Food Chemistry 192 (2016) 825–830

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of tocopherols and sitosterols in seeds and nuts by QuEChERS-liquid chromatography

M. Milagros Delgado-Zamarreño *, Cristina Fernández-Prieto, Myriam Bustamante-Rangel, Lara Pérez-Martín

Departamento de Química Analítica, Nutrición y Bromatología, Universidad de Salamanca, 37008 Salamanca, Spain

ARTICLE INFO

Article history:
Received 4 March 2014
Received in revised form 10 July 2015
Accepted 20 July 2015
Available online 21 July 2015

Chemical Compounds studied in this article:
Alpha-tocopherol (PubChem CID: 2116)
Delta-tocopherol (PubChem CID: 92094)
Gamma-tocopherol (PubChem CID: 92729)
Beta-sitosterol (PubChem CID: 222284)
Brassicasterol (PubChem CID: 5281327)
Campesterol (PubChem CID: 173183)
Stigmasterol (PubChem CID: 5280794)

Keywords:
QuEChERS
LC
Tocopherols
Phytosterols
Nuts

ABSTRACT

In the present work a simple, reliable and affordable sample treatment method for the simultaneous analysis of tocopherols and free phytosterols in nuts was developed. Analyte extraction was carried out using the QuEChERS methodology and analyte separation and detection were accomplished using HPLC–DAD. The use of this methodology for the extraction of natural occurring substances provides advantages such as speed, simplicity and ease of use. The parameters evaluated for the validation of the method developed included the linearity of the calibration plots, the detection and quantification limits, repeatability, reproducibility and recovery. The proposed method was successfully applied to the analysis of tocopherols and free phytosterols in samples of almonds, cashew nuts, hazelnuts, peanuts, tiger nuts, sun flower seeds and pistachios.

1. Introduction

Nuts are a food group that should form part of healthy nutrition, such as in the Mediterranean diet. In general, nuts are energy-dense and provide 23.4 to 26.8 kJ/g of food, with a high fat content (45–75% of weight), although such fat is mostly unsaturated fat (Vadivel, Kunyanga, & Biesalski, 2012). Nuts are also rich sources of a wide range of nutrients and bioactive compounds such as vitamins, phytosterols, and many different flavonoids. Recent studies have demonstrated that the bioactive constituents of whole nuts have cardioprotective (Urpi-Sarda et al., 2012; Casas-Agustench, López-Uriarte, Ros, Bulló, & Salas-Salvadó, 2011), hypocholesterolemic (Mukuddem-Petersen, Oosthuizen, & Jerling, 2005), antiobesity (Martínez-González & Bes-Rastrollo, 2011), anticancer (Yang, Liu, & Halim, 2009), and antioxidant effects (López-Uriarte et al., 2010), all mediated by several different mechanisms.

Vitamin E belongs to a group of structurally similar compounds known as tocopherols and tocotrienols. They are chroman phenolic derivatives, with a long hydrocarbon chain; they are saturated, in the case of tocopherols, or unsaturated, resulting in tocotrienols. Within each of these groups, the vitamers α, β, γ and δ, differ in the number and position of the methyl group on the aromatic ring. All of them show biological activity, α-tocopherol having the greatest potency of all of them. The main function of vitamin E is its natural antioxidant capacity; it is considered to be one of the most efficient biological antioxidants in breaking the chain reaction of free radicals.

Phytosterols are bioactive components of all plant foods, similar in terms of both function and structure to cholesterol. In plants, more than 200 different types of phytosterols have been reported, the most abundant being β-sitosterol, campesterol and stigmasterol (Brufau, Canela, & Rafecas, 2008). Phytosterols decrease the levels of serum low-density lipoprotein (LDL cholesterol), and

* Corresponding author at: Pza. de los Caídos s/n, 37008 Salamanca, Spain.
E-mail address: mdz@usal.es (M.M. Delgado-Zamarreño).

http://dx.doi.org/10.1016/j.foodchem.2015.07.083
0308-8146/

hence protect against cardiovascular diseases. Other beneficial activities related to phytosterols include their anti-cancer properties and their anti-atherosclerotic, anti-inflammatory, and anti-oxidative effects (Lagarda, García-Llatas, & Farré, 2006).

