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The increase in oxidative stability of sunflower oil enriched with *Nigella sativa* L. Seed extracts

Faten Ammari · Christophe B. Y. Cordella ·
Néziha Boughanmi · Douglas N. Rutledge

Abstract *Nigella sativa* L. seeds are rich sources of phenolic compounds. The aim of this study was to evaluate the efficiency of Nigella seed extract as natural antioxidant compared with butylated hydroxytoluene (BHT) during accelerated oxidation of edible vegetable oils at 120 °C. The modifications during heating were monitored by 3D-front-face fluorescence spectroscopy. The fluorescence spectra were obtained with excitations from 280 to 500 nm and emission wavelengths from 300 to 550 nm and analysed using independent components analysis. Decomposition products formed during heating were also evaluated by means of ^{1}H-NMR spectroscopy and classic 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.

Keywords *Nigella sativa* L. · BHT · Sunflower oil stability · 3D-front-face fluorescence spectroscopy · ^{1}H-NMR spectroscopy · Independent components analysis

Introduction

Edible vegetable oils such as sunflower oil, corn oil, soya oil, and olive oil have an important place in human nutrition. These oils are consumed as salad dressing oils, cooking oils or frying oils.

During frying, a gradual deterioration of oil quality occurs due to oxidative decomposition reactions [1]. Lipid oxidation not only lowers quality and nutritional value of foods, it is also suspected of having consequences for the health of the consumer, such as aging, membrane damage, heart disease, and cancer [2].

Lipid oxidation causes changes in the type and concentration of the molecular species present in food. This is undesirable as it may lead to the development of off-flavours and result in potentially toxic compounds. For this reason, we investigated changes of fluorescence characteristics of sunflower oil resulting from thermal oxidation and the influence of a plant extract and a synthetic antioxidant on the rate of the oxidation.

Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and *tert*-butyl hydroquinone (TBHQ), are widely used in the food industry because they are effective and less expensive than natural antioxidants. In fact, Labuza [3] noted that antioxidants can increase shelf life of foods by 15–200 %. Their safety, however, has been questioned. Ito et al. [4] reported BHA to be carcinogenic in animal experiments. Under these circumstances, research on and development of safer natural antioxidants are therefore essential. According to Pokorny [5] when compared to synthetic antioxidants, many natural antioxidants have the following advantages:

(i) These natural antioxidant are often extracted from or mixed with foods that have been consumed by people for hundreds of years, which is not the case for synthetic antioxidants;

(ii) They are more readily accepted by consumers;

F. Ammari (✉) · N. Boughanmi
Faculté des Sciences de Bizerte Jarzouna, Université 7 Novembre Carthage, 7021 Tunis, Tunisia
e-mail: ammarifatouna@gmail.com

F. Ammari · C. B. Y. Cordella · D. N. Rutledge
INRA/AgroParisTech UMR1145 Génial, Laboratoire de Chimie Analytique, 16 rue Claude Bernard, 75005 Paris, France

(iii)   They are considered to be safe;

(iv)   Safety tests are not usually required by legislation;

(v)   These antioxidants not only stabilize the edible oils but may also add to the nutraceutical value of the oil.

Among herbs and spices, most attention has focussed on rosemary as a source of antioxidants. Black seed (*Nigella sativa* L.) is another plant used as a source of antioxidant. It is an annual herbaceous plant of the Ranunculaceae family [6] growing in Mediterranean countries. It has been traditionally used, especially in the Middle East and India, for the treatment of asthma, cough, bronchitis, headaches, rheumatism, fever, influenza and eczema [7] and for its antitumoral, antihistaminic, antidiabetic, anti inflammatory and antimicrobial activities.

In this study, *Nigella sativa* L. seed extract was used to enrich sunflower oil with a view to improving its thermal resistance during accelerated oxidation at 120 °C over 4 h. The modifications that occur in oil samples were monitored by front-face fluorescence spectroscopy (FFFS), ^{1}H-NMR spectroscopy and by determination of viscosity and anisidine values (AV).

In fact, fluorescence spectroscopy is gaining importance in food analysis and several authors have demonstrated that fluorescence is a good indicator of lipid oxidation in food systems. This technique, which does not require any sample preparation, has been applied to many food products, including cereal products, poultry meat and milk samples.

^{1}H-NMR spectroscopy has been largely used to determine edible oil composition and to evaluate their authenticity and quality. However, the approach of studying the antioxidant capacity of natural extracts during accelerated oxidation of edible vegetable oils by using both these techniques (front-face spectroscopy and ^{1}H-NMR spectroscopy) is rather novel and no such investigations have been carried out.

