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Evaluation of oxidative stability of vegetable oils by monitoring the tendency to radical formation. A comparison of electron spin resonance spectroscopy with the Rancimat method and differential scanning calorimetry

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Abstract

Electron spin resonance spectroscopy (ESR), based upon the spin trapping technique, was applied at 60 °C for evaluating the oxidative stability of 11 vegetable oil samples, including sunflower oil, rapeseed oil and their mixtures. The ESR results were compared with the oxidative stability values provided by the Rancimat method and differential scanning calorimetry (DSC) at 100 °C. High linear correlations were found between ESR and Rancimat ($r = 0.963$), and between ESR and DSC ($r = 0.979$), suggesting that ESR can predict the oxidative stabilities provided by the Rancimat method, as well as by DSC, within a wide range of stability values. In order to examine the capability of each method to evaluate antioxidant activity, different concentrations of α-tocopherol were tested in purified sunflower oil and purified rapeseed oil. Minor differences among the three methods were found when low concentrations of α-tocopherol were used, whereas similar responses were obtained at the highest antioxidant concentration assayed. In comparison with the Rancimat method and DSC, the ESR method is concluded to be useful as a method employing milder conditions and shorter time, and which can be applied for the evaluation of oxidative stability of oils containing volatile antioxidants and other lipid systems containing water.

Keywords: ESR; DSC; Rancimat; Oxidative stability

1. Introduction

Lipid oxidation is a major cause of deterioration of fats and oils, leading to losses of quality and nutritional value, and the development of unpleasant flavours. Oxidative stability is known as the resistance to oxidation under defined conditions and is expressed as the period of time required to reach an end point which can be selected according to different criteria (e.g. development of rancidity), but usually corresponds to a sudden increase in oxidation rate. As oxidation normally proceeds very slowly until this point is reached, this time period is known as the induction period (IP).

Numerous methods, using accelerated oxidation conditions, have been developed for the evaluation of oxidative stability (Rossell, 1994; Wan, 1995). Elevated temperatures in the presence of oxygen or air, in excess, are applied to obtain results in reasonably short periods of time. The oil stability index (OSI) method, also commonly known as the Rancimat method, allows oxidative stability to be determined automatically under standardised conditions (AOCS, 1992). This method is widely used in the fats and oils industry and it can be applied by using two commercially available instruments: the Rancimat from Methrohm Ltd. (Herisau, Switzerland) and the Oxidative Stability Instrument from Omniom Inc. (Rockland, MA). The end point is that corresponding to a sudden rise of volatile acids generated from the oil samples heated at high temperature under constant aeration. These compounds are trapped in water and monitored by electro-conductivity.

Differential scanning calorimetry (DSC) has been applied as an oxidative stability method by several

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authors. Cross (1970), the first researcher who applied DSC, used isothermal conditions with oxygen flow. The IP was taken as the time where a rapid exothermic reaction between oil and oxygen occurred. DSC has shown a very good correlation with other accelerated methods, such as the active oxygen method (Cross, 1970) and the OSI method by applying the Oxidative Stability Instrument (Tan, Man, Selamat, & Yusoff, 2002).

Electron spin resonance (ESR) spectroscopy is of great value for the study of the early stages of lipid oxidation. Recently, ESR spectroscopy, based upon the spin trapping technique, has been developed to evaluate the oxidative stability of different food lipids as a measure of the resistance to free-radical formation. The IP was defined as the period during which radicals are formed very slowly before a sharp linear increase is observed. Unlike most rapid methods, oxidative stability is determined under mildly accelerated oxidation conditions by applying significantly low temperatures (Thomsen, Kristensen, & Skibsted, 2000). This method has also been used for beer (Uchida, Suga, & Ono, 1996) and raw milk (Kristensen, Andersen, & Skibsted, 2002), and it has even been used for quantitative determination of radicals to evaluate the tendency to oxidation of mayonnaise (Thomsen, Vedstesen, & Skibsted, 1999). In addition, it has been applied to evaluate potential antioxidants in beer (Andersen, Outtrup, & Skibsted, 2000), as well as in mayonnaise (Thomsen, Jacobsen, & Skibsted, 2000). In spite of its growing applications, this method has still not been compared with other methods for evaluating oxidative stability of oils.

The aim of the present study was to evaluate the oxidative stability of 11 vegetable oil samples, including sunflower oil, rapeseed oil and their mixtures, by monitoring the tendency to radical formation by ESR spectroscopy, and to compare the ESR results with the oxidative stability values provided by the Rancimat method and DSC. These three methods assess very different aspects of the oxidative process. Rancimat and DSC are based on generation of volatiles and thermal release, respectively, indicating the onset of advanced oxidation (termination), while ESR spectroscopy assesses the formation of the free radicals originating in the early stages of oxidation, indicating the onset of primary oxidation (initiation). In order to examine the capability of each method to evaluate antioxidant activity, different concentrations of α-tocopherol were tested in purified sunflower oil and purified rapeseed oil.

2. Materials and methods

2.1. Chemicals

N-*tert*-Butyl-α-phenylnitrone (PBN) (purity ⩾97%) was obtained from Fluka Chemie Gmbh (Buchs, Germany) and used as received. All other chemicals and reagents were of analytical grade and were used without further purification.