Tocopherols and sterols are usually determined separately in foods, using different techniques (Kalogeropoulos et al., 2010; Chirinos et al., 2013). Liquid chromatography, in the normal (Lee & Lee, 2006; Lee et al., 2012) or reversed phase (Bustamante-Rangel, Delgado-Zamarreño, Sánchez-Pérez, & Carabias-Martínez, 2007; San Andrés, Otero, & Vera, 2011) modes, is the technique most frequently used in tocopherol analysis. For phytosterols, gas chromatography, with flame ionization (Laakso, 2005; Iafelice, Verardo, Marconi, & Caboni, 2009) or mass spectrometry (Santos et al., 2007; Lu, Zhang, Wu, & Shi, 2007) detection of the trimethylsilyl ether derivatives, is the most widely employed technique. Sample preparation for the analysis of both tocopherols and phytosterols generally includes solvent extraction (Wall, 2010; Pinheiro-Sant'Ana et al., 2011), almost always preceded by a saponification step (Lanina, Toledo, Sampels, Kamal-Eldin, & Jastrebova, 2007; Rocco & Fanali, 2009; Da Costa, Ballus, Teixeira-Filho, & Teixeira Godoy, 2010) or Soxhlet extraction (Kornsteiner, Wagner, & Elmadfa, 2006). Recently, other methods such as solid-phase extraction (Grigoriadou, Androulaki, Psomiadou, & Tsimidou, 2007), solid-phase microextraction (Balme & Gülaçar, 2012), pressurized liquid extraction (Delgado-Zamarreño, Bustamante-Rangel, García-Jiménez, Sánchez-Pérez, & Carabias-Martínez, 2006; Jonnala, Dunford, & Dashiell, 2006), supercritical fluid extraction (Vági et al., 2007; Nyam, Tan, Lai, Long, & Che Man, 2010) and microwave extraction (Xiao, Yuan, & Li, 2013) have been used. To our knowledge, there are few references in which simultaneous analysis of tocopherols and phytosterols has been carried out (López Ortíz, Prats Moya, & Berenguer Navarro, 2006; Zarrouk, Carrasco-Pancorbo, Zarrouk, Segura-Carretero, & Fernández-Gutiérrez, 2009; Slavin & Yu, 2012). In these works, sample treatment included saponification and extraction of the analytes, and separation was performed using HPLC.

The duration of these extractions range from 30 min to overnight. Moreover, in some cases a previous saponification step is carried out. The saponification step is always followed by the extraction of tocopherols or phytosterols from the unsaponifiable matter, evaporation of the extraction solvent, and reconstitution in a new solvent compatible with the chromatographic system. In most cases, the solvents used for analyte extraction of were *n*-hexane, diethyl ether or chloroform, which not environmental friendly.

The aim of this work was to develop a simple, reliable and affordable sample treatment method for the simultaneous analysis of tocopherols and phytosterols from nuts, avoiding a saponification step and using an environmental friendly solvent. Analyte extraction was carried out using the QuEChERS methodology, developed by Anastassiades, Lehotay, Stajnbaher, and Schenck (2003), consisting of acetonitrile extraction followed by dispersive solid-phase extraction (d-SPE). Analyte separation and detection were achieved by HPLC–DAD. The use of this methodology for the extraction of naturally occurring substances provides advantages such as: speed, simplicity and ease of use with respect other methods.

2. Experimental

2.1. Chemicals

Tocopherols – δ-tocopherol (δ-TOC, CAS RN 119-13-1), γ-tocopherol (γ-TOC, CAS RN 54-28-4), α-tocopherol (α-TOC, CAS RN 10191-41-0), sterols – brassicasterol (BRA, CAS RN 474-67-9), campesterol (CA, CAS RN 474-62-4), stigmasterol (ST, CAS RN 83-48-7) and β-sitosterol (β-SIT, CAS RN 83-46-5), with 2,6-di-tert-butyl-4-methylphenol (BHT, CAS RN 128-37-0) as an internal standard – were purchased from Sigma–Aldrich (Steinheim, Germany). Stock solutions of each standard were prepared in methanol at concentrations ranging from 500 and 1000 mg L^{-1} and stored at −18 °C. Standard solutions were prepared daily by diluting the stock solutions in methanol.