Materials and methods

Nigella 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 [8, 9].

Oil samples

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

Chemicals

Butylated hydroxytoluene, Folin–Ciocalteu reagent, gallic acid and quercetin were purchased from Sigma (Saint-Quentin Fallavier, France).

Characterization of *Nigella sativa* L. extract

UV/Vis Determination of polyphenol and o-diphenol content

Total polyphenol content was analysed as described by Singleton et al. [10]. The phenolic compounds were isolated from the oily seed extract by a mixture of water/methanol (25/75). Dichloromethane was added to transfer the oily phase to the lower phase and thus facilitate the collection of the supernatant. 800 µL of tenfold diluted Folin–Ciocalteu reagent were added. The mixture was allowed to stand at room temperature for 2 min. A volume of 1 mL of 75 % (w/v) sodium carbonate was added to the mixture and then mixed gently. The absorbance was read at 760 nm using a simple beam UV/visible spectrophotometer (UV–VIS Roucaire SHIMADZU UV-1205). The experiment was carried out in triplicate and the means and standard deviations calculated. The standard calibration curve was plotted using gallic acid and the results expressed as gallic acid equivalents (GAE), in milligrams per kg of oily extract.

The content of *o*-diphenols was determined according to Gutfinger [11]. 5 mL of phenolic extract obtained from the oily extract of *Nigella sativa* L. were mixed with 1 mL of the 5 % solution of sodium molybdate dehydrate in ethanol/water (1:1, v/v) and shaken vigorously. After 15 min, the absorbance was measured at 370 nm.

Determination of total flavonoids

Total flavonoid contents were measured according to Dewanto et al. [12]. 250 µL of the phenolic extract was mixed with 75 µL $NaNO_2$ (5 %). After 6 min, 150 µL of 10 % aluminium chloride were added, and 5 min later 500 µL of NaOH (1 M) were added. Finally, the mixture was adjusted to 2.5 mL with distilled water. The absorbance versus prepared blank was read at 510 nm. Total flavonoid content was expressed as mg quercetin equivalents (QE) per Kg of oily extract through the calibration curve with quercetin.

Determination of chlorophyll and carotenoid compounds

Chlorophylls and carotenoids were determined colorimetrically following the method of Minguez-Mosquera et al. [13]. The maximum absorption at 670 nm is related to the chlorophyll fraction and that at 470 nm is related to carotenoid fraction. The values applied for the specific coefficients of extinction were E0 = 613 for pheophytin as a major component in the chlorophyll fraction and E0 = 2,000 for lutein as a major component in the carotenoid fraction. Thus, the pigment contents were calculated as follows:

$$\text{Chlorophyll (mg/kg)} = \left(A_{670} \times 10^6\right)/(613 \times 100 \times d)$$

$$\text{Carotenoid (mg/kg)} = \left(A_{470} \times 10^6\right)/(2000 \times 100 \times d)$$

where A is the absorbance and d is the spectrophotometer cell thickness (1 cm).

Thermal stability test

Sunflower oil, free of additives, was used as the substrate for oxidation studies. Non enriched oils and oils enriched with 800 ppm of *Nigella sativa* L. extract were heated at 120 °C for 4 h to mimic cooking and frying conditions. For a comparative study, the synthetic antioxidant (BHT) was also tested at the legal limit of 200 ppm [9]. One 10 mL aliquot of each sample was taken every 15 min up to 180 min. A final aliquot was taken at 240 min.

Front face fluorescence spectroscopy measurements parameters

Fluorescence landscapes (3D spectra) were measured directly on the samples without prior chemical treatment except addition of *Nigella* extract, 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 (recorded every 2 nm) and excitation wavelengths 280–500 nm (recorded every 2 nm). A photomultiplier (PM) voltage of 420 V was used to avoid detector saturation. The "Forcing" option was also applied in order to limit the emission range so that data acquisition started above the excitation frequency, thus avoiding to the region of the Rayleigh scattering.

Thus, under these conditions, a 3D excitation–emission spectrum was acquired for each sample which covered the signals due to antioxidants and oxidation products.

Viscosity and anisidine value (AV) 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 represents the content of secondary oxidation products such as α and β-alkenals and of all those compounds able to react with *p*-anisidine reagent. AV were determined by the standard 2504 IUPAC method [14] by measuring absorbance at 350 nm using a simple beam UV/visible spectrophotometer (UV–VIS Roucaire SHIMADZU UV-1205).