2.2. Samples

Different vegetable oil samples ($n = 11$) were used in this study. Rapeseed oil (RO), acquired from Aarhus Olie A/S (Aarhus, Denmark), sunflower oil (SO), acquired from a local supermarket, and their mixtures, in proportions 3:1, 1:1 and 1:3 (w/w), respectively, were used. RO and SO were purified by alumina column chromatography, according to Fuster, Lampi, Hopia, and Kamal-Eldin (1998) in order to obtain oils devoid of their naturally occurring tocopherols, peroxides and trace metals. Different amounts of DL-α-tocopherol (Merck, Darmstadt, Germany) were added to the stripped oils to obtain six additional samples containing 100, 200 and 400 ppm of α-tocopherol and which were denoted as RO*100, RO*200 and RO*400 for the stripped-RO samples and as SO*100, SO*200 and SO*400 for the stripped-SO samples, respectively.

2.3. Chemical analyses

2.3.1. Fatty acid composition

Fatty acid composition was analysed by gas–liquid chromatography after derivatisation to fatty acid methyl esters with 2 M KOH in methanol at room temperature according to the IUPAC standard method (IUPAC, 1992). A 5890 A-II chromatograph (Hewlett-Packard Co., San Fernando, CA) with a split injector operating with a 10:1 split ratio at 250 °C, a HP-FFAP capillary column, 25 m × 0.20 mm i.d. and film thickness 0.33 μm (Hewlett-Packard Co., San Fernando, CA), and a flame ionization detector at 300 °C was used. The analyses were performed using Helium (0.95 ml min^{-1}) as the carrier gas and applying the following temperature programme: 50 °C held for 1 min, 15 °C min^{-1} to 180 °C, 5 °C min^{-1} to 220 °C held for 10 min.

2.3.2. Analysis of tocopherols

Quantification of α- and γ-tocopherol was carried out by reverse-phase liquid chromatography (HPLC) with fluorescence detection by using external standard solutions of known concentrations of α- and γ-tocopherol. Oil samples (25 mg) were dissolved in 3 ml of ethanol/1-butanol (75:25, v/v) and directly analysed. An HPLC analytical column (125 mm × 4.0 mm) (Agilent Technologies, Karlsruhe, Germany) packed with a C18 phase and containing a mean particle size of 5 μm was used. Methanol/water (94:6, v/v) was used as mobile phase with a flow of 1 ml min^{-1}. The fluorescence detector operated with the excitation wavelength set at 292 nm and the emission wavelength at 330 nm.

2.3.3. Peroxide value

Peroxide value (PV) determination was carried out by iodometric assay (AENOR, 1991).

2.4. Rancimat tests

A Rancimat instrument, model 679, from Metrohm Nordic ApS (Glostrup, Denmark) was used in this study. The tests were carried out with 2.00 ± 0.01 g of oil at 100 °C and with an air flow of 20 l h^{-1}.

2.5. DSC analysis

DSC experiments were performed with a DSC 820 from Mettler Toledo (Schwerzenbach, Switzerland) under isothermal conditions (100 °C) and with an air flow of 60 ml min^{-1}. The samples (5.00 ± 0.25 mg) were weighed into 40 ml aluminium DSC crucibles which were closed with lids with a hole (1 mm i.d.) drilled in the centre in order to allow the oil to be in contact with the air stream. An empty crucible, hermetically sealed, was used as reference.

2.6. ESR spectroscopy analysis

The spin trapping method was applied to monitor the formation of free radicals. PBN, dissolved in the oils (1 mg g^{-1} oil) by stirring, was used as spin trap. Ten aliquots of each oil sample containing PBN were weighed (1.00 ± 0.05 g) in ESR tubes (700-PQ-7, heavy wall; Wilmad Glass, Buena, NJ) and placed in a water bath at 60 °C in darkness. The tubes were successively taken from the bath in 30-min periods and immediately placed in an ECS 106 ESR spectrometer (Bruker, Rheinstetten, Germany) to be analysed in real time. The parameters used in all ESR measurements were as follows: microwave power, 20 mW; modulation amplitude, 1.0 G; modulation frequency, 100 kHz; conversion time, 41 ms; and time constant, 82 ms. Formation of PBN-adducts was monitored by the peak-to-peak amplitude of the first line of the ESR signal, which was determined by the Winepr software programme (Bruker, Rheinstetten, Germany). A more detailed description of the method can be found in a previous report (Thomsen et al., 2000).

2.7. Statistical analysis

The ESR method applied in the present study requires that the measurements are performed in real time during the analysis. Hence, not more than three or four samples can be simultaneously assessed. Available ESR tubes, frequency of sampling and time taken to evaluate each sample-containing tube must be taken into account. Therefore, if double or triple determinations are performed, only one type of sample can be analysed. In this study, the three stability methods were concurrently applied to groups of samples, namely, RO and SO; RO*100, RO*200 and RO*400; SO*100, SO*200 and SO*400; and the different mixtures of RO and SO, using single ESR measurements and replicates ($n = 2$) in the Rancimat and DSC analyses. Once this series was completed, experimental results were verified by repeating each experiment including the preparation of the samples. These two series of experiments were denoted as 1st and 2nd experiment, respectively.

Statistical data analysis was performed by using SAS 8.2 software (SAS Institute Inc., Cary, NC, USA). Statistical comparisons between means were made applying the Student's t test. Significance was defined at $P < 0.05$ unless otherwise indicated. The SAS REG procedure was applied in the linear regression analysis.

3. Results and discussion

3.1. Characterisation of the oil samples

Fatty acid composition, tocopherol content and peroxide value of the oils used in the present study are given in Table 1. RO and SO showed the typical fatty acid profile of low-erucic rapeseed oils and high-linoleic sunflower oils, respectively. The contents of α- and γ-tocopherol were within normal range and the low PVs found were indicative of fresh refined oils. After purification, the oils showed, in comparison with the non-purified oils, no difference in fatty acid compositions, and contained undetectable amounts of tocopherol (< 0.1 mg kg^{-1} oil) and PVs lower than 0.5 meq O_2 kg^{-1} oil.