The organic solvents – acetonitrile (ACN), methanol (MeOH) and ethanol (EtOH) – were of HPLC grade and were supplied by Merck (Darmstadt, Germany). Anhydrous magnesium sulfate was from Sigma–Aldrich. Sodium chloride was from Scharlau (Barcelona, Spain). Primary secondary amine sorbent (PSA) was from Supelco (Bellefonte, PA, USA), sorbent C18 was from Sigma, and silica was from Merck. Ultra-high quality (UHQ) water was obtained with a Wasserlab (Spain) water purification system. All other chemicals used were of analytical reagent grade.

2.2. Samples

The samples analyzed, raw seeds and nuts, were purchased in local markets. The samples were peeled, in case of almonds, hazelnuts, peanuts, sunflower seeds and pistachios, and all samples (approx. 100 g) were ground with a KnifetecTM 1905 from Foss (Barcelona, Spain) just before analysis.

Extraction, using the optimized amount in each case, was carried out on an SBS automatic inversion mixer (ABT-2) (Barcelona, Spain). The extracts were filtered through 0.22 μm PVDF (polyvinylidene fluoride) syringe filters (Scharlau).

2.3. Instrumentation

LC–DAD analyses were performed on a HP 1100 Series chromatograph from Agilent (Waldbronn, Germany) equipped with a binary pump, a membrane degasser, an autosampler, and a six-port valve. Chromatographic separation was achieved under isocratic conditions, using a Zorbax Eclipse XDB-C18 (4.6 × 150 mm, 5 μm) column (Agilent). The mobile phased consisted of a mixture of methanol and UHQ water (98:2, v/v), at a flow rate of 1.3 mL min^{-1}. The analytical column was thermostatted at 25 °C, and the injection volume was 20 μL.

Detection was carried out on a Diode Array Detection (DAD) set at 205 nm. The full spectra were recorded in the 190–400 nm range. The system was controlled by HP ChemStation software, which also performed data collection from the DAD and quantitative measurements.

2.4. Sample preparation (QuEChERS extraction method)

For sample preparation with the QuEChERS extraction method, 1.0 to 3.0 g (depending on the kind of sample) of ground sample was placed in a 50-mL plastic centrifuge tube with a screw cap. Extraction was carried out as follows: first, 10 mL of MeOH was added and the mixture was shaken for 5 min using an automatic inversion mixer at room temperature. Following this, a mixture of 4 g of magnesium sulfate and 1 g of sodium chloride was added. The tube was immediately shaken vigorously for 1 min to prevent the formation of $MgSO_4$ conglomerates, and centrifuged at 3000 rpm for 5 min. Then, 3.0 mL of the methanol fraction was subjected to dispersive SPE using a mixture of 450 mg of $MgSO_4$ and 75 mg of PSA, again shaking vigorously and centrifuging for 5 min at 3000 rpm. Finally, the extract was filtered through a 0.22 μm PVDF syringe filter before injection into the chromatographic system.

3. Results and discussion

3.1. Optimization of LC–DAD

The first study carried out here addressed the optimization of the mobile phase. Different mixtures of MeOH/H_2O and ACN/H_2O were tested as mobile phases, and in nearly all cases mixtures of methanol afforded better resolution than acetonitrile. Methanol percentages of 95–100% were tested to achieve the best separation. The optimum percentage of mobile phase to obtain the best resolution in the least time was MeOH:H_2O (98:2, v/v). It should be noted that, using a C18 silica bonded phase β-tocopherol and γ-tocopherol were not resolved, and nor were stigmasterol and campesterol, as can be seen in Fig. 1.

3.2. Optimization of sample treatment

The implementation of a QuEChERS extraction method for isolating these analytes in food matrices involves the optimization of the different variables that affect the extraction and cleaning processes. It is also important to note that the analytes studied are naturally present in nut samples.

The extraction method used for these experiments was carried out as described in Section 2.4 Sample preparation (QuEChERS extraction method) in the experimental section. The parameters studied to optimize extraction were the type of extraction solvent, the amount of sample, the type of agitation, the extraction time, and the clean-up step.

3.2.1. Optimization of the type of extraction solvent

Considering the low polarity of the analytes, the solvents tested were ACN, MeOH, EtOH and ACN/MeOH (1:2, v/v). As can be seen in Fig. 2, the solvent with which the highest signal was obtained was EtOH. However, the chromatograms obtained did not have adequate resolution. In contrast, injections of the extracts obtained using methanol exhibited much better defined peaks, and hence this was chosen as the extraction solvent.

