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Development of a chemiluminescent method for the evaluation of total hydroperoxide content of edible oils

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ABSTRACT

One of the most important quality indices of edible oils is the total hydroperoxide content expressed as peroxide value (PV). Oils with high hydroperoxide content show a degree of oxidation and, hence, lower quality. For these reasons, the development of methods for the evaluation of this quality index of edible oils is required.
The aim of the present work is to develop, a rapid, accurate, sensitive, simple and low cost chemiluminescence (CL) method for the determination of the total hydroperoxide content of different kinds of olive oils and other types of edible oils. The CL method proposed is based on the chemiluminescent reaction of alkaline luminol and the hydroperoxides of oil, catalyzed by Fe(III) using 1-propanol as the reaction solvent. Calibration curves of the CL intensity as a function of concentration of di-tert-butyl peroxide, used as an external peroxide standard, and of different types of edible oils were prepared. In all the cases the correlation coefficient (R) of the regression lines was satisfactory ($R > 0.996$). The precision of the method expressed in terms of repeatability and reproducibility was also satisfactory, as repeatability in terms of mean %RSD was 4.8% and reproducibility in terms of mean %RSD was 8%. The method was applied for the evaluation of total hydroperoxide content of olive oils, corn oils, sunflower oils, sesame oils and soybean oils, within the concentration range of 0.1–9.0%v/v and the obtained results were compared with those of the official method for peroxide value. Finally, the different types of olive oils and seed oils have been classified according to their estimated total hydroperoxide content.

1. Introduction

Edible oils are a significant component of human nutrition, due to their beneficial and protective health effects and their important nutritional value. Especially, in many Mediterranean countries, like Greece, olive oil plays a major role in the everyday life, market and diet of the consumers (Tsimidou, Blekas, & Boskou, 2003). Its high commercial and nutritional values and quality are ascribed to its composition, mainly to the high content of unsaturated fatty acids and other micronutrients, such as vitamins, carotenes and polyphenols (Navas & Jiménez, 2007).

A critical factor which affects and determines the shelf-life, the nutritional value and the price of olive oils is lipid oxidation. Lipid oxidation is the main process that leads to the quality deterioration, degradation and off flavor formation in olive oils and other edible oils (Le Dréau, Dupuy, Artaud, Ollivier, & Kister, 2009). The oxidative deterioration of oils occurs in two stages: (a) formation of lipid hydroperoxides and (b) decomposition of lipid hydroperoxides that leads to different secondary products such as aldehydes, ketones and alcohols which affect the organoleptic characteristics of oils (Yu, van de Voort, & Sedman, 2007). The formation of hydroperoxides occurs when oils are exposed to an oxygen-containing atmosphere. The autoxidation is even higher when heat and air flow are employed and in the presence of several metal ions. It has been proposed that autoxidation occurs via a free-radical chain mechanism initiated by the abstraction of a hydrogen atom from a bis-allylic methylene group present in polyunsaturated fatty acids by reactive oxygen and nitrogen species (Miyamoto et al., 2007). Bearing in mind that fatty acids are mainly esterified to triglycerides, a variety of lipid hydroperoxides can be formed. Monohydroperoxides are mainly formed (>90% of total hydroperoxides) in either the 1(3)- or 2-triacylglycerol position, while bis-hydroperoxides and tri-hydroperoxides could be formed only as minor products (Neff, Frankel, & Miyashita, 1990a; Neff, Frankel, & Miyashita, 1990b). In most of the cases, the hydroperoxide moiety is located on the esterified linoleic acid in triacylglycerols, therefore the differences in concentration of fatty acids between different types of oils result in differences of lipid hydroperoxides formed in oxidized oils (Miyazawa, Kunika, Fujimoto, Endo, & Kaneda, 1995).

The risk of oils to undergo oxidative deterioration can be monitored by measuring the initial concentration of hydroperoxides present in oils. The most common way of estimating the degree of oxidation and total hydroperoxide content of oils is by measuring the peroxide value (PV). The official method of the European Union for the determination of PV is an iodometric method based on the titration of the iodine, liberated from potassium iodide by the hydroperoxides present in the oil, using sodium thiosulphate solution as titrant (Commission Regulation (EEC), No. 2568r91). The official method shows several disadvantages because it is an empirical, time consuming and quite hazardous method for the environment and human health, due to the use of high amounts

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of organic solvents (Armenta, Garrigues, & de la Guardia, 2007; Ruíz, Ayora Cañada, & Lendl, 2001). In addition the method is not very accurate, sensitive and reproducible and it depends on many experimental parameters, such as reaction time, temperature and exposure to atmospheric oxygen and light. The different times and rates of reaction of different types of hydroperoxides which result in incomplete liberation of iodine, the additional liberation of iodine by oxygen and light, the possible absorption of an amount of generated iodine at unsaturated bonds of lipids and the influence of matrix effect and side reactions of the sample are few of the factors that could lead to erroneous results (Kardash-Strochkova, Tur'yan, & Kuselman, 2001; Nouros, Georgiou, & Polissiou, 1999; Saad, Wai, Peng Lim, & Saleh, 2006; Tian & Dasgupta, 1999; Yildiz, Wehling, & Cuppett, 2001).

A variety of new analytical methods has been developed for the determination of total hydroperoxide content of oils, as alternatives to the official method. Different analytical techniques such as fluorimetry (Akasaka, Suzuki, Ohrui, & Meguro, 1987; Chotimakorn, Ohshima, & Ushio, 2005; Peinado, Toribio, & Peresz-Bendito, 1986; Pérez-Ruiz, Martínez-Lozano, Tomás, & Val, 1993a), infrared spectrometry (IR) (Armenta et al., 2007; Ma, van de Voort, Ismail, & Sedman, 1998; Ma, van de Voort, Sedman, & Ismail, 1997; Mailer, 2004; Moh, Che Man, van de Voort, & Abdullah, 1999; Ruíz et al., 2001; Russin, van de Voort, & Sedman, 2004; Setiowaty, Che Man, Jinap, & Moh, 2000; van de Voort, Ismail, Sedman, Dubois, & Nicodemo, 1994; Yildiz et al., 2001; Yu et al., 2007), ultraviolet spectrometry (UV) (Hornero-Méndez, Pérez-Gálvez, & Mínguez-Mosquera, 2001; Nourooz-Zadeh, Tajaddini-Sarmadi, Birlouez-Aragon, & Wolff, 1995; Pérez-Ruiz, Martínez-Lozano, Tomás, & Val, 1993b; Shantha & Decker, 1994; Talpur, Sherazi, Mahesar, & Bhutto, 2010) and electrochemistry (Adhoum & Monser, 2008; Hara & Totani, 1988; Kardash-Strochkova et al., 2001; Toniolo, Comisso, Bontempelli, & Schiavon, 1996) have been used. Total hydroperoxide content of oils has been also determined in flow conditions using flow injection analysis (FIA) (Thomaidis & Georgiou, 1999) coupled to spectrophotometric detection (Bonastre, Ors, & Peris, 2004; Dhaouadi, Monser, Sadok, & Adhoum, 2006; Nouros et al., 1999; Piñeiro Avila, Salvador, & de la Guardia, 1997), fluorimetric detection (Akasaka, Takamura, Ohrui, Meguro, & Hashimoto, 1996; Sohn, Taki, Ushio, & Ohshima, 2005) and electrochemical detection (Mannino, Cosio, & Wang, 1994; Saad et al., 2006) or after a chromatographic separation coupled to spectrophotometric (Bauer-Plank & Steenhorst-Slikkerveer, 2000; Park, Terao, & Matsushita, 1981), electrochemical (Song, Chang, & Park, 1992) and mass spectrometric detection (Steenhorst-Slikkerveer, Louter, Janssen, & Bauer-Plank, 2000). Chemiluminescence (CL) has also been used for the determination of hydroperoxide content in oils (Bezzi, Loupassaki, Petrakis, Kefalas, & Calokerinos, 2008; Bunting & Gray, 2003; Miyazawa, Fujimoto, Kinoshita, & Usuki, 1994; Rolewski, Siger, Nogala-Kałucka, & Polewski, 2009; Stepanyan, Arnous, Petrakis, Kefalas, & Calokerinos, 2005; Szterk & Lewicki, 2010) due to its ability to monitor radical reactions, which involve reactive oxygen species. The developed CL methods for the determination of oxidative stability and hydroperoxide content of oils have been extensively reviewed recently (Christodouleas, Fotakis, Papadopoulos, Dimotikali, & Calokerinos, 2012). In general terms, until now total hydroperoxide content has been determined in heated oils (Rolewski et al., 2009; Szterk & Lewicki, 2010). In unheated samples, the hydroperoxide content of oils has been evaluated using CL reactions based on: i) luminol using hemin as catalyst and a mixture of acetone–ethanol as a reaction medium (Bezzi et al., 2008) and ii) lucigenin using a mixture of methanol–chloroform as reaction mixture (Bunting & Gray, 2003).

The purpose of the present work is the development of an accurate, reproducible, sensitive, robust, rapid and low cost CL method for the evaluation of total hydroperoxide content of different types of edible oils. The CL system which has been chosen is based on the CL reaction of luminol and oil hydroperoxides, using Fe(III) as a catalyst and 1-propanol as the reaction solvent. In order to achieve the best CL signal, the concentration of luminol, the concentration of sodium hydroxide and the concentration of Fe(III) working solutions were optimized. The developed method was applied to the determination of lipid hydroperoxide content of various types of edible oils and the obtained results were compared with those of the official iodometric procedure.

2. Materials and methods

2.1. Instrumentation

A LKB Wallac static chemiluminometer was used for the CL measurements.

2.2. Materials

All chemicals were of analytical reagent grade. Aqueous solutions were prepared with de-ionized water. 1-Propanol was obtained from Panreac (Spain), luminol from Fluka (USA), and di t-butyl peroxide solution (98%w/v) and starch soluble from Sigma-Aldrich (USA). Chloroform, glacial acetic acid, ferric nitrate, sodium hydroxide, potassium iodide and Titrisol sodium thiosulfate solution were all obtained from Merck (Germany). All types and brands of oil samples were obtained from local supermarkets in Athens, Greece.

Luminol stock solution (1.00×10^{-3} M) was prepared in an aqueous alkaline solution (pH = 12). This solution can be kept in the refrigerator and remain stable for a month. Working solutions of alkaline luminol were prepared in 1-propanol by appropriate dilution. Sodium hydroxide stock solution (1.00 M) was prepared in de-ionized water. Fe(III) stock solution (0.100 M) was prepared in an aqueous acid solution (0.2 M of HNO_3) by dissolving $Fe(NO_3)_3 \cdot 9H_2O$. Working solutions of Fe(III) were prepared in 1-propanol by appropriate dilution. Di-t-butyl peroxide solutions of different concentrations were prepared daily by the appropriate dilution of 98%w/v di-t-butyl peroxide solution to 1-propanol. Edible oil solutions of different concentrations were prepared daily with the appropriate dilution of untreated oil samples to 1-propanol.

3. Experimental

3.1. CL procedure

In the polystyrene test tube, 250 μL of 1-propanol (blank sample) or di-t-butyl peroxide solution or oil solution of different concentrations was mixed with 100 μL of 5.0×10^{-7} M Fe(III) solution. Then the tube was inserted in the luminometer and 250 μL of alkaline luminol working solution (luminol 1.0×10^{-6} M, sodium hydroxide 0.0320 M) was added with a syringe. CL intensity reaches maximum within few seconds and CL signal is recorded.

3.2. Iodometric procedure

The iodometric procedure is applied according to the official method of the European Union (Commission Regulation (EEC), No. 2568r91). In brief, 2.0 g of oil was diluted in 10 mL of chloroform and then 15 mL of acetic acid and 1 mL of saturated aqueous solution of potassium iodide were added. The mixture was shaken for one minute and was left for exactly five minutes away from the light at a temperature from 15 to 25 °C. Then, 75 mL of distilled water was added and the liberated iodine was titrated using a sodium thiosulphate solution, 0.002 N, and starch solution as indicator. The peroxide value of oils was calculated using the equation: $\mathrm{PV\ (meq/kg)} = \frac{V\ \mathrm{(mL)} \times T\ \mathrm{(meq/mL)} \times 1000}{m\ \mathrm{(g)}}$, where V is the volume of the solution of sodium thiosulphate consumed in the titration, T is the titer of the sodium thiosulphate solution and m is the mass of the oil used.