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Vitamin E oxidation and tocopheroxyl radical stabilization in bleached rice bran oil

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ARTICLE INFO

Article history:
Received 24 June 2012
Received in revised form
25 April 2013
Accepted 28 April 2013

Keywords:
EPR
Antioxidants
Oils
Tocopheroxyl radicals
Rice bran oil
Oil bleaching

ABSTRACT

Changes in the free radical components of rice bran oil before and after bleaching using a modified kaolin were investigated by electron paramagnetic resonance (EPR) spectroscopy. Before bleaching the EPR spectrum of the oil consisted of a single weak peak with *g*-value and linewidth similar to those of melanoidins, common colored components of seeds and husks. After bleaching, this was replaced by an EPR spectrum with ^{1}H hyperfine structure characteristic of the α-tocopheroxyl radical, thus indicating some vitamin E oxidation during the bleaching process. This radical was exceptionally stable compared to its half-life in biological or simple chemical systems. The high stability of the α-tocopheroxyl radical indicates that reactions in the bleached oil are extremely slow, and that this oil has exceptional stability properties.

1. Introduction

Although natural rice bran oil has several constituents that are regarded as having health benefits, it has limited stability to oxidation and its overall color is considered unacceptable to consumers (Chotimarkorn, Benjakul, & Silalai, 2008; Zubair, Anwar, Ashraf, & Uddin, 2012). Thus bleaching agents are used to improve both its shelf-life and color quality. Adsorbents commonly used for vegetable oil decolorization include activated clays, activated carbon, and various silica-based products, but the relatively low cost of activated clays makes them the most attractive class of adsorbent. Bentonite (montmorillonite) is the most popular clay used for stabilization, color removal, and purification of plant-derived oils (Gonzalez Pradas, Villafranca Sanchez, Socias-Viciana, & Gallego Compo, 1994; Boukerroui & Quali, 2002), but the use of magnesiosilicate minerals, such as sepiolite (Sabah, 2007; Sabah, Cinar, & Celik, 2007) and attapulgite (Huang, Liu, Liu, & Wang, 2007) has also been documented. In addition, a recent investigation showed that a kaolin (a non-swelling aluminosilicate) modified by a combination of physical and chemical treatments had a decolorization capacity for rice bran oil that was comparable to that of a commercial bleaching clay (Worasith, Goodman, Jeyachoke, & Thiravetyan, 2011a). However, apart from measuring the color intensity by UV/visible spectroscopy, the emphasis in that paper was on understanding pigment removal by the modified kaolin.

Attempts are now being made to obtain a better understanding of rice bran oil bleaching using modified kaolins, and a detailed characterization of the changes induced in the clay mineral by various physical and chemical treatments,

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http://dx.doi.org/10.1016/j.fbio.2013.04.006

has been recently published (Worasith, Goodman, Neampan, Jeyachoke, & Thiravetyan, 2011b). An initial objective of the present paper was to explore the possible application of electron paramagnetic resonance (EPR) spectroscopy as an alternative method for quantifying the bleaching of rice bran oil; rice bran, in common with seed coats and husks from other plant species, (Magill, Deighton, Pritchard, Benson, & Goodman, 1994; Nalgreiter, Reichenauer, Goodman, & Bolhàr-Nordenkampf, 2005; Szöcs, 2005) has stable free radical centers from the melanoidin pigment, and this was expected to be at least partially extracted into the rice bran oil and to contribute to its color. However, our preliminary (unpublished) results showed that instead of there simply being a decrease in the free radical signal intensity following bleaching, the rice bran oil produced a distinctly different EPR spectrum. The bleaching process for vegetable oils involves heating in air, and there is, therefore, the possibility of oxidation processes taking place during this treatment. Such processes would be expected to proceed via free radical reactions, even though stable free radicals are not generally observed in bleached vegetable oils. Thus the observation of the formation a stable free radical in bleached rice bran oil is unusual.

The objective of the present research was to optimize the EPR spectrum for this free radical, and to use its parameters to obtain a chemical identification of the radical or radicals responsible.

2. Materials and methods

Rice bran oil was obtained from the Thai Edible Oil Co.,Ltd., Bangkok, Thailand. It was washed with water at 90–95 °C, and dried under vacuum at 115 °C for 15 min before conducting the bleaching treatment. The kaolin, from Ranong Province in southern Thailand, was supplied by the Had Som Pan Co., Muang Ranong, Thailand then purified, ground and chemically modified as described by Worasith et al. (2011a) using either 2 M sulfuric acid or 0.7 M oxalic acid. Characterization of the final product revealed that a substantial fraction of the kaolin was converted to a poorly-crystalline Si-rich material (Worasith et al., 2011b).

