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Water-contained surfactant-based vortex-assisted microextraction method combined with liquid chromatography for determination of synthetic antioxidants from edible oil

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ABSTRACT

For the first time, a novel water-contained surfactant-based vortex-assisted microextraction method (WSVAME) was developed for the extraction of two synthetic antioxidants (t-butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA)) from edible oil samples. The novel microextraction method is based on the injection of an aqueous solution of non-ionic surfactant, Brij-35, into the oil sample in a conical bottom glass tube to form a cloudy solution. Vortex mixing was applied to accelerate the dispersion process. After extraction and phase separation by centrifugation, the lower sediment phase was directly analyzed by HPLC. The effects of the four experimental parameters including volume and concentration of extraction solvent (aqueous solution of Brij-35), percentage of acetic acid added to the oil sample and vortex time on the extraction efficiency were studied with a full factorial design. The central composite design and multiple linear regression method were applied for the construction of the best polynomial model based on experimental recoveries. The proposed method showed good linearity within the range of 0.200–200 μg mL^{-1}, the square of correlation coefficient higher than 0.999 and appropriate limit of detection (0.026 and 0.020 μg mL^{-1} for TBHQ and BHA, respectively), while the precision for inner-day was ≤3.0 (n = 5) and it was ≤3.80 (n = 5) for inter-day assay. Under the optimal condition (30 μL of 0.10 mol L^{-1} Brij-35 solution as extraction solvent and vortex time 1 min), the method was successfully applied for determination of TBHQ and BHA in different commercial edible oil samples. The recoveries in all cases were above 95%, with relative standard deviations below 5%. This approach is considered as a simple, sensitive and environmentally friendly method because of biodegradability of the extraction phase and no use of organic solvent in the extraction procedure.

1. Introduction

Oxidation of food is a detrimental process, leads to rancidity and degrades the composition, causing loss of nutritional value. To prevent food degradation, the addition of antioxidants plays a significant role [1]. Using of the synthetic phenolic antioxidants (SPAs), t-butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA), lieu to natural antioxidant in fat-containing foods because of their more chemical stability, low cost and availability are preferred [2,3]. BHA has been used in food products with some restrictions, since the late 1950s [4]. More recently, TBHQ have been added to the list of permitted antioxidants in foods in many countries [5,6]. Nevertheless, studies have shown that use of the synthetic phenolic antioxidants at a high dosage, causes carcinogenesis in animals [7–11]. In fact the conjugated aromatic ring of these compounds is able to release free radicals [12] that cause cancer and tumors [11,13,14]. In this way, for the quality control procedure in the food industries, determination of synthetic antioxidant content in foodstuffs is necessary and managed by law in many countries.

Various analytical techniques had been developed to determine the amount of SPAs in foodstuffs samples. There are gas chromatography (GC) [15,16], GC-mass spectrometry (GC-MS) [17,18], high performance liquid chromatography [19,20], HPLC-mass spectrometry (HPLC-MS) [21,22], voltammetry [23,24] and micellar electrokinetic capillary chromatography [25,26]. Additionally due to the complexity of sample matrix and low concentration level of the antioxidant in the samples, the preconcentration and cleaning up steps are necessary [27]. Different pretreatment methods such

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as solid-phase extraction [26,28], liquid–liquid extraction (LLE) [29,30] and cloud-point extraction (CPE) [31,32] had been used for this purpose. In 2006, dispersive liquid–liquid microextraction (DLLME) as a high-performance, powerful, rapid and inexpensive microextraction method was developed by Assadi and coworkers [33]. In the basic principle of this method, dispersion of extraction solvent within the aqueous solution was assisted by disperser solvent [34–37]. The main disadvantage of the early employed DLLME procedure is the reduction of the extraction efficiency due to the low partition coefficient of the analytes between organic and aqueous phases in the presence of the organic disperser solvent [38,39]. Additionally the organic disperser and extraction solvent which used in the traditional DLLME method are relatively toxic and environmentally unfriendly. To overcome to these defects, the use of the surfactants in sample preparation techniques has been interested. Recently, Sarafraz-Yazdi [40] and Moradi and Yamini [41] reviewed the recent developments in surfactant-based extraction methods. In these methods surfactants were used as an emulsifier, surfactant rich phase or an extraction medium, ion pair reagents, hemimicelle or admicelle extraction. Among the mentioned methods, the using of surfactant-rich phase in extraction techniques has the main advantages of safety, low cost, ability to concentrate solutes, easily availability of surfactant, and low toxicity compared with classical organic solvents [40]. Nevertheless conventional liquid phase microextraction methods almost employed organic solvents or surfactant rich solutions as extraction phase for isolation of the analytes from the aqueous sample. In the present study for the first time a microextraction approach (WSVAME method) based on the using of aqueous solution of non-ionic surfactant, Brij-35, as extraction solvent was used for isolation and preconcentration of synthetic antioxidants from edible oil samples. In this method the extraction solvent was the tiny droplet of water-contained Brij-35 micelles dispersed in a nonpolar sample only by vortex agitation. A methodology based on the central composite design (CCD) and response surface modeling (RSM) [42–45] was applied to investigate the effect of the experimental factors on the extraction efficiency (recovery) and finding the best experimental extraction condition.