3.2. Oxidative stability by the Rancimat method

The IPs obtained by the Rancimat method were within the range 8.05–19.2 h, and the coefficients of variation (CV) were lower than 3.3% (Table 2).

3.3. Oxidative stability by DSC

Purified oxygen is the oxidant commonly applied in DSC analyses. However, as can be observed in Fig. 1, showing a representative DSC curve, in this study, air (60 ml/min) was found to be sufficient to determine the IP. The rise in heat flow at the end of the IP was fast enough to obtain results with good repeatability (CV lower than 8.4%). The IP values obtained by DSC ranged between 2.76 and 15.66 h and were significantly lower ($P < 0.0001$) than those obtained by the Rancimat method (Table 2). These results are in agreement with those reported by Tan et al. (2002) who suggested that these differences may have been due to a higher surface-to-volume ratio used in DSC.

Table 1
Fatty acid composition, α- and γ-tocopherol contents and peroxide value (PV) of the oil samples studied[a]

	RO	RO*100	RO*200	RO*400	SO	SO*100	SO*200	SO*400	RO:SO 3:1	RO:SO 1:1	RO:SO 1:3
Fatty acids[b] (%)											
C16:0	5.05	4.98	4.98	5.20	5.65	5.75	5.73	5.73	5.35	5.52	5.71
C16:1	0.19	0.19	0.19	0.20	0.08	0.09	0.13	0.08	0.17	0.14	0.11
C18:0	1.69	1.78	1.72	1.69	3.32	3.45	3.45	3.45	2.13	2.52	2.96
C18:1	59.58	60.35	60.39	60.55	27.05	27.07	27.03	27.02	51.25	43.03	34.75
C18:2	20.88	20.52	20.55	20.67	62.51	62.23	62.25	62.23	31.40	42.07	52.48
C18:3	9.77	9.23	9.22	8.91	0.13	0.13	0.14	0.21	7.45	4.81	2.46
C20:0	0.59	0.61	0.61	0.54	0.26	0.26	0.26	0.27	0.46	0.39	0.32
C20:1	1.42	1.46	1.46	1.35	0.30	0.29	0.29	0.29	1.09	0.84	0.56
C22:0	0.35	0.37	0.37	0.37	0.69	0.73	0.72	0.73	0.38	0.46	0.54
C22:1	0.49	0.51	0.51	0.50	nd	nd	nd	nd	0.32	0.22	0.11
α-Toc[c] (mg kg^{-1})	252 (2.2)	92 (0.2)	194 (3.3)	386 (2.2)	537 (11.9)	90 (1.8)	181 (3.7)	404 (7.6)	309 (3.2)	366 (2.1)	426 (1.2)
γ-Toc[c] (mg kg^{-1})	314 (2.7)	nd	nd	nd	20 (0.7)	nd	nd	nd	244 (1.8)	170 (0.6)	97 (1.3)
PV[d] (meq O_2 kg^{-1})	1.4 (0.07)	0.4 (0.21)	0.4 (0.14)	0.3 (0.14)	1.6 (0.07)	0.5 (0.07)	0.4 (0.14)	0.3 (0.21)	1.3 (0.07)	1.4 (0.14)	1.5 (0.21)

nd: not detected.
[a] For abbreviations see Section 2.
[b] Results correspond to mean values of duplicate samples.
[c] Results are expressed as means (standard deviations) of duplicate samples.
[d] Results are expressed as means (standard deviations) of duplicate samples.

Table 2
Induction periods determined by Rancimat, differential scanning calorimetry (DSC) and electron spin resonance (ESR) spectroscopy

Samples[a]	1st Experiment[b]			2nd Experiment[b]		
	Rancimat[c] (h)	DSC[c] (h)	ESR (min)	Rancimat[c] (h)	DSC[c] (h)	ESR (min)
SO	12.90 (0.14)	7.36 (0.11)	122.78	11.95 (0.21)	6.92 (0.03)	95.35
SO*100	8.05 (0.18)	2.96 (0.25)	64.15	8.24 (0.05)	2.76 (0.17)	62.67
SO*200	10.09 (0.16)	3.39 (0.01)	65.46	10.45 (0.21)	3.07 (0.05)	62.82
SO*400	11.95 (0.07)	4.38 (0.34)	95.30	12.00 (0.28)	4.36 (0.01)	103.00
RO	19.05 (0.35)	15.66 (0.31)	280.93	19.25 (0.35)	15.45 (0.04)	296.90
RO*100	9.19 (0.01)	4.34 (0.21)	60.00	9.12 (0.30)	4.41 (0.08)	60.00
RO*200	11.45 (0.35)	4.84 (0.20)	60.00	11.20 (0.00)	5.13 (0.27)	63.96
RO*400	12.45 (0.07)	6.01 (0.01)	115.66	12.65 (0.21)	6.04 (0.40)	90.00
RO:SO 3:1	17.60 (0.14)	13.48 (0.28)	264.59	17.20 (0.28)	13.33 (0.02)	240.00
RO:SO 1:1	15.90 (0.42)	11.47 (0.25)	200.40	15.15 (0.07)	11.23 (0.25)	195.40
RO:SO 1:3	13.70 (0.14)	9.46 (0.11)	163.93	13.90 (0.00)	9.33 (0.31)	157.70

[a] For abbreviations see Section 2.
[b] See Section 2.7.
[c] Expressed as means (standard deviations) of duplicate determinations.

