High sensitive and efficient detection of edible oils adulterated with used frying oil by electron spin resonance

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ABSTRACT

Edible oil adulteration exposes a high risk to consumer's health and is strictly regulated for food industry. However, efficient and reliable detection of food oil adulteration, particularly for detection of adulteration of commonly vegetable oils (rapeseed oil, peanut oil, sunflower oil, corn oil and olive oil), is not an easy task. In this paper, a high sensitive and reliable method of electron spin resonance (ESR) spin-trapping technique was introduced. α-phenyl-N-tert-butylnitrone (PBN) was used as spin trap to discriminate the adulteration of qualified vegetable oils, very intense spectra revealing a hyperfine splitting made up of a triplet of doublets ($a^N = 14.88$G and $a^H = 1.81$G) were applied. The initial signal intensities and increasing rate of the UFO were both higher than the qualified oils. All five qualified vegetable oils with higher adulteration proportions had higher initial signal intensities detected at 2 min, which could be assigned to thermal oxidation products that were present in adulterated oils. Initial signal intensities and adulteration proportions (<20%) were treated for quantitative analysis by linear regression. The simple linear equations were built and the coefficients of determination (R^2) were all above 0.94 except PO (0.8920). Therefore, the proposed ESR spin-trapping technique could be applied to discriminate adulteration of qualified vegetable oils.

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1. Introduction

Deep-fat frying is a welcome food process at high temperature, fried foods with optimum temperature and time are properly cooked. However, during deep-fat frying, plant oils with high amounts of unsaturated fatty acids have high susceptibility to thermal oxidation (Choe & Min, 2007; Perkins & Erickson, 1996; Zhang, Saleh, Chen, & Shen, 2012). Many unexpected reaction products, such as polymers, trans-fatty acids and compounds with oxygen-containing groups, which are generated during deep-fat frying process (Eunok & Min, 2006; Yin, Xu, & Porter, 2011), are difficult to remove by refining process. Nowadays, qualified vegetable oils adulteration has been a big problem for the health of consumers.

Many detection methods have been applied to assess the adulteration of qualified vegetable oils based on the physical properties or chemical components of qualified vegetable oils. There are lots of methods, including dielectric spectroscopy (Cataldo, Piuzzi,

Cannazza, Benedetto, & Tarricone, 2010; Hu, Toyoda, & Ihara, 2010), nuclear magnet resonance (Agiomyrgianaki, Petrakis, & Dais, 2010; Zhang, Saleh, & Shen, 2013), high performance liquid chromatography (Cunha & Oliveira, 2006), gas chromatography (Hajimahmoodi et al., 2005), mass spectrometry (Lerma-García, Herrero-Martínez, Ramis-Ramos, & Simó-Alfonso, 2008), near infrared spectroscopy (Downey, Mcintyre, & Davies, 2002; Zhang, Cheng, Sun, Hu, Shen, & Wu, 2012), differential scanning calorimetry (Chiavaro et al., 2008), etc. However, all these methods are labor intensive, time-consuming sample preparation and some high toxic chemical reagent for laboratory stuff. Qing zhang et al. discriminate the adulteration of edible vegetable oil by low field nuclear magnetic, the limit of detection of different oils were all greater than 10, and even limit of detection of rapeseed oil was 31% (Zhang et al., 2013). It might not be sensitive and efficient while the adulteration proportions were low.

ESR or EPR (electron paramagnetic resonance) has developed a lot with many advantages, such as convenience, fastness, simplicity and objectiveness, which can be used in the detection of adulteration of qualified vegetable oils. ESR is a valuable tool in detection and quantification of lipid free radicals because of its unique

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magnetic properties of radicals with unpaired electron (Ottaviani, Spallaci, Cangiotti, Bacchiocca, & Ninfali, 2001). However, the steady-state concentration of lipid free radicals that detected directly are below the lowest limit (10^{-9}–10^{-8} M) (Andersen & Skibsted, 2002). Detection of lipid free radicals can be approached indirectly by the spin-trapping technique, which is based on the reaction of free radicals with diamagnetic compounds (spin traps) to form much more stable spin adducts to accumulate at detectable concentrations ($>10^{-7}$–10^{-6} M) (Andersen, Velasco, & Skibsted, 2005). The spin reagent nitrones are most widely used spin traps in helping the formation of stable notroxyl radicals, the unpaired electrons are located on the notroxide function. α-phenyl-*N-tert*-butylnitrone (PBN) is a most extensively used spin trap in detecting free radicals formed via lipid oxidation of food and biological material because of its high lipophilic and reactivity characteristics. Recently, spin-trapping technique, based on PBN as the spin trap, has been widely used for its specificity and high sensitivity on studying the oxidative stability of lipids in food (Jerzykiewicz, Ćwieląg-Piasecka, & Jezierski, 2013; Ottaviani et al., 2001; Szterk, Stefaniuk, Waszkiewicz-Robak, & Roszko, 2011).

