Assessing the Extent of Oxidation in Thermally Stressed Vegetable Oils. Part I: Optical Characterization by Photothermal and Some Conventional Physical Methods

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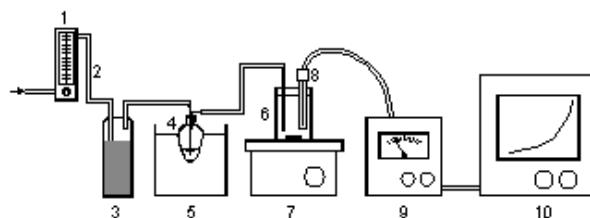
UV absorption, hyphenated HPLC-dual beam thermal lens spectrometry, photoluminescence, optothermal window (OW) method at the NIR wavelengths (using xenon lamp and optoparametric oscillator (OPO) as the excitation sources), FTIR and Raman spectroscopies, refractometry, dielectric spectroscopy and a widely adopted Rancimeter approach were studied in terms of the potential of each procedure to assess the extent of changes induced in safflower oil subjected to accelerated oxidation under well controlled conditions (ten hours long at 130° C and presence of air flow 300 ml/min). Thus obtained "analytical indices" for optical characterization of thermally stressed oil, were then compared in terms of mean value and variance); the correlation coefficients for all analytical indices were also determined.

 Since the oxidative breakdown causes degradation of edible oils, there is a need for optical and thermal techniques capable of characterizing these products; such data are useful in predicting behavior of oils during the storage [1-3]. However, deterioration of oils is a complex process and thus far no single method was established to represent all the oxidative qualities. The most widely used approach is the so called Rancimeter (Fig. 1) which implies a deliberate, accelerated promotion of oxidation in edible oil by exposing it to well controlled, severe "stress" conditions (high temperature and presence of air). The quantity being measured by Rancimeter is the induction time (also termed oxidative stability index OSI), i.e. the moment of a notable change in electric conductance (caused by organic acids accumulated in oil as a result of oxidation) of a distilled water. A comprehensive, coordinated study was undertaken in an attempt to explore feasibility of some of the photothermal methods for optical and thermal characterization of thermally stressed oil. The results of the study are summarized in the two back-to-back papers. Part I (this very paper) is concerned with examining the potential of hyphenated HPLC-dual beam thermal lens spectrometry (in visible and infrared) for monitoring changes in safflower oil (moderate stability) subjected to a ten hours long thermal treatment. The feasibility of optothermal

window (OW) was also investigated using Xe-lamp and optoparametric oscillator (for excitation at NIR wavelengths) but with olive oil (this oil is more stable than the safflower oil) that were treated in the same manner as the safflower oil. The result, that is to say the "analytical index" used to optically characterize safflower oil was then compared to data acquired from the same

oil by a number of conventional methods.

Fig. 1   The Rancimeter apparatus : flowmeter(1), pressurized air(2), drying agent(3), vessel with test sample(4), thermostat bath(5), glass vessel filled with distilled water(6), magnetic stirrer(7), electrode(8), readout unit(9) and display(10).

The extent to which various analytical indices agree statistically with each other was also investigated extensively (mean values,

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variance). The correlation coefficients for each analytical index were computed.

