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Study of the heat stability of sunflower oil enriched in natural antioxidants by different analytical techniques and front-face fluorescence spectroscopy combined with Independent Components Analysis

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ABSTRACT

The aim of this study was to find objective analytical methods to study the degradation of edible oils during heating and thus to suggest solutions to improve their stability. The efficiency of Nigella seed extract as natural antioxidant was compared with butylated hydroxytoluene (BHT) during accelerated oxidation of edible vegetable oils at 120 and 140 °C. The modifications during heating were monitored by 3D-front-face fluorescence spectroscopy along with Independent Components Analysis (ICA), ^{1}H NMR spectroscopy and classical physico-chemical methods such as anisidine value and viscosity. The results of the study clearly indicate that the natural seed extract at a level of 800 ppm exhibited antioxidant effects similar to those of the synthetic antioxidant BHT at a level of 200 ppm and thus contributes to an increase in the oxidative stability of the oil.

1. Introduction

Lipid oxidation is one of the major factors resulting in losses in fatty food quality by the formation of products with negative effects on taste, aroma and nutritional value of the food, which are associated with many types of biological damages in living tissues and may increase the risk of cardiovascular disease [1,2]. Antioxidants are major ingredients that protect the quality of oils and fats by retarding oxidation [3]. Synthetic antioxidants are used within regulated limits to reduce deterioration, rancidity and oxidative discoloration [4]. Butylated hydroxyl anisol (BHA) and butylated hydroxyl toluene (BHT) are two widely used synthetic antioxidants but are quite volatile and decompose easily at high temperatures [5].

Questions have been raised concerning the safety and toxicity of such synthetic antioxidants in relation to their metabolism and possible absorption and accumulation in organ and tissues [6,7]. Therefore, the search for and the development of other antioxidants from natural plant materials is highly desirable.

In this study, *Nigella sativa* L. (Ranunculaceae) seed extract was used to enrich sunflower oil with a view to improving its thermal resistance during accelerated oxidation at 120 °C and 140 °C over 3 h. The modifications that occur in oil samples were monitored by front-face fluorescence spectroscopy (FFFS), by ^{1}H NMR spectroscopy and by measurements of viscosity and anisidine values.

Spectroscopic techniques such as Fourier transform infrared spectroscopy have been largely used for the determination of oil degradation [8–10]. However, vibrational techniques lack sensitivity in comparison to fluorescence spectroscopy.

Fluorescence spectroscopy has been successfully used for olive oil characterization and adulteration assessment [11–13].

The advantages of this technique are its speed of analysis, lack of solvents and reagents, and requirement of only small amounts of sample. In addition, it is a noninvasive, a highly selective and sensitive technique. In fact, the sensitivity of fluorescence is 100–1000 times higher than that of the absorption techniques, enabling to measure concentrations down to parts per billion levels technique. The front-face (FF) technique was shown to be the method providing the most accurate results for the fluorescence of edible oils in nondiluted samples [14,15].

The interpretation of fluorescence spectral data is complex due to the presence of many fluorophores and by changes caused by variation in the sample matrix, etc. In this paper, Independent

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Components Analysis (ICA) was applied to the 3D-front-face fluorescence spectra to facilitate monitoring the antioxidant effect of Nigella extract during heat treatment.

Independent Components Analysis is a blind source separation (BSS) technique developed to extract the pure underlying signals from a set of signals where they are mixed in unknown proportions. The general ICA model is [16,17]:

$$\mathbf{X} = \mathbf{A}.\mathbf{S}$$

where **X** is the matrix of observed signals (the spectra in our case), **S** is the matrix of unknown "pure" source signals and **A** is the mixing matrix of unknown coefficients, related to the corresponding concentrations. Based on the Central Limit Theorem, ICA assumes that statistically independent source signals have intensity distributions that are less Gaussian than are their mixtures [16,17]. For this reason, ICA aims to maximize the non-gaussianity of the extracted signals.

This method is now widely applied in the signal processing fields, such as biomedical signals [18], image processing [19] and financial data analysis [20]. Its applications in processing analytical signals, including NIR [21], MIR [22], fluorescence spectroscopy [23,24], photoacoustic spectroscopy [25], GC/MS [26] and electron paramagnetic resonance (EPR) [27] have also been reported.