2. Experimental

2.1. Material and reagents

The deionized, double distilled, filtered (through a 0.45 μm filter (Millipore membranes, Bedford MA, USA)) and degassed water was used. The blank oil sample free of any additive (SPAs) was prepared from the Salej Syrup manufacture (Babolsar, Iran). Six different oil samples (sunflower, soybean, grape seed oil, sesame, almond and olive) were purchased from local markets. Butylated hydroxyanisole (BHA, >98%) were purchased from Merck (Darmstadt, Germany), and t-butyl hydroquinone (TBHQ, 97%) were bought from Sigma–Aldrich (Steinheim, Germany). Methanol (HPLC grade), acetic acid (AcOH) and tetra hydrofuran (THF) were obtained from Merck. The non-ionic surfactant Brij-35 [Polyoxyethylene lauryl ether $(C_2H_4O)_{23}$-$C_{12}H_{25}OH$] was purchased from Fluka (Buchs, Switzerland) and a stock solution (0.10 mol L^{-1}) was prepared in water. A stock solution containing both of TBHQ and BHA at $1 \times 10^{+5}$ μg mL^{-1} was prepared in methanol. Other solutions for calibration carve were prepared by dilution of the stock in Brij-35 solution (0.10 mol L^{-1}). All solutions were stored at 4 °C.

2.2. Instrumentation

The chromatographic analysis was performed with a HPLC system consisted of a model 515 solvent delivery system from Waters (Milford, MA, USA), equipped with model 7725i manual injector fitted with a 20 μL loop (Rheodyne, Cotati, CA, USA) and Perkin–Elmer LC-95 UV detector (Norwalk, CT, USA) set at 280 nm. Analytes were separated by isocratic elution on a C_{18} column (250 × 4.6 mm, 5 μm) from Dr. Maisch (Beim Brueckle, Germany). The mobile phase consisted of methanol: water: THF (70/29/1, v/v/v) was filtered through a 0.45 μm Millipore filter (Bedford, MA, USA), degassed under vacuum and passed through the column at flow rate of 1.0 mL min^{-1} at room temperature. Sample shaking was done on a vortex agitator (Fisher Scientific, USA).

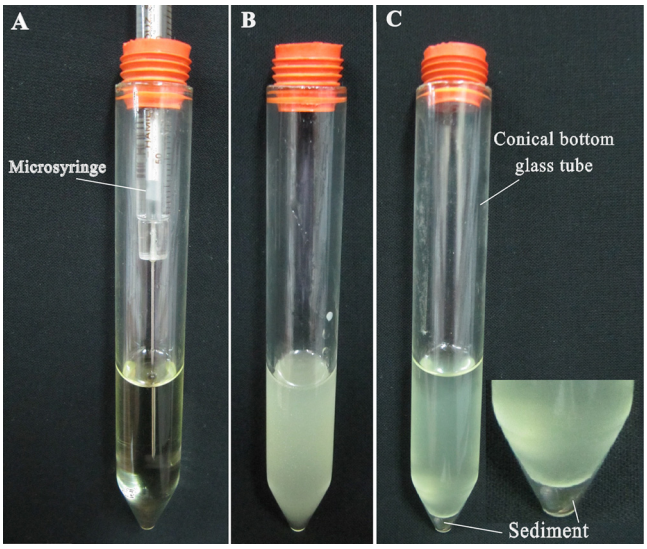

Fig. 1. Photography of different steps in WSVAME method: (A) injection of extraction solution (Brij-35 solution) into the oil sample; (B) formation of cloudy solution after vortex agitation; (C) phase separation after centrifugation.

2.3. Extraction procedure

For WSVAME, 5.0 mL oil sample was placed in a 10 mL conical glass test tube fitted with a plastic cap. 30 μL of 0.10 mol L^{-1} Brij-35 aqueous solution as extraction solvent was injected into the sample solution using 250 μL microsyringe rapidly (Fig. 1). The mixture was then vigorously shaken by a vortex agitator for 1 min. The resulting cloudy solution was centrifuged at 3000 rpm for 1 min. Then the sediment aqueous phase in the bottom of the conical test tube was transferred for subsequent analysis by HPLC using a 20 μL sample loop.

2.4. Calculation of extraction recovery and enrichment factor

The extraction recovery (ER) was defined as the percentage of the total analyte (n_0) extracted into the aqueous sediment phase (n_{sed}) and the enrichment factor (EF) was defined as ratio of the analyte concentration in the aqueous phase (C_{sed}) to the analyte concentration in the oil phase (C_0). Accordingly, calculation of the extraction recovery was carried out using the following equations:

$$ER\% = \frac{n_{sed}}{n_0} = \frac{C_{sed}V_{sed}}{C_0V_{sample}} \times 100 \quad (1)$$

$$EF = \frac{C_{sed}}{C_0} \quad (2)$$

$$ER\% = EF \times \frac{V_{sed}}{V_{sample}} \times 100 \quad (3)$$

where, V_{sed} and V_{sample} are the aqueous phase and the oil sample volumes, respectively. C_{sed} is determined from a calibration curve which was obtained using direct injection of standard solutions.