Experimental

Cold pressed, unrefined safflower oil (Reform Natufood) was obtained from the local supermarket. Fatty acid composition, determined by GC analysis is as follows: C16:0 (6.8 %), C18:0 (2.5 %), C18:1 (14.9 %), C18:2 (74.3 %) trace minor quantities of C12:0, C14:0, C20:0 and C22:0. Thermal stress was achieved by immersing a 150 ml reservoir filled with the safflower oil in a bath maintained at 130° C. The pressurized air was continuously flown (300 ml/min) through the reservoir [4]. The peroxide value and the iodine value (this latter was determined by the Wijs reagent) of fresh safflower were 11 meq O_2 and 146 respectively. Samples of oxidized oils were taken for the analysis at 2, 4, 6, 8 and 10 hours after the onset of oxidative treatment. The UV absorption measurement in oxidized oils (25000 times dilution in iso-octane) was carried out at 232 nm (for conjugated dienes) and 268 nm (conjugated trienes) using Varian 634 spectrophotometer. The isocratic, reverse-phase HPLC (4.6x250 mm Vydac 2145TP54 C18 column) and a double beam thermal lens spectrometry (TLS) with the c.w. argon laser (478 nm) were combined and used to monitor (in a HPLC cell 1 cm long) bleaching of carotenoids as a result of a heat-catalyzed oxidation. The set-up for photoluminescence studies (at 20 Hz) included twenty LED's for excitation at 460 nm, the 675 nm interference filter, and a dual phase lock-in amplifier. Direct (i.e. without any dilution) OW (300 μm thick sapphire plate used as a sensor) measurements on oils were performed at NIR and MIR wavelengths (corresponding to those of functional groups) using Xe-lamp and optoparametric oscillator (OPO). Dominant spectral features (C-H stretch in a 3030 to 2800 cm^{-1}, C=O carbonyl absorption close to 1750 cm^{-1}, and a band at 965 cm^{-1} C=C-H bending vibration of trans double bonds) on oils were also investigated here by the FTIR [5] and Raman spectroscopy. Additionally, changes due to the oxidative treatment (at 1750 cm^{-1} and 965 cm^{-1}) were also studied by a HPLC-dual beam TLS technique but with the CO and CO_2 lasers as excitation sources; the length of the HPLC cell was about 200 μm .

Since oxidation is expected to generate polar compounds, both, the dielectric constant and a dielectric relaxation of thermally stressed safflower oils were studied at 18°C; the sample was allowed to relax for several hours at this temperature before initiating data acquisition. A three-terminal stainless steel capacitor was immersed in a thermostat bath, and a system calibrated using the values for dielectric constants of air, cyclohexane and chlorobenzene. The complex part of electrical conductance of a capacitor was measured at frequencies between 20 Hz and 1 MHz and 75 kHz and 10 MHz. The Abbe 2L Bausch & Lomb was used to measure refractive indices of oils at 25°C. Finally, induction time of thermally stressed oil was determined by means of a home made Rancimeter apparatus coupled to the x-t recorder.

Results and Discussion

The UV absorbance at 232 nm and 268 nm was found to increase in time; no evidence for saturation was observed after 10 hours long treatment. Results collected by a highly sensitive HPLC-TLS method (eluent 90% MeOH-10%THF) indicate a rapid decay of all carotenoids as the oxidation proceeds [6]; both, the height and the area of HPLC peaks are time dependent. The cis-β carotene appears however to be more stable than α and trans-β carotenes. The concentration of trans-β carotene in fresh safflower oil (initially 100 ppb) decreases appreciably already after two hours of heating. Unfortunately, relatively large intrinsic infrared absorption of the eluent (acetonitrile and chloroform), precludes, at least for the time being, that high sensitivity of the HPLC-TLS scheme could also be achieved at wavelengths of the CO and CO_2 lasers.

As to photoluminescence, intensity (proportional to a content of chlorophyll) of the signal was found to decrease with time; most pronounced changes are observed after four hours of treatment. The OW detector when used both with the Xe-lamp source as well as with the OPO, was demonstrated capable of detecting changes at 3005 cm^{-1} (characteristic for unsaturation) induced during early stage of oxidative treatment. For longer oxidation intervals, the OW-OPO detected appearance of a band centered close to 3440 cm^{-1}, which is likely due to a formation of polymers. The reader is reminded that unlike what was done in case of all other methods, OW studies were performed only on olive oil.. Inspection of FTIR and the Raman spectra revealed changes at 3010 cm^{-1} and 3011.5 cm^{-1} respectively. The effect of oxidation (possibly indicating generation of free fatty acids; similar effect was also observed at 3324 cm^{-1} (OH stretching band of carboxyl group) during OPO-OW experiments for oil subjected to a long treatment) is detectable at 1746 cm^{-1} in FTIR spectrum and at 1748 cm^{-1} in Raman spectra of oils.