Several ICA algorithms exist, such as FastICA, Joint Approximate Diagonalization of Eigenmatrices (JADE), Infomax ICA, Mean-field ICA (MF-ICA), Kernel ICA (KICA), often based on different definitions of independence and using different procedures to extract the Independent Components [17]. In this paper, the JADE algorithm was used [28]. JADE performs a joint diagonalization of matrices extracted from the fourth-order cumulants calculated from the data and does not involve gradient searches, thus avoiding the convergence problems encountered with other procedures.

It is shown here how ICA may be useful to simplify the interpretation of the data.

2. Materials and methods

2.1. N. sativa L. extract preparation (natural antioxidant)

Nigella seeds were washed and then dried in a hot-air oven at 40 °C. The dried seeds were ground into a fine powder in a mill. The material that passed through an 80 mesh sieve was retained for use. Ten grams of ground seeds were extracted with 100 ml of ethanol overnight in a shaker (Heidolph REAX 2) at room temperature. The extract was filtered and the residue was re-extracted under the same conditions. The combined filtrate was evaporated in a rotary evaporator (Rotavapor R110, Büchi, Switzerland) at below 40 °C. The extract obtained after evaporation of ethanol was used as the natural antioxidant [29,30].

2.2. Oil samples

Sunflower oil was a commercial brand bought in the French marketplace.

2.3. Experimental parameters and software

Non-enriched sunflower oils and sunflower oils enriched with 800 ppm of *N. sativa* L. extract were heated at 120 and 140 °C for 3 h. For comparison, the synthetic antioxidant (BHT) was also tested at a legal limit of 200 ppm [30]. The samples taken after each hour of heating during 3 h are then analyzed by 3D-front-face fluorescence.

Fluorescence landscapes (3D spectra) were measured directly on the samples without prior chemical treatment, using a Xenius spectrofluorometer (SAFAS, Monaco) equipped with a xenon lamp source, excitation and emission monochromators and a front-face sample-cell holder. Measurements were carried out using acryl cuvettes. The instrumental settings were: bandwidths 10 nm, emission wavelengths 300–550 nm (every 2 nm) and excitation wavelengths 280–500 nm (every 2 nm). A photomultiplier (PM) voltage of 420 V was used to avoid detector saturation. The "Forcing" option was also used in order to limit the emission range so that data acquisition started 15 nm beyond the excitation wavelength, thus avoiding interference from Rayleigh scattering.

The data consisting of 3D fluorescence spectra were exported in ASCII format for data treatment using MATLAB version 7.0.4 (The MathWorks, Natick, USA).

2.4. Viscosity and anisidine value Measurements

A Houillon viscometer (S. Lauda, LAUDA France S.A.R.L.) was used to study the kinematic viscosity of the oils after heat treatment. Measurements were performed in duplicate at 40 °C using "Houillon" capillary tubes.

Anisidine values were determined by the standard 2504 IUPAC method (IUPAC, 1987) by measuring absorbance at 350 nm using a single beam U.V./visible spectrophotometer (UV–vis Roucaire SHIMADZU UV-1205). Measurements were carried out in duplicate.

2.5. ^1H NMR spectroscopy

The oil samples were placed in 5 mm diameter NMR tubes for the analysis. TSP (Trimethylsilyl propanoic acid) was dissolved in deuterated water, placed in a 200 μL NMR capillary and used as a chemical shift reference. The spectra were acquired using a Bruker 300 MHz spectrometer with a relaxation delay of 3 s, an pulse angle of 90° and 64 scans.

The baseline corrections of the spectra were performed manually and the chemical shifts are expressed in δ scale (ppm). Each sample was analyzed three times.

3. Chemometrics methods: choice of the number of ICA components

The choice of the optimal number of components to use in ICA is one of the crucial points in the analysis. In this work, two methods were used in order to determine the number of independent components.

The first method is based on the Durbin–Watson (DW) criterion which was applied to the residual matrices after removing the contributions of the calculated signals to find out which ones had a low signal/noise ratio and could therefore be assumed to no longer contain informative ICs. The DW statistic is a criterion which is classically used to test for the correlation of residuals after a regression [31] but which has been proposed as a measure of the signal/noise ratio of the loadings and regression vectors obtained by multivariate analysis of signals, in order to determine the optimal dimensionality of multivariate models [32,33]. The basic justification for the use of this criterion is that uninformative loadings and over fitted regression vectors contain more random noise. If the DW value is close to zero, the vector is structured and so the factor is significant and can be retained, while if the DW value is close to 2, the vector is noisy and can be discarded [32].

