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Oxidative stability of DHA phenolic ester

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ABSTRACT

Docosahexaenoic acid vanillyl ester (DHA-VE) was synthesized from docosahexaenoic acid ethyl ester (DHA-EE) and vanillyl alcohol by a solvent-free alcoholysis process catalysed by *Candida antarctica* lipase B. Oxidative stability of pure DHA-VE and the crude reaction medium consisting of 45% DHA-VE and 55% DHA-EE were compared with that of DHA-EE under various storage conditions. Oxidation progress was followed by determination of conjugated dienes and FTIR measurements. Analyses showed that DHA-EE was rapidly oxidised under all storage conditions in comparison with DHA-VE and crude reaction medium, whatever the temperature and the storage time. The grafting of vanillyl alcohol appeared as a powerful way to stabilize DHA against oxidation. Thanks to their stability, both DHA-VE and the crude reaction medium, allowing the production of the ester, offer huge potential as functional ingredients.

1. Introduction

DHA is required for brain and eyesight development of both foetus and infant (Swanson, Block, & Mousa, 2012). For adults, DHA is involved in maintaining the normal functioning of brain and nervous system (Domenichiello, Chen, Trepanier, Stavro, & Bazinet, 2014; Kuratko, Barrett, Nelson, & Salem, 2013; Picq, Bernoud-Hubac, & Lagarde, 2013). DHA deficiency has been found in several disorders, such as Alzheimer's disease, schizophrenia, dyslexia, and some cases of attention-deficit or hyperactivity disorder (Cunnane, Chouinard-Watkins, Castellano, & Barberger-Gateau, 2013; Wu et al., 2014). Because mammals have limited ability to synthesize polyunsaturated fatty acids (PUFA), these must be supplied in the diet. The American Heart Association generally recommends a daily intake of n-3 PUFA, up to 400–500 mg of EPA and DHA, which may be reached by consuming at least two servings of oily fish per week or cod liver fish oil or encapsulated n-3 PUFA ethyl esters (EEs) (Lichtenstein et al., 2006). However, the practical use of such lipids for a preventive purpose is often limited because of their high susceptibility to oxidation, producing hydroperoxides and aldehydes responsible for the undesirable rancidity off-flavour (Arab-Tehrany et al., 2012; St. Angelo, Vercellotti, Jacks, & Legendre, 1996). In addition to these organoleptic limitations, nutritional aspects also have to be considered, as the consumption of high amounts of oxidised products is suspected to cause oxidative stress, and then to induce diseases, such as cancer, diabetes and rheumatoid arthritis (Lin, Lai, Lin, & Chiang, 2000).

The oxidative degradation of unsaturated lipids has been the subject of many studies and still today remains one major scientific topic for both researchers and industries. Under mild conditions and according to the autoxidation mechanism, molecular oxygen reacts with unsaturated fatty acid radicals, leading to primary oxidation products, namely peroxides and hydroperoxides, and then to secondary oxidation products, such as ketone and aldehyde compounds responsible for rancidity. This degradation pathway is complex and variable from one oil to another due to its dependency on fatty acid composition and conditions causing oxidation. Different strategies have been described in the literature and applied in the industry to reduce this phenomenon. A common way consists in using synthetic or natural antioxidants (Roby, Sarhan, Selim, & Khalel, 2013b; Staszewski, Pizones Ruiz-Henestrosa, & Pilosof, 2014; Wang et al., 2011). For the past few

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years, natural antioxidants, such as phenolic compounds, have increasingly been suggested as interesting alternatives to synthetic materials (Kindleysides, Quek, & Miller, 2012). The antioxidant activity of such compounds generates a lot of interest and many works have been carried out aiming to benefit from their properties in a wide range of applications (Roby, Sarhan, Selim, & Khalel, 2013a). Most often, phenols have simply been added to lipid phases in order to protect them against oxidation (Mosca, Diantom, Lopez, Ambrosone, & Ceglie, 2013; Rubilar et al., 2012). However, there are limits to this approach, since the antioxidant capacity of phenols strongly depends on their location relatively to the lipid phase (Liu, Jin, & Zhang, 2014). Another approach has been proposed that consists in combining lipids with phenols into a single structure, by forming an ester bond (Kobata, Kawaguchi, & Watanabe, 2002). The main objective was the production of lipophilic derivatives of phenols, aiming to enhance their antioxidant activity in lipid systems. Mbatia, Kaki, Mattiasson, Mulaa, and Adlercreutz (2011) found that incorporating rutin or vanillyl alcohol into a PUFA-enriched extract, through an esterification process, improved the hydrophobicity of phenolic compounds and then enhanced their antioxidant activity in lipid phases. Similar results were obtained in emulsions and biological systems with esters combining epigallocatechin and stearic, eicosapentaenoic or docosahexaenoic acids (Zhong & Shahidi, 2012).

For a given system, the choice of the right antioxidant solution requires the capacity to make a qualitative and quantitative assessment of oxidation progress. To this end, many methods have been developed, based on the determination of primary and secondary oxidation products (Barriuso, Astiasaran, & Ansorena, 2013; Shahidi & Zhong, 2005). The most commonly used consists in measuring the concentration of hydroperoxide (peroxide value, PV); nevertheless PV is not necessarily a good indicator to follow PUFA oxidation because of the high instability of hydroperoxides issued from their degradation (Cho, Miyashita, Miyazawa, Fujimoto, & Kaneda, 1987). Another method, based on the measure of absorbance at specific wavelengths, namely 232 and 270 nm, allows evaluating the content of conjugated dienes (CD) and trienes (CT) (Frankel, 2005). The above-mentioned methods have found widespread applications as routine tests to determine oxidative deterioration of lipids. However they provide a single index, which does not give information on actual chemical composition of the product, and then provides limited insight of the problem. Several years ago, the determination of epoxy compounds in thermo-oxidised oils was reported (Velasco, Berdeaux, Marquez-Ruiz, & Dobarganes, 2002). Epoxy compounds are easy to detect and this allows characterisation of low oxidation levels in various oils and food lipids; they are nevertheless time-consuming tests. In this context, vibrational spectroscopy has been considered as a useful tool for fast measurement of lipid oxidation because of its high capacity to provide detailed information about molecular systems. FTIR has been successfully used to monitor oil oxidation under moderate and accelerated conditions (Vandevoort, Ismail, Sedman, & Emo, 1994). Main changes in FTIR bands were interpreted and related to oxidation mechanism (Brys et al., 2013). Parameters related to oil oxidation were quantified, based on the determination of specific compounds, such as peroxides (Guillen & Cabo, 2000; Ruiz, Canada, & Lendl, 2001), anisidine (Dubois, vandeVoort, Sedman, Ismail, & Ramaswamy, 1996), volatile compounds (Ahro, Hakala, Kauppinen, & Kallio, 2002) and malonaldehyde (Mirghani, Man, Jinap, Baharin, & Bakar, 2002).