^{1}H-NMR spectroscopy

The oil samples were placed in 5 mm diameter NMR tubes for the analysis. TSP (Trimethylsilyl propanoic acid) was used as a chemical shift reference. The spectra were obtained in a Bruker 300 MHz spectrometer by using a relaxation delay of 3 s and 64 scans.

The baseline corrections of the spectra were manually performed. Each sample was analysed three times.

Chemometrics methods

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

The fluorescence spectra, with the excitation wavelengths along the *x*-axis and the emission along the *y*-axis, were concatenated to give an excitation–emission cube with the oils samples at different heating times along the *z*-axis.

Independent component analysis

Independent component analysis is a blind source separation (BSS) techniques developed with the aim of extracting the pure underlying signals from a set of signals mixed in unknown proportions [15]. The general ICA model is [16]:

$$X = a.s$$

where $\mathbf{X}$ is the matrix of observed spectra, $\mathbf{S}$ is the matrix of unknown "pure" source spectra and $\mathbf{A}$ 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 must have intensity distributions that are less Gaussian than are their mixtures [15]. We applied ICA to the data set of unfolded 3D fluorescence spectra using the JADE algorithm [17].

Independent component analysis has already been used by Bouveresse et al. [16] as a pre-treatment method of 3D fluorescence data in order to eliminate Rayleigh and Raman scattering effects overlapping spectral regions containing interesting chemical information. In our case, this problem is already solved thanks to the use of the "forcing" option. In fact, the use of this option in the data acquisition software removes artefacts from the recorded spectra, at the beginning. In the present article, we intend to demonstrate the usefulness of ICA as a means to extract the proportions and the signals of the underlying fluorophore from complex fluorescence spectra.

Results and discussion

UV spectrophotometric analysis

Since plant phenolics constitute one of the major groups of compounds that act as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in Nigella seed extract. Flavonoids, as one of the most diverse and widespread groups of natural compounds, are probably the most important natural phenolics [18]. These compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties.

All analytical determinations were performed in triplicate. Values were expressed as the mean $\pm$ standard deviation (X $\pm$ SD).

Nigella seed extracts contain a higher content of polyphenol (253.6 $\pm$ 11.1 mg GAE/Kg) and o-diphenol (144.7 $\pm$ 2.1 mg GAE/Kg). These results are compatible with those obtained by Cheikh-Rouhou et al. [19] who found a total phenolic content of Nigella seed oil of 245 $\pm$ 9 mg GAE/Kg for Tunisian seeds and 309 $\pm$ 12 mg GAE/Kg for Iranian seeds.

Moreover, the extract is rich in flavonoids (103.7 $\pm$ 10.0 mg QE/Kg) known to be free radical scavengers and as an inhibitors of hydrolytic and oxidative enzymes [20]. The content of chlorophylls and carotenoids is also an important quality parameter. In fact, the presence of these pigments influences the auto-oxidation and photo oxidation of the oils. Chlorophylls exert an antioxidant action in the dark but an oxidant effect in the light [21] and thus may enhance oil deterioration, whereas carotenoids prevent it by quenching the reactive oxygen species [22].

According to Cheikh-Rouhou et al. [19], the chlorophyll content in Tunisian seed oil is 6.04 ppm compared to 2.26 ppm for Iranian seed oil. In our extract, the chlorophyll content is 4.3 $\pm$ 0.1 ppm and the carotenoid content is 2.8 $\pm$ 0.1 ppm.

