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Graphene solid phase extraction (SPE) of synthetic antioxidants in complex food matrices

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ABSTRACT

Synthetic phenolic antioxidants such as propyl gallate (PG) and butylated hydroxyanisole (BHA) are food additives whose levels in foodstuff must be controlled. The purpose of this study is to evaluate, for the first time, the usefulness of graphene as sorbent for the isolation of PG and BHA from complex food sample matrices, by a solid phase extraction (SPE) method. The influence of the eluent type and volume, amount of graphene, sample volume and concentration of antioxidants in the extraction process has been evaluated. Once the process was optimized, extraction yields of 81 and 95% were obtained for PG and BHA respectively. Quantification of the antioxidants was carried out by RP-HPLC with detection at 290 nm, in isocratic mode, using acetonitrile/1% H_3PO_4 (60/40 v/v) as mobile phase. The proposed method was applied to the quantification of both antioxidants in pre-cooked spaghetti and hard bouillon cube samples. In the spaghetti matrix, which contains both antioxidants, PG and BHA levels were found to be below established legal limits. The hard bouillon cubes, free from both antioxidants, were spiked with different amounts of PG and BHA, and recoveries very close to 100% were attained.

1. Introduction

Antioxidants, a subgroup of preservatives, are essential to extend the life of many foods. They form a group of compounds that can inhibit oxygen-dependent lipid oxidation, usually by scavenging and thereby neutralizing free radicals. This chain-breaking process can occur in either the initiation or propagation phase of lipid oxidation. Two or more antioxidants working together can enhance the antioxidant activity of one or both in a phenomenon known as synergism (Pokorny, 2007; Nanditha and Prabhasankar, 2008; André et al., 2010; Carocho et al., 2014). The most commonly used antioxidants with *quantum satis* status are among others, ascorbic acid and derivatives, tocopherols, lecithins, or salts of lactic, maleic and tartaric acid, the so-called natural antioxidants. On the other hand, the most common synthetic antioxidants added to foods are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG), ethoxyquin (EQ), and *tert*-butylhydroquinone (TBHQ).

BHA has been used since the 1970s as an antioxidant in food, but there are numerous studies that describe its toxicity. In 2011, the European Food Safety Authority (EFSA) reviewed the literature and published a revised Acceptable Daily Intake (ADI) that was unlikely to be exceeded by the population (EFSA, 2011). BHT, due to its similarity with BHA, has had a similar path with many studies indicating its carcinogenicity and detrimental effect on health. The EFSA also reviewed the daily intake of BHT, placing it at 0.05 mg/kg bw (EFSA, 2012), which is low compared to BHA (0.5 mg/kg bw). Propyl gallate, ADI = 0.5 mg/kg bw (EFSA, 2014), is used to prevent rancidity in meat products, and may also act in synergism with BHA and BHT, but not with TBHQ. Since its discovery in 1948, it has controversial and antagonistic effects, as reported by many authors. Some claim to have chemotherapeutic and nephroprotective effects among other beneficial activities, while others point to its effect as a xenoestrogen, a precursor of contact dermatitis and mutagenic inducer, and that its antioxidant potential may, under certain conditions, become pro-oxidant (Amadasi et al., 2009; Chen et al., 2011a, 2011b; Tian et al., 2012). Ethoxyquin (EQ) is a quinolone-based antioxidant and its addition to foodstuff is not permitted; it is only used in domestic and farm feed. It has been reported that this compound induces dermatitis in animals and humans, as well as being a promoter of certain types of cancer. Although there is no immediate danger of EQ for humans, there is still a latent risk, arising from the excess present in the ingested animal tissue (EFSA, 2013).

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The European Union (EU), through the European Food Safety Authority, has been very interested in creating a single database that contains all the approved additives, as well as their allowable daily intake (ADI) (Regulation (EC) No 1333/2008, Commission Regulation No 1129/2011).

The determination of synthetic antioxidants in food samples requires a treatment step prior to sample analysis to eliminate possible interferences, isolate analytes in a suitable medium, clean-up of the sample matrix and pre-concentration of the antioxidants. The most common method to extract these compounds is solvent extraction, which can be either Liquid–Liquid Extraction (LLE) or liquid-solid extraction (LSE). In some matrices are also described Solid-Phase Extraction (SPE) and Solid-Phase Micro Extraction (SPME) (Karovičov & and Šimko, 2000; Tombesi and Freije, 2002; André et al., 2010; Serra et al., 2013; Guiberteau et al., 2015; Boyaci et al., 2015). The technique chosen depends in each case on the aggregation state of the sample, the matrix and the analytes.

Solid phase extraction (SPE) is probably the most commonly used extraction technique for sample treatment due to the large number of commercially available sorbents with different characteristics and the low solvent consumption required. In addition, SPE is more rapid and environmentally friendly (due to solvent saving) than LLE. Generally, a high pre-concentration factor is obtained using only a few millilitres of organic solvent. This technique can also be coupled with on- and off-line detection techniques. A variety of sorbents have been developed to facilitate convenient processing of different types of samples. While traditional reverse phase, normal phase, gel filtration and ion exchange sorbents are well established and widely used, multi-functionalized sorbents such as the ion exchange-reverse phase combination, immunosorbent materials by the covalent immobilization of antigens or antibodies, molecularly imprinted polymers (MIPs), metallic nanoparticles, mixed metal-organic structures, carbon nanomaterials, sol-gel process synthesized materials, magnetic particles and ionic liquids, are still being developed (Poole, 2003; Chen et al., 2008; Fontanals et al., 2010; Wen et al., 2014; Fumes et al., 2015; Plotka-Wasylka et al., 2016; Andrade-Eiroa et al., 2016a, 2016b). Regarding synthetic antioxidants, studies on the extraction of foodstuffs by SPE are very scarce (Ziakov & and Brandšteterov & , 2002; Delgado-Zamarreño et al., 2007; Kang et al., 2014.

