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ORIGINAL PAPER

A New Chemiluminescence Method for Detecting Lipid Peroxides in Vegetable Oils

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Abstract The peroxide value (PV) of several vegetable oils oxidized at 70 °C was measured spectrophotometrically using a new chemiluminescence (CL) method. The reaction in CL was based on lipid hydroperoxides decomposition with HO^- ions in two different medium. The first reaction was carried out in a DMF/KOH/oil mixture, while in the second reaction acridine was used in addition. A linear correlation between PVs determined spectrophotometrically and measured with the CL method was found.

Keywords Chemiluminescence · Peroxide value · Electronic excited state · Oxidation · Lipid hydroperoxides

Introduction

Chemiluminescence (CL) is an electromagnetic wave emission phenomenon usually occurring in the visible or near infrared range, caused by a chemical reaction. When the reaction takes place in living organisms and results in the emission of light, the process is known as bioluminescence (BL). [1]. To induce this kind of photon emission the following conditions have to be met [2]:

1. Electronic excited state of a condensed medium (gas, liquid, solid state, biological cell) molecules,
2. radiance relaxation state of those molecules (photon emission $E = h \times v$).

Production of the electronic excited state product (P^*) is a result of chemical excitation. The product in the excited state (P^*) is formed in the reaction of substrate A (usually it is a precursor of the CL reaction) with a substrate B (usually an oxidizer). Sometimes some reaction cofactors (C) are required for the reaction to proceed [1]. The reaction can be briefly written as follows:

$$A + B + C \rightarrow P^* \rightarrow P + h \times v \quad (1)$$

Direct CL takes place when a considerable part of the reaction enthalpy (ΔH) is transformed into the reaction products (P^*) electronic excited energy, and subsequently leads to photon emission ($h \times v$). The essence of the process is the phase of excitation $\Delta H \rightarrow P^*$, but the following conditions have to be fulfilled: $E_{hv} < \Delta H + \Delta H^{\#}$, where $\Delta H^{\#}$ is the standard enthalpy of activation [2].

Sometimes P^* does not undergo direct light deactivation but the energy excess is transferred to some other molecule, e.g., to a fluorophore, F. If an energy acceptor molecule F is characterized by a significantly high quantum efficiency of fluorescence then a sensibilizated CL process may appear.

Extremely low light emissions in the range of 10^{-1}–10^4 $h \times v$ s^{-1} cm^{-1}, a wide spectral range of 200–1,100 nm, and the high dynamics of the changes in chemical reactions intensity requires very fast and sensitive light detection systems and sophisticated detection techniques. Photomultiplier tubes (PMTs) and charged-coupled devices (CCD) are the most often used as light detectors. These kinds of detectors coupled to a suitable high-tech electronics give a very sensitive tool able to detect and analyze even ultra low photon emissions. The high

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sensitivity of this techniques can be achieved with single proton counting (SPC) methods. Older methods, which are practically not in use anymore, were based on direct current measurement, which was proportional to light intensity, or were based on photographical techniques (the CL diagram was visible on film) [1].

Chemiluminescence is used practically to determine the stability of lipids and the dynamics of the lipids oxidation precess. As a consequence of unsaturated lipids double-bonds oxidation, peroxide radicals are generated (LOO^{*}). Reactions between peroxide radicals leads to the formation of products in the electronic excitation state ($L = O^{*}$), which are able to emit light [3–5]. The reaction is as follows:

$$LOO^{\cdot} + LOO^{\cdot} \rightarrow LOH + L = O^{*} + {}^{3}O_2 + h \times v \qquad (2)$$

The intensity of the light emitted during the oxidation process under given experimental conditions illustrates lipid oxidation kinetics and could also be used as a measure of the amounts of hydroperoxides generated in the reaction medium [3].

Chemiluminescence is also used to investigate the presents of lipid hydroperoxides formed during the lipid oxidation process [6–11]. The CL reaction gives us an opportunity to detect very low concentrations of lipid hydroperoxides (range of tens of pmol in 100 µl sample volume) [6]. The common method used for detection of lipid hydroperoxides is catalyzed by both enzymatic or non-enzymatic protein where the catalyst reacts with lipid hydro peroxide.

Reorganization of peroxides leads to peroxidation of luminol and/or isoluminol with simultaneous light emission [6–10].