The second method is the "*ICA-by-Blocks" method* which consists in splitting the data matrix into *B* blocks of samples of approximately equal size (equal numbers of rows). For each of these predefined blocks, *Amax* ICA models are computed, with 1–*Amax* ICs. ICs corresponding to true source signals should be found in all representative subsets of samples, or row blocks, of the full data matrix. Such true ICs calculated from different blocks should be strongly correlated. The noisy ICs will then have lower correlations with the ICs extracted from other blocks [34].

4. Results and discussion

The data corresponding to sunflower oil heated at different times for a given × 111) three-way cubic array of ten spectra (one spectra for each oil: as-is, with BHT or with Nigella extract after three different heating times (1 h, 2 h and 3 h) plus the unheated oil as the reference sample), with 126 emission wavelengths and 1 1 1 excitation wavelengths. So as to simplify the interpretation of the data, the two elementary cubes (corresponding to the two different temperatures: 120 and 140 °C) were gathered together giving a (20 × 126 × 111) three-way cubic array. The final cube of data was unfolded to create a (20 × 13 986) matrix.

4.1. Choice of the number of ICA components

Independent Components Analysis, with 1–10 Independent Components (ICs), was applied to the unfolded matrix. These models were used to calculate ten approximations of the initial unfolded data matrix. Residuals matrices were then calculated by subtracting these approximations from the initial matrix. As progressively more ICs are extracted from the matrix, the resulting residuals become progressively noisier. The Durbin–Watson (DW) criterion was applied to each spectrum of the matrix of residuals in order to detect when all the informative signals had been removed, leaving just noise.

Fig. 1a shows the DW values for each unfolded residuals matrix for each sample, on abscissa, plotted as a function of the number of Independent Components, on ordinate. It can be seen that ICs are noisy after subtracting more than three ICs.

These results are in agreement with those of the ICA-by-Blocks method (Fig. 1b) which was applied to the same data set with $B=2$ blocks and $Amax=10$. In fact, the correlation plot presented in Fig. 1b shows that after extracting three ICs, the curves of correlation coefficients go down. Thus, three ICs will be used in the final Independent Components Analysis model.

4.2. Independent Components Analysis

An Independent Components Analysis (ICA) with 3 Independent Components (ICs) was applied to the unfolded matrix (20 × 13986).

It can be seen that at each temperature the "scores" on IC1 (Fig. 2a) and on IC2 (Fig. 3a for the enriched samples (with Nigella seeds extract or with BHT) evolve less as a function of heating time than for the unenriched Controls. These IC scores are related to the oxidation products [13,23,35] as can be seen by examining the corresponding IC signals folded back into excitation-emission matrices (Figs. 2b and 3b). The decrease in the scores during heat treatment can be explained by the degradation of the already existing oxidation products and the formation of new ones. As an example, oils contain polyunsaturated fatty acids which undergo spontaneous peroxidation by way of thermal oxidation reactions which leads initially to the formation of hydroperoxides. The lipid hydroperoxides can undergo further oxidation, eventually