3.2.2. Optimization of sample amount

QuEChERS methodology proposes sample amounts between 5 and 10 g, to which water is added, depending on the humidity content (Anastassiades et al., 2003). Taking into account that nut samples are fairly dry, the extraction of some water-wetted samples was tested in order to increase the contact between the matrix and the solvent, but the amount of analytes extracted decreased considerably. It is possible that the polarity of methanol in the presence of humidity increased too much and hence the extraction of low-polarity analytes decreased.

Thus, extracts of all the sample types were analyzed in their natural state without the addition of water, weighing amounts ranging between 0.5 and 5 g. Saturation of the extraction solvent was observed in almost all cases. In light of the results, it was determined that the optimum sample amount for sunflower seeds, peanuts, cashews and tiger nuts should be 3 g; for almonds, 2 g, and finally, for pistachios and hazelnuts, 1 g.

3.2.3. Optimization of type of agitation

In order to achieve the best extraction efficiency, different methods were used to place the sample and the extraction solvent in contact. For this, the following agitation systems were tested: an automatic inversion mixer, a thermostatted shaking tray, an ultrasound bath at room temperature and thermostatted, an ultrasound probe, and vortexing. In the systems that allow thermostat control – shaking tray and ultrasound bath – the temperature selected was 50 °C. This temperature was chosen taking into account the thermolability of some analytes. A study of different modes of agitation was performed for all the samples, with varying results, depending on each matrix. However, it was noted that the automatic inversion mixer allowed the extraction of the greatest number of analytes as well as the highest amount of them. As an example Fig. 3 shows the influence of the agitation mode on the signal corresponding to β-sitosterol for tiger nut samples.

3.2.4. Optimization of extraction time and clean-up

The QuEChERS methodology allows analyte extraction in about 1 min. Taking into account that the analytes studied were already

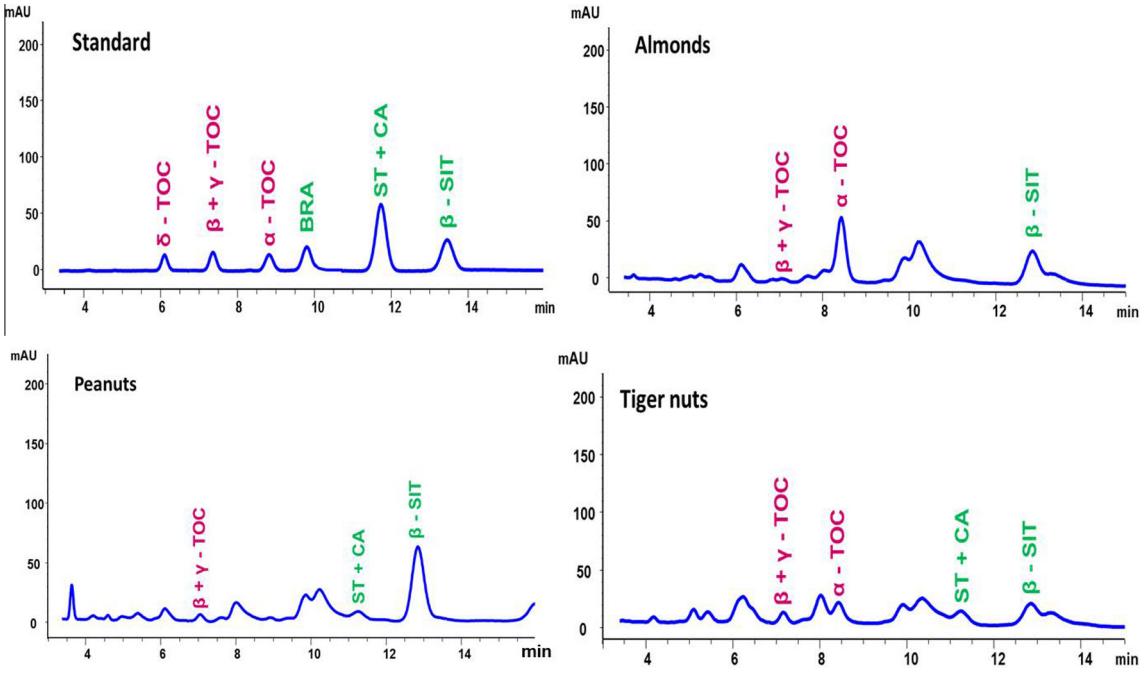

Fig. 1. Chromatogram of a standard solution of tocopherols and sterols. Chromatograms obtained after application of the method to a sample of almonds, peanuts and pistachios.