3.4. Oxidative stability by ESR

Evaluation of oxidative stability by ESR is based on the measure of the free radicals formed in the early stages of oxidation. In this respect, oxidative stability is defined as the resistance to the formation of radicals, and is expressed as the period of time during which radicals are formed very slowly before a sharp linear increase is observed (Thomsen et al., 2000). Radicals are very reactive species with very short life times, and usually the use of spin trapping agents, which react with free radicals to form more stable radicals (spin adducts), is necessary to detect radicals within the ESR time scale. In the present study, PBN was used as spin trap and the formation of PBN-adducts was monitored for determining oxidative stability.

For comparative purposes, all the samples were assessed at the same temperature (60 °C) in the ESR

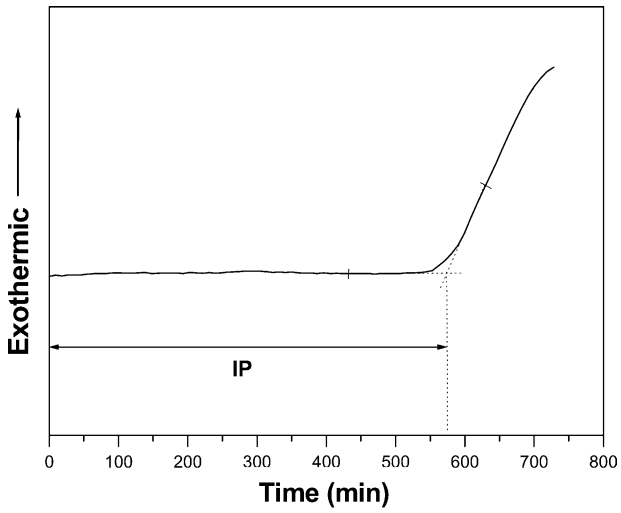

Fig. 1. Determination of the induction period by differential scanning calorimetry. Isothermal curve at 100 °C with air flow at 60 ml min^{-1} for sample RO:SO 1:3.

analyses. However, each oil sample should be assayed at an optimal temperature in order to determine the IP in a suitable range within the fixed time scale of the method (Thomsen et al., 2000). As a result, for the most stable samples, an IP was not observed, such as for RO (Fig. 2), or was not reliably determined due to the lack of points at the end of the experimental time scale, such as for RO:SO 1:1 and RO:SO 3:1 samples (data not shown). The rest of the samples showed a definite IP, and it was observed that the amount of the trapped radicals at the end of the IP was approximately the same for all these samples. This finding was of great utility for evaluating oxidative stability of the most stable samples showing either a non-definite IP or none. Thus, for RO:SO 1:1, RO:SO 3:1 and RO samples, the IP was determined as the time required to reach the amount of radicals observed at the end of the IP of the other samples assayed. Fig. 2 shows some examples of the ESR curves and how IP was determined.

For the oxidation curves obtained by the Rancimat method and by DSC, as well as by other stability methods based on the determination of the onset of accelerated oxidation, it is generally accepted that, at the accelerated stage, the oxidation rate depends only on oxygen availability and temperature. In contrast, for the ESR curves, it was found that the rate of spin adduct formation also depends on the stability of the samples before and after the IP (Fig. 2). This led to problems in obtaining accurate IP values in the most stable samples due to small changes in the slopes of the oxidation curves, as can be observed for SO (Fig. 2). This fact further supports the necessity of applying optimal temperatures, depending on the stability of each sample. However, this pattern of the ESR curves allows other alternative parameters to be considered for evaluating oxidative stability under the same experimental conditions, such as the increment of signal at fixed time (Thomsen et al., 2000) and the time required to reach a determined increment of signal, as that applied in the present study for RO:SO 1:1, RO:SO 3:1 and RO samples.

Table 2 lists the results corresponding to the two sets of assayed samples, i.e. those of the 1st and 2nd experiment, obtained by the three methods studied. Except for the SO sample, the IPs determined by Rancimat in the 1st experiment were not significantly ($P < 0.05$) different from those found in the 2nd experiment, and the same was true for the DSC results. By contrast, significantly ($P < 0.05$) lower values were found by both Rancimat and DSC for SO in the 2nd experiment, probably due to slight oxidative alteration of oil, occuring during handling and

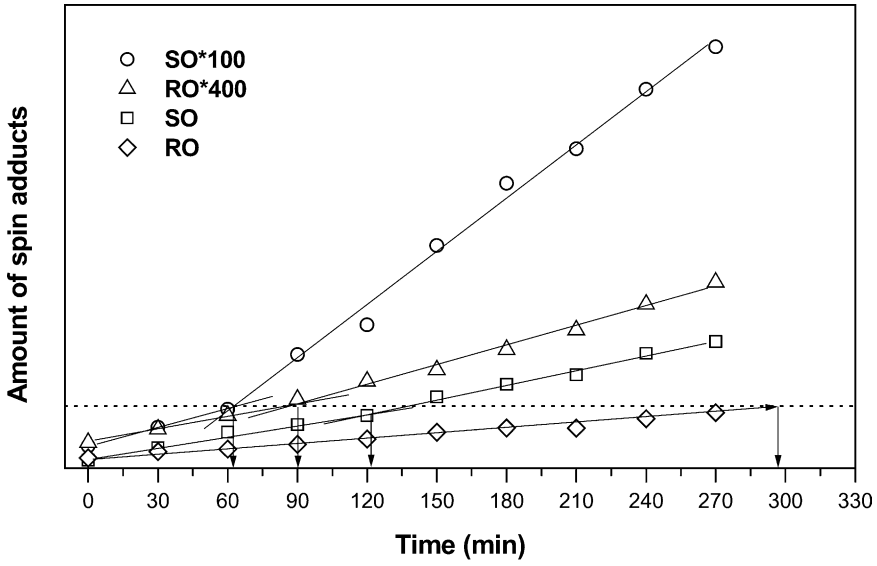

Fig. 2. Determination of the induction period (IP) by electron spin resonance spectroscopy. The dotted line indicates the amount of radicals that was considered to determine the IP of RO sample.

