Microsoft Corporation, Redmond, WA, USA) was used for data analysis.

3. Results

3.1. Characterization of IFA and FA

The compounds obtained by O, L and Ln intercalation were characterized by XRPD patterns and TGA. The solvent used in the synthetic procedure, water or water/ethanol mixture, did not dramatically affect the X-ray diffraction patterns and the thermal profile of the intercalated compounds; thus, the data related to the samples obtained from water/ethanol will be discussed. The powder diffraction data provide information about the interlayer distance and thereby indirectly gives information about the arrangement of chains in the interlayer region. Figure 1 reports the XRPD of the LDH intercalated with O, L and Ln compared with the pristine LDH-NO₃. The interlayer distance of the LDH increases markedly upon intercalation of the organic anions with respect to that of LDH-NO₃, which is 8.8 Å. Note that the pattern of LDH-NO₃ shows two reflections at low 2 theta angles ascribable to the diffraction of the reticular planes (003) and (006); the first, related to LDH metal atomic planes, gives the interlayer distance between the layers. Conversely, the first and the second peaks of the XRPD patterns of the intercalated LDH correspond to the (006)and (009) reticular planes, respectively. The interlayer distance in these compounds may be obtained by multiplying the distance of (006) planes by two; thus, interlayer distances of 30.8 Å, 30.0 Å, 30.0 Å for MgAl-O, MgAl-L and MgAl-Ln, respectively, were obtained. The patterns of MgAl-O and MgAl-L showed a small reflection at 9.9 2theta, corresponding to the nitrate phase, suggesting that nitrate anions are not completely exchanged by the organic anions. This reflection is lacking in the MgAl-Ln X-ray pattern, attesting that in this compound, a more efficient exchange occurred. However, the presence of residual nitrate anions cannot be excluded due to the formation of solid solution in which the nitrate phase is solubilized in the Ln phase [27]. The interlayer distances of the LDH intercalated with anions deriving from FA are close to that of the LDH intercalated with stearate (30.6 A) [17,28], the corresponding saturate anion. The simulation of the arrangement of the stearate anions between the LDH sheets suggested the formation of a partially interdigitated monolayer. It can be supposed that this arrangement is also adopted from O, L and Ln.



Figure 1. X-ray diffraction patterns of LDH-NO₃ (**a**) and of MgAl-O (**b**), MgAl-L (**c**) and MgAl-Ln (**d**) prepared in $H_2O/EtOH$ as solvent.

In order to study the thermal behavior of the intercalation compounds, TGA of the samples prepared in H₂O/EtOH were performed and reported in Figure 2. The results were similar for all the considered samples (MgAl-O; MgAl-L; MgAl-Ln). The first weight loss, ranging from 20 °C to 150 °C, was due to the loss of the hydration water. After this, a series of processes occurred: exothermic degradation of the organic moieties prevailed and took place concomitantly with the de-hydroxylation of the brucite layers and the decomposition of residual nitrate anions, leading to the formation of mixed metal oxides [29–32]. Notably, the position of the TGA curve, and the curves of MgAl-L and MgAl-Ln shifted towards high temperature, suggesting that the combustion of organic anions is delayed with respect to that of O in MgAl-O. The DTA curves are also different; the exothermic events in MgAl-L and MgAl-Ln occurred in a broader temperature range because the LDH lamellae produced a barrier effect to oxygen diffusion, protecting the organic species from the degradation. Moreover, it can be assumed that the packing of L and Ln into the interlayer region is more efficient to hinder the oxygen diffusion.



Figure 2. TGA and DTA curves of the indicated samples prepared in $H_2O/EtOH$ as a solvent compared with LDH-NO₃. Operative conditions: air, 10 °C/min.

The water contents obtained in MgAl-O, MgAl-L and MgAl-Ln were 5.7%, 4.0% and 4.6%, respectively. Manzi et al. (2009) prepared MgAl-LDH containing oleate, and the water content of this compound, calculated by TGA, was 7.1% (air, 20 $^{\circ}$ C/min, 50–800 $^{\circ}$ C).

The amount of anion intercalated was obtained by TGA considering the presence of nitrate to balance the positive charge of the lamellae, and the obtained values are reported in Table 1. In MgAl-O and MgAl-L, the percentage of exchanged nitrate ranged between 62% and 68%, whereas in MgAl-Ln, it was about 78%, and it seemed that $H_2O/EtOH$ solvent slightly favors the intercalation process. The higher amount of nitrate found by TGA in MgAl-O and MgAl-L than in MgAl-Ln is in agreement with the XRPD data.