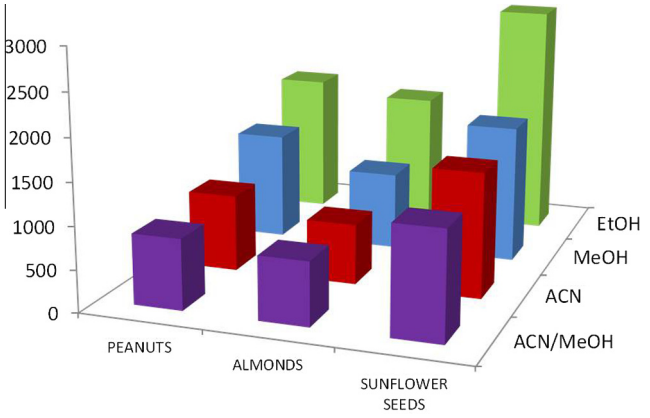

Fig. 2. Influence of solvent extraction on the analytical signal of β-sitosterol.

present naturally in the samples, it was tested whether increases in the extraction time might increase the efficiency of extraction. Thus, extractions were carried out using times ranging from 1 to 10 min. It was observed that the extraction time did not affect the analytical signal very much, except in the case of peanuts, where higher analytical signals were reached in 5 min. Thus, this was chosen as the optimal time for all samples.

Finally, to study the d-SPE step, different solid sorbents, including PSA, C18 and silica sorbents, were evaluated using the procedure described in Section 2.4. The results were very similar in all cases. In light of these, PSA was selected as the clean-up solid sorbent. It should be noted that the time taken for the treatment of all samples was less than 20 min.

3.3. Method validation

In order to apply the method for the determination of tocopherols and phytosterols in real samples of seeds and nuts, a validation procedure was used. The parameters evaluated for the validation of the method were recovery, repeatability and reproducibility. Quantification was performed on the basis of the internal standard method. Several analytes were tested as internal standards: cholesterol, PMC (2,2,5,7,8-pentamethyl-6-chromanol), BHA (Butylated Hydroxyanisole), apigenin and BHT (Butylated Hydroxytoluene). The latter was chosen as the IS because, under the working conditions, it was eluted sufficiently separated from the rest of analytes.

Table 1
Analytical characteristics of the method.

Analyte	Ordinate at the origin	Slope ($L\ mg^{-1}\ 10^{-2}$)	R^2	LOD[a] ($mg\ L^{-1}$)	LOQ[b] ($mg\ L^{-1}$)
δ-TOC	−0.01 ± 0.02	2.62 ± 0.07	0.999	0.21	0.69
γ-TOC	−0.02 ± 0.03	3.6 ± 0.1	0.998	0.04	0.15
α-TOC	0.004 ± 0.016	3.40 ± 0.07	0.999	0.08	0.26
BRA	−0.003 ± 0.004	0.503 ± 0.006	0.999	0.74	2.47
ST + CA	0.02 ± 0.03	0.047 ± 0.05	0.980	0.51	1.70
β-SIT	−0.02 ± 0.02	0.315 ± 0.007	0.999	0.82	2.75

[a] OD: Detection limit for a ratio of S/R = 3.
[b] LOQ: Quantification limit for a ratio of S/R = 10.

3.3.1. Calibration curves and detection limits

Calibration graphs were obtained by triplicate injections of standard mixtures of the target tocopherols and phytosterols containing the IS, at concentration levels ranging from 0.2 to 70 $mg\ L^{-1}$ for tocopherols and from 1 to 100 $mg\ L^{-1}$ for phytosterols except for β-sitosterol, which ranged from 20 to 500 $mg\ L^{-1}$. These ranges were chosen taking into account the expected levels in the samples analyzed. The concentration of the IS was 20 $mg\ L^{-1}$. The results for the intercept, slope and correlation coefficient (R^2) are summarized in Table 1. A good linear correlation coefficient ($R^2 > 0.998$) was observed between the peak area ratio and the standard concentration.