the time taken between the two experiments. The fact that these slight differences in oxidation level were not observed in the samples prepared from SO could be attributed to the dilution of peroxides in the preparation of the mixtures with RO and to their removal during the purification step. Taking this into consideration, the results obtained by ESR in the two experiments can be compared with each other with the exception of the SO sample. Thus, the IP values obtained by ESR in the 2nd experiment were quite similar to those found in the 1st experiment. However, higher differences would have been expected, taking into account not only the error involved in the repetition of experimental conditions, but also the graphic determination of the IP, which depends on a linear fit of the points, on the change in the slope, on the range within the time scale, as commented above, and, finally, on the sampling time which was in this study relatively long (30 min) in comparison with the low differences found between the two experiments. In this respect, it is important to stress that, despite the radical formation in each oil sample was followed up by measuring independent aliquots, the experimental points fitted quite well to straight lines (Fig. 2).

Despite using a much smaller surface-to-volume ratio and a lower temperature, the ESR method provided IPs which were markedly shorter than the IPs determined by the Rancimat method, as well as by DSC (Table 2). These results are related to the fact that these three methods assess very different aspects of the oxidative process. The ESR method assesses the formation of the free radicals originating in the early stages of oxidation, indicating the onset of primary oxidation (initiation), while the Rancimat and DSC methods are based on generation of volatiles and thermal release, respectively, which are indicative of the onset of advanced oxidation (termination). Therefore, even under the same oxidation conditions, the IPs determined by ESR would be expected to be shorter than the IPs provided by the Rancimat and DSC methods.

From a study on the evaluation of antioxidants in beer by ESR spin-trapping, it was suggested that, after the IP, the rate of spin adduct formation is indicative of the capacity of the system to generate radicals since antioxidants are completely exhausted during the lag phase (Andersen et al., 2000). In the present study, it was found that, once the IP was passed, the rate at which spin adducts were formed was not only dependent of the degree of unsaturation, but also of the concentration of α-tocopherol (Fig. 3), as will be discussed below. Unlike what is found for beer, these results indicate that antioxidants were not exhausted during the lag phase determined by ESR, which is reasonable considering the moderate oxidation conditions applied in the ESR analysis and the much longer IPs obtained at 100 °C by the Rancimat method, as well as by DSC. Therefore, it is suggested that the IP obtained by the ESR method is only indicative of the onset of the oxidative process, and that the onset of advanced oxidation, where the complete exhaustion of antioxidants occurs, is far from the time scale of the method.

3.5. Evaluation of antioxidant activity of α-tocopherol

Fig. 4 shows oxidative stability as a function of α-tocopherol concentration. In the range of concentrations assayed, the IP obtained by Rancimat increased with the concentration of antioxidant for both stripped SO and stripped RO. DSC showed poor changes in IP when the amount of tocopherol was increased from 100 to 200 ppm, whereas a considerable rise, similar to that found in the Rancimat, was observed from 100 to 400 ppm for both stripped SO and stripped RO. Furthermore, it should be noted that the increase in stability detected by DSC as a result of increasing the concentration of α-tocopherol was lower than that caused by the decrease of the degree of unsaturation when comparing SO with RO samples. Consequently, for the range of antioxidant concentrations assayed, the stabilities provided by DSC were mainly influenced by the degree of unsaturation and, as a result, the stripped RO samples were in general more stable than the stripped SO samples ($SO^*100 < SO^*200 < SO^*400 \approx RO^*100 < RO^*200 < RO^*400$). In contrast, the increase of stability caused by α-tocopherol, as detected by the Rancimat method, was higher than that caused by the decrease in the degree of unsaturation, and, consequently, the stability order predicted by Rancimat ($SO^*100 < RO^*100 < SO^*200 < RO^*200 < SO^*400 < RO^*400$) was the result of a higher contribution of the antioxidant concentration. These differences may be due to the different oxidation conditions applied in Rancimat and DSC, namely, the surface-to-volume ratio and air flow. It is commonly accepted that under different oxidation conditions different variables are expected to control the pathway and rate of oxidation. In this line, in a comparative study between DSC under non-isothermal conditions and the Oxidograph method, the latter based on measurements of oxygen consumption, Simon, Kolman, Niklová, and Schmidt (2000) found that the results obtained by these two methods were very similar for rapeseed oil, whereas shorter IPs were obtained in the DSC analysis for sunflower oil. These authors suggested that, in the case of sunflower oil, oxidation was mostly controlled by oxygen diffusion in the Oxidograph method where, with a lower surface-to-volume ratio in comparison with DSC, the rate of oxygen consumption was greater than its supply to the sample by diffusion. Therefore, it is likely to find differences between two methods in which different oxygen-availability conditions can occur as a result of the influence of the intrinsic variables, especially the