In recent years, carbon nanomaterials have been shown to be a new class of sorbents due mainly to their large surface area and the ability to modify their surface, both covalently and non-covalently, making them very versatile materials that can interact with a wide variety of compounds. Since its discovery, graphene (G) has attracted much interest because of its two-dimensional structure, and its extraordinary electronic, thermal and mechanical properties (Stoller et al., 2008; Singh et al., 2011). Taking into account the outstanding properties of G, it is reasonable to think that it can become a high-performance sorbent for SPE. Firstly, G has a very large surface area that leads to a high adsorption capacity. In particular, both sides of graphene sheets are available for molecule adsorption, whilst for carbon nanotubes and fullerenes, steric hindrance occurs when molecules access their inner walls. Secondly, G can be readily modified with functional groups, especially through graphene oxide (GO) that has many reactive groups, functionalization that can further enhance the selectivity in SPE. In 2011, G was first used as a sorbent in SPE (Liu et al., 2011); it was packed in an extraction cartridge and a sample comprising eight chlorophenols was passed, which were eluted with alkaline methanol. Recently, many studies on the use of G as sorbent in SPE have been reported (Liu et al., 2012; Sitko et al., 2013; Ibrahima et al., 2016).

Regarding to the detection and quantification of antioxidants, high performance liquid chromatography (HPLC) determination of synthetic antioxidants has become one of the most widely used analytical procedures (Karovičová and Šimko, 2000; Chen et al., 2011a, 2011b; Lin et al., 2011; Biparva et al., 2012; Serra et al., 2013) due to its important advantages such as simple sample treatment, capacity to change the polarity of the mobile phase during analysis to separate antioxidants of different polarity, short analysis time, high reproducibility and adequate detection limits.

The goal of this work is to assess, for the first time, the effectiveness of graphene as sorbent for the extraction of synthetic antioxidants, PG and BHA, from complex food sample matrices.

2. Materials and methods

2.1. Reagents and samples

AvanGRAPHENE, G powder with lamellar structural morphology comprising less than 6 layers with a thickness ≤ 2 nm, was provided by Avanzare Innovación Tecnológica, SL (Logroño, Spain). Acetonitrile (ACN) and methanol (MeOH), HPLC grade, purity ≥ 99.9%, were obtained from Scharlab (Barcelona, Spain). The rest of chemical reagents were of analytical grade and used as received. Butylated hydroxyanisole (BHA), $(CH_3)_3CC_6H_3(OCH_3)OH$, M_w = 180 g/mol, ρ = 1.06 g/cm^3, mp = 48 °C, purity ≥ 98.5%, propyl gallate (PG), 3,4,5-$(HO)_3C_8H_2CO_2CH_2CH_2CH_3$, M_w = 212 g/mol, ρ = 1.21 g/cm^3, mp = 146 °C, purity ≥ 98%, and orthophosphoric acid (H_3PO_4), M_w = 98 g/mol, ρ = 1.68 g/cm^3, purity 85–90%, were obtained from Fluka (Buchs, Switzerland). Trichloroacetic acid (TCA), ρ = 1.63 g/cm^3, purity ≥ 99% was purchased from Panreac (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q system (*Millipore* Co., Milford, MA, USA). A pre-cooked spaghetti carbonara, a very complex sample containing BHA and PG (labelled in the package), milk, flour and other ingredients in powder form, and a food condiment, vegetable bouillon cube free from the indicated antioxidants, were purchased from a local market (Mercadona SA, Alcalá de Henares, Madrid, Spain). Four samples of each type were tested to perform a statistical analysis.

Stock solutions of the antioxidants 400 mg L^{-1} were prepared by weighing the appropriate amount and filling up to a final volume of 25 mL with ACN. From these solutions, concentrations in the range of 0.08–15 mg L^{-1} were used to determine the analytical characteristics by the chromatographic method, whilst 0.8, 1.4 and 2.0 mg L^{-1} were prepared for the solid phase extractions.

2.2. Instrumental techniques

Reverse phase high performance liquid chromatography (RP-HPLC) measurements were carried out on a chromatographic system equipped with an isocratic LC pump 250 (Perkin-Elmer, Madrid, Spain), a manual six-port Rheodyne injection valve with a 20 μL loop, a Jet-Stream Plus column thermostat (Knauer, Berlin, Germany), and a 785A programmable UV/VIS absorbance detector (Applied Biosystems, Madrid, Spain). The acquisition and processing of the chromatographic data was carried out using TotalChrom v6.3.2 software (Perkin-Elmer, Madrid, Spain). The analytical column was Chromaphase RP-18, 150 × 4.6 mm, and particle size of 5 μm, from Scharlab (Barcelona, Spain). The degasification of the mobile phases was carried out in an Ultrasounds-3000683 ultrasonic bath (Selecta SA, Barcelona, Spain).

A vacuum system Viseprep TM (Supelco, Madrid, Spain) was used for the solid phase extraction processes. Four types of 3-mL SPE cartridges were evaluated: graphene and three commercial cartridges: non-polar C_{18} 100 mg, 55 μm (Strata Phenomenex, Torrance, CA, USA), Oasis® HLB (hydrophilic-lipophilic balance) 60 mg, 30 μm (Waters, Milford, MA, USA) and polar silica (Supelco, Madrid, Spain) 500 mg, 50 μm.

Scanning Electron Microscopy (SEM) micrographs were obtained with a Zeiss DSM-950 SEM (Carl Zeiss, Oberkochen, Germany), operating at an acceleration voltage of 15.0 kV, with a magnification of 30,000 x.