Other CL reactions, used for detection of lipid hydroperoxides, are based on the oxidation reaction of several other molecules with lipid peroxides, i.e., TCPO, lucigenine. The advantage of CL with the use of TCPO in comparison to the luminol/isoluminol based method, when used for detection of lipid hydroperoxides and determination of its concentration is the possibility of carrying out the reaction in a non-aqueous medium. The CL with TCPO requires the use of Mn^{2+} ions as catalysts and additional fluorescence molecules to work as secondary photon emitters [11]. Reaction of lucigenine oxidation with peroxides also leads to the emission of light quanta, and this could also be used to detect the presence of lipid peroxides in the reaction medium. The main advantages of this method is the possibility of conducting the reaction in a non-aqueous medium without the use of a catalyst. [12, 13].

One of the most interesting methods used for the detection of hydroperoxides is based on their reaction with sodium hypochlorite (NaOCl) [7]. The reaction of lipid hydroperoxides with sodium hypochlorite leads to the formation of oxygen singlet molecules in an electronic excitation state, with the subsequent light emission occurring after the electron relaxation process. This method could be used as a very fast, cheap and easy procedure for determining lipid hydroperoxides formed during lipid oxidation [7, 14, 15].

The aim of this study was to develop an inexpensive and easy CL reaction that could be used for the determination of lipid hydroperoxides in a non-aqueous medium and without the necessity of using expensive protein catalysts.

Experimental

Apparatus

A static chemiluminometer built by the first-named author was used for the CL measurement. The chemiluminometer (Fig. 1) was equipped with a piston pump (2). The chemiluminometer was also equipped with a mechanical agitator (5) and a thermostat (8). The sample solution to be analyzed was injected with a syringe (3) into the Reodyne valve sample loop (4). The solvent from the reservoir (1) was used to pump the analyte to a test tube (7) of a chemiluminometer (6). Photons emitted from the test tube were counted by a PMT located in the second section of the chemiluminometer (11). Both sections were connected to a Teflon optical port (9). The PMT was cooled to −20 °C with a thermoelectric cell (10). The PMT was supplied with a high-stability power supply operated at 1,000 V. The analytical signal from the PMT was changed to numeric values (12, 13, 14) and sent to a computer system. The PMT used in this experiment was especially designed for counting photons in the range of 180–700 nm.

Materials

Low-linolenic cold-pressed linseed oil (CPLO), refined rapeseed oil (RRO) and refined palm olein (RPO) were obtained in a local food market. Hexane, chloroform, methanol, 36% hydrochloric acid, acetone, *N*,*N*′-dimethylformamide, potassium hydroxide were supplied by POCH (Gliwice, Poland). Acridine, $FeCl_2$, ammonium thiocyanate (NH_4SCN) was obtained from Sigma–Aldrich (Bellefonte, PA, USA).

Oil Oxidation and Dilution

The vegetable oil samples were submitted to accelerated oxidation at a temperature of 70 °C. This oxidation was performed in triplicate. Oil subsamples (200 mg) sampled