Α	Intercalation	[Mg _{0.63} Al _{0.3}	mmol A/g		
	Solvent	x	у	n	LDH
Oleate	H ₂ O	0.23	0.14	0.44	1.64
	$H_2O:EtOH(1:1, v/v)$	0.24	0.13	0.45	1.68
Linoleate	H ₂ O	0.25	0.12	0.32	1.76
	H ₂ O:EtOH (1:1, <i>v/v</i>)	0.25	0.12	0.30	1.76
α-Linolenate	H ₂ O	0.28	0.09	0.38	1.88
	H ₂ O:EtOH (1:1, <i>v/v</i>)	0.29	0.08	0.38	1.92

Table 1. Molar fractions of O, L and Ln intercalated in LDH-NO3 using different solvents.

The selectivity of LDH-NO₃ towards the different anions was studied by equilibrating the solid with an equimolar solution of O, L and Ln. The XRPD, the TGA and DTA of MgAl-OLLn are shown in Figure 3. The interlayer distance of 30.6 Å is very close to those of the intercalated single anions, and does not give information on the type of anions present in the interlayer region. The DTA profile is different from the previous results, suggesting the contribution of more than one anion.



Figure 3. XRPD (a); TGA and DTA, operative conditions: air, 10 °C/min (b) of MgAl-OLLn.

TGA and HRGC-FID data were combined to determine the content of each anion in MgAl-OLLn (IFA), obtaining the following empirical formula: $[Mg_{0.63}Al_{0.37}(OH)_2](O)_{0.1}$ (L)_{0.11}(Ln)_{0.14}(NO₃)_{0.02}·0.33H₂O. The LDH showed a higher selectivity towards Ln, as expected from the data of the intercalation of single anions. This slightly preferential intercalation could be ascribed to higher interaction among the Ln anions into the interlayer region than the solution.

3.2. Oxidative Stability

FFA and IFA mixtures were heated to evaluate their thermal stability and to investigate the eventual protective role of LDH on the oxidative processes. Then, aliquots of the thermally stressed mixtures (FFA and IFA samples) were withdrawn periodically after seven days for analysis. The primary and secondary oxidative products, in addition to the FA composition, were evaluated. The evaluation of the primary oxidation products was carried out using the FOX spectrophotometric assay. Figure 4 shows the evolution of peroxide values (PV, meq/kg) during the heating of FFA and IFA samples. The determination of PV is the typical and official method to evaluate the primary oxidative lipid process, including in vegetable foods [8]. Fatty acid oxidation has been recognized as a free radical chain reaction, with the abstraction of hydrogens at the allylic position of the double bond of unsaturated fatty acids to form hydroperoxides and free radicals on a new fatty acid. Hydroperoxides are considered the primary oxidation products; therefore, the PV parameter represents an indicator of initial fat autoxidation [8,9].



Figure 4. Peroxide values (PV, mean value \pm SD) of FFA (free fatty acid; OLLn) and IFA (intercalated fatty acid; MgAl-OLLn) series during heating.

The results showed that the hydroperoxide content was always lower for the IFA series than for the corresponding FFA, at different heating times. These results are very significant because they demonstrate the protective effect of LDH on the oxidation.

In this work, the secondary oxidative compounds of the thermally stressed FA mixture were evaluated using SPME-HRGC-MS analysis, a procedure used in a previous paper to evaluate the volatile profile of vegetable foods and FA oxidative status [6,33].

Table 2 shows the main volatile categories detected for FFA and IFA series. Aldehydes were the most represented compounds in FFA and IFA series, among the many categories of volatiles. Alcohols and ketones were the second most represented category in FFA and IFA series, respectively. Moreover, alkylfurans and hydrocarbons were also detected, together with carboxylic acids in FFA series.

Table 2. Main categories of volatile compounds in FFA and IFA during heating (area percentage, mean value \pm SD).

		Time (days)						
		7	14	21	28	35	42	
Aldehydes	FFA IFA	$\begin{array}{c} 71.3 \pm 3.2 \\ 80.1 \pm 2.8 \end{array}$	$60.2 \pm 2.0 \\ 58.7 \pm 1.8$	$56.6 \pm 1.8 \\ 49.1 \pm 1.1$	$52.6 \pm 1.3 \\ 49.7 \pm 1.4$	$53.8 \pm 1.5 \\ 49.7 \pm 1.3$	$50.6 \pm 1.6 \\ 45.8 \pm 1.4$	
Ketones	FFA IFA	$\begin{array}{c} 2.4\pm0.5\\ 11.9\pm1.0\end{array}$	$6.6 \pm 0.8 \\ 16.2 \pm 1.0$	$\begin{array}{c} 4.1\pm0.5\\ 24.0\pm1.0\end{array}$	$\begin{array}{c} 3.3\pm0.6\\ 23.6\pm0.9\end{array}$	$\begin{array}{c} 3.4\pm0.6\\ 23.8\pm0.9\end{array}$	$\begin{array}{c} 3.6\pm0.5\\ 26.3\pm1.0\end{array}$	
Alcohols	FFA IFA	$\begin{array}{c} 12.6 \pm 1.1 \\ 6.0 \pm 0.5 \end{array}$	$\begin{array}{c} 11.8\pm0.9\\ 4.0\pm0.4\end{array}$	$\begin{array}{c} 12.7\pm0.9\\ 5.3\pm0.1\end{array}$	$\begin{array}{c} 12.0\pm0.8\\ 5.4\pm0.2\end{array}$	$\begin{array}{c} 11.8\pm0.7\\ 4.9\pm0.4\end{array}$	$\begin{array}{c} 10.3\pm0.1\\ 4.5\pm0.1\end{array}$	
Alkylfurans	FFA IFA	$\begin{array}{c} 2.9\pm0.3\\ 0.8\pm0.0\end{array}$	$\begin{array}{c} 5.8\pm0.5\\ 8.7\pm0.3\end{array}$	$\begin{array}{c} 6.0\pm0.2\\ 9.7\pm0.3\end{array}$	$5.6 \pm 0.1 \\ 10.2 \pm 0.3$	$\begin{array}{c} 4.9\pm0.5\\ 9.8\pm0.3\end{array}$	$\begin{array}{c} 5.2\pm0.2\\ 13.1\pm0.5\end{array}$	
Hydrocarbons	FFA IFA	$\begin{array}{c} 0.5\pm0.0\\ 1.2\pm0.1 \end{array}$	$\begin{array}{c} 1.6\pm0.5\\ 7.2\pm0.7\end{array}$	$\begin{array}{c} 2.5\pm0.5\\ 5.2\pm0.2\end{array}$	$\begin{array}{c} 1.8\pm0.5\\ 4.6\pm0.5\end{array}$	$\begin{array}{c} 1.7\pm0.2\\ 5.7\pm0.3\end{array}$	$\begin{array}{c} 3.3\pm0.3\\ 6.1\pm0.4\end{array}$	
Carboxylic acids	FFA IFA	0.7 ± 0.0	1.5 ± 0.4 -	2.7 ± 0.1	7.2 ± 0.3	6.7 ± 0.4	9.2 ± 0.6	
Others	FFA IFA	9.7 ± 0.9 -	$\begin{array}{c} 12.5 \pm 1.0 \\ 5.2 \pm 0.4 \end{array}$	$15.6 \pm 1.1 \\ 6.7 \pm 0.5$	$17.3 \pm 1.2 \\ 6.4 \pm 0.5$	$\begin{array}{c} 17.5\pm1.2\\ 6.2\pm0.4\end{array}$	$\begin{array}{c} 17.8\pm1.2\\ 4.2\pm0.3\end{array}$	

FFA, free fatty acid; IFA, intercalated fatty acid; -, not detected; , unidentified compounds.

Among alcohols, 1-penten-3-ol and 1-pentanol, typical autoxidation products of linoleic and α -linolenic acids [8], were produced during the heat treatments of FFA and IFA, whereas 3-exen-2-one and 3,5-octadien-2-one were detected among ketones in both series.

The results of Table 2 show that the maximum value for the aldehyde category was observed after seven days of treatment, both for FFA and IFA series; then, this value decreased with the increase in the heating time (42 days).

Figure 5 shows the aldehydes grouped as saturated (S), monounsaturated (M), and polyunsaturated (P) for FFA and IFA series. It can be observed that monounsaturated aldehydes are the most represented in the FFA category, whereas saturated aldehydes are the most represented in the IFA category.



Figure 5. Aldehyde content (area %, mean value \pm SD) in FFA and IFA series during the thermal treatment. S, saturated; M, monounsaturated; P, polyunsaturated.

Aldehydes, among all the compounds derived from the oxidation process, are considered the most important breakdown products and the largest contributors to flavor [33,34]. It has been reported that saturated aldehydes are more stable than unsaturated aldehydes [35], and that unsaturated aldehydes can be further oxidized and additional volatile products may be formed [34].

Regarding the aldehydes in both FFA and IFA series, hexanal, octanal, nonanal, and decanal were detected among saturated aldehydes, whereas 2-butenal, *trans*-2-pentenal, *trans*-2-hexenal, *trans*-2-eptenal, *trans*-2-octenal, *trans*-2-nonenal, *trans*-2-decenal and *trans*, *trans*-2,4-estadienal, *trans*-2,4-eptadienal and *trans*, *trans*-2,4-nonadienal were the main unsaturated aldehydes.

Propanal, pentanal, and heptanal were detected among the compounds identified in saturated aldehydes from IFA. Propanal is a typical volatile compound from α-linolenic acid oxidation, whereas pentanal and heptanal originated from the decomposition of linoleate 13- and 11-hydroperoxides. Hexanal, octanal, and nonanal are the typical volatile compounds of oleic acid oxidation, in particular, from 8-O[•], 10-O[•], and 11-O[•] [34–36].

Among saturated compounds, hexanal was particularly represented in the IFA series, but also in the FFA series. Morales et al. (1997) [37] reported that the hexanal:nonanal ratio could be an appropriate way to detect the beginning of oxidation and its evolution; they found that the ratio changed quickly from the highest values for high-quality extravirgin olive oil to the lowest for oxidized oils. In this paper, as an interesting result, the hexanal:nonanal ratio decreased for the FFA series, whereas it increased for the IFA series (data not shown), indicating a better protection of IFA with respect to FFA.

As an example, Figure 6a–c shows the trend of octanal, *trans*-2-octenal, and *trans*, *trans*-2,4-heptadienal during heating, showing lower percentage contents in IFA samples compared to the FFA series. *trans*-2-Octenal was formed from the decomposition of linoleic acid 11-hydroperoxide. Xu et al. (2017) [36] reported, for example, that *trans*-2-octenal percentage content increased with oxidation in soybean and peanut oils, although it was not found in the rapeseed and linseed oils. This difference was linked to the different FA composition; in fact, linoleic acid was more abundant in soybean and peanut oils, with respect to the other two edible oils. *trans*, *trans*-2,4-heptadienal was formed by the decomposition of α -linolenic acid 12-hydroperoxide. It has been reported that its content increased with oxidation in linseed oils, in particular, for linseed oil where α -linolenic acid is the main FA [36].

Regarding alkylfurans, 2-pentylfuran was the main alkylfuran detected in FFA, whereas 2-ethylfuran was the main for IFA. High amounts of 2-pentylfuran were detected in edible oils with relatively high linoleic acid [35].

For further investigation, HRGC-FID analysis was performed in order to evaluate the FA percentage composition during the thermal treatment of IFA and FFA series, and the results are reported in Figure 7.



Figure 6. Cont.



Figure 6. Content (area percentage, mean value \pm SD) of octanal (**a**), *trans*-2-octenal (**b**), and *trans*, *trans*-2,4-heptadienal (**c**) in FFA and IFA series during heating.



Figure 7. Fatty acid composition (area percentage, mean value \pm SD) of FFA and IFA series during the thermal treatment.

Regarding the FFA series, the FA percentage contents of oleic, linoleic and α -linolenic acids were fairly constant for the first 21 days; after that, linoleic and α -linolenic acid percentage contents decreased, reaching values of 21.5% and 7.5%, respectively, after 42 days. Clearly, the increase in oleic acid percentage content is a consequence of the percentage decrease in linoleic and α -linolenic acids.

The trend observed for the FFA mixture was expected because it is known that the unsaturation degree is inversely related to the oxidative stability of FA. Very interesting results were obtained for the IFA mixture during the heating; in fact, minimal changes in the FA percentage content were observed. In particular, after 42 days of thermal treatment, the linoleic acid percentage content was almost similar to the initial value. Regarding the percentage content of α -linolenic acid, only a slight decrease was observed, and it explains the slight increase in oleic acid percentage content. These results indicate that intercalation could represent a valid approach to protect unsaturated FAs from oxidative processes.

4. Conclusions

Layered compounds, belonging to the family of biocompatible layered double hydroxides, are versatile hosts of a wide variety of molecular anions with biological activity. The obtained results showed a high percentage of incorporation of all three FA with the preference of α -linolenate in respect to oleate and linoleate. The data indicated that inorganic sheets offer protection to labile biomolecules such as long-chain FAs with different unsaturation degrees. The results of oxidation products and the FA percentage composition showed a higher stability of IFA with respect to FFA samples. This study has provided scientific evidence for the practical applications of improvements in the oxidative stability of oleic, linoleic, and α -linolenic acid during thermal treatment. LDH offer interesting perspectives for developing new innovative inorganic–organic materials to be applied in food, nutraceutical, pharmaceutical, and cosmeceutical fields.

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