So, Nigella seeds could be considered as a potential source of natural phenolic compounds that, as well as conferring a specific flavour to the oil, may have a positive
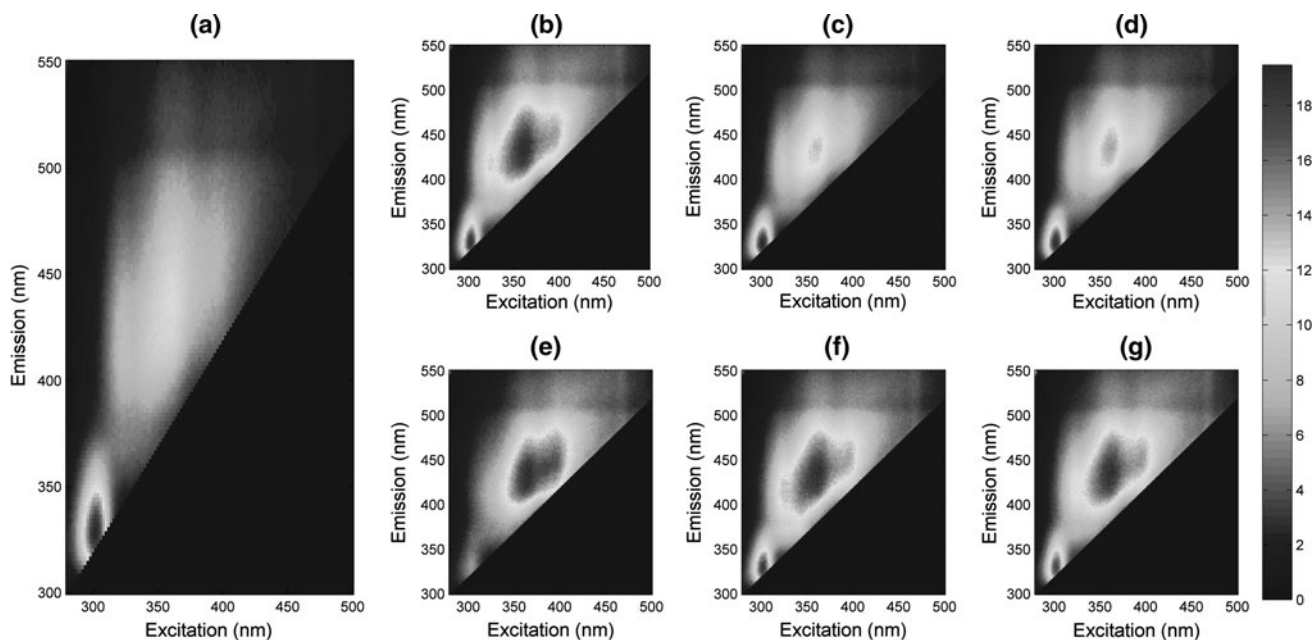
Fig. 1 Effect of thermal oxidation at 120 °C on fluorescence spectra after 180 min of heating [unheated sunflower oil (**a**), non enriched sunflower oil (**b**), sunflower sunflower oil enriched with BHT (**c**) and oil enriched with Nigella extract (**d**)] and after 240 min of heating [non enriched sunflower oil (**e**), sunflower oil enriched with BHT (**f**) and sunflower oil enriched with Nigella extract (**g**)]

effect in the prevention of coronary heart disease and cancer [23].

Front face fluorescence spectroscopy analysis

The unheated oil and the oils heated at different times, with and without the Nigella extract or the BHT antioxidant, exhibit two main bands with excitation at about 290–315 nm and emission at about 320–360 nm, for one, and excitation at about 320–420 nm and emission at about 400–500 nm, for the other.

Figure 1 shows the spectra of enriched (with BHT or Nigella extract) and non-enriched oils heated at 120 °C for 180 and 240 min. This figure shows that for a given period of heating (180 min or 240 min), the intensity of the signals due to oxidation products (excitation region 320–420 nm and emission 400–500 nm) in non-enriched oils is greater than that in the enriched oils, indicating that the quantity of oxidation products is greater in non-supplemented samples. It can also be clearly seen that after 240 min, the peak due to the polyphenols and tocopherols naturally present in the oil (excitation region 290–315 nm and emission 320–360 nm)