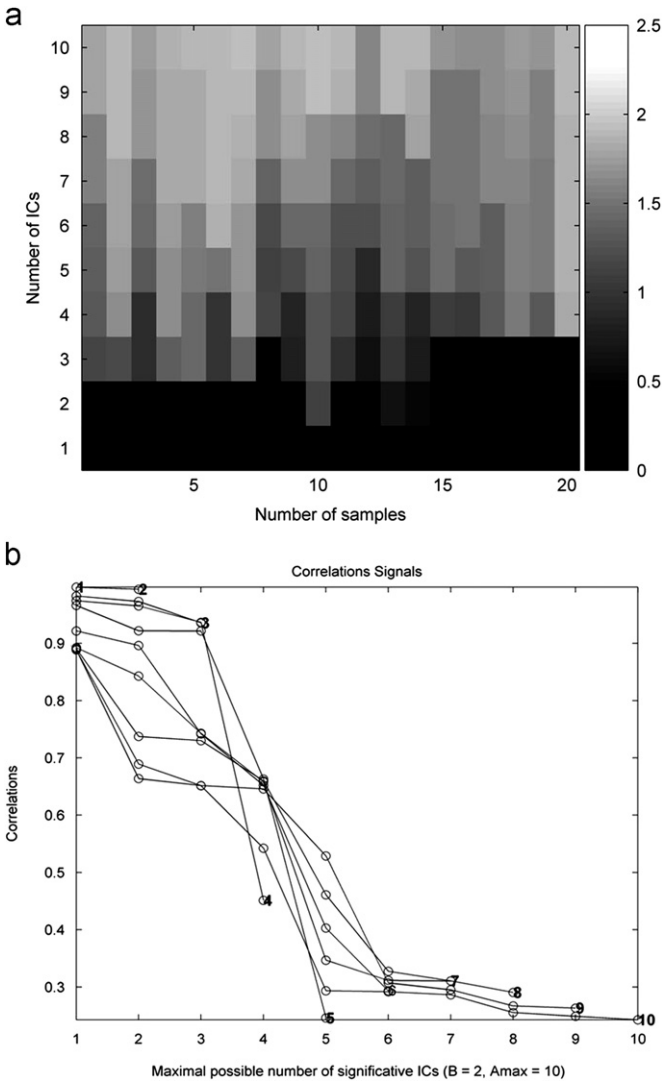

Fig. 1. (a) Durbin–Watson values of the residual matrices for increasing numbers of extracted IC signals plotted as a function of the sample number. (b) Correlations between signals extracted from the two blocks.

forming secondary oxidation products such as aldehydes, ketones and other species via a complex series of radical reactions.

IC3 (Fig. 4b) is related to the antioxidants naturally present in the oil (polyphenols and tocopherols) [13–15]. The IC3 scores plot (Fig. 4a shows that these compounds decrease during heating but remain at a higher level in the enriched samples.

Loss of naturally occurring antioxidants, such as vitamin E (tocopherol), during heating of oils may be attributed to their decomposition, the radical scavenging reactions of the antioxidants and evaporation [36].

All ICs show that during heating, the scores corresponding to the oils enriched with Nigella extract or with BHT evolve in the same way, which confirms that the addition of the extract plays the same role as the addition of synthetic antioxidant. Finally, one can observe that each IC signal plot presents a specific wavelength zone which allows the interpretation of the differences observed in the corresponding IC scores plot between Control, BHT-spiked and Nigella-spiked samples.

We can clearly notice that the reaction of degradation of the oxidation products already existing in the refined oils is slower at 120 °C than at 140 °C. Indeed, for all the ICs, the slope values (calculated before the break in the slope: between 0 and 120 min for IC1 and IC3 at 140 °C and for all the points for the other

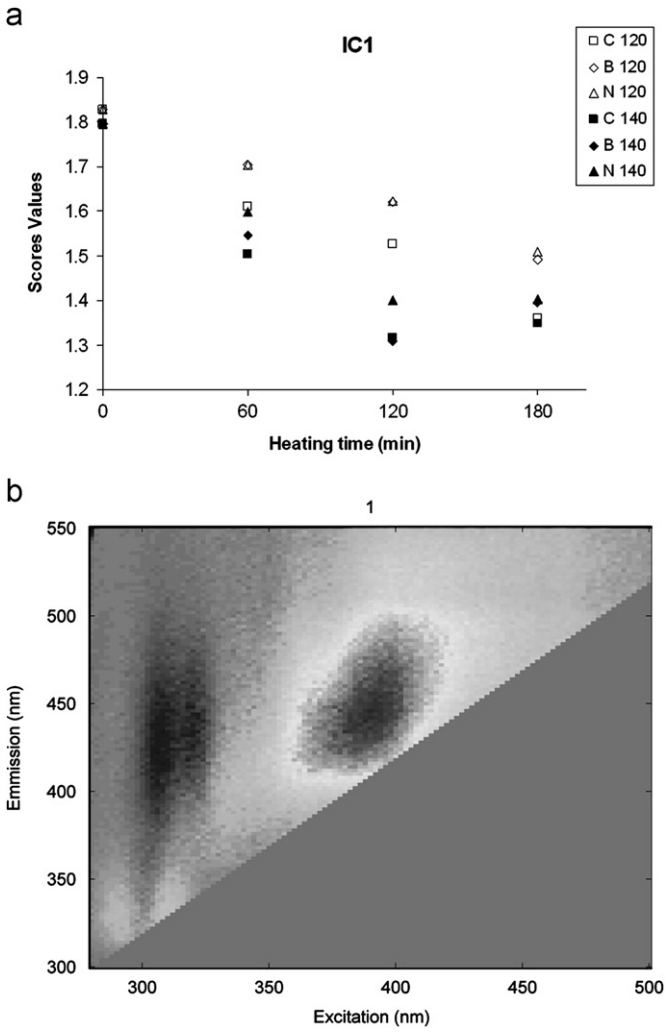

Fig. 2. (a) Scores and (b) signals of IC1 (In the Figure legend, C=Control samples, B=BHT-spiked sample, and N=Nigella-spiked samples, heated either at 120 or 140 °C).

