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Determination of synthetic phenolic antioxidants in edible oils using microvial insert large volume injection gas-chromatography

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ABSTRACT

Three synthetic phenolic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butyl hydroquinone (TBHQ), were determined in different edible vegetable oil samples. The analyses were carried out by gas chromatography–mass spectrometry (GC–MS) using microvial insert large volume injection (LVI). Several parameters affecting this sample introduction step, such as temperatures, times and gas flows, were optimised. Quantification was carried out by the matrix-matched calibration method using carvacrol as internal standard, providing quantification limits between 0.08 and 0.10 ng g^{-1}, depending on the compound. The three phenolic compounds were detected in several of the samples, BHT being the most frequently found. Recovery assays for oil samples spiked at two concentration levels, 2.5 and 10 ng g^{-1}, provided recoveries in the 86–115% range.

1. Introduction

The growth of the modern food industry has been accompanied by the development of new food preservatives that ensure longer product shelf lives. One of the main problems in food conservation is rancidity, which is related to the oxidation of unsaturated fatty acids, a process that takes place through the formation of free radicals by oxygen, leading to chain reactions. While the use of modified atmospheres in the manufacturing process or vacuum packs for storage purposes may limit such degradation phenomena, it is impossible to exclude all traces of oxygen in food (Coma, 2008).

In order to ensure the proper conservation of fat-containing food products, compounds that are able to stop radical chain reactions are needed. It is for this reason that synthetic antioxidants such as butylated hydroxyanisole (BHA, E320), butylated hydroxytoluene (BHT, E321) and *tert*-butyl hydroquinone (TBHQ, E319) are added to food as antioxidant additives. These phenolic compounds are fat-soluble and reactive against radicals and some of their resulting degradation intermediates, thus being able to stop degradation by means of their reaction with the free radicals, limiting the propagation of their chain reaction (Shahidi, 2000). The radicals formed as a result of these reactions are quite stable, due to the presence of their aromatic ring as well as the steric hindrance imposed by the large *tert*-butyl group substituents (Brigati, Lucarini, Mugnaini, & Pedulli, 2002).

Phenolic antioxidants are quite effective in preventing fat degradation, being added to fat-containing foods, like oils, fried potatoes or nuts. In addition, these compounds have an antimicrobial effect against Gram positive bacteria (Gutiérrez-Larraínzar, Rúa, De-Arriaga, del Valle, & García-Armesto, 2013). BHA is especially resistant against pH variations, while TBHQ is mostly used in foods submitted to thermal treatments because of its good thermal stability. When used together, BHA and BHT have a synergic effect, which further increases their protective potential (Madhavi, Deshpande, & Salunkhe, 1996).

However, the safety of these compounds has been of great concern, since they may cause allergic reactions, including asthma and hives, in sensitive subjects (Simon, 2003). Animal laboratory tests have demonstrated their enzymatic induction capability, and there is some evidence that at high diet doses of BHT may act as a carcinogenic agent, leading to the development of lung and liver cancer; indeed, its presence in the body can increase the effect of other carcinogenic compounds (Laningan & Yamarik, 2002). BHA is not a mutagenic agent *in vivo* or *in vitro*, but may affect the metabolism of some mutagenic compounds (Williams, Iatropoulos, & Whysner, 1999). Both BHA and BHT may act as endocrine disruptors (Pop et al., 2013), whose longer-term effects can include infertility and growth problems. The use of synthetic antioxidants and the levels added to food are subject to regulations in the European Union, the

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maximum permitted concentration in edible oils being 200 mg kg^{-1}, for any synthetic phenolic antioxidant or their combinations (Directive, 2006/52/CE).

Vegetable oils are complex chemical mixtures, whose health benefits and composition depend on the vegetable, seed or nut from which it is extracted. Being this food-type the major source of lipids and micronutrients in the human dietary intake, its quality control is of great concern (Gunstone, 2011, Chapter 1). The determination of phenolic antioxidants in edible oils has been accomplished using specific electrochemical probes (Lin, Ni, & Kokot, 2013; Surareungchai & Kasiwat, 2000) and different separation techniques, like micellar electrokinetic capillary chromatography (Delgado-Zamarreño, González-Maza, Sánchez-Pérez, & Carabias Martínez, 2007) and liquid chromatography (LC) coupled with different detection methods, such as fluorescence (Amlashi, Hadjmohammadi, & Nazari, 2014; Oishi, Matsuda, Nojiri, & Saito, 2002), UV–Visible (Chen, Xia, Liu, & Yang, 2011; Chen et al., 2013; Wang et al., 2012) or mass spectrometry (MS) (Tsuji, Nakanoi, Terada, Tamura, & Tonogai, 2005; Xiu-Qin, Chao, Yan-Yan, Min-Li, & Xiao-Gang, 2009). Gas chromatography–mass spectrometry (GC–MS) (Ding & Zou, 2012; Guo, Xie, Yan, Wan, & Wu, 2006; Tsuji et al., 2005; Yang, Lin, & Choong, 2002) has also been used.

Methods for the determination of these antioxidants in edible oils frequently include a clean-up step using liquid–liquid extraction (LLE) (Amlashi et al., 2014; Ding & Zou, 2012; Guo et al., 2006; Xiu-Qin et al., 2009). Novel microextraction procedures, such a cloud-point extraction (CPE) (Chen et al., 2011, 2013), or water-containing surfactant-based vortex assisted microextraction (Amlashi et al., 2014) have also been proposed as greener alternatives to classic LLE. Usually, only a few microliters of the extraction solvent are finally introduced in the instrumental set-up for the determination of the analytes, which, considering the low expected concentration of these compounds in oil samples, results in a great loss of the attainable sensitivity.