Fig. 2 **a** Scores and **b** signals of IC1
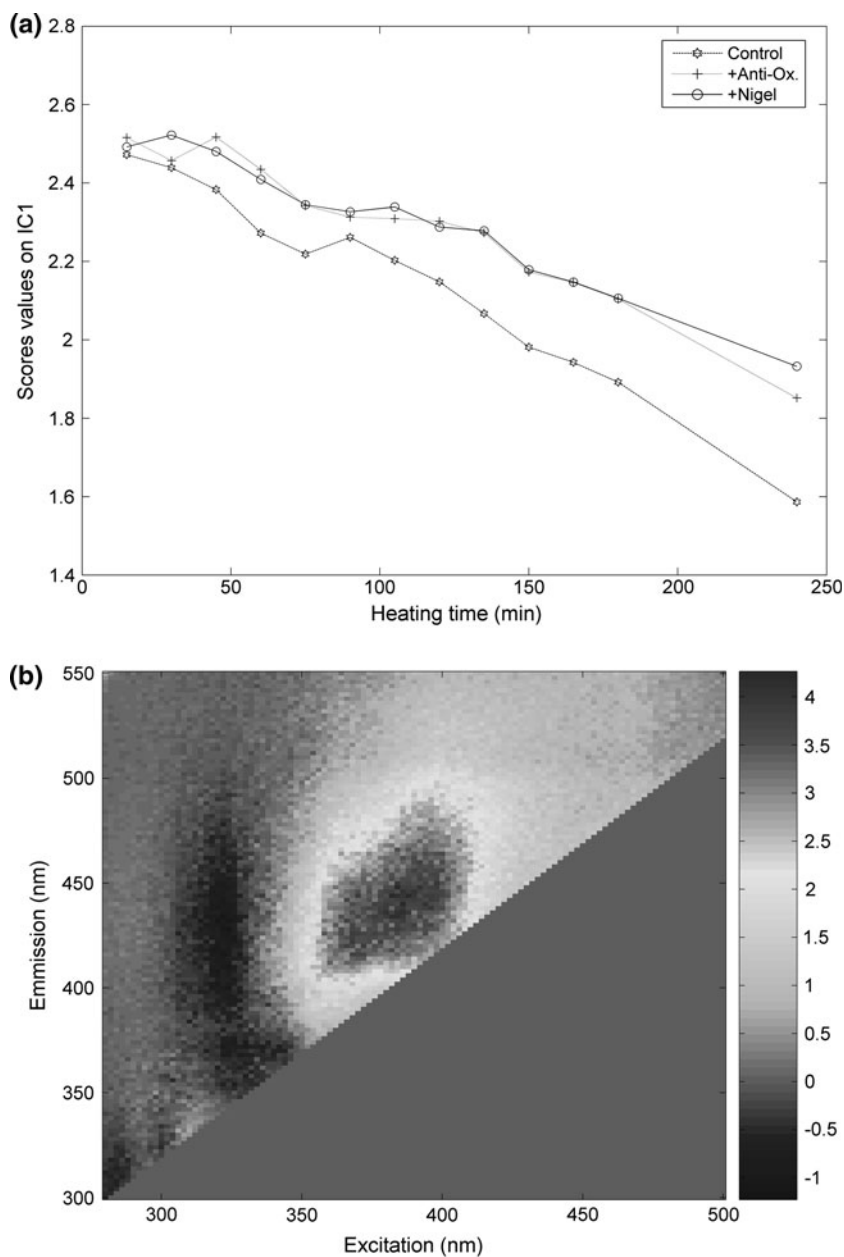
Fig. 3 **a** Scores and **b** signals of IC4
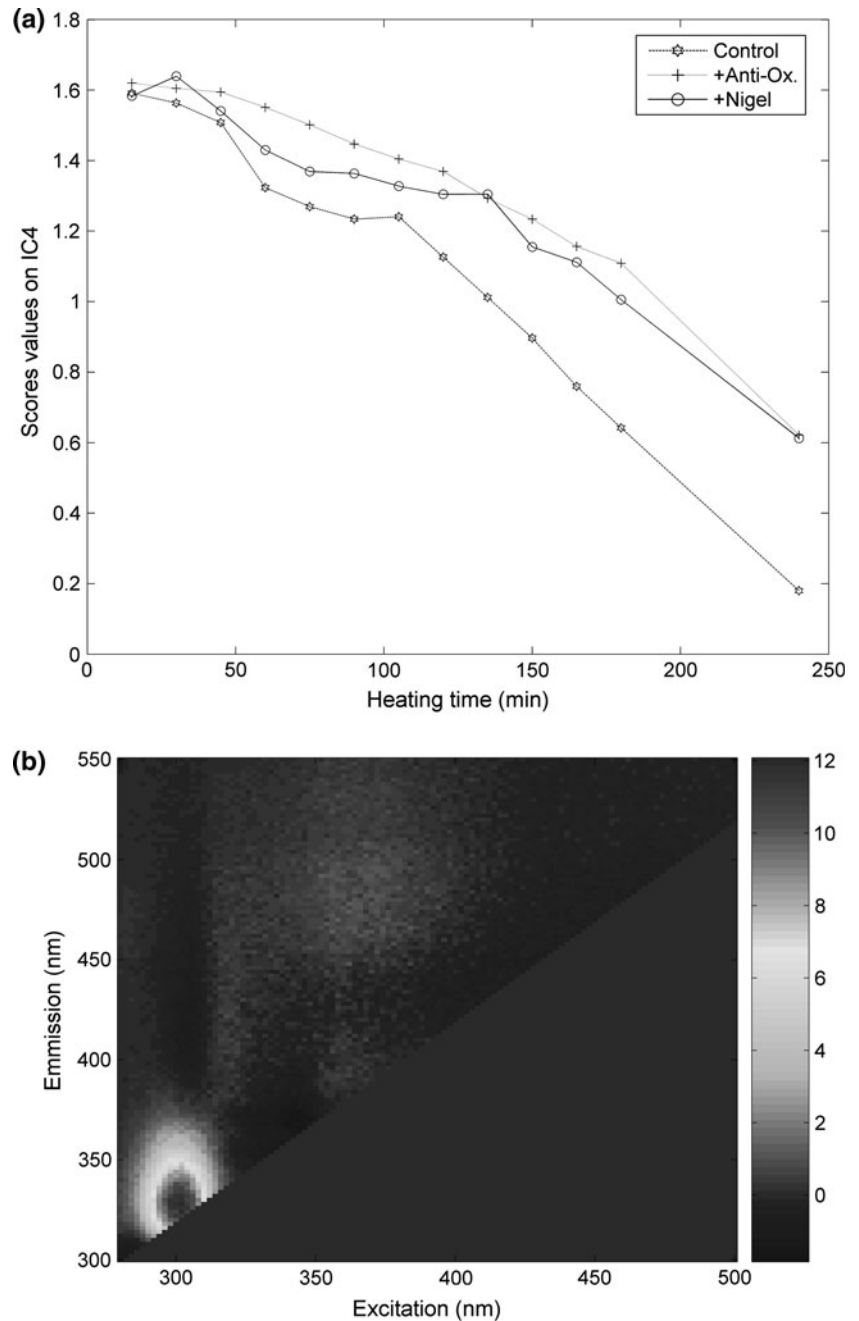
Table 1 Chemical interpretation of IC signals calculated by the JADE algorithm