This work aimed: (i) to study the effect of enzymatic esterification of DHA with vanillyl alcohol on the oxidative stability of DHA, using different spectroscopic methods, and (ii) to model oxidation kinetics under different storage conditions.

2. Materials and methods

2.1. Chemicals

Lipase B, from Candida Antarctica immobilised on a macroporous acrylic resin (Novozym 435®, Novo Industry), was used to catalyse acylation reactions. DHA ethyl ester (docosahexaenoic acid ethyl ester, abbreviated as DHA-EE) and vanillyl alcohol (4-hydroxy-3-methoxybenzyl alcohol) were purchased, respectively, from KD-Pharma (Bexbach, Germany, purity 95%) and Sigma–Aldrich Chemicals (St. Louis, MO, purity 98%). All solvents were of analytical grade.

2.2. Sample preparation

DHA vanillyl ester (DHA-VE) was obtained from an enzymatic esterification between DHA-EE (sample 1) and vanillyl alcohol. Vanillyl alcohol is a powerful aroma constituent of many food products. This phenol has been proved to be as intense as vanillin, the most abundant component in vanilla (*Vanilla planifolia*) (Perez-Silva et al., 2006). In addition to this interesting olfactory property, vanillyl alcohol exhibits a high radical-scavenging capacity that was suggested to be responsible for its anticonvulsive and antioxidant properties (Shyamala, Naidu, Sulochanamma, & Srinivas, 2007).

The reaction was achieved in the evaporation flask of a rotary evaporator, at 37 °C, under a 500 mbar pressure, with a 250 rpm rotation speed. Vanillyl alcohol (0.5 g) was added to 10 ml of DHA-EE three times, every 24 h (fed batch process). The ethanol, formed as a by-product of the reaction, was continuously eliminated from the medium, moving the reaction equilibrium in favour of DHA-VE production. To stop the reaction, the enzyme was separated from the reaction medium by filtration. At the end of the synthesis, the reaction medium (sample 3) contained 45% DHA-VE. Flash chromatography was applied to get pure DHA-VE (sample 2).

2.3. Accelerated oxidation test

5 g of each sample (1, 2 and 3) were weighed into screw-capped glass tubes (10 ml) and stored at 100, 20 and 4 °C, in darkness. Oxidative stability was monitored throughout storage until significant differences between the treated samples were observed. Samples were periodically taken for further analysis.

2.4. Determination of conjugated dienes

Conjugated dienes (CD) are considered as primary products of oxidation. For each sample, the conjugated diene value was determined as previously described (Weber, Bochi, Ribeiro, Victorio, & Emanuelli, 2008). A 0.5 mg sample was dissolved in 10 ml of n-hexane, then diluted or concentrated to obtain an absorbance between 0.1 and 0.8. The solution must be perfectly clear. The absorbance was measured at 234 nm, using *n*-hexane as a blank. Temperature effect on oxidation rate was illustrated by means of the Arrhenius equation:

$$\ln(k) = \ln A - E_a/RT$$

where k is the reaction rate, A is the kinetic constant, E_a is the activation energy (kJ mol^{-1}), R is the molar gas constant (8.3145 J K^{-1} mol^{-1}), and T is the absolute temperature (K). Activation energy and kinetic constants were determined, respectively, from the slopes and the intercepts of the lines generated by regressing ln (k) vs. $1/T$ (linear regression).

2.5. FTIR Instrumentation

Infrared spectra were recorded on a Tensor 27 mid-FTIR spectrometer (Bruker, Karlsruhe, Germany) with a deuterated triglycine sulphate (DTGS) detector, operating under Opus software. A ZnSe ATR sampling accessory from Spectra Tech (Shelton, CT) was used for total attenuated reflection measurements. The diaphragm was set to 4 mm and the scanning rate was fixed to 10 kHz. 256 scans were performed for both reference and samples.

2.6. Spectral acquisition

A small amount of each sample, approximately 30 µl, was deposited in the attenuated total reflectance (ATR) ZnSe crystal, and avoiding the presence of air by circulating nitrogen. All spectra were recorded from 4000 to 800 cm^{-1}. To avoid high noise levels, the spectra were collected with a resolution of 4 cm^{-1} to give a data point spacing of approximately 1.9 cm^{-1}, after Fourier transform and zero-filling. Spectra recorded with higher resolution gave similar frequency data for all samples, but higher noise level, and their registration took more time. Assignment of bands was done by comparison with literature spectral data and with reference compounds spectra included in the software spectral library. Height and area of each band were measured and calculated by using the essential FTIR software. This procedure avoided experimental errors associated with the subjectivity of external operators. After each operation, the crystal was thoroughly cleaned up, washed with ethanol and water, and then dried.

2.7. Statistical analyses

All analytical values represent the means ± SD of triplicate analyses. Two results were considered to be statistically significant when $p < 0.05$. All data were processed using the Microsoft Excel package.

3. Results and discussion

3.1. Conjugated dienes (CD)

Conjugated dienes are primary oxidation products, resulting from the rearrangement of polyunsaturated lipid double bonds. CD determination is a measure of oxidation state, making it a good indicator of oil stability and antioxidant effectiveness (Iqbal & Bhanger, 2007). An increase of absorbance at 234 nm is correlated with lipid degradation, due to CD formation during the first stage of oxidation.