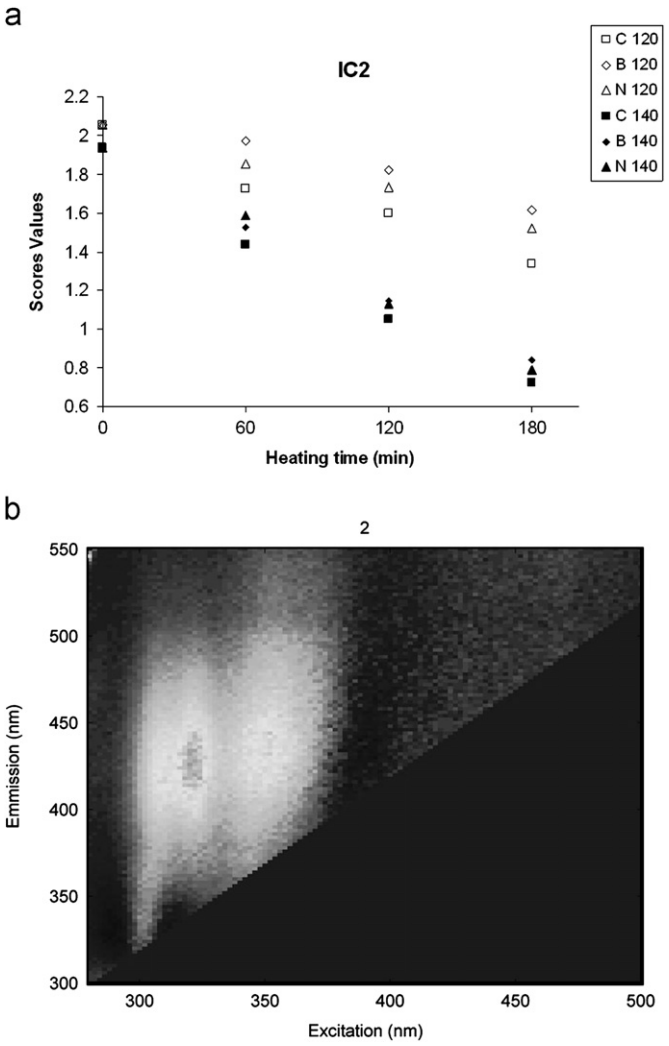

Fig. 3. (a) Scores and (b) signals of IC2 (In the Figure legend, C=Control samples, B=BHT-spiked sample, and N=Nigella-spiked samples, heated either at 120 or 140 °C).

curves) at 120 °C are lower than those at 140 °C (Table 1). We can also observe in Table 1 that the addition of antioxidants (synthetic or natural ones) delays this reaction and that this effect is more visible at 120 °C. In fact, at this temperature the slopes of all the ICs are evidently lower for the enriched samples (with BHT or with nigella extract) in comparison with 140 °C where the difference between the supplemented samples and the non-supplemented ones becomes small with the exception of the nigella-spiked oil on the IC1.

At 140 °C, after 60 min of heating, the effect of the Nigella extract becomes less apparent because the antioxidants present in the oil which protect the already existing oxidation products (refined oil) disappear which leads to the formation of many others degradation products.

Thus, Nigella extract at the level of 800 ppm exhibits an antioxidant effect similar to that of synthetic antioxidant BHT at 200 ppm.

4.3. Physicochemical properties (viscosity and anisidine values)

During heating, the oil rapidly undergoes oxidation, polymerization and other chemical changes which result in an increase in viscosity [37]. An increase in viscosity of heated oil is therefore a sign of oil deterioration through polymerization, oxidation, hydrolysis and isomerisation [38].

As shown in the supplementary data (SD) (Fig. S1a), at 120 °C, viscosity increases slowly. However, the values of viscosity of the oils enriched with BHT or Nigella remain (slightly) lower than those of Control oils.

At 140 °C (Fig. 5a, the difference between enriched and Control samples becomes clearly discernable. In fact, the viscosity values increase significantly in the non-supplemented samples, indicating that these samples are much degraded (the differences between the *Y*-scales of Fig. S1a (SD) and Fig. 5a must be considered).

The anisidine values (AV) evolves in the same way as the viscosity, as shown in Fig. S1b (SD) and Fig. 5b.