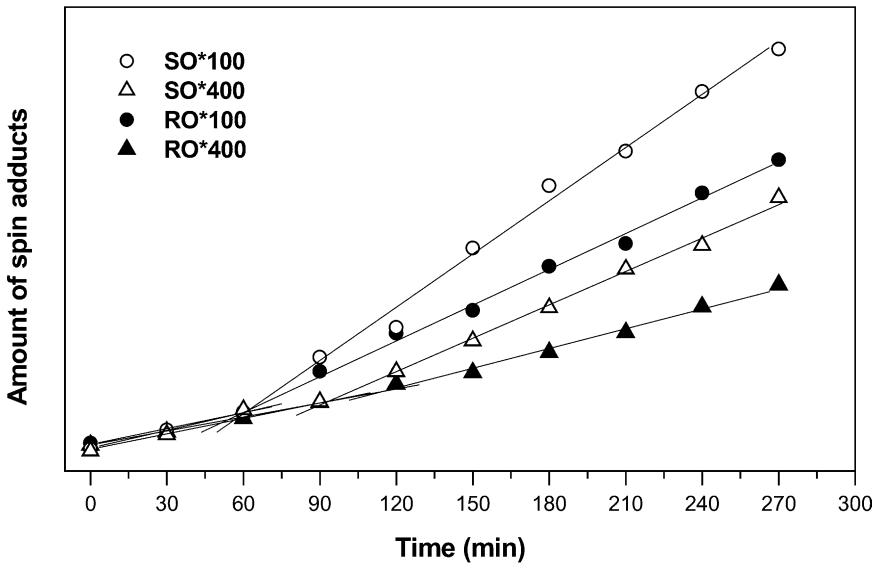

Fig. 3. Influence of the degree of unsaturation and α-tocopherol concentration on the formation of PBN-spin adducts.

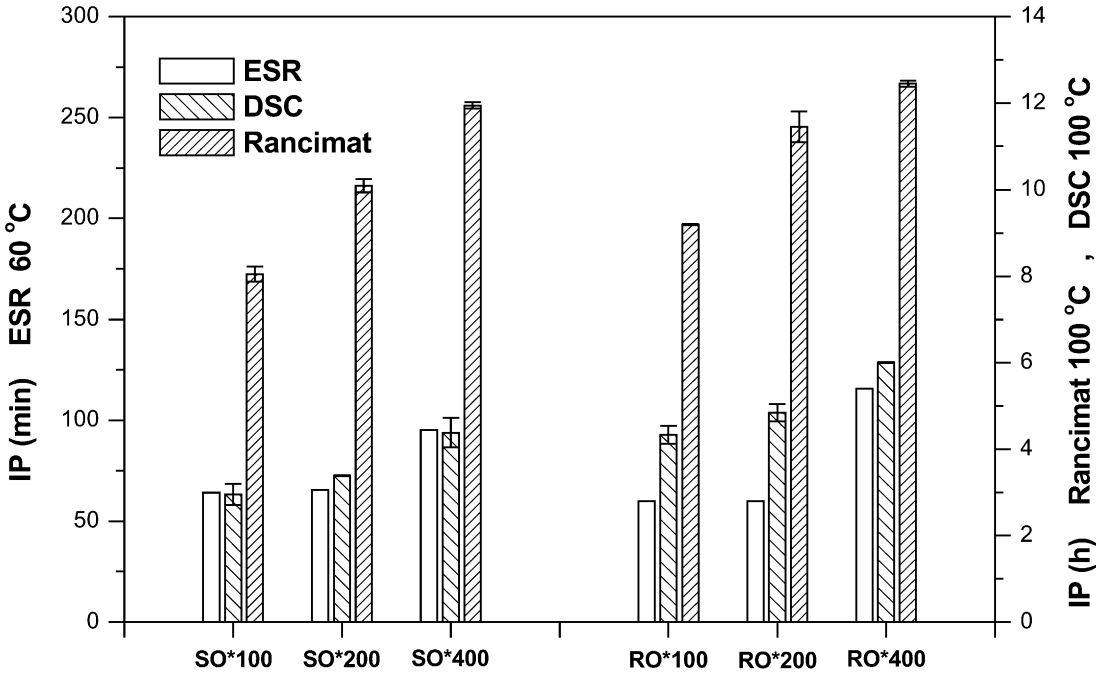

Fig. 4. Evaluation of antioxidant activity of α-tocopherol by ESR, DSC and Rancimat. Error bars denote standard deviation ($n = 2$).

degree of unsaturation and the antioxidant concentration, on the rate of oxidation.

The IPs obtained by ESR showed no increase of stability from 100 to 200 ppm of α-tocopherol, while an increase from 100 to 400 ppm, comparable with that obtained by Rancimat and DSC, was found for both stripped SO and stripped RO. Considering that the relative concentrations in moles of α-tocopherol and PBN were approximately 1:4, 2:4 and 4:4, respectively, one might think that the fact that no change was observed between 100 and 200 ppm of α-tocopherol was due to a concentration effect, as PBN and α-tocopherol compete for the lipid radicals generated, and that the antioxidant effect would be observed at higher concentrations of α-tocopherol, e.g. under equimolecular conditions. However, according to Barclay and Vinqvist (2000), unlike α-tocopherol, PBN does not act as a chain-breaking antioxidant by effectively trapping peroxyl radicals. These authors claim that PBN reacts with peroxyl radicals very slowly to form spin adducts which are known to be very unstable and only detectable at very low temperatures (Chiba & Kaneda, 1984; Janzen, Krygsman, Lindsay, & Haire, 1990). As a result, there are other reactions that compete effectively with the

trapping of peroxyl radicals by PBN, such as the trapping of alkyl and alkoxyl radicals (Barclay & Vinqvist, 2000; Janzen et al., 1990). In consequence, the detected spin adducts are expected to arise from trapping alkyl and alkoxyl radicals. Furthermore, as the reactions between oxygen and alkyl radicals, on the one hand, and between alkoxyl radicals and the lipid-substrate molecules, on the other, are known to be very fast, the trapping of alkyl and alkoxyl radicals by PBN is thought to occur at concentrations which do not influence the course of lipid oxidation. In fact, formation of radicals took place from time zero in stripped SO and stripped RO, both containing PBN, and, consequently, an IP was not observed (data not shown). In contrast, the presence of 100 ppm of α-tocopherol not only decreased the ESR signal to a great extent, but also a definite IP was observed for stripped SO (SO*100) and stripped RO (RO*100). These results indicate that PBN did not act as an antioxidant by showing an IP under the oxidation conditions used in the ESR method.