For adsorption of colored materials from the oil, unbleached rice bran oil (9.8 g) was placed in a flask in an oil bath at 90 °C, and the modified kaolin (0.2 g) added. After 30 min the clay and oil were separated by centrifugation at 2500 rpm for 10 min, and the oil was then filtered (Whatman no. 40) under vacuum. The bleached oil was stored at laboratory environment temperature (~25 °C) in glass bottles with a small volume of air in the headspace for ~1 month before investigation by EPR spectroscopy.

EPR spectra were recorded as 1st derivatives of the microwave absorption at ambient temperature using a Bruker AMX CW spectrometer operating at X-band frequencies with a Gunn diode as microwave source; the spectrometer was equipped with a high sensitivity resonator and in-line frequency counter. Spectra were acquired from 100 μL samples in 3 mm i.d. tubes, and accumulated in 1024 points using a 5 mT sweep width, 5 mW microwave power, 100 kHz modulation frequency, and either 0.2 or 0.1 mT modulation amplitude. Other acquisition parameters are specified in the figure caption. During spectral acquisition, fine tuning of the resonator was performed automatically before the beginning of each scan. Two basic parameters are obtained from the EPR spectra of free radicals; a constant known as the *g*-value, from the position of the center of the spectrum, and hyperfine structure (hfs) from interactions between the unpaired electron and any nuclei with non-zero spin. With organic free radicals hfs usually arises from the ^{1}H isotope (nuclear spin $I=½$), since the main isotopes of C and O have zero spin. Hfs patterns consist of 2I+1 peaks for each atom, and the separations between individual peaks are proportional to the unpaired electron density on that atom.

For all of the results reported here, the spectral interpretations were tested and parameters refined by simulation using the Bruker Simfonia software, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) ($g=2.0036$) was used as an external standard for *g*-value calibration.

3. Results and discussion

The EPR spectrum of rice bran oil before bleaching is shown in Fig. 1(a). It consists of a single peak with $g=2.0040$ and 1st derivative peak-to-peak width of ~0.5 mT. Thus it is similar to the spectra from many materials of biological origin (e.g. humic substances (Cheshire, Goodman, McPhail, & Sparling, 1985), seed testa (Hepburn, Goodman, McPhail, Matthews, & Powell, 1986; Magill et al., 1994), roasted coffee (Pascual, Goodman, & Yeretzian, 2002)) that contain melanoidins, the high molecular weight colored materials formed by the Maillard reaction (e.g. Rizzi, 2003). The spectra obtained from the oil after bleaching with kaolin modified using sulfuric acid are shown under low and high resolution in Fig. 1(b and c). A spectrum similar to that in Fig. 1(b) was obtained from the rice bran oil bleached using kaolin modified with oxalic acid, but this sample was not investigated further. The spectrum in Fig. 1(b) contains substantial hfs, which can be simulated by six equivalent ^{1}H coupling constants of 0.545 mT. This septet pattern is similar to the (low resolution) spectrum produced in biological systems by the oxidation of α-tocopherol (Kalyanaraman, Darley-Usmar, Struck, Hogg, & Parthasrathy, 1995; Laranjinha & Cadenas, 1999), the major form of vitamin E in rice bran oil (Bruscatto et al., 2009), and a similar spectrum has been reported to be generated during the early stage of the thermal decomposition of grape seed oil (Vicente, Deighton, Glidewell, Empis, & Goodman, 1995).

When the spectrum of the bleached oil was recorded with a smaller modulation amplitude (to give higher spectral resolution), additional hfs was resolved (Fig. 1c), and a simulation using the hfs values reported by Gregor, Grabner, Adelwöhrer, Rosenau, and Gille (2005) for the α-tocopheroxyl radical is shown in Fig. 1(d). Although the fit is not perfect, it is very close, and strongly suggests that the spectrum in Fig. 1(c) corresponds mainly to the α-tocopheroxyl radical, the EPR spectrum of which is distinctly different from those derived from other components of vitamin E (Lehtovuori & Joela, 2002).

The bleaching treatment resulted in the removal of ~80% of the chlorophyll from a typical rice bran oil (Table 1) and an