4.4. ^{1}H NMR spectroscopy analysis

^{1}H NMR can be employed to determine minor components of the oils which are related to oil quality. Although, at a first glance, an ^{1}H NMR spectrum of edible oil only shows the signals corresponding to the major components in the oil, the presence of some other components may be detected by analyzing the small signals that appear in the baseline.

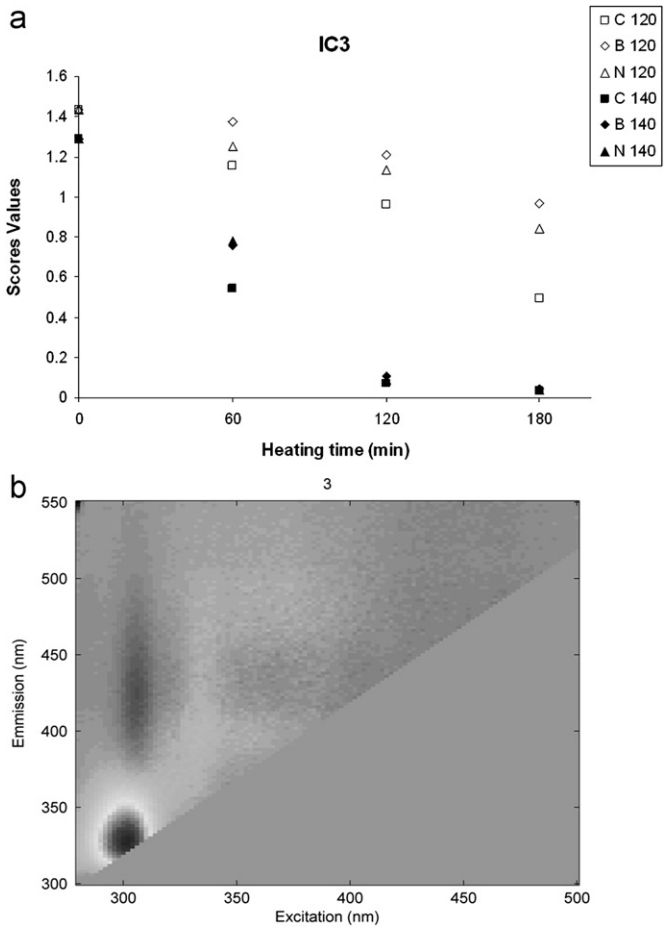

Fig. 4. (a) Scores and (b) signals of IC3 (In the Figure legend, C=Control samples, B=BHT-spiked sample, and N=Nigella-spiked samples, heated either at 120 or 140 °C).

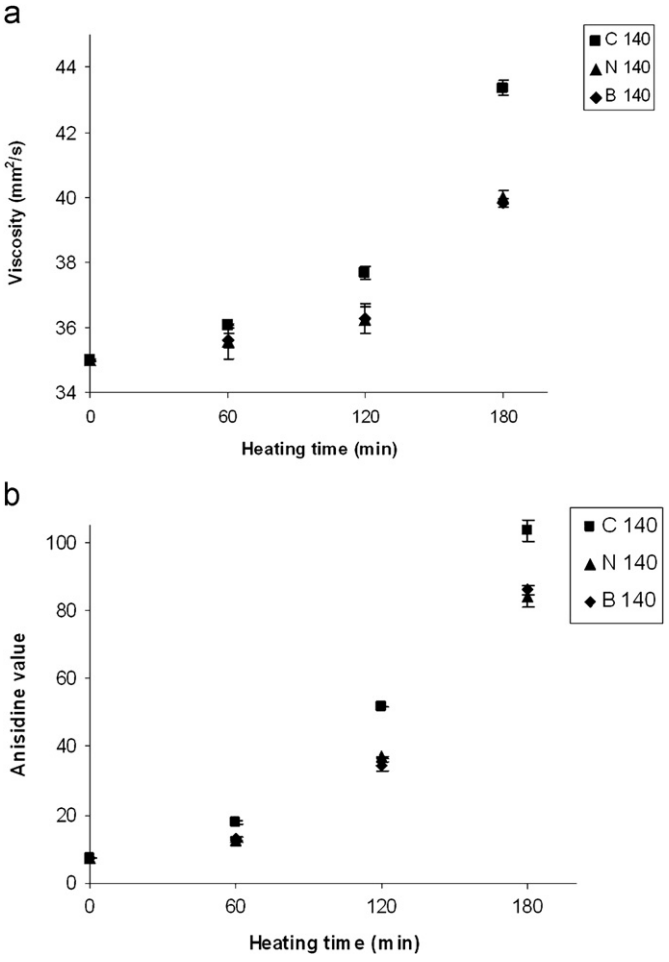

Fig. 5. Evolution of physicochemical properties of oil during heating (a) Viscosity at 140 °C, (b) anisidine values at 140 °C.