As commented above, the rate at which PBN-adducts were formed after the IP depended on the α-tocopherol concentration, and it was found that the higher the antioxidant concentration the slower the adduct formation (Fig. 3). In this respect, PBN accounts for the capacity of α-tocopherol to trap peroxyl radicals which propagate the reaction chain by forming new radicals in the reaction with the lipid-substrate molecules. These results were of utility to evaluate the antioxidant effect of α-tocopherol by considering the amount of radicals trapped at the end of the analysis (270 min). Thus, the ESR signal decreased by approximately 30 and 40% in SO and by 20 and 40% in RO when the concentration of α-tocopherol was increased from 100 to 200 and from 100 to 400 ppm, respectively. Based upon this, the stability order predicted by ESR (SO*100 < SO*200 < RO*100 < SO*400 ≈ RO*200 < RO*400) was different from that predicted by Rancimat, as well as by DSC.

Although the three methods under study showed an increase in stability with the amount of α-tocopherol, and a similar response was found for the highest antioxidant concentration assayed, some slight differences at low concentrations of tocopherol resulting in the three methods predicting a different stability order among the purified oils treated with α-tocopherol. However, the three methods ranked the rest of the samples assayed in the same order of oxidative stability (Table 2).

3.6. Linear correlations

As shown in Fig. 5, the IPs determined by the ESR method showed high linear correlations with the IPs provided by the Rancimat method ($r = 0.963$) and with those by DSC ($r = 0.979$). However, as different responses were given by each method for the purified oils with low α-tocopherol concentrations, it is observed that, in the range of low stabilities, the points deviate from linearity. On the other hand, a high linear correlation ($r = 0.967$) was also found between the IP values determined by the Rancimat method and DSC, which is in agreement with the results previously reported by other authors (Cross, 1970; Tan et al., 2002).

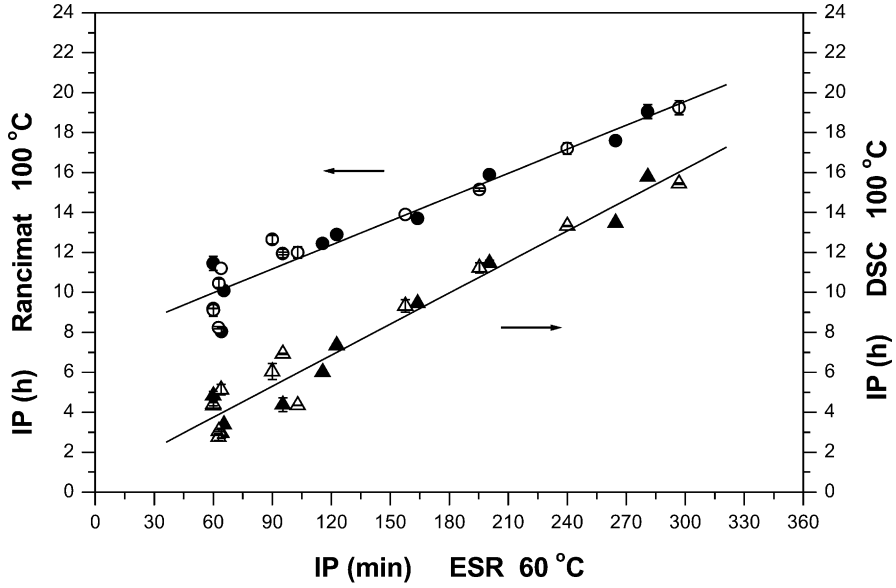

Fig. 5. Linear correlations between the induction periods (IPs) determined by ESR and by the Rancimat method ($IP_{Rancimat}$ (h) = 7.6 + 0.040 IP_{ESR} (min); $r = 0.963$), and between the ESR IPs and those determined by DSC (IP_{DSC} (h) = 0.6 + 0.052 IP_{ESR}(min); $r = 0.979$). Full and hollow symbols correspond to the results obtained in the 1st and 2nd experiments, respectively. Regression analysis was carried out considering all the data ($n = 22$) corresponding to the 1st and 2nd experiment. Error bars express standard deviation ($n = 2$).

In spite of the different criteria considered to evaluate oxidative stability and the different experimental conditions applied (temperature and/or oxygen availability), the results obtained show that the three methods studied can predict the same relative oxidative stabilities. Depending on the influence of the intrinsic variables, such as the degree of unsaturation and the antioxidant concentration, on the oxidation rate, some of the different variables applied in each method could be either determining of the rate of oxidation, allowing differences among the methods to be observed, or could otherwise not have a definite effect on the final results. What is here exposed may also apply when the results of oxidative stability given by accelerated methods are used to predict oxidative stability under other oxidation conditions (shelf life), as different variables can control the pathway and rate of oxidation. In consequence, unless the IPs are related to measured shelf life, accelerated methods only provide useful comparative data on the susceptibility to oxidation of different oils under certain conditions rather than representative data of shelf life.