The aim of this study was to investigate an analytical method based on ESR spin-trapping technique, for qualitative and quantitative analysis of the vegetable oils adulteration.

2. Materials and methods

2.1. Chemicals

α-phenyl-*N-tert*-butylnitrone (PBN) was purchased from J & K Chemical Technique (Shanghai, China). It was stored under freezing conditions. PBN was used as received without purification. All other chemicals used were all of analytical grade.

2.2. Samples preparation

Oils used as samples in this study were commonly consumed commercial rapeseed oil (RO), soybean oil (SO), peanut oil (PO), corn oil (CO) and olive oil (OO), which were purchased from the local markets. They were all qualified and pure products. The UFO was collected from western fast food restaurant in the local. Origin of the UFO was mainly rapeseed oil.

The UFO was discolored by activated clay due to the deeply dark color. The discoloration procedure was cited as follows: Heating the UFO at 100 °C with adding 5% activated clay and stirring slowly for 30 min under vacuum. Then, the mix was filtrated by centrifuging at 5000 r/m for 10 min, and the supernatant was collected.

2.3. Measurement of oil chemistry properties

Acid values (AV) of the studied oils were measured by alkaline titration according to the AOCS official method (Cd 3a-63). The acid value was expressed as mg of KOH per gram of oil sample. Peroxide values (POV) of the UFO and five qualified vegetable oils were performed exactly according to the AOCS official method (AOCS Cd 8-53). Total polar compounds (TPC) were determined according to the IUPAC Standard Method 2.507 (Dobarganes, Velasco and Dieffenbacher, 2000).

2.4. Samples preparation and procedures for ESR analysis

UFO was proportionally added into the five commonly commercially edible vegetable oils, respectively. The adulteration proportions were 1%, 2%, 5%, 8%, 10%, 20%, 50%, 80%, 100% with five edible vegetable oils set as control.

ESR detection was carried out at 140 °C using a Bruker EMXplus-10/12 spectrometer (Bruker, Germany) operating at 9.85 GHz with a variable temperature control unit (Bruker ER 4141 VT-I). 100 μL oil samples and 20 μL PBN in toluene (150 mM) were mixed transferred into quartz tubes with 4 mm diameter by stirring at room temperature, the final concentration of PBN in the system was about 25 mM. The tubes were inserted into the resonant cavity when the selected temperature was reached. ESR spectrum was recorded at per 2 min intervals after the tubes inserted at 140 °C. Analyses were performed without the presence of light. The parameters which were kept constant in all ESR measurements in each determination and only receiver gain were adjusted if necessary. The instrumental settings applied were as follows: center Field, 3360 G; sweep width, 100 G; resolution, 1024 points; microwave power, 20 mW; modulation amplitude, 1.0 G; modulation frequency, 100 kHz; conversion time, 1.28 ms; time constant, 20.48 ms.

In the experimental condition, various concentrations of PBN with given amount of oil samples gave the same results as the 25 mM concentration, indicating there is no saturation of radical trapping capacity. Oil samples and toluene solution of PBN were tested as a control. In some cases, a small ESR signal from these reference samples were subtracted from the EPR signal obtained by experimental spectra. All analyses were performed in triplicate. All the data showed statistical significance.

2.5. Data analysis of ESR spectrum

Computer simulation of the experimental ESR spectra was applied for calculation of g value and hyperfine coupling constants, which were calculated by Bruker Xenon software after optimizing signal-to-noise ratios. The precision in the EPR parameters is ~3%.

3. Results and discussion

3.1. Chemical properties of the vegetable oils and UFO

Chemical indices of vegetable oils could partly reflect qualities of vegetable oils. The POVs, AVs and TPCs (total polar compounds) of the UFO and the studied qualified vegetable oils were shown in Table 1. The POVs, AVs and TPCs of the five qualified vegetable oils and UFO showed obvious differences by means of SPSS, respectively. POVs of UFO were far higher than five qualified vegetable oils, which mean large amounts of hydroperoxides existed in the UFO, while five qualified vegetable oils were all in good conditions. AVs of all studied oils were less than 0.5 mg/g except UFO. During a long-time high temperature treatment, hydrolytic reaction took place which lead to higher AVs. The percentage of polar compounds gives information of the compounds having higher polarity than that of triacylalycerols, such as polymerized triacylglycerols, oxidized triacylglycerols, diacylglycerols, and fatty acids, being a good indicator of the quality of oils. The level of TPC of UFO was higher than 24%, which was the required quality limit for using again. The TPCs of the five qualified vegetable oils were all less than 2%, indicating that they were all kept in good reservation conditions.

High level of AV, POV and TPC demonstrated that the quality of the UFO was beyond the continued utilization range. The UFO contained large amounts of hydrolyzed, oxidized and polymerized compounds produced during the deep-frying process, contributing to the bad quality. However, the detection of AV, POV and TPC were complicated. Besides, they were a waste of time and solvents. A rapid and sensitive method should be developed.