Detection and quantification limits were calculated on the basis of a signal-to-noise ratio (S/N) of 3 and 10 respectively. The detection limits ranged from 0.04 to 0.82 $mg\ L^{-1}$, the quantification limits ranged from 0.15 to 2.75 $mg\ L^{-1}$ for γ-tocopherol and β-sitosterol, respectively.

In the absence of certified or standard materials, the method was validated by measuring the percentage of recovery after the addition of known amounts of standard to the same samples: almonds, peanuts and tiger nuts. Recovery studies performed in triplicate were carried out by spiking samples of nuts, before sample treatment, with the seven analytes studied at concentration levels close to those present in the original samples, and the IS. When tocopherols or phytosterols were not detected in the samples, the fortification level was 20 $mg\ L^{-1}$. In all cases, the recovery values obtained were satisfactory, ranging from 71% to 116% (Table 2).

The repeatability and reproducibility of the method were evaluated. Repeatability was evaluated as the intra-day precision, by analyzing samples of almonds, peanuts and tiger nuts in quadruplicate on the same day, following the optimized procedure. Reproducibility was checked as the precision on different days

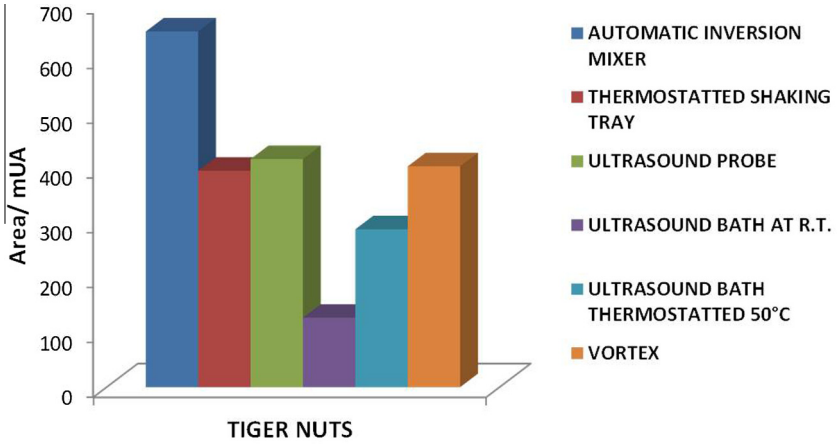

Fig. 3. Influence of agitation mode on the analytical signal corresponding to the β-sitosterol for tiger nut samples.

Table 2
Recovery, repeatability and reproducibility of the method.

Analyte	Almonds			Peanuts			Tiger nuts		
	Intra-day[a]	Inter-day[b]	Recovery (%)	Intra-day[a]	Inter-day[b]	Recovery (%)	Intra-day[a]	Inter-day[b]	Recovery (%)
γ-TOC	5	17	73 ± 4	3	13	71 ± 4	2	8	80 ± 5
α-TOC	3	17	72 ± 3	–	–	71 ± 5	4	8	78 ± 2
ST + CA	–	–	116 ± 9	4	6	116 ± 10	4	5	80 ± 7
β-SIT	2	15	98 ± 7	2	10	116 ± 9	3	4	74 ± 8

[a] Repeatability: *intraday* precision as RSD%.
[b] Reproducibility: *interday* precision as RSD%.

Table 3
Contents of analyte (mg/100 g) of different seed and nut samples.

	mg/100 g				
	β + γ-TOC	α-TOC	BRA	ST + CA	β-SIT
Almonds	0.7 ± 0.7	11.8 ± 0.5	n.d.	n.d.	107 ± 4
Cashew nuts	1.5 ± 0.5	n.d.	n.d.	n.d.	73 ± 3
Hazelnuts	n.d.	6 ± 1	n.d.	n.d.	161 ± 9
Peanuts	1.3 ± 0.5	n.d.	n.d.	11 ± 3	137 ± 3
Tiger nuts	1.3 ± 0.5	2.2 ± 0.3	n.d.	11 ± 3	49 ± 3
Sunflower seeds	n.d.	11.6 ± 0.4	n.d.	25 ± 3	244 ± 6
Pistachios	16 ± 1	n.d.	40 ± 1	n.d.	427 ± 34

(inter-day) in quadruplicate. In all cases the relative standard deviation (RSD) values obtained for almonds, peanuts and tiger nuts were lower than 10%, for the repeatability study, and below 20% for reproducibility. These are very acceptable values for these types of complex sample.