Regardless length of the oxidative treatment, real part ε' of a dielectric constant was found frequency independent up to approximately 100 kHz. The imaginary dielectric constant ε'' shows peaks at some tens of MHz. The dependence of a dielectric constant on a length of treatment was measured at 10 kHz; at such high frequency dielectric constant can be considered as being equal to static (d.c.) value. However, polarization effects on electrodes make the measurement of static dielectric constant unreliable. Results of studies show only a slight change in a value dielectric constant during first two hours following the onset of oxidative treatment; a gradual increase (probably due to accumulation of polar compounds) is observed thereafter. In addition to dielectric constant, real part of conductance was also measured. In low frequency range (well below frequencies affected by dielectric relaxation), the conductance was in a sub nS range, i.e. noise level of the equipment, and showed no correlation with the length of the oxidative treatment.

Refractive index of safflower oil increases almost linearly with longer heating, which can probably be attributed to the conjugation and polymerization. Finally, induction time of the safflower oil was determined (Rancimeter) by drawing a tangent to the plot showing a time dependent electrical conductance of a distilled water (including organic compounds such as free fatty acids). The tangent was then extrapolated to the baseline; and the induction time calculated by converting (via known speed of chart recorder) the intersect at the time axis; this gives induction time of approximately two hours for oil used in this study.

The analytical indices, indicative for optical characterization, were first compared in terms of their arithmetic mean values. In doing so, signals obtained from oxidized oil were normalized by dividing them with those acquired from the untreated oil. Such a normalization process not only ensures that the initial value will be dimensionless and consistently equal to a unity, but also provides the information about the "sensitivity" of a specific analytical index (and hence the particular method). The most sensitive analytical index is the one yielding the maximal signal increase for a given length of oxidative treatment, and has a highest mean (Fig. 2). One of the numbers ranging between 1 and 20 was assigned to a particular analytical index for optical characterization (see Tables 1 and 2).

Results of the analysis of the variance (one-way ANOVA procedure, an extension of the two-sample t test) used as a statistical method, are shown in Table 1. The comparison was made for each pair of analytical indices. The empty cells in Table 1 imply the absence of significant differences between analytical indices that are being compared; i.e. each of indices will statistically yield the same result. The meaning of minus signs appearing along and below a diagonal is self-explanatory. The asterisk (*) refers to significant differences (here at a 0.05

level); as an example, the symbol * appearing at the intersection of row 1 and column carrying a headline 2, implies the statement (certainty 95%) that the result of absorbance measurement at 232 nm (analytical index 1) will differ from that of the absorbance measurement at 268 nm (analytical index row 2).
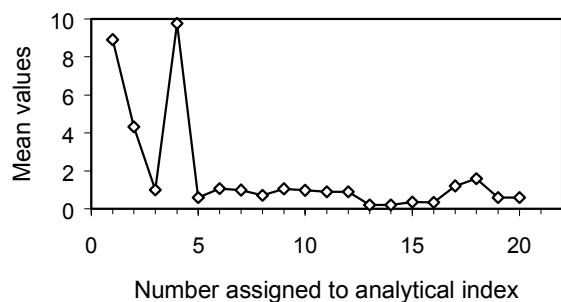

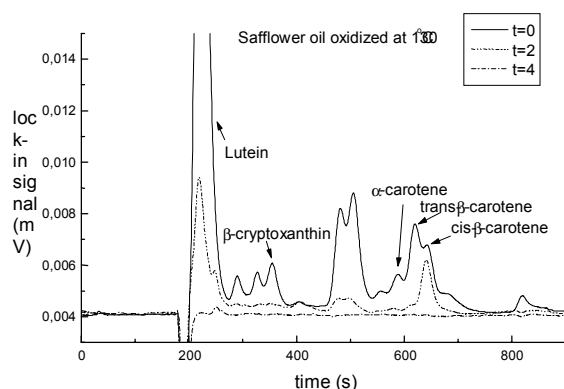Fig. 2   Mean values for various analytical indices.