IC	Wavelength region	Possible fluorophores	References
1	λex = 350–440 nm, λem = 400–500 nm	Oxidation products (oxidation of conjugated dienes and trienes)	[16, 24–26]
2	λex = 320–370 nm, λem = 350–450 nm		
3	λex = 300–330 nm, λem = 380–490 nm		
4	λex = 290–315 nm, λem = 320–360 nm	Antioxidants (tocopherols + polyphenols)	[24, 27, 28]
5	λex = 280–300 nm, λem = 300–340 nm		

has decreased markedly in the non-enriched sample, whereas it persists in the supplemented ones.

Independent components analysis

The data are initially in a (40 × 126 × 111) 3-ways cubic array of 40 spectra, with 126 emission wavelengths and 111 excitation wavelengths. The cube of data was unfolded to create a (40 × 13986) matrix. An independent components analysis (ICA) with five independent components (IC) was applied to this matrix.

IC1 (Fig. 2), IC2 and IC3 (not shown) of the enriched samples (with Nigella seeds extract or with BHT), corresponding to primary and secondary oxidation products [16, 24–26], evolve less as a function of heating time. In fact, oils containing polyunsaturated fatty acids undergo spontaneous peroxidation by way of thermal oxidation reactions involving ground-state oxygen, which leads initially to the formation of lipid hydroperoxide products. The lipid hydroperoxides can undergo further oxidation, eventually forming aldehydes, ketones and other species via a complex series of radical reactions.

IC4 (Fig. 3) and IC5 (not shown) corresponding to the naturally present antioxidants (polyphenols and tocopherols) [24, 27, 28] decrease during heating. After 4 h of heat treatment, the difference between enriched oils (with Nigella seeds extract or with BHT) and non-enriched oils becomes greater. In fact, the antioxidant peak decreases markedly in non-enriched samples, whereas it persists in the enriched ones.

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 [29]. The presence of vitamin E protects the fats to some extent from oxidation by combining with radicals and stopping the chain reactions by which the free radicals multiply. In this process, vitamin E is oxidized especially by hydroperoxides, which are oxidation products of unsaturated fatty acids [30].

All ICs show that during the 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 wavelengths zone which allows the interpretation of the differences observed in the corresponding IC scores plot between Control, BHT-spiked and Nigella-spiked samples (See Table 1).