Through the present study we have for the first time examined the capability of ESR spin-trapping for evaluating oxidative stability of oils in a wide range of stability values. The high correlations found between ESR and two methods based on the determination of the onset of advanced oxidation suggest that oxidative stability can be evaluated as a measure of the resistance to the formation of the first radicals generated during the early steps of oxidation. Detection of radicals at this stage of the oxidative process allows mild conditions to be used in a rapid method for evaluation of oxidative stability. The high temperatures and the constant stream of air required for both Rancimat and DSC may cause the loss of volatile components and limit the use of these methods for the evaluation of oils containing volatile antioxidants (Frankel, 1993). In contrast, the milder oxidation conditions applied in the ESR method allow the evaluation of oxidative stability of these oils, as well as the study of oxidation in other lipid systems containing water, such as oil-in-water emulsions, where previous separation of the lipid phase would be needed under the conditions applied in the Rancimat and DSC methods. To this end, new studies aimed at evaluating oxidative stability of other lipid systems, such as oil-in-water emulsions and oils encapsulated in a solid matrix of carbohydrates and/or proteins, by the direct application of the ESR method, are in progress.

In comparison with the Rancimat method and DSC, the ESR method is concluded to be useful as a method employing milder conditions and shorter time, and which can be applied for the evaluation of oxidative stability of oils containing volatile antioxidants and other lipid systems containing water.

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References

AENOR. (1991). *Standard method UNE 55-023-73. Catálogo de Normas UNE*. Madrid, Spain: Asociación Española de Normalización.

Andersen, M. L., Outtrup, H., & Skibsted, L. H. (2000). Potential antioxidants in beer assessed by ESR spin trapping. *Journal of Agricultural and Food Chemistry*, *48*, 3106–3111.

AOCS. (1992). *Method Cd 12b-92. Official methods and recommended practices of the American Oil Chemists' Society (5th ed.)*. Champaign, Illinois: American Oil Chemists' Society ed. Firestone. D.

Barclay, L. R. C., & Vinqvist, M. R. (2000). Do spin traps also act as classical chain-breaking antioxidants? A quantitative kinetic study of phenyl tert-butylnitrone (PBN) in solution and in liposomes. *Free Radicals in Biology and Medicine*, *28*(7), 1079–1090.

Chiba, T., & Kaneda, T. (1984). ESR spectra of peroxy radicals derived from unsaturated esters. *Agricultural and Biological Chemistry*, *48*, 2593–2594.

Cross, C. K. (1970). Oil stability: a DSC alternative for the active oxygen method. *Journal of the American Oil Chemists Society*, *47*, 229–230.

Frankel, E. N. (1993). In search of better methods to evaluate natural antioxidants and oxidative stability in food lipids. *Trends in Food Science and Technology*, *4*, 220–225.

Fuster, M., Lampi, A., Hopia, A., & Kamal-Eldin, A. (1998). Effects of α- and γ-Tocopherols on the autooxidation of purified sunflower triacylglycerols. *Lipids*, *33*, 715–722.

IUPAC. (1992). *Standard method 2.301*. Standards methods for the analysis of oils, fats and derivatives (7th ed.). Blackwell, Oxford, England: International Union of Pure and Applied Chemistry (1st supplement. to the 7th ed.).

Janzen, E. G., Krygsman, P. H., Lindsay, D. A., & Haire, D. L. (1990). Detection of alkyl, alkoxyl, and alkylperoxyl radicals from the thermolysis of azobis(isobutyronitrile) by ESR/spin trapping. Evidence for double spin adducts from liquid-phase chromatography and mass spectroscopy. *Journal of the American Chemists Society*, *112*, 8279–8284.

Kristensen, D., Andersen, M. L., & Skibsted, L. H. (2002). Prediction of oxidative stability of raw milk using spin trapping electron spin resonance spectroscopy. *Milchwissenschaft*, *57*(5), 255–258.

Rossell, J. B. (1994). Measurement of rancidity. In J. C. Allen, & R. J. Hamilton (Eds.), *Rancidity in foods* (pp. 22–53). Glasgow: Chapman & Hall.

Simon, P., Kolman, L., Niklová, I., & Schmidt, S. (2000). Analysis of the induction period of oxidation of edible oils by differential scanning calorimetry. *Journal of the American Oil Chemists Society*, *77*, 639–642.

Tan, C. P., Che Man, Y. B., Selamat, J., & Yusoff, M. S. A. (2002). Comparative studies of oxidative stability of edible oils by differential scanning calorimetry and oxidative stability index methods. *Food Chemistry*, *76*, 385–389.

Thomsen, M. K., Jacobsen, C., & Skibsted, L. H. (2000). Mechanism of initiation of oxidation in mayonnaise enriched with fish oil as studied by electron spin resonance spectroscopy. *European Food Research and Technology*, *211*, 381–386.

Thomsen, M. K., Kristensen, D., & Skibsted, L. H. (2000). Electron spin resonance spectroscopy for determination of oxidative stability

of food lipids. *Journal of the American Oil Chemists Society*, *77*(7), 725–730.

Thomsen, M. K., Vedstesen, H., & Skibsted, L. H. (1999). Quantification of radical formation in oil-in-water food emulsions by electron spin resonance spectroscopy. *Journal of Food Lipids*, *6*, 149–158.

Uchida, M., Suga, S., & Ono, M. (1996). Improvement for oxidative flavor stability of beer- rapid prediction method for beer flavor stability by electron spin resonance spectroscopy. *Journal of the American Society of Brewing Chemists*, *54*, 205–211.

Wan, P. J. (1995). Accelerated stability methods. In K. Warner, & N. A. M. Eskin (Eds.), *Methods to assess quality and stability of oils and fat-containing foods* (pp. 179–189). Champaign, Illinois: American Oil Chemists' Society.