Amirav and Dagan (1997) developed direct sample introduction (DSI), a rapid, sensitive, simple and inexpensive procedure in the context of large volume injection (LVI). When this technique is applied to liquid samples it is also known as microvial insert thermal desorption or microvial insert large volume injection (Du & Qian, 2008). The liquid sample, with a volume up to 150 μL, is placed in a glass microvial and introduced into the programmed temperature vaporiser (PTV) or into a thermodesorption unit (TDU) attached to the PTV inlet. Non-volatile interfering matrix components are retained in the vial, which can be removed and discarded after the assay, while volatile compounds are vaporised and transferred to the GC column for separation (Hoh & Mastovska, 2008). DSI has previously been used for the determination of pesticides (Lehotay, Lightfield, Harman-Fetcho, & Donoghue, 2001; Mastovska, Dorweiler, Lehotay, Wegscheid, & Szpylka, 2010) and odour-related compounds (Chen, Wang, & Xu, 2013; Du & Qian, 2008). The use of microvial insert large-volume injection allows the introduction into the chromatographic system of larger volumes of the edible oil extract, and, in addition, avoids contamination problems related to the co-extracted oil matrix components.

In this work, we present the results obtained in the analysis of twelve vegetable edible oil samples, obtained from different plants, for the determination of three synthetic phenolic antioxidants, BHA, BHT and TBHQ, using a microvial insert large-volume injection GC–MS method.

2. Materials and methods

2.1. Chemicals and reagents

Butylated hydroxytoluene (2,6-bis(1,1-dimethylethyl)-4-methylphenol, BHT), butylated hydroxyanisole (3-*tert*-butyl-4-hydroxyanisole, BHA), *tert*-butyl hydroquinone (2-(1,1-dimethylethyl)-1,4-benzenediol, TBHQ) and carvacrol (5-isopropyl-2-methylphenol) were obtained from Sigma (St. Louis, MO). Stock solutions (1000 mg L^{-1}) were prepared by dissolving the commercial products in methanol, and kept at −18 °C in darkness. Acetonitrile (ACN), hexane and methanol were obtained from Sigma.

2.2. Instrumentation

The sample introduction system was composed of a thermal desorption unit (TDU-2) equipped with an autosampler (MPS-2) and programmed temperature vaporisation (PTV) cooled injector system (CIS-4) provided by Gerstel (Mullheim an der Ruhr, Germany). The experimental conditions used for the sample introduction system are summarised in Table 1.

GC analyses were performed on an Agilent 6890N (Agilent, Waldbronn, Germany) gas chromatograph coupled to an Agilent 5973 quadrupole mass spectrometer equipped with an inert ion source. Under the selected conditions (Table 1) the compounds eluted at retention times between 6.2 and 8.5 min, corresponding to carvacrol (IS) and TBHQ, respectively (Table 2). The compounds were quantified in the selected ion monitoring (SIM) mode in order to improve the detection limits using different ions (Table 2). Identification was confirmed by the retention time of the target ion and the qualifier-to-target ion ratios for each compound.

An ultrasonic processor UP 200 H (Dr. Hielscher, Teltow, Germany), with an effective output of 200 W in liquid media equipped with a titanium sonotrode (7 mm i.d.), was used for oil sample extraction. An EBA 20 centrifuge (Hettich, Tuttlingen, Germany) was used to disrupt the sample emulsions formed.

2.3. Samples and analytical procedure

A total of 12 edible oil samples, including corn, olive, sunflower, sesame and peanut, were obtained from local supermarkets. Samples were kept in darkness at 4 °C until analysis.

A 1-mL aliquot of oil sample spiked with the IS, 0.25 mL of hexane and 0.25 mL of ACN were placed in a glass centrifuge tube. Carvacrol was added as internal standard to the oil, at a concentration of 25 ng mL^{-1} after confirming that the samples were free of this compound. The resulting mixture was submitted to ultrasound by means of a probe for 20 cycles of 0.6 s at 60% amplitude. The resulting emulsion was centrifuged for 2 min at 3000 rpm, and

Table 1
Experimental conditions of the TD-GC–MS procedure.

Thermal desorption unit	
Mode	Solvent vent
Venting time	5 min
Venting pressure	2 MPa
Temperature programme	85 °C held 5 min 85–250 °C at 190 °C min^{-1}, held 1 min
Desorption flow	100 mL min^{-1}
Cooled injector system	
Mode	Solvent venting
Liner	Packed silanised glass wool, 2 mm i.d.
Temperature programme	15–275 °C (5 min) at 540 °C min^{-1}
GC–MS	
Capillary column	HP-5MS, 5% diphenyl–95% dimethylpolysiloxane (30 m × 0.25 mm, 0.25 μm)
Carrier gas	Helium (1 mL min^{-1})
Oven programme	80 °C, held 1 min 80–230 °C at 15 °C min^{-1}, held 1 min
Transfer line temperature	300 °C
Quadrupole temperature	150 °C
Ion source temperature	230 °C
Ionisation	Electron-impact mode (70 eV)