3.4. Application of the method to real samples

To assess the applicability of the proposed method, seven commercial samples of nuts were analyzed in triplicate. Quantification of the tocopherols and phytosterols was performed using the internal standard method. The results obtained from analysis of all the nut samples, in mg/100 g, are shown in Table 3. As may be seen, β-sitosterol was found at higher levels in all samples. Tiger nuts contained a major number of analytes naturally, although the highest contents of tocopherols and phytosterols were found in almonds and sun flower seed and pistachios respectively. δ-Tocopherol was not detected in any of the samples.

4. Conclusions

The proposed method included analyte extraction using the QuEChERS method, followed by LC-DAD. This methodology permits the determination of tocopherols and free phytosterols in their natural form in seeds and nuts. The QuEChERS extraction method was applied for the isolation of analytes naturally present in these food samples. This method includes a step extraction process with a clean-up step using d-SPE extraction with PSA. The extraction method was easy to use, making it highly suitable for complex matrices such as foods. The proposed method is precise, selective and rapid. It is important to note that this sample treatment has never been used in these types of analyte and sample.

Acknowledgments

The authors acknowledge financial support from Programa Propio de Financiación de Grupos de Investigación de la Universidad de Salamanca Ref.: USAL2015-16 and L.P.M. is also grateful to Regional Government of Castilla-León and European Social Fund for a doctoral fellowship.

References

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing cetonitrile extraction/partition and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*, 412–431.

Balme, S., & Gülaçar, F. O. (2012). Rapid screening of phytosterols in orange juice by solid-phase microextraction on polyacrylate fibre derivatisation and gas chromatographic–mass spectrometric. *Food Chemistry, 132*, 613–618.

Brufau, G., Canela, M. A., & Rafecas, M. (2008). Phytosterols: physiologic and metabolic aspects related to cholesterol-lowering properties. *Nutrition Research, 28*, 217–225.

Bustamante-Rangel, M., Delgado-Zamarreño, M. M., Sánchez-Pérez, A., & Carabias-Martínez, R. (2007). Determination of tocopherols and tocotrienols in cereals by pressurized liquid extraction–liquid chromatography–mass spectrometry. *Analytica Chimica Acta, 587*, 216–221.

Casas-Agustench, P., López-Uriarte, P., Ros, E., Bulló, M., & Salas-Salvadó, J. (2011). Nuts, hypertension and endothelial function. *Nutrition Metabolism and Cardiovascular Diseases, 21*, S21–S33.

Chirinos, R., Zuloeta, G., Pedreschi, R., Mignolet, E., Larondelle, Y., & Campos, D. (2013). Sacha inchi (*Plukenetia volubilis*): A seed source of polyunsaturated fatty acids, tocopherols, phytosterols, phenolic compounds and antioxidant capacity. *Food Chemistry, 141*, 1732–1739.

Da Costa, P. A., Ballus, C. A., Teixeira-Filho, J., & Teixeira Godoy, H. (2010). Phytosterols and tocopherols content of pulps and nuts of Brazilian fruits. *Food Research International, 43*, 1603–1606.

Delgado-Zamarreño, M. M., Bustamante-Rangel, M., García-Jiménez, M., Sánchez-Pérez, A., & Carabias-Martínez, R. (2006). Off-line coupling of pressurized liquid extraction and LC/ED for the determination of retinyl acetate and tocopherols in infant formulas. *Talanta, 70*, 1094–1099.

Grigoriadou, D., Androulaki, A., Psomiadou, E., & Tsimidou, M. Z. (2007). Solid phase extraction in the analysis of squalene and tocopherols in olive oil. *Food Chemistry, 105*, 675–680.

Iafelice, G., Verardo, V., Marconi, E., & Caboni, M. F. (2009). Characterization of total, free and esterified phytosterols in tetraploid and hexaploid wheats. *Journal of Agricultural and Food Chemistry, 57*, 2267–2273.

Jonnala, R. S., Dunford, N. