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Analytical Methods

High-throughput analysis of lipid hydroperoxides in edible oils and fats using the fluorescent reagent diphenyl-1-pyrenylphosphine

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ABSTRACT

A fluorometric method for the determination of hydroperoxides (HP) in edible oils and fats using the reagent diphenyl-1-pyrenylphosphine (DPPP) was developed and validated. Two solvent media containing 100% butanol or a mixture of chloroform/methanol (2:1, v/v) can be used to solubilise lipid samples. Regardless of the solvent used to solubilise the sample, the DPPP method was precise, accurate, sensitive and easy to perform. The HP content of 43 oil and fat samples was determined and the results were compared with those obtained by means of the AOCS Official Method for the determination of peroxide value (PV) and the ferrous oxidation-xylenol orange (FOX) method. The proposed method not only correlates well with the PV and FOX methods, but also presents some advantages such as requiring low sample and solvent amounts and being suitable for high-throughput sample analysis.

1. Introduction

Lipids undergo oxidation reactions in the presence of common catalytic systems such as light, heat, enzymes, metals and metalloproteins (Frankel, 1998; Shahidi & Zhong, 2005). The most common process of oxidation of edible fats and oils in bulk is autoxidation. In this, and other oxidation processes, there is a continuous formation of primary oxidation compounds, particularly lipid hydroperoxides (HP). These lipid HPs lack odour and flavour, but their instability leads to further free radical and oxidation reactions and thus a high variety of non-volatile and volatile compounds (Frankel, 1998). Overall, these latter secondary oxidation compounds are the major cause of lipid deterioration and are responsible for the development of off-flavours and rancidity in foods (Dobarganes & Velasco, 2002; Frankel, 1998; Shahidi & Zhong, 2010). Furthermore, lipid oxidation decreases the nutritional value and safety of food (Billek, 2000; Esterbauer, Schaur, & Zollner, 1991; Shahidi & Zhong, 2010). Different oxidation compounds can be absorbed and, although it is difficult to discern them from those produced *in vivo*, once in the organism they play a role in the development of different disorders and health conditions, including cardiovascular disease (Cohn, 2002; Staprans, Hardman, Pan, & Feingold, 1999), Alzheimer's disease (Corsinovi, Biasi, Poli, Leonarduzzi, & Isaia, 2011), cancer (Kanazawa, Sawa, Akaike, & Maeda, 2002) and ageing (Bokov, Chaudhuri, & Richardson, 2004; Pandey & Syed, 2010).

Therefore, the determination of HP is an important quality parameter in the food industry and is also very useful for assessing the progression of oxidation in *in vitro* and *in vivo* experiments. Consequently, the determination of HPs is of considerable interest and explains why suitable analytical methods are being developed (Dobarganes & Velasco, 2002; Shahidi & Zhong, 2010). Because they are a highly reactive species, their determination is challenging. However, there is a wide range of methods (e.g. iodometry, spectrophotometry, spectroscopy, fluorometry, etc.), some of which use separation techniques (e.g. GC and HPLC), that can be used to determine the HP content of foods and biological samples (Barriuso, Astiasaran, & Ansorena, 2013; Bou, Codony, Tres, Decker, & Guardiola, 2008; Dobarganes & Velasco, 2002; Frankel, Neff, & Weisleder, 1990). With respect to fats and oils, the AOAC and AOCS provide official methods to determine peroxide value (PV), which is defined in terms of milliequivalents of peroxide per kg of lipid (AOAC, 2000; AOCS, 2006). These classical titration methods are based on the reduction of the HP group (ROOH) by iodide ion and have the advantage of being simple and inexpensive. However,

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these iodometric methods have some disadvantages such as being highly empirical, presenting interference and having a high detection limit (0.2 μmol H_2O_2) (Jessup, Dean, & Gebicki, 1994; Nielsen, Timm-Heinrich, & Jacobsen, 2003).

A simple alternative to these official methods that has a broad applicability to foodstuffs and biological samples is the ferrous oxidation-xylenol orange (FOX) method. This method consists of the spectrophotometric measurement of the chromophore complex formed after reaction of xylenol orange with ferric ions previously oxidised by the HPs present in the sample (Bou et al., 2008). Likewise, the spectrophotometric method, which is based on the standard method of the International Dairy Federation (International Dairy Federation, 1991; Shantha & Decker, 1994), utilises the oxidation of ferrous ions to ferric ions in acidic media by HP; the ferric ions then react with thiocyanate to form a chromophore complex, instead of being formed by xylenol orange. Consequently, there is a high correlation between these two methods (Burat & Bozkurt, 1996; Nielsen et al., 2003; Shantha & Decker, 1994). They require common laboratory instruments and can be used routinely. In addition, they are specific for HP, require low sample and solvent amounts, and are fairly sensitive; all of these factors explain why they are so widely used. However, they have the disadvantage of being affected by various factors such as the presence of chelators and other chromophores, and have also shown low reproducibility (Bou et al., 2008).

Akasaka, Sasaki, Ohrui, and Meguro (1992) were the first to describe a simple method to determine lipid HP in oils and foods by means of the reagent diphenyl-1-pyrenylphospine (DPPP). This is a non-fluorescent phosphine molecule that reacts with HP to form DPPP oxide, which emits a strong fluorescence. The reaction is specific for HP and the fluorescence intensity of DPPP oxide is directly proportional to the amount of HP (Akasaka & Ohrui, 2000). Various publications have showed that DPPP is useful for the determination of HP in biological samples using flow injection and HPLC post-column methods (Akasaka & Ohrui, 2000; Akasaka, Ohrui, & Meguro, 1993; Akasaka, Takamura, Ohrui, Meguro, & Hashimoto, 1996; Meguro, Akasaka, & Ohrui, 1990). The high sensitivity and specificity of these methods is considerable and useful for some specific purposes in fields such as human health research. Nevertheless, these more sophisticated methods have some drawbacks such as the need for expensive apparatus and highly trained personnel, and this limits their implementation in the food industry. The industry often demands cheap and robust routine methods for the purposes of quality control and/or regulation. In terms of lipid HP determination, the food industry is more concerned with being able to determine the overall HP content easily and with having highly sensitive methods with a high-throughput of samples.

Therefore, the aim of this work was to develop and validate a routine method for the determination of total HP in edible oils and fats by means of the fluorescent reagent DPPP. In addition, the performance of this method was compared with the AOCS Official Method and the FOX method, which are probably the most frequently used titrimetric and spectrophotometric methods for HP determination.

2. Materials and methods

2.1. Materials

Cumene hydroperoxide (80% purity) (CHP) and 2,6-di-*tert*-butyl-4-methylphenol (BHT) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Dipheny-1-pyrenylphosphine (DPPP) was obtained from Cayman Chemical Co. (Ann Arbor, MI, USA). HPLC-grade 1-butanol was obtained from Panreac (Barcelona, Spain), HPLC-grade methanol from Carlo Erba (Barcelona, Spain) and chloroform from Scharlau (Sentmenat, Barcelona, Spain). Bi-distiled water was obtained using a Milli-Q® Gradient System (Millipore Co., Billerica, MA, USA).

2.2. Samples

A total of 43 samples were used for the study. The samples were among the world's most commonly used vegetable oils or animal fats for edible purposes (USDA, 2013). The samples were: 4 coconut oils, 3 canola oils, 3 corn oils, 2 high-oleic sunflower oils, 3 palm kernel oils, 3 palm oils, 3 soybean oil and 3 sunflower oils, all of which were donated by Lipidos Santiga. These oils were different because they belonged to different batches and were stored at room temperature thus explaining the different extents of oxidation. The remaining oils and fats were: 4 different brands of butter, 3 different brands of lard, 3 different brands of fish oil capsules, 1 grapes seed oil, 1 hazelnut oil, 3 different brands of olive oils, 1 peanut oil, 1 safflower oil, 1 sesame oil, and 1 walnut oil, all of which were purchased from local shops.

The lipid fraction of the butters was extracted as follows: the butters were melted at 80 °C, and the supernatant was separated and immediately centrifuged for 3 min at 1500*g*. The upper phase was separated again and centrifuged for 3 min at 2700*g*. The lard was melted at 55 °C and filtered through a Whatman No. 54 filter paper. All samples were placed in 10 mL-vials with minimum head space and kept at −80 °C until analysis.

2.3. Determination of the lipid hydroperoxide content by means of diphenyl-1-pyrenylphospine (DPPP)

A new method for the determination of HP in oils and fats using fluorescent probe DPPP was developed. The method was conducted under subdued light conditions, and positive displacement pipettes were used throughout the study. Two versions of the method were used depending on the solubility of the samples in the solvent media. The solubility of the different samples is shown in Table 1.

2.4. DPPP method version 1 (DPPP1)

An appropriate sample size, between 1 and 5 g of sample, was dissolved in butanol containing 4 mM BHT (between 10 and 50 mL depending on the expected peroxide content) to lie within

Table 1
Solubility of oils and fats in pure butanol and chloroform/methanol (2:1, v/v).

Lipid	Butanol	Chloroform/methanol
Butter	Insoluble	Soluble
Canola oil	Soluble	Soluble
Coconut oil	Partly soluble*	Soluble
Corn oil	Soluble	Soluble
Fish oil	Soluble	Soluble
Grapes seed oil	Soluble	Soluble
Hazelnut oil	Soluble	Soluble
High-oleic sunflower oil	Soluble	Soluble
Olive oil	Soluble	Soluble
Palm kernel oil	Insoluble	Soluble
Palm oil	Insoluble	Soluble
Peanut oil	Soluble	Soluble
Pork lard	Insoluble	Soluble
Safflower oil	Soluble	Soluble
Sesame seed oil	Soluble	Soluble
Soybean oil	Soluble	Soluble
Sunflower oil	Soluble	Soluble
Walnut oil	Soluble	Soluble

* 7 ml of solvent is the minimum volume required to dissolve 1 g of sample.