Table 1
Slopes of IC curves at 120 °C and 140 °C.

	C	B	N
Slope ($\times 10^{-3}$) 120 °C			
IC1	−2.6	−1.9	−1.8
IC2	−4.0	−2.4	−3.0
IC3	−5.2	−2.6	−3.3
Slope ($\times 10^{-3}$) 140 °C			
IC1	−4.0	−4.1	−3.3
IC2	−6.7	−6.1	−6.3
IC3	−10.1	−9.8	−10.0

Among all these signals, some of the most interesting are those that correspond to volatile compounds. In particular, the detection of aldehydic compounds is relatively easy because this part of the spectrum is free of other signals. Sacchi et al., Segre et al., and Mannina et al. [39–41] showed that the ^{1}H NMR spectra of edible oils may exhibit signals of linear alkanals, which appear as a singlet at δ 9.70 ppm; branched alkanals that appear as a doublet at δ 9.61 ppm; branched alkenals, appearing as a doublet at δ 9.54 ppm; and *trans* 2-alkenals, whose presence may be detected as a doublet at δ 9.45 ppm. These signals are related to both the positive fruity and green flavor of virgin oils and the negative rancid off-flavors produced during oil deterioration.

Fig. S2 (SD) shows the peaks due to aldehydes between 9.3 and 9.9 ppm in enriched samples and Controls heated at the same temperature for the same time (e.g. 2 h at 120 °C) [42–44]. We

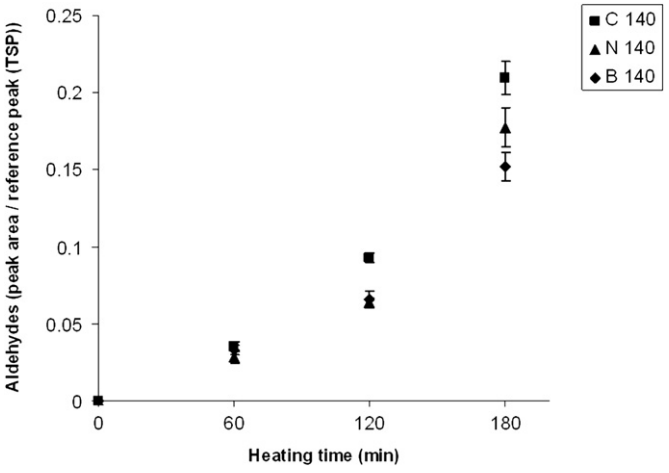

Fig. 6. Evolution of total surface of aldehyde peaks in enriched and Control oil samples during heat treatment for 3 h at 140 °C.

can see that peaks of aldehydes in the Controls are more intense than in the samples enriched with BHT or with Nigella extract.

The sum of the areas under all aldehyde peaks in each spectrum is plotted as a function of the heating time. The evolution of this total peak area (Fig. S3 (SD) and Fig. 6) shows that the total quantity of aldehydes formed during the oxidation

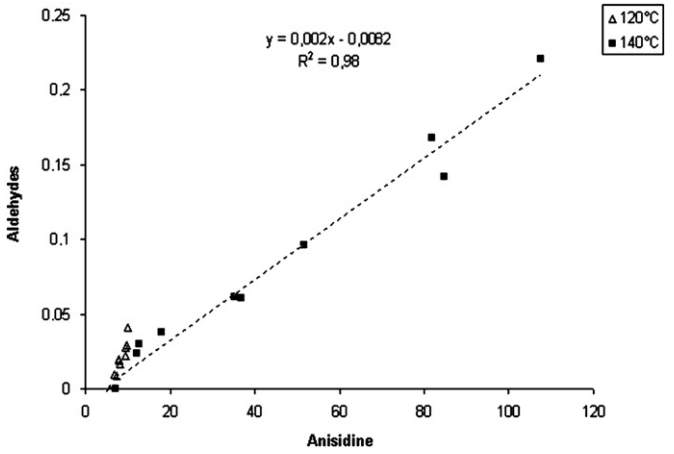

Fig. 7. Relation between total aldehydes peak surface (NMR) and anisidine values.