Fig. 3   The HPLC-TLS chromatogram of carotenoids in thermally stressed safflower oil recorded at 40 HZ. The laser power at 488 nm was 60 mW.

In conclusion, inspection of Table 2 reveals a relatively high correlation for analytical indices numbered as 1 to 6, while for indices 11 to 14 correlation is satisfactory. Highest correlation was observed for photoluminescence, dielectric constant and ΔK. The reason for omitting correlation coefficients for analytical indices assigned numbers 15 to 20 is too low (two and three) number of data available for analysis; carotenoids apparently almost completely decompose after four hours of oxidative treatment (Fig. 3). Remembering that under given experimental conditions the induction time of thermally stressed safflower oil (measured by Rancimeter) is two hours, it is worth noting that monitoring the fate of carotenoids by isocratic HPLC-TLS at visible wavelengths, is very likely an indicator for early degradation stages in oil. The OPP-OW approach proved useful in tracing loss of unsaturation at 3005 cm^{-1}. Although this information was derived from results obtained in studies of olive oil, it is clear that suitability of this approach when investigating safflower oil will be even more pronounced, as this latter oil is less stable than the olive oil. A new experiment that will focus on HPLC-TLS and OW studies of the thermally stressed safflower oil is anticipated; however oil samples for analysis will be taken at five minutes intervals, rather than two hours as it was the case in this study.

References

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Table 1 Results of the variance analysis performed for the parameters indicative for optical characterization.

	parameter/technique indicative for optical characterization	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	absorbance of dienes at 232 nm	-	*	*		*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
2	absorbance of trienes at 268 nm	-	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*			*	*
3	refractometry	-	-	-	*																
4	ΔK	-	-	-	-	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
5	photoluminescence	-	-	-	-	-															
6	dielectric constant	-	-	-	-	-	-														
7	FTIR 3009.9 cm^{-1}	-	-	-	-	-	-	-													
8	FTIR 987.5 cm^{-1}	-	-	-	-	-	-	-	-												
9	FTIR 1745.5 cm^{-1}	-	-	-	-	-	-	-	-	-											
10	Raman 1748 cm^{-1}	-	-	-	-	-	-	-	-	-	-										
11	Raman 3011.5 cm^{-1}	-	-	-	-	-	-	-	-	-	-	-									
12	Raman 972.5 cm^{-1}	-	-	-	-	-	-	-	-	-	-	-	-								
13	lutein peak height	-	-	-	-	-	-	-	-	-	-	-	-	-							
14	lutein peak area	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
15	trans-β carotene peak height	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
16	trans-β peak area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				
17	cis-β carotene peak height	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
18	cis-β peak area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
19	α carotene peak	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
20	α carotene peak area	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

* The mean difference is significant at the 0.05 level.

Table 2.Correlation between specific analytical index (normalized signals were used) and the length of oxidative treatment.

	analytical index	correlation factor
1	absorbance of dienes at 232 nm	0.84
2	absorbance of trienes at 268 nm	0.87
3	refractometry	0.94
4	ΔK	0.96
5	photoluminescence	0.96
6	dielectric constant	0.96
7	FTIR 3009.9 cm^{-1}	0.05
8	FTIR 987.5 cm^{-1}	0.66
9	FTIR 1745.5 cm^{-1}	0.26
10	Raman 1748 cm^{-1}	0.25
11	Raman 3011.5 cm^{-1}	0.70
12	Raman 972.5 cm^{-1}	0.75
13	lutein peak height	0.75
14	lutein peak area	0.77