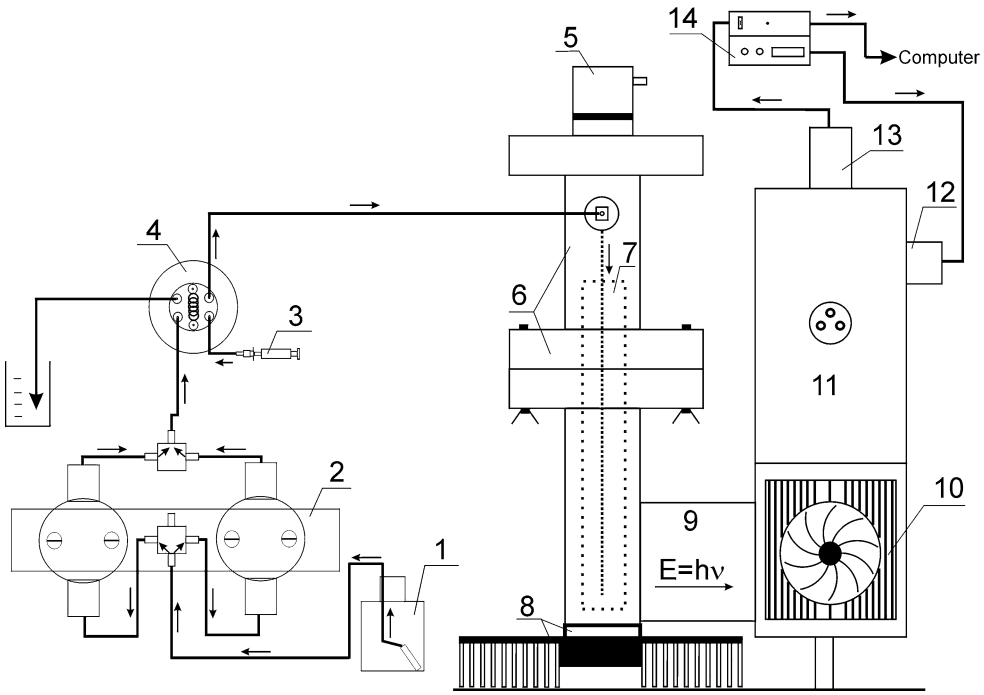

Fig. 1 Schematic diagram of chemiluminometer used in this work (description in the text)

after oxidation were diluted to final volume of 5 ml with a hexane/acetone (1:1 v/v) solution.

Peroxide Value Determination (PV)

A modified method proposed by Shanta et al. (1994) [16] was used for the determination of PV. Briefly, 200 µl of oil solution was mixed with 5 ml of methanol:chloroform:HCl = 1:1:0.012 solution (v/v). Subsequently 100 µl of ammonium thiocyanate aqueous solution and 100 µl of 0.4% solution of $FeCl_2$ were added. The reaction was conducted at room temperature. After 5 min absorbance at a wave length of 480 nm was measured. Spectrophotometer readings were set to zero using a sample containing all reagents except of oil (blank sample). The lipid hydroperoxides concentration was expressed as PV [17].

Chemiluminescence Reaction in DMF/KOH/Oil

To a test tube, 2 ml of KOH in DMF saturated solution (3 g KOH in 200 ml of DMF) was added and mixed with 200 µl of the oil solution to be analyzed. Changes in the chemiluminescence intensity (CI) were recorded for 3 min.

Chemiluminescence Reaction in DMF/KOH/Acridine/Oil

To the test tube 2 ml of KOH in DMF saturated solution (3 g KOH in 200 ml of DMF) and 50 µl of acridine solution (50 mg of acridine in 5 ml of hexane/acetone mixture 1:1, v/v) were added. Then 200 µl of the oil solution to be analyzed was added. Changes in CI were recorded for 3 min.

Mathematics and Statistical Analysis

Peroxide values and CL were measured in triplicates. Mean PV and its standard deviation were used to prepare charts.

The peroxide value was calculated using following equation:

$$\mathrm{PV} = \frac{12.5 \times \mathrm{ABS}}{0.1058}[\mathrm{mgO_2} \cdot 100\mathrm{g}^{-1}] \quad (3)$$

where ABS is an absorbance reading from the spectrophotometer.

Figure 2 shows a typical CL diagram of CI of an example of a vegetable oil in a DMF/KOH/oil mixture. The CI was calculated as an area under the curve showing CL recorded against time.

$$\int_{13}^{180} v \cdot \mathrm{d}t \quad (4)$$

where v, frequency [Hz]; t, time [s].

A paired sample-comparison test was used to compare results of experiments ($\alpha = 0.05$). Regression analysis was used to compare the CL with results obtained by spectrophotometric analysis ($\alpha = 0.05$).

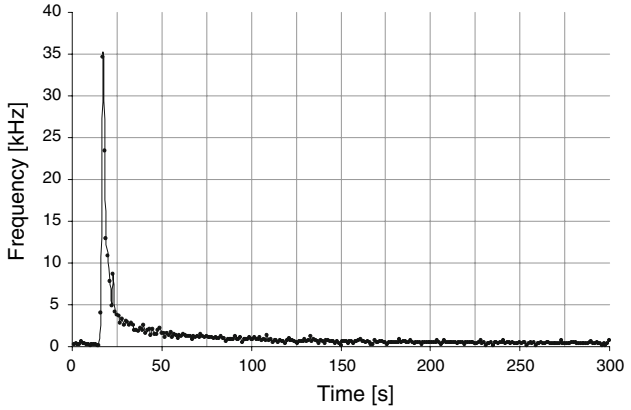

Fig. 2 Typical example of CL intensity versus time diagram of an oil sample

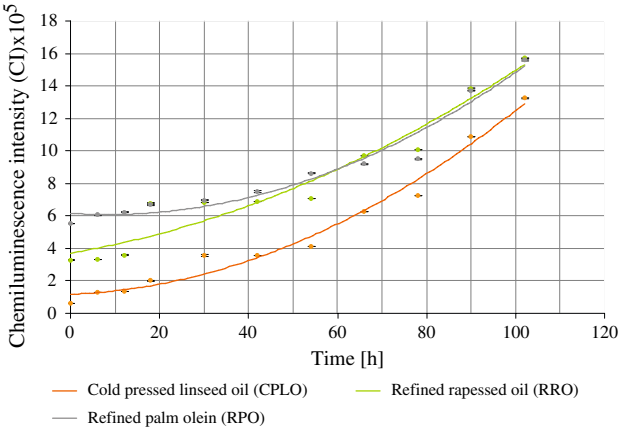

Fig. 4 Chemiluminescence of various vegetable oils held at 70 °C for various times in a DMF/KOH/oil reaction

Results and Discussion

Changes in PV during vegetable oils oxidation at a temperature of 70 °C are shown in Fig. 3. Increases in the PV was observed for all types of oils. In the case of RRO and RPO there was no statistical difference observed in the PV. As a consequence, it might be stated that the dynamics of the oxidation reaction for both oils was similar. A statistically significant difference was determined in the changes of PV during oxidation of CPLO and RRO/RPO. However, these oils were less liable to oxidation in comparison to RRO or RPO.

The CL diagrams for accelerated vegetable oil oxidation in a DMF/KOH/oil reaction medium are shown on Fig. 4. It is evident that non-oxidized oils also show CL, the highest values were observed for RPO (CI = 552 × 10^3 Hz s), RRO (CI = 327 × 10^3 Hz s) and the lowest for CPLO (CI = 59 × 10^3 Hz s). A statistically significant increase in CL was observed during the oxidation process of vegetable oils. The most intensive changes in CL were observed in the case of RPO, secondly in RRO and the lowest less intensive changes were observed in the case of CPLO.

Figure 5 shows a correlation between CL in a DMF/KOH/oil reaction medium and the PV measured spectrophotometrically. Linear correlation between CL and PV was found for all the investigated oils. The best correlation was found in the case of CPLO ($r = 0.9822$), next for RPO ($r = 0.9346$) and the lowest one in the case of RRO ($r = 09057$).

The time changes of CL during oil oxidation were also determined in the DMF/KOH/oil reaction medium but with the addition of acridine (fluorescence molecule). The results obtained are presented in Fig. 6. The CL of non-oxidized oils was detectable as in previous experiments. The highest CI was observed for RPO (CI = 573 × 10^3 Hz s), next RRO (CI = 427 × 10^3 Hz s) and the smallest for CPLO (CI = 122 × 10^3 Hz s). Statistically

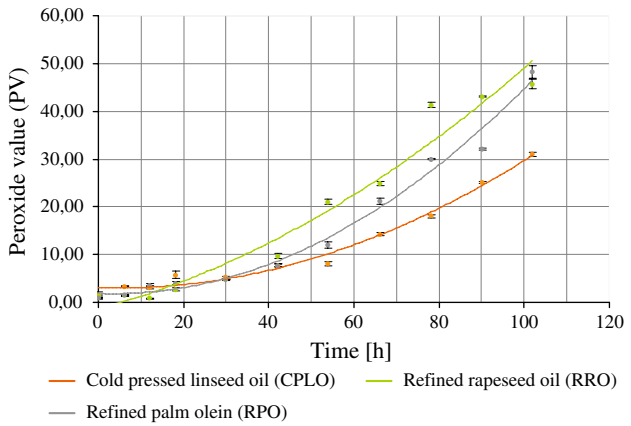

Fig. 3 The peroxide value of various vegetable oils at 70 °C for various times

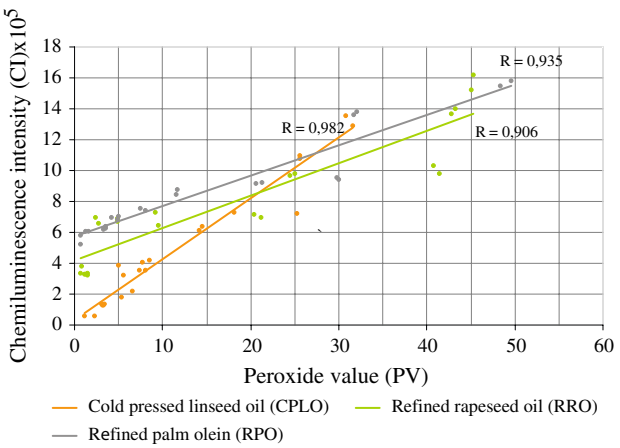

Fig. 5 Linear regression of chemiluminescence in DMF/KOH/oil reaction versus the peroxide value

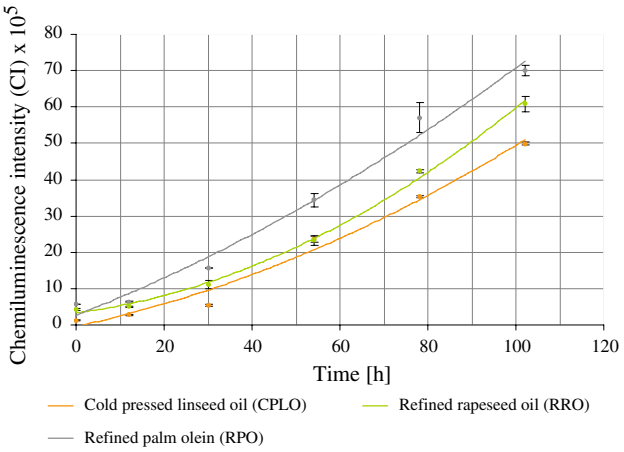

Fig. 6 Chemiluminescence of various vegetable oils held at 70 °C for various times of the DMF/KOH/acridine/oil reaction

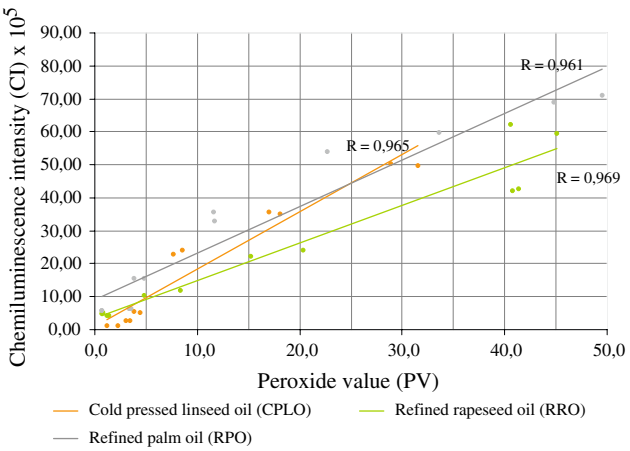

Fig. 7 Linear regression of chemiluminescence in a DMF/KOH/acridine/oil reaction versus the peroxide value

significant differences were found between the CI of the oil samples studied.

Data showing the correlation between PVs and CLs determined in DMF/KOH/acridine/oil reaction medium are presented in Fig. 7. Correlation coefficients for all three oil samples analyzed were high and exceeded a value of 0.96. With this reaction medium, the correlation between CL and PV is much stronger in comparison to a reaction medium containing no fluorescence molecule.

Discussion

Low-linolenic CPLO has low oxidation stability [18–20] in comparison to RPO and RRO [21]. Refined palm olein showed the highest oxidative stability as a consequence of its low-unsaturated fatty acid composition, i.e., C16:0 (palmitic acid) ca 38%, C18:1 (oleic acid) ca 42% and C18:2 (linoleic acid) ca 11% [21]. Refined rapeseed oil has a less advantageous composition because it contains C16:0 ca 5%, C18:1 ca 58% and C18:2 ca 19% [21, 22]. Low linolenic cold pressed linseed oil contains a large amount of C18:2 ca. 74%, which has an extreme influence on its oxidative stability [18–20]. Results presented in this work (Fig. 3) show that CPLO has the smallest PV, hence the highest oxidative stability. Lipid hydroperoxides are relatively stable products of oxidation [23, 24]. The stability of lipid hydroperoxides depends on the fatty acids profile and the temperature of the oxidation reaction. It is well known that herring oil and other fish oils with a high proportion of polyunsaturated fatty acids (PUFA) have a low oxidative stability. However, during their oxidation the PV tends to decrease. It results from the faster decomposition of lipid hydroperoxides in secondary reactions than the speed if their formation. Because CPLO is composed of linoleic acid, peroxides originating from that acid mostly show a higher reactivity than those formed during the oxidation of oleic acid which has the highest contribution in the composition of RRO and RPO. This is probably the main reason for the smaller PV in the case of CPLO in comparison to RRO and RPO.

Papadopoulos et al. (2002) [25] studied the CL of various vegetable oils in a strongly solvating ion alkaline medium. Dimethyl sulfoxide (DMSO) was used as the solvating agent. The authors found that the CL depends on the kind of oil used for the oxidation experiments. The strongest CL signal was observed for corn oil, soy oil and sunflower oil while the weakest signal was recorded for virgin olive oil. Moreover, it was suggested that the CL signal was influenced by the presence of fluorescent molecules and some other antioxidants from the reaction medium.

An ions solvating medium in the presence of H_2O_2 (DMSO/NaOH/H_2O_2) is used in the EPR spectroscopy to generate non-Fenton reaction reactive oxygen species (ROS) [26–29]. The reaction can be presented as follows:

$$\begin{aligned}
&H_2O_2 + HO^- \rightarrow HO_2^- + H_2O \\
&H_2O_2 + HO_2^- \rightarrow O_2^{\cdot -} + {}^{\cdot}OH + H_2O \\
&{}^{\cdot}OH + H_2O_2 \rightarrow {}^{\cdot}O_2H + H_2O \\
&DMSO + {}^{\cdot}OH \rightarrow {}^{\cdot}CH_3 + CH_3SO(OH) \\
&{}^{\cdot}CH_3 + H_2O_2 \rightarrow CH_4 + {}^{\cdot}O_2H
\end{aligned} \quad (5)$$

Based on the data presented in this work (Figs. 4 and 5) and the literature as well, it might be stated that the CL in DMF/KOH/oil medium is a result of the presence of lipid hydroperoxides and fluorescence molecules. The use of DMF instead of DMSO is characterized by similar physicochemical properties [30, 31], although it has a lower solvating ability. It was assumed that solvent exchange to DMF had no significant influence on the course of the

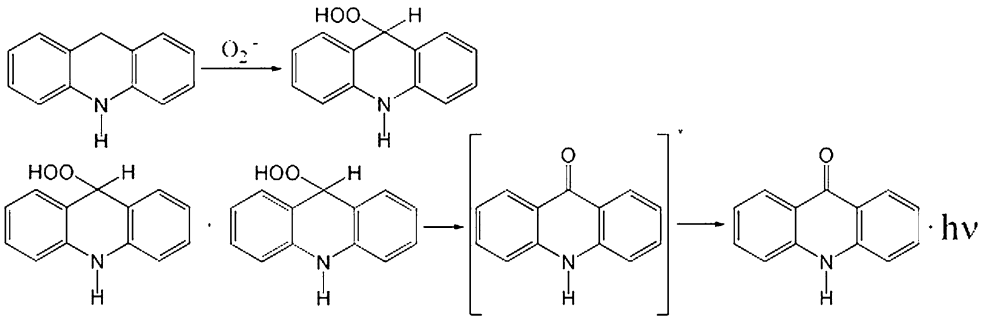

Fig. 8 Propose chemiluminescence reaction in a DMF/KOH/lipid system with acridine adding

reaction. The probable mechanism of the CL reaction (DMF/KOH/oil) used in this work was:

$$\begin{aligned}
&LOOH + {}^{-}OH \rightarrow LOO^{-} + H_2O \\
&LOO^{-} + LOOH \rightarrow {}^{\cdot}OH + LOL + O_2^{\cdot -} \\
&LOOH + {}^{\cdot}OH \rightarrow LOO^{\cdot} + H_2O \\
&LOO^{\cdot} + LOO^{\cdot} \rightarrow L = O^{*} + LO^{\cdot} + O_2 \rightarrow L \\
&= O + h \times v \geq 300 kJ/mol \qquad (6) \\
&DMF + {}^{\cdot}OH \rightarrow {}^{\cdot}CH_3 + HOCNCH_3OH \\
&{}^{\cdot}CH_3 + LOOH \rightarrow CH_4 + LOO^{\cdot} \\
&LOO^{\cdot} + LOO^{\cdot} \rightarrow L = O^{*} + LO^{\cdot} + O_2 \rightarrow L \\
&= O + h \times v \geq 300 kJ/mol
\end{aligned}$$

Chemiluminescence observed for the analyzed oils (Figs. 2 and 4) is probably the result of the reaction between radicals after HO^{-} addition. Molecules formed in an electronic excited state ($L = O^{*}$) interacted with the fluorescence molecules present in low concentration in the oils [32–37]. As a result, various levels of CL were observed in non-oxidized oils.

Addition of fluorescence molecules to the reaction mixture had a positive influence on the CL process (Fig. 6). Correlation between the CI in DMF/KOH/acridine/oil and PV determined spectrophotometrically was significantly better than that observed for CI in the absence of fluorescence molecules (Fig. 7). It proves that CL carried out in a basic-metal ions solvating medium is related to the presence of lipid hydroperoxidates and fluorescence molecules. The proposed mechanism of the CL reaction intensified by acridine might look as it is presented in Fig. 8 [38].

The super oxide anion radical $O_2^{\cdot -}$ taking part in acridine oxidation is generated as the result of the reorganization of lipid hydroperoxidates after HO^{-} addition to the reaction medium. In the studied reaction, there is no photoreaction occurring between acridine and DMF because CL after addition of $HO^{-}$was very weak. HO^{-} ion was not enough to catalyze the reaction between aciridine and DMF. These reactions require the presence of peroxide.

Conclusion

Results of this work shows that proposed CL reactions can be used to determine lipid hydroperoxidates in vegetable oils. This chemiluminescent method we have developed is much more precise and reproducible, with high sensitivity than a traditional spectrophotometric-based method. Correlation between PVs determined spectrophotometrically and by CL is high, however depends on the type of oil used. The sensitivity of the method was able be increased significantly by the addition of fluorescence molecules to the reaction mixture. The method can be used for the fast determination of oil characteristics including determination of the lipid peroxides value and the presence of fluorescence molecules.

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References

1. Garcia-Campańa AM, Baeyens WRG, Hang XA, Garcia-Campańa AM, Baeyens WRG (eds) (2001) Chemiluminescence in analytical chemistry. Marcel Dekker, New York, pp 41–100
2. Kowalczyk A, Anzenbacher P, Hudećek J, Sławiński J (1989) Biospektroskopia 3. In: Twardowski J (ed) PWN, Warszawa, 107–230
3. Timms RE, Roupas P (1982) The application of chemiluminescence to the study of the oxidation of oils and fats. Lebensm Wiss Technol 15:372–377
4. Albertini R, de Luca G, Palladini G, Passi A, Melzi d'Eril GV, Abuja PM (1999) Mechanistic aspects of the relationship between low-level chemiluminescence and lipid peroxides in oxidation of low-density lipoprotein. FEBS Lett 459:47–50
5. Catala A (2006) An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. Int J Biochem Cell Biol 38:1482–1495
6. Yamamoto Y, Brodsky MH, Baker JC, Ames BN (1987) Detection and characterization of lipid hydroperoxides at picomole levels by high-performance liquid-chromatography. Anal Biochem 160:7–13
7. Yamamoto Y, Niki E, Tanimura R, Kamiya Y (1985) Study of oxidation by chemiluminescence. IV. Detection of low levels of lipid hydroperoxides by chemiluminescence. J Am Oil Chem Soc 62:1248–1250

8. Ashida S, Okazaki S, Tsuzuki W, Suzuki T (1991) Chemiluminescent method for the evaluation of antioxidant activity using lipid hydroperoxide-luminol. Anal Sci 7:93–96
9. Kobayashi N, Kaneda H, Kano Y, Koshino S (1994) Behavior of lipid hydroperoxides during mashing. J Am Soc Brew Chem 52:141–145
10. Bunting JP, Gray DA (2003) Development of a flow injection chemiluminescent assay for the quantification of lipid hydroperoxides. J Am Oil Chem Soc 80:951–955
11. Stepanyan V, Arnous A, Petrakis C, Kefalas P, Calokerinos A (2005) Chemiluminescent evaluation of peroxide value in olive oil. Talanta 65:1056–1058
12. Li W-G, Miller FJ, Zhamg HJ, Spitz DR, Oberley LW, Weintraub NL (2001) H_2O_2-induced O_2 production by a non-phagocytic NAD(P)H Oxidase causes oxidant injury. J Biol Chem 276: 29251–29256
13. Navas MJ, Jimenez AM (2007) Chemiluminescent methods in olive oil analysis. J Am Oil Chem Soc 84:405–411
14. Panasenko OM, Evgina SA, Driomina ES, Sharov VS, Sergienko VI, Vladimirov YuA (1995) Hypochlorite induces lipid peroxidation in blood lipoproteins and phospholipid liposomes. Free Radic Biol Med 19:133–140
15. Shams El Din AM, Mohammed RA (1998) Kinetics of reaction between hydrogen peroxide and hypochlorite. Desalination 115:145–153
16. Shantha NC, Decker EA (1994) Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. J Assoc Offic Anal Chem Int 77:421–424
17. Hornero-Méndez D, Pérez-Gálvez A, Mínguez-Mosquera MI (2001) A rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid content. J Am Oil Chem Soc 78:1151–1155
18. Mallegol J, Gardette JL, Lemaire J (2000) Long-term behavior of oil-based varnishes and paints. Fate of hydroperoxides in drying oils. J Am Oil Chem Soc 77:249–255
19. Rudnik E, Szczucinska A, Gwardiak H, Szulc A, Winiarska A (2001) Comparative studies of oxidative stability of linseed oil. Thermochimica Acta 370:135–140
20. Stenberg C, Svensson M, Johansson M (2005) A study of the drying of linseed oils with different fatty acid patterns using RTIR-spectroscopy and chemiluminescence (CL). Ind Crop Prod 21:263–272
21. Xu X-Q, Tran VH, Palmer M, White K, Salisbury P (1999) Chemical and physical analyses and sensory evaluation of six deep-frying oils. J Am Oil Chem Soc 76:1091–1099
22. Kowalski B, Ratusz K, Miciula A, Krygier K (1997) Monitoring of rapeseed oil autoxidation with a pressure differential scanning calorimeter. Termochimica Acta 307:117–121
23. Choe E, Min DB (2006) Mechanisms and factors for edible oil oxidation. Compr Rev Food Sci Food Saf 5:169–186
24. Fox JN, Stachowiak GW (2007) Vegetable oil-based lubricants—a review of oxidation. Tribol Int 40:1035–1046
25. Papadopoulos K, Triantis T, Tzikis CH, Nikokavoura A, Dimotikali D (2002) Investigations of the adulteration of extra virgin olive oils with seed oils using their weak chemiluminescence. Anal Chim Acta 464:135–140
26. Csanyi LJ, Nagy L, Galbacs ZM, Horvath I (1983) Alkali-induced generation of superoxide and hydroxyl radicals from aqueous hydrogen peroxide solution. Z Phys Chem Neue Folge Bd 138:107–117
27. Dong DJ, Vandegrift GF (1997) Alkaline peroxide processing of low-enriched uranium targets for exp 99 Mo production–decomposition of hydrogen peroxide. Nucl Sci Eng 126:213–223
28. Yoshimura Y, Inomata T, Nakazawa H, Kubo H, Yamaguchi F, Ariga T (1999) Evaluation of free radical scavenging activities of antioxidants with an H_2O_2/NaOH/DMSO system by electron spin resonance. J Agric Ford chem 47:4653–4656
29. Polovka M, Brezová V, Staśko A (2003) Antioxidant properties of tea investigated by EPR spectroscopy. Biophys Chem 106:39–56
30. Olea-Azar C, Terraza CA, Guerrero J (2002) Electron spin resonance studies in acrylamide polymerization using rhenium complexes as photosensitizers. Bol Soc Chil Quím 47:221–225
31. Malavolta L, Poletti EF, Silva EH, Schreier S, Nakaie CR (2008) Application of electron paramagnetic resonance spectroscopy for validation of the novel (AN + DN) solvent polarity scale. Int J Mol Sci 9:1321–1332
32. Goodacre R, Kell DB, Bianchi G (1993) Rapid assessment of the adulteration of virgin olive oils by other seed oils using pyrolysis mass spectrometry and artificial neural networks. J Sci Food Agric 63:297–307
33. Neeman I, Joseph D, Biggley WH, Seliger HH (1985) Induced chemiluminescence of oxidized fatty acids and oils. Lipids 20:729–734
34. Metsa-Ketela T, Montfoort A, Kunnas T (1988) Platelet chemiluminescence induced by linoleic and arachidonic acids. Basic Life Sci 49:233–238
35. Velding GA, Garssen GJ, Slappendel S, Bolding JF (1977) Chemiluminescence during lipoxygenase-catalysed oxygenation of linoleic acid. Biochem Biophys Res Commun 78:424–428
36. Watanabe T, Shirai N, Okada H, Honda Y, Kuwahara M (2001) Production and chemiluminescent free radical reactions of glyoxal in lipid peroxidation of linoleic acid by the ligninolytic enzyme, manganese peroxidase. Eur J Biochem 268:6114–6122
37. Kyriakidis N, Skarkalis P (2000) Fluorescence spectra measurement of olive oil and other vegetable oils. J AOAC Int 83:1435–1439
38. Papadopoulos K, Triantis T, Dimotikali D, Nikokavouras J (2001) Evaluation of food antioxidant activity by photostorage chemiluminescence. Anal Chim Acta 433:263–268