CD measurements within DHA-EE, DHA-VE and crude reaction medium, were followed during storage at different temperatures. Results are shown on Fig. 1. As expected, CD contents increased with time during the storage, whatever the sample and the temperature, indicating an oxidative degradation. CD values were globally higher for DHA-EE than for DHA-VE and the crude reaction medium. At 20 and 4 °C, DHA-VE and crude reaction medium remained almost stable for a few weeks, whereas DHA-EE get oxidised during the first 2 weeks of storage. DHA-VE appeared to be the most stable compound whereas the crude reaction medium exhibited a midway behaviour between pure DHA-VE and pure DHA-EE. For any storage temperature, two stages were observed in the degradation process. In the first stage, CD values slowly increased, and then this trend accelerated in the second stage. CD values obtained at 4 and 20 °C were not significantly different. For all samples, the second stage of oxidation seemed to start after 8 weeks of storage at 20 °C, in comparison with 10 weeks of storage at 4 °C, suggesting a higher stability of the samples at low temperature. At 100 °C, oxidation was observed to start in the early hours of storage, and then rapidly progressed over time. These results are in accordance with previous studies demonstrating that the increase of temperature speeds up oxidative degradation (Iqbal & Bhanger, 2007; Wang et al., 2011). Furthermore, the high stability of DHA-VE compared with DHA-EE was demonstrated, showing

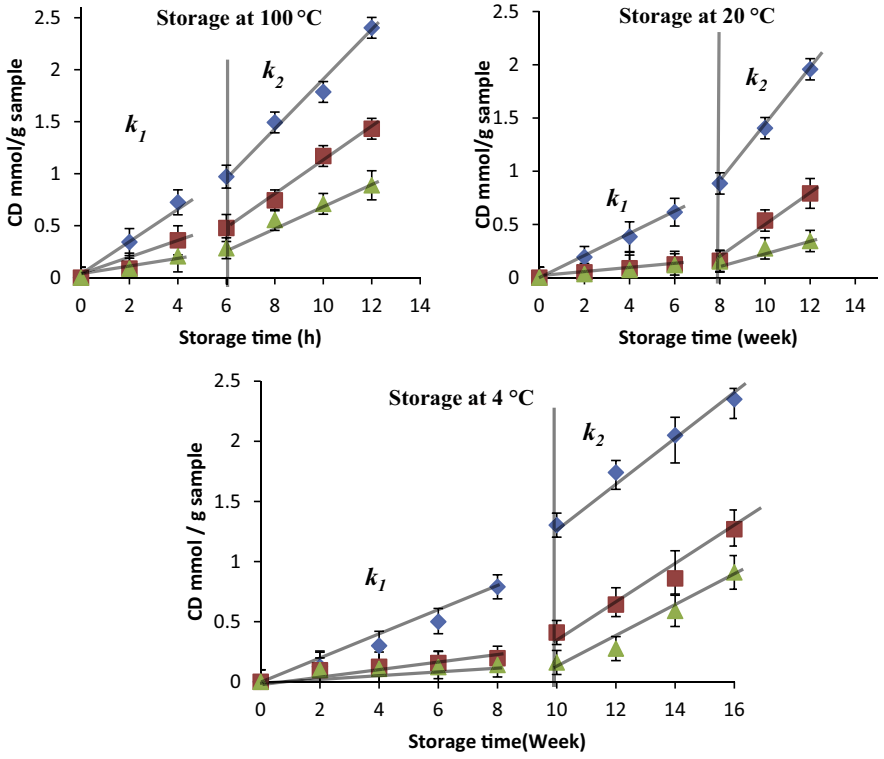

Fig. 1. Conjugated dienes at 234 nm of sample oxidations under different conditions using UV–Visible spectrometry analysis. Values obtained are the averages ± standard deviation of 3 replicates. DHA-EE ◆, synthesis reaction medium ■ and DHA-VE ▲.

the interest of combining highly oxidizable lipids with phenolic compounds, *via* the esterification process.

The Arrhenius model describes the relationship between reaction rate and temperature. Sample oxidation, at low and high temperatures, may pass through different steps or reaction pathways, depending on activation energy (Nagy & Turanyi, 2012). Moreover, temperature affects reaction kinetics but also oxygen diffusivity and solubility. The apparent reaction kinetics are dependent on the multiparametric system and the Arrhenius model integrates all these factors to produce a global model. In Fig. 1, two linear steps of oxidation kinetic appear and Fig. 2 provides parameters for the Arrhenius equation (kinetic constant and activation energy) for each of them. At any studied temperature, the reaction rates (k_1 and k_2) were lower for DHA-VE than for DHA-EE and reaction medium; So, oxidation of DHA-VE was the slowest at any temperature. DHA-EE was highly sensitive to oxidation in comparison with DHA-VE.

The activation energy (E_a) is proportional to the slope of the Arrhenius equation (Slope = $-E_a/R$). E_a for DHA-EE was lower than those of DHA-VE for both the first and second step of oxidation. This means that the DHA-VE oxidation kinetics were more activated by increasing temperature than was the DHA-EE oxidation. So, at ambient temperature and below, DHA-VE was much more stable than was DHA-EE. As temperature increased up to 100 °C, oxidation kinetics became closer and the protective effect of the phenol was less efficient.

3.2. Fourier transforms infrared spectroscopy (FTIR)

3.2.1. General

Samples were analysed during storage by infrared spectroscopy. Both intensity and frequency of the bands were assessed, aiming to identify structural changes due to oxidation. FTIR spectra of fresh samples (DHA-EE, DHA-VE and reaction medium) are shown in Fig. 3. DHA-EE and DHA-VE spectra comprised the characteristic bands of the two pure compounds: 3600–3100 cm^{-1} due to O—H bond stretching of vanillyl alcohol hydroxyl groups, 3013 cm^{-1} due to =C—H stretching of DHA *cis*-double bonds and 1738 cm^{-1} due to C=O stretching of DHA-EE and DHA-VE ester groups. Reaction medium spectra were characteristic of a mixture of residual DHA-EE and DHA-VE resulting from esterification (Belhaj, Arab-Tehrany, & Linder, 2010; Vongsvivut et al., 2012).

During storage, some changes in FTIR spectra were observed, indicating the oxidative degradation of the samples. Main changes were the emergence of a broad band near 3445 cm^{-1} in the case of DHA-EE and the broadening of bands from 3600 to 3100 cm^{-1} in the case of DHA-VE and crude reaction medium, indicating the formation of hydroperoxide oxidation products. A slight decrease of the band around 3013 cm^{-1} was observed, due to disappearance of DHA *cis*-double bonds. In addition, the frequency of the C=O band around 1737 cm^{-1} was observed to decrease with time. All these changes were indicative of the oxidative degradation of lipids and are detailed below (Ahro et al., 2002; Akhtar, Jacquot, & Desobry, 2014; Belhaj et al., 2010).

3.2.2. Changes in the spectral region 3100–3600 cm^{-1}

Significant changes occurred, during storage, in the 3600–3100 cm^{-1} spectral region, whatever the sample (Fig. 4). In the case of DHA-EE, a band appeared and increased at 3457 cm^{-1}, from the first month of storage at 20 and 4 °C and from the first two hours of storage at 100 °C. This was indicative of the presence of hydroxyl groups newly formed resulting from the formation of hydroperoxides. A similar trend was observed by Belhaj et al. (2010) during

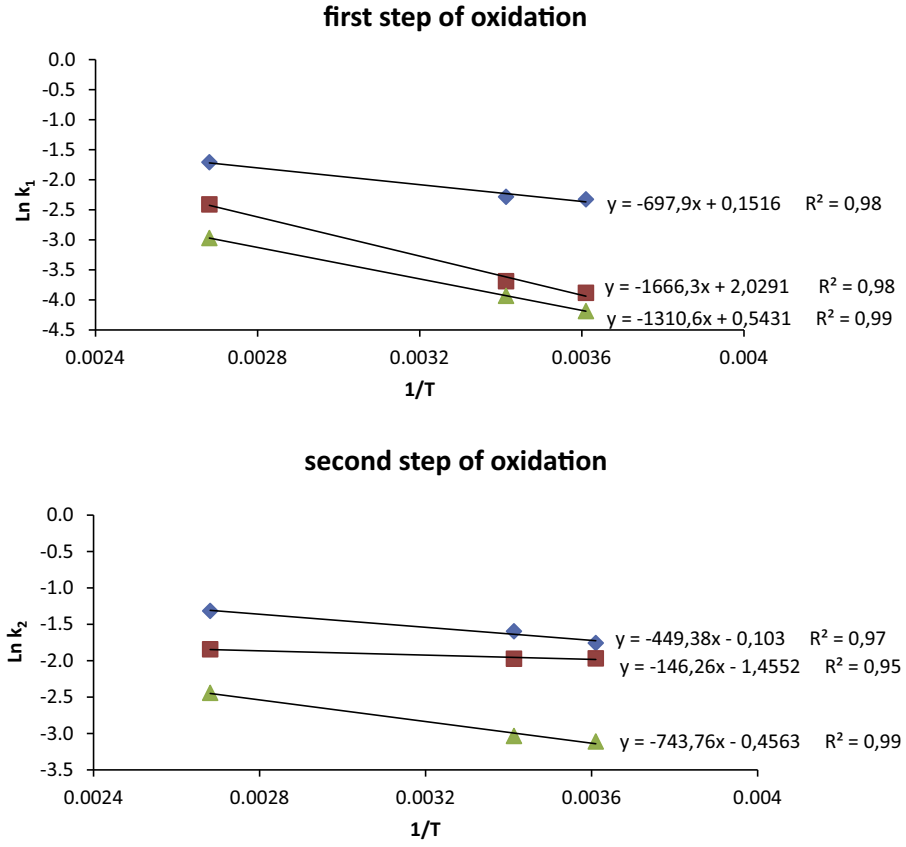

Fig. 2. Regression parameters for Arrhenius relationships between the reaction rate and temperature for various samples. DHA-EE ◆, synthesis reaction medium ■ and DHA-VE ▲.

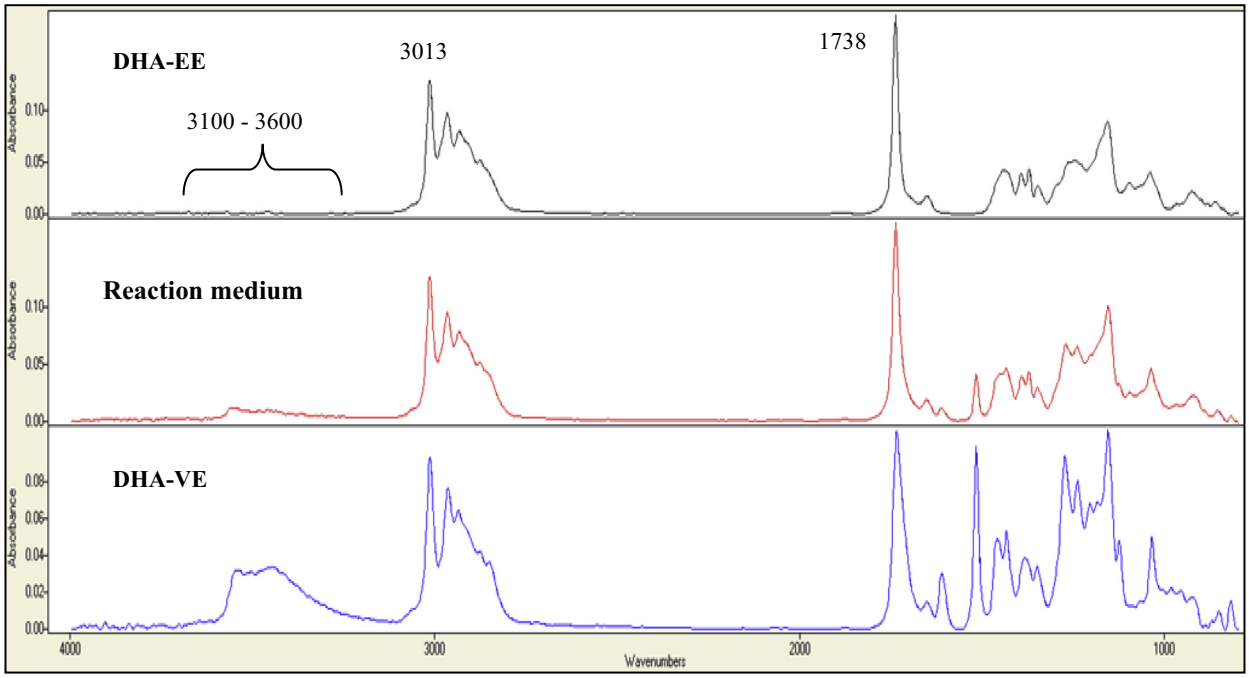

Fig. 3. FTIR spectra of fresh sample of DHA-EE, DHA-VE and synthesis reaction medium.

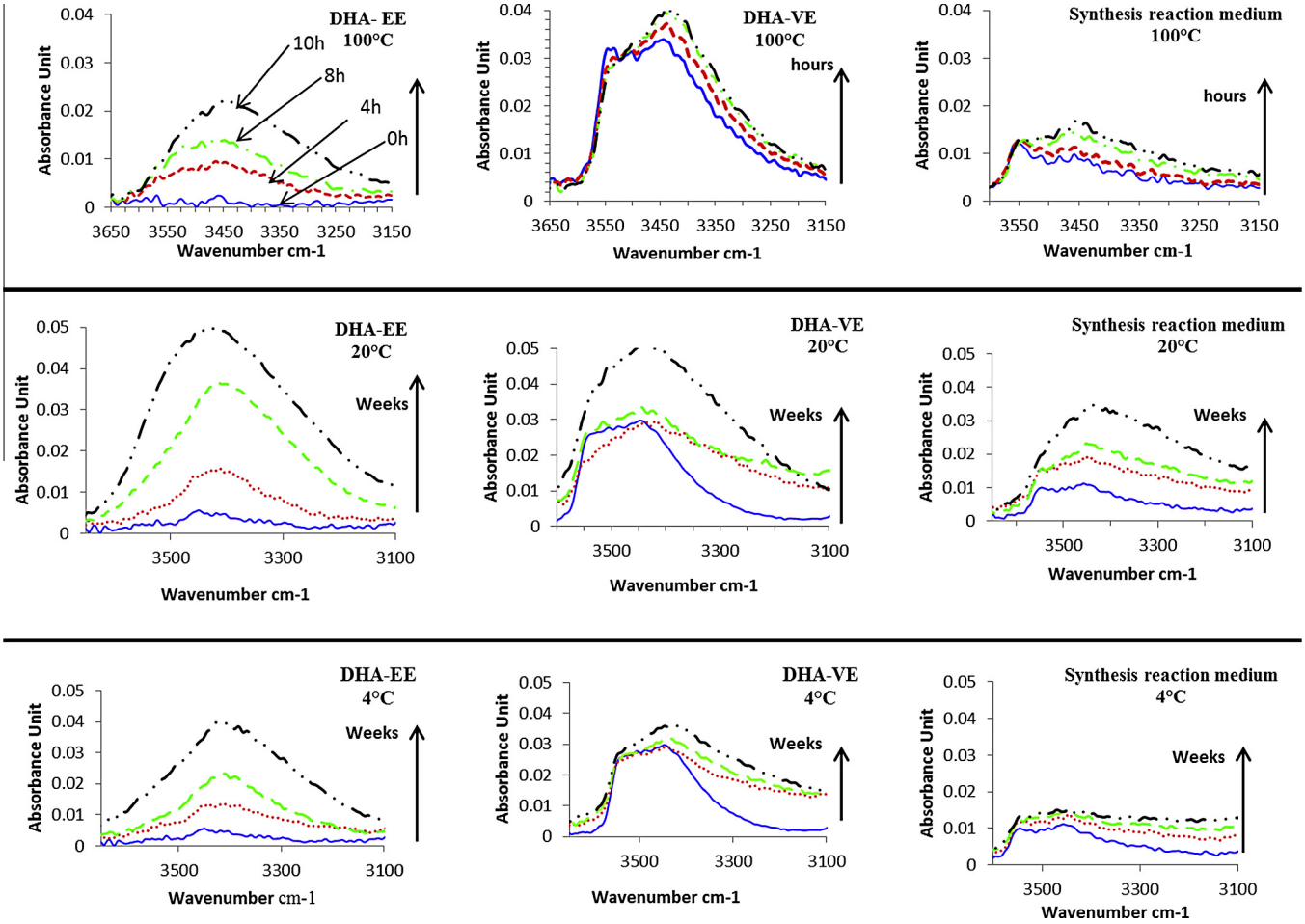

Fig. 4. Changes produced in the region between 3650 and 3100 cm^{-1} of the infrared spectrum of samples stored during 3 months. t = 0 month ——, t = 1 month ·····, t = 2 months – – and t = 3 months — · · at 4 °C and 20 °C and of samples stored at 100 °C t = 0 h ——, t = 4 h ·····, t = 8 h – – and t = 10 h — · ·.

the storage of salmon oil and was attributed to oxidative degradation of polyunsaturated lipids. In the case of pure DHA-VE, intensification and broadening of the bands were observed with time from the second month of storage at 4 and 20 °C. These changes were significant during the first two hours of storage at 100 °C and were explained by overlapping of original bands of DHA-VE and new absorption bands due to hydroperoxides generated during the oxidation process. Just as before, FTIR spectra of the crude reaction medium evolved intermediately between DHA-EE and pure DHA-VE. Whatever the storage temperature, DHA-VE and DHA-EE appeared, respectively, as the most and the least stable samples towards oxidation. The crude reaction medium exhibited a midway stability. As expected, for all samples, a storage temperature of 4 °C was shown to decrease oxidation rate in comparison with 20 °C. All these results were in agreement with the above-mentioned CD results (see Fig. 1).

3.2.3. Changes in the band at 3013 cm^{-1}

In addition to changes observed in the region related to OH bond vibrations, FTIR spectra showed a significant decrease in the intensity of the band at 3013 cm^{-1}, whereas bands around 2850 cm^{-1} intensified. These bands relate, respectively, to =C—H bond stretching vibration of *cis*-double bonds and symmetric stretching vibration of saturated CH_2 groups. From a practical point of view, the time until the band at 3013 cm^{-1} begins to decrease could be considered as an indicator of oxidation (Guillen & Cabo, 2002). This trend was clearly observed in the DHA-EE sample stored at 100 °C, whereas no significant change was noticed for the DHA-VE sample (Fig. 5A). At storage temperatures of 4 and 20 °C, the ratio between the absorbance of the band at 2853 cm^{-1} and that of the band at 3013 cm^{-1} (A2854/A3013) was followed with time as an indicator of oxidation progress (Fig. 5B). In fact the increase of this ratio was due to disappearance of *cis*-double bonds of lipids during oxidation, favouring the formation of saturated bonds (Vandevoort et al., 1994). No significant change was observed for DHA-VE and crude reaction medium samples stored at 4 °C. At this temperature, DHA-EE became oxidised after 10 weeks of storage. Storage at 20 °C was shown to increase oxidation rate, leading to the degradation of DHA-VE, crude reaction medium and DHA-EE after 9, 4 and 3 weeks, respectively. Unsurprisingly, high storage temperature was shown to favour oxidation.

3.2.4. Changes in the spectral region 1800–1600 cm^{-1}

The spectral region 1600–1800 cm^{-1} provides information about secondary oxidation products, especially aldehydes responsible for rancidity. More specifically, a shift of the band related to ester carbonyl bond towards lower wavenumbers is indicative of lipid oxidation, due to the formation of aldehydes (Muik, Lendl, Molina-Diaz, & Ayora-Canada, 2005; Rohman & Man, 2013). Fresh samples exhibited a characteristic band around 1738 cm^{-1}, corresponding to ester carbonyl bond stretching. The frequency of this band was followed during storage, as shown in Fig. 6. For all samples and storage conditions, a shift of the C=O stretching band was observed with time. Main shifts were obtained for DHA-EE, whereas similar trends were noticed for DHA-VE and the crude reaction medium. Once again, the phenomenon was accentuated by high temperatures. The frequency remained almost stable in the case of DHA-VE and the crude reaction medium stored at 4 or 20 °C. By contrast, the frequency significantly decreased with time in the case of DHA-EE, even at low temperature. Besides these changes, other authors have reported the appearance of a band at 1654 cm^{-1} that was assigned to α,β-unsaturated aldehydes and ketones as secondary oxidation products (Vandevoort et al., 1994). In the present study no significant change was observed at this wavenumber.

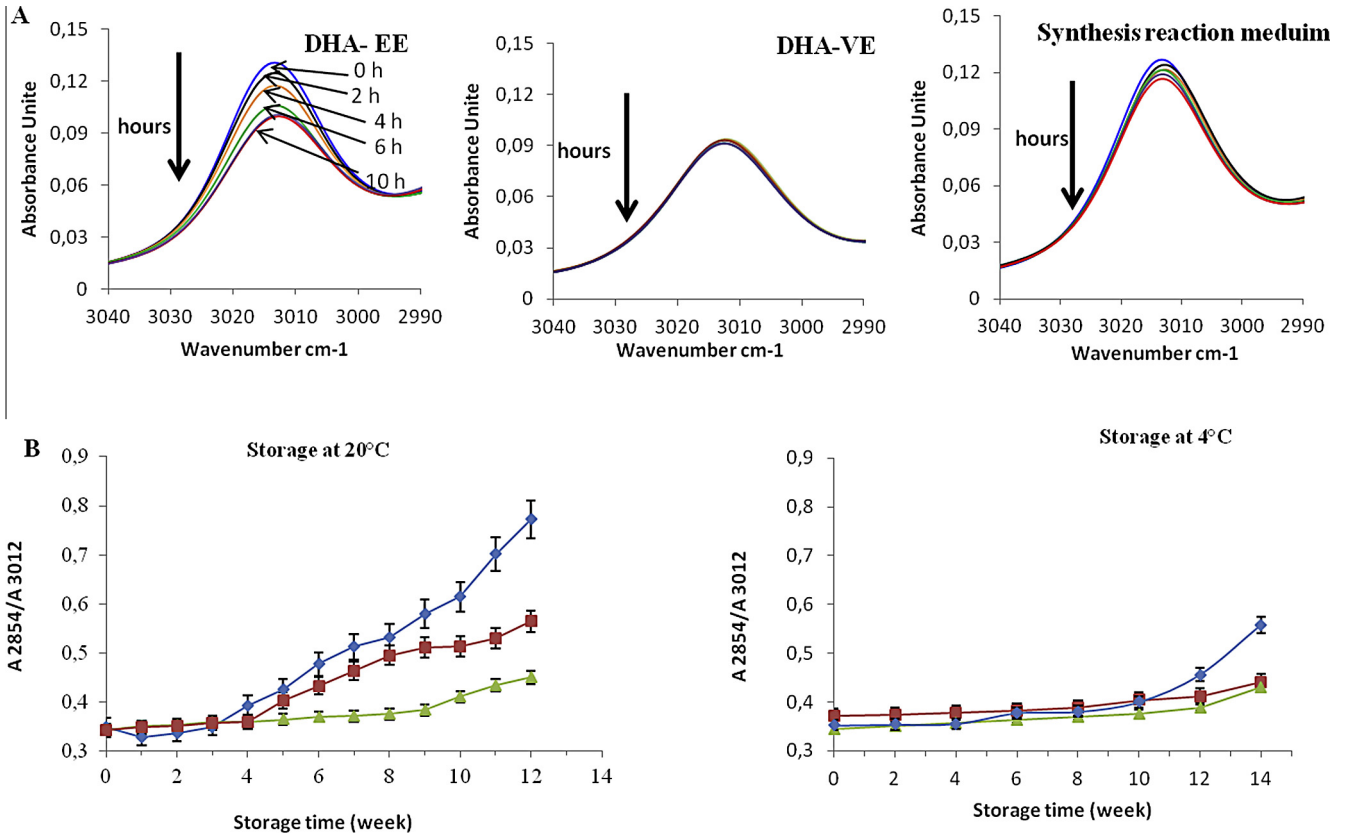

Fig. 5. (A) Changes produced in the band at 3013 cm^{-1} of the infrared spectrum of samples stored at 100 °C. (B) The variation in A2853/A3012 ratio during the oxidation process at 4 °C and 20 °C of samples DHA-EE ◆, synthesis reaction medium ■ and DHA-VE ▲.

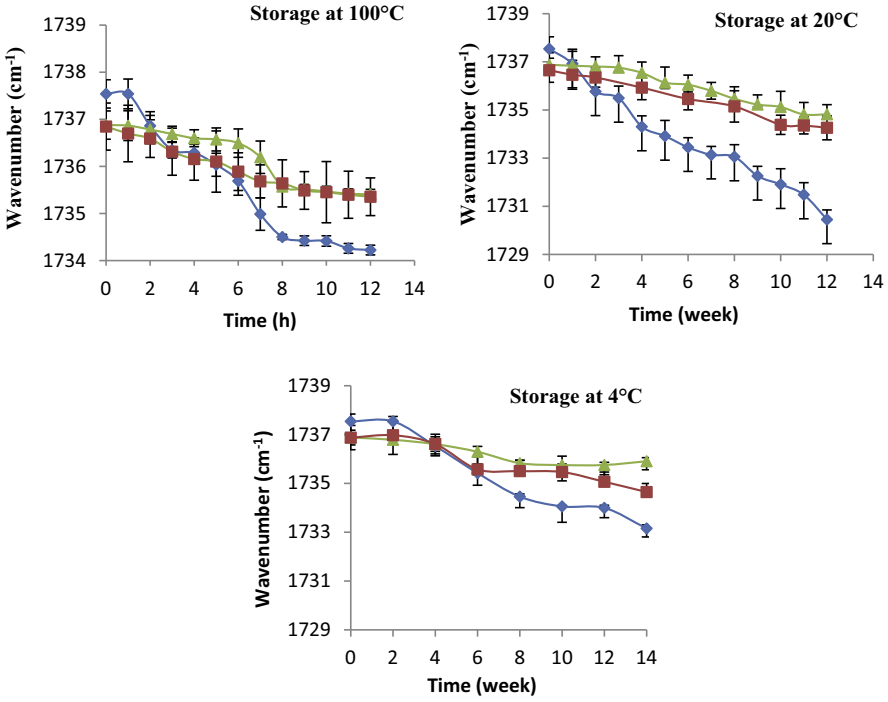

Fig. 6. Evolution of wavenumber values of C=O band of DHA-EE ◆, synthesis reaction medium ■ and DHA-VE ▲ under different oxidative conditions.

4. Conclusion

In this study, different spectroscopic methods were used to monitor the oxidative stability of DHA-based esters: DHA ethyl ester is a commercially available form of DHA; DHA vanillyl ester results from the enzymatic alcoholysis reaction between DHA-EE and vanillyl alcohol. Conjugated diene determination is a widespread and inexpensive technique, providing information about the first stage of oxidation that leads to primary oxidation products. In the present work, CD determination was particularly effective for studying the thermal and temporal stability of DHA-based compounds. Furthermore, FTIR is now well-known for its high efficiency, to follow structural changes in complex evolving systems. The different regions of FTIR spectra provide useful information about functional groups and their chemical environment. From a practical point of view, this rapid and nondestructive method does not require any sample or chemical preparation, and it allows significant time- and cost-savings in comparison with classical analyses. In this work, FTIR was shown to be an efficient tool for following lipid oxidation, thanks to significant changes in the frequency and the magnitude of characteristic bands. More specifically, the intensity of the band related to =C—H bond stretching vibration of *cis*-double bonds at 3013 cm^{-1} depended on the degree of unsaturation of the samples, and then was used as a marker for DHA oxidation. Another sensitive indicator was the ratio between the absorbance at 3013 cm^{-1} and the absorbance at 2853 cm^{-1} that corresponded to the vibration of saturated C—H bonds.

Unsurprisingly, the oxidative stability of the compounds was negatively affected by increasing temperature and storage time. All results indicated a higher stability of DHA-VE in comparison with DHA-EE, showing the interest of combining this highly oxidizable lipid with vanillyl alcohol in a single structure. According to FTIR data, oxidation was delayed until 8 weeks in the case of pure DHA-VE stored at 20 °C against 2 weeks in the case of pure DHA-EE. A midway stability was determined for the crude reaction medium made of 45% DHA-VE and 55% DHA-EE. Main advantages of such a medium are, first, a high stability, despite a significant content of DHA, and second, easy preparation and use that do not require any purification step. Phenolic esters of DHA undoubtedly appear as promising derivatives that could make easier the use of polyunsaturated lipids in food preparations.

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References

Ahro, M., Hakala, M., Kauppinen, J., & Kallio, H. (2002). Headspace FT-IR analysis of rapeseed oil oxidation. *Applied Spectroscopy, 56*, 217–222.
Akhtar, M. J., Jacquot, M., & Desobry, S. (2014). Effect of HPMC-anthocyanin packaging color and oxygen permeability on salmon oil preservation. *Food and Bioprocess Technology, 7*, 93–104.
Arab-Tehrany, E., Jacquot, M., Gaiani, C., Imran, M., Desobry, S., & Linder, M. (2012). Beneficial effects and oxidative stability of omega-3 long-chain polyunsaturated fatty acids. *Trends in Food Science & Technology, 25*, 24–33.
Barriuso, B., Astiasaran, I., & Ansorena, D. (2013). A review of analytical methods measuring lipid oxidation status in foods: A challenging task. *European Food Research and Technology, 236*, 1–15.
Belhaj, N., Arab-Tehrany, E., & Linder, M. (2010). Oxidative kinetics of salmon oil in bulk and in nanoemulsion stabilized by marine lecithin. *Process Biochemistry, 45*, 187–195.
Brys, J., Wirkowska, M., Gorska, A., Ostrowska-Ligeza, E., Brys, A., & Koczon, P. (2013). The use of DSC and FT-IR spectroscopy for evaluation of oxidative stability of interesterified fats. *Journal of Thermal Analysis and Calorimetry, 112*, 481–487.
Cho, S.-Y., Miyashita, K., Miyazawa, T., Fujimoto, K., & Kaneda, T. (1987). Autoxidation of ethyl eicosapentaenoate and docosahexaenoate. *Journal of the American Oil Chemists Society, 64*, 876–879.
Cunnane, S. C., Chouinard-Watkins, R., Castellano, C. A., & Barberger-Gateau, P. (2013). Docosahexaenoic acid homeostasis, brain aging and Alzheimer's

disease: Can we reconcile the evidence? *Prostaglandins Leukotrienes and Essential Fatty Acids, 88*, 61–70.
Domenichiello, A. F., Chen, C. T., Trepanier, M. O., Stavro, P. M., & Bazinet, R. P. (2014). Whole body synthesis rates of DHA from alpha-linolenic acid are greater than brain DHA accretion and uptake rates in adult rats. *Journal of Lipid Research, 55*, 62–74.
Dubois, J., vandeVoort, F. R., Sedman, J., Ismail, A. A., & Ramaswamy, H. R. (1996). Quantitative Fourier transform infrared analysis for anisidine value and aldehydes in thermally stressed oils. *Journal of the American Oil Chemists Society, 73*, 787–794.
Frankel, E. N. (2005). Methods to determine extent of oxidation. In E. N. Frankel (Ed.), *Lipid oxidation*, 2nd ed., (p. 106). Oxford, Cambridge, Philadelphia, New Delhi: Woodhead Publishing in Food Science, Technology and Nutrition.
Guillen, M. D., & Cabo, N. (2000). Some of the most significant changes in the Fourier transform infrared spectra of edible oils under oxidative conditions. *Journal of the Science of Food and Agriculture, 80*, 2028–2036.
Guillen, M. D., & Cabo, N. (2002). Fourier transform infrared spectra data versus peroxide and anisidine values to determine oxidative stability of edible oils. *Food Chemistry, 77*, 503–510.
Iqbal, S., & Bhanger, M. I. (2007). Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry, 100*, 246–254.
Kindleysides, S., Quek, S. Y., & Miller, M. R. (2012). Inhibition of fish oil oxidation and the radical scavenging activity of New Zealand seaweed extracts. *Food Chemistry, 133*, 1624–1631.
Kobata, K., Kawaguchi, M., & Watanabe, T. (2002). Enzymatic synthesis of a capsinoid by the acylation of vanillyl alcohol with fatty acid derivatives catalyzed by lipases. *Bioscience, Biotechnology, and Biochemistry, 66*, 319–327.
Kuratko, C. N., Barrett, E. C., Nelson, E. B., & Salem, N. (2013). The relationship of docosahexaenoic acid (DHA) with learning and behavior in healthy children: A review. *Nutrients, 5*, 2777–2810.
Lichtenstein, A. H., Appel, L. J., Brands, M., Carnethon, M., Daniels, S., Franch, H. A., et al. (2006). Diet and lifestyle recommendations revision 2006 – A scientific statement from the American Heart Association Nutrition Committee. *Circulation, 114*, 82–96.
Lin, B. F., Lai, C. C., Lin, K. W., & Chiang, B. L. (2000). Dietary oxidized oil influences the levels of type 2 T-helper cell-related antibody and inflammatory mediators in mice. *British Journal of Nutrition, 84*, 911–917.
Liu, L., Jin, C., & Zhang, Y. (2014). Lipophilic phenolic compounds (Lipo-PCs): emerging antioxidants applied in lipid systems. *RSC Advances, 4*, 2879–2891.
Mbatia, B., Kaki, S. S., Mattiasson, B., Mulaa, F., & Adlercreutz, P. (2011). Enzymatic synthesis of lipophilic rutin and vanillyl esters from fish byproducts. *Journal of Agricultural and Food Chemistry, 59*, 7021–7027.
Mirghani, M. E. S., Man, Y. B. C., Jinap, S., Baharin, B. S., & Bakar, J. (2002). Rapid method for determining malondialdehyde as secondary oxidation product in palm olein system by Fourier transform infrared spectroscopy. *Phytochemical Analysis, 13*, 195–201.
Mosca, M., Diantom, A., Lopez, F., Ambrosone, L., & Ceglie, A. (2013). Impact of antioxidants dispersions on the stability and oxidation of water-in-olive-oil emulsions. *European Food Research and Technology, 236*(2), 319–328.
Muik, B., Lendl, B., Molina-Diaz, A., & Ayora-Canada, M. J. (2005). Direct monitoring of lipid oxidation in edible oils by Fourier transform Raman spectroscopy. *Chemistry and Physics of Lipids, 134*(2), 173–182.
Nagy, T., & Turanyi, T. (2012). Determination of the uncertainty domain of the Arrhenius parameters needed for the investigation of combustion kinetic models. *Reliability Engineering & System Safety, 107*, 29–34.
Perez-Silva, A., Odoux, E., Brat, P., Ribeyre, F., Rodriguez-Jimenes, G., Robles-Olvera, V., et al. (2006). GC–MS and GC-olfactometry analysis of aroma compounds in a representative organic aroma extract from cured vanilla (*Vanilla planifolia* G. Jackson) beans. *Food Chemistry, 99*, 728–735.
Picq, M., Bernoud-Hubac, N., & Lagarde, M. (2013). Synthesis and biological interest of structured docosahexaenoic acid-containing triacylglycerols and phospholipids. *Current Organic Chemistry, 17*, 841–847.
Roby, M. H. H., Sarhan, M. A., Selim, K. A. H., & Khalel, K. I. (2013a). Antioxidant and antimicrobial activities of essential oil and extracts of fennel (*Foeniculum vulgare* L.) and chamomile (*Matricaria chamomilla* L.). *Industrial Crops and Products, 44*, 437–445.
Roby, M. H. H., Sarhan, M. A., Selim, K. A. H., & Khalel, K. I. (2013b). Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. *Industrial Crops and Products, 43*, 827–831.
Rohman, A., & Man, Y. B. C. (2013). Application of FTIR spectroscopy for monitoring the stabilities of selected vegetable oils during thermal oxidation. *International Journal of Food Properties, 16*, 1594–1603.
Rubilar, M., Morales, E., Saez, R., Acevedo, F., Palma, B., Villarroel, M., et al. (2012). Polyphenolic fractions improve the oxidative stability of microencapsulated linseed oil. *European Journal of Lipid Science and Technology, 114*, 760–771.
Ruiz, A., Canada, M. J. A., & Lendl, B. (2001). A rapid method for peroxide value determination in edible oils based on flow analysis with Fourier transform infrared spectroscopic detection. *Analyst, 126*, 242–246.
Shahidi, F., & Zhong, Y. (2005). Lipid oxidation: Measurement methods. In *Bailey's industrial oil and fat products*. John Wiley & Sons Inc.
Shyamala, B. N., Naidu, M. M., Sulochanamma, G., & Srinivas, P. (2007). Studies on the antioxidant activities of natural vanilla extract and its constituent compounds through in vitro models. *Journal of Agricultural and Food Chemistry, 55*, 7738–7743.
St. Angelo, A. J., Vercellotti, J., Jacks, T., & Legendre, M. (1996). Lipid oxidation in foods. *Critical Reviews in Food Science and Nutrition, 36*, 175–224.
Staszewski, M. V., Pizones Ruiz-Henestrosa, V. M., & Pilosof, A. M. R. (2014). Green tea polyphenols-β-lactoglobulin nanocomplexes: Interfacial behavior, emulsification and oxidation stability of fish oil. *Food Hydrocolloids, 35*, 505–511.
Swanson, D., Block, R., & Mousa, S. A. (2012). Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Advances in Nutrition, 3*, 1–7.
Vandevoort, F. R., Ismail, A. A., Sedman, J., & Emo, G. (1994). Monitoring the oxidation of edible oils by fourier-transform infrared-spectroscopy. *Journal of the American Oil Chemists Society, 71*, 243–253.
Velasco, J., Berdeaux, O., Marquez-Ruiz, G., & Dobarganes, M. C. (2002). Sensitive and accurate quantitation of monoepoxy fatty acids in thermoxidized oils by gas–liquid chromatography. *Journal of Chromatography A, 982*(1), 145–152.
Vongsvivut, J., Heraud, P., Zhang, W., Kralovec, J. A., McNaughton, D., & Barrow, C. J. (2012). Quantitative determination of fatty acid compositions in micro-encapsulated fish-oil supplements using Fourier transform infrared (FTIR) spectroscopy. *Food Chemistry, 135*(2), 603–609.
Wang, H., Liu, F., Yang, L., Zu, Y., Wang, H., Qu, S., et al. (2011). Oxidative stability of fish oil supplemented with carnosic acid compared with synthetic antioxidants during long-term storage. *Food Chemistry, 128*, 93–99.
Weber, J., Bochi, V. C., Ribeiro, C. P., Victorio, A. M., & Emanuelli, T. (2008). Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. *Food Chemistry, 106*, 140–146.
Wu, F. J., Xue, Y., Liu, X. F., Xue, C. H., Wang, J. F., Du, L., et al. (2014). The protective effect of eicosapentaenoic acid-enriched phospholipids from sea cucumber *Cucumaria frondosa* on oxidative stress in PC12 cells and SAMP8 mice. *Neurochemistry International, 64*, 9–17.
Zhong, Y., & Shahidi, F. (2012). Lipophilised epigallocatechin gallate (EGCG) derivatives and their antioxidant potential in food and biological systems. *Food Chemistry, 131*, 22–30.