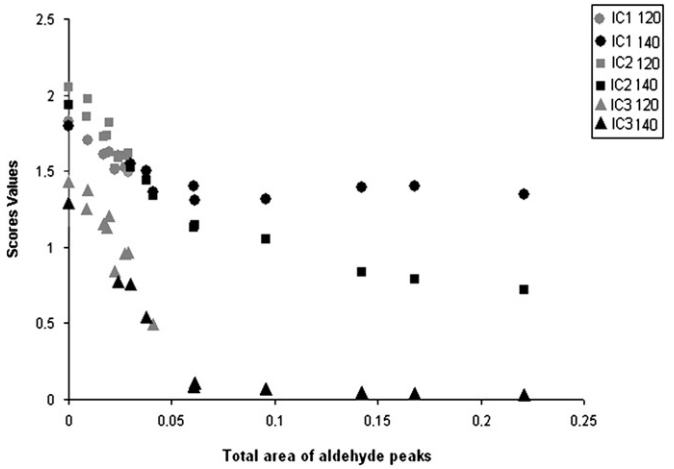

Fig. 8. Evolution of ICA Scores and total surface of NMR aldehyde peaks.

of the Controls is greater than the quantity formed in the enriched oils. This is particularly visible at 140 °C; at 120 °C, the values, although different, remain quite close (The differences between the Y-scales of Fig. S3 (SD) and Fig. 6 must be considered).

After 3 h of heat treatment at 140 °C, the quantity of aldehydes increases significantly (e.g: for the control samples this value passes from 0.03 after 1 h of heat treatment to 0,22 after 3 h).

The oxidation of unsaturated fatty acids is important in the development of rancidity and "off-flavors" in edible oils and fats. The secondary reactions occurring during the oxidation produce shorter-chain carbonyl compounds, mainly saturated and unsaturated aldehydes which, although present in trace amounts, are responsible for the characteristic flavors, both desirable and undesirable, of fat-containing foods [45].

It is clear that the anisidine and viscosity values and the aldehyde region of the ^{1}H NMR spectra are not very affected at 120 °C by the addition of the Nigella extract as the oil is not very oxidized at this temperature. However, at 140 °C, the oxidation of oil is accelerated and the antioxidant effect of Nigella extract is clearly discernable.

The similarity of the results for anisidine and aldehydes can be explained by the fact that these two parameters assess the formation of secondary oxidation products during heat treatment.

Fig. 7 shows the relation between the total aldehydes by ^{1}H NMR spectroscopy and the spectrophotometric determination of the anisidine values. One can clearly see that these two methods

Table 2
Slopes of IC curves.

Slope	
IC1	−8
IC2	−14
IC3	−21

are highly correlated (The regression line in the figure is for the curve at 140 °C).

We can also see that the amount of aldehydes formed at 120 °C is much lower than that formed at 140 °C.

Fig. 8 presents the evolution of the ICA scores values as a function of the total surface of the NMR aldehyde peaks, which can be used as an indicator of the degree and rate of oil oxidation. These curves show that the oxidation compounds corresponding to the fluorophore described on IC1 degrade more slowly than those described on IC2.

The evolution of the IC3 scores, corresponding to the antioxidants naturally present in the oil, shows that they degrade more rapidly than the oxidation products in IC1 and IC2. In fact, if we compare the initial slopes between 0 and 0.06 on the abscissa (Fig. 8) we can see that the slope attributed to the IC3 is greater (Table 2).

Once the antioxidants in IC3 disappear, the total aldehyde surface increases more quickly. In fact, we notice on Fig. 8 that antioxidants (IC3) disappear completely when the amount of aldehydes reaches 0.05. In Fig. 6b, we observe that starting for that value (0,05), aldehydes are formed more quickly.

After the degradation of antioxidants which tend to inhibit or to slow down the oxidation chain reactions, the oxidation products either remain stable or continue to decline but more slowly (see IC2 and IC3 in Fig. 8). This could be explained by the formation of new degradation products.

5. Conclusion

In this study, in addition to the classical methods, such as the determination of anisidine and viscosity values, fluorescence spectroscopy and NMR spectroscopy were shown to be easy, reliable and practical analytical methods to study the degradation of edible oils during heating and to highlight the antioxidant effect of Nigella seed extract, which improves the thermal stability and the shelf-life of the oils, and thus could be an interesting alternative to the use of synthetic antioxidants.

ICA is a powerful tool that allows the decomposition of the front-face 3D-fluorescence spectra and the extraction of the signals of individual fluophores which facilitate their interpretation.

Appendix A. Supplementary information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2012.05.059.

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