Thus, Nigella extract at the level of 800 ppm exhibited antioxidant effect similar to that of synthetic antioxidant BHT at 200 ppm.
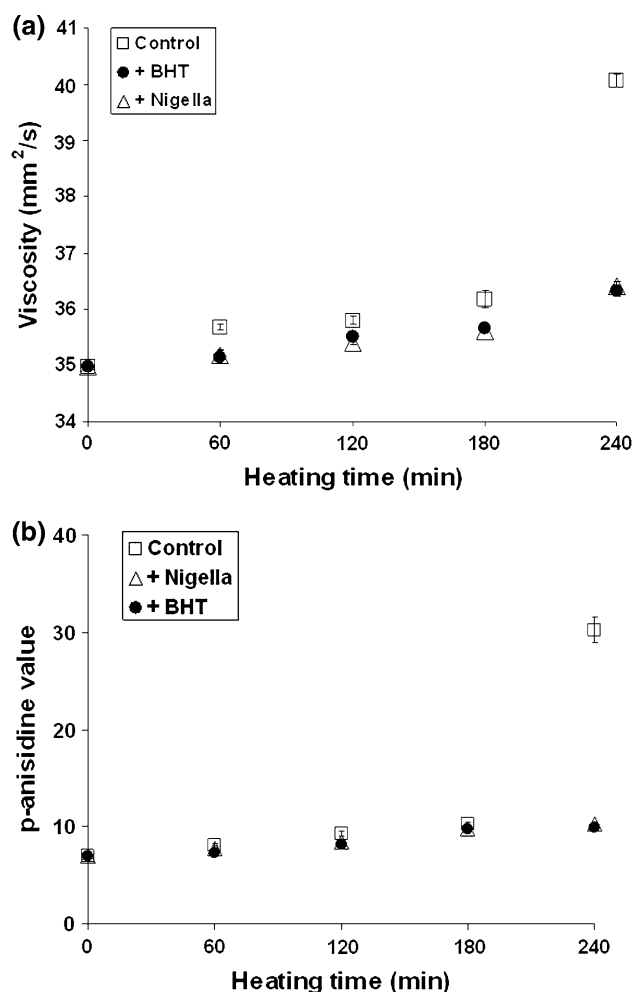
Fig. 4 Evolution of physicochemical properties of oil during heating **a** viscosity and **b** anisidine values (*square* non-enriched oils, *triangle* oils enriched with Nigella extract, *filled circle* oils enriched with BHT)

Physicochemical properties (viscosity and AV)

The oil rapidly undergoes oxidation, polymerization and other chemical changes which also result in an increase in viscosity of the heated oil [31]. The changes in viscosity of heated oil are the signs of oil deterioration. The oil may thicken and become more viscous as it is heated. This is due to the process of polymerization and also to oxidation, hydrolysis and isomerization [32]. Thickening reduces the rate of heat transfer and it takes longer to cook and color the food. It also increases oil absorption, and this effect will carry over into the finished products.

As shown in Fig. 4a, during the first 3 h of the heating, viscosity increases slowly. However, the values of viscosity of the oils enriched with BHT or Nigella remain lower than those of non enriched oils.

After 4 h of heating, the viscosity of non-enriched samples increases abruptly whereas that of the enriched

Fig. 5 Increase of the peak intensity of aldehydes during heating (control oil)
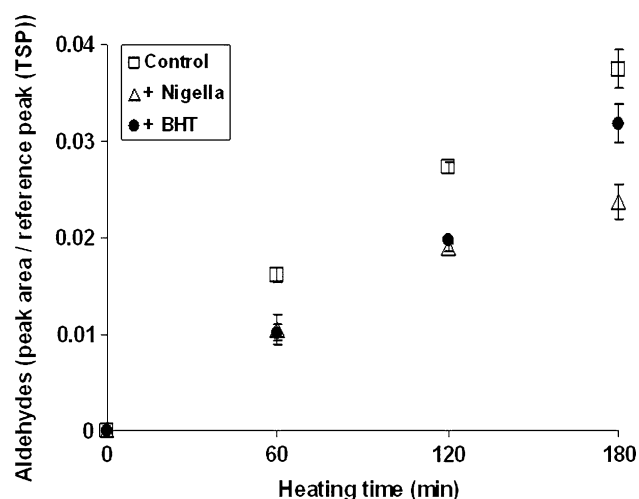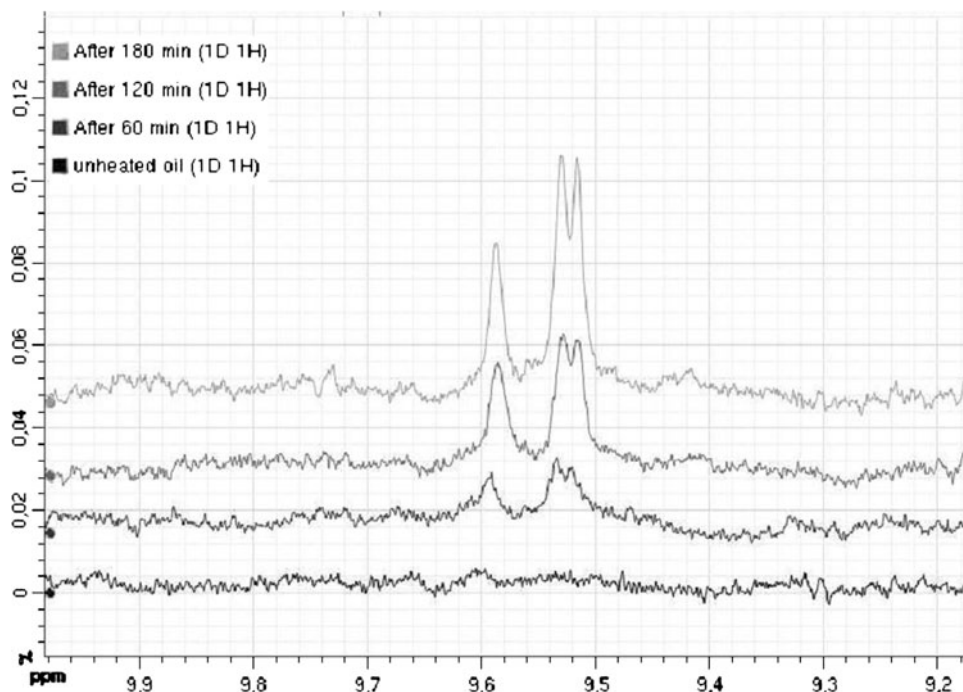
Fig. 6 Evolution of aldehydes in enriched and non-enriched samples during heat treatment (*square* non-enriched oils, *triangle* oils enriched with Nigella extract, *filled circle* oils enriched with BHT)

oils continues to increase very slightly. The AV evolve in the same way as the viscosity, as shown in Fig. 4b.

^{1}H-NMR spectroscopy analysis

Figure 5 shows the progressive appearance of peaks due to aldehydes which give signals in the ^{1}H-NMR spectrum between 9.3 and 9.9 ppm [33–35]. The sum of the areas under all aldehyde peaks in the spectra is plotted as a function of heating time. The evolution of this total peak area (Fig. 6) shows that the quantity of aldehydes formed

during the oxidation of non-enriched oils is greater than that formed in oils with added BHT or Nigella.

The oxidation of unsaturated fatty acids is important in the development of rancidity and "off flavours" 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 flavours, both desirable and undesirable, of fat-containing foods [36].

During heat treatment, oxidation products already existing in refined oils undergo degradation reactions providing new oxidation products. Primary oxidation products such as hydroperoxides can lead to the formation of secondary ones as aldehydes and ketones. It is what we observe using fluorescence spectroscopy where oxidation products are degraded during heating while remaining clearly lower for enriched samples. ^{1}H-NMR spectroscopy results are in agreement with those of fluorescence ones. In fact, aldehydes, secondary oxidation products, increase during heating what results in an increase in the peak area.

The results of the 3D-fluorescence and ^{1}H-NMR spectroscopies are seen here to reflect better the evolution of the oils and their relative stabilities than the AV and viscosity, especially during the first hours of heating. These classical methods give a global characterisation of the oils. Therefore, they offer only global information about the changes occurring during the oxidation process.

Fluorescence spectroscopy has the advantage over NMR of being a much less expensive and more robust technique

which generates signals that are more easily analysed by chemometric methods. The decomposition of the 3D-fluorescence spectra allows the extraction of individual signals to characterise the evolution of different fluorophores.

Therefore, by combining 3D-fluorescence spectroscopy with chemometric methods, as in this study, the complete heating process can be monitored on-line, and changes to improve the quality and to extend the frying life of the oil can be studied.

Conclusion

The decomposition of the front-face 3D-fluorescence spectra by ICA allows the extraction of the signals of individual fluorophores. The evolution of the contribution of the various fluorophores to the spectra during the heating process can then be monitored.

The use of ICA to analyse 3D- front face fluorescence spectra facilitated the characterisation of the effect of antioxidants during the thermal evolution of samples with or without Nigella extract, and with or without synthetic antioxidant.

Based on the results of this study, Nigella seed extract is seen to be a good source of antioxidant compounds. The addition of such an extract to edible oils may improve their thermal stability and shelf-life. Thus, Nigella extract could be an interesting alternative to the use of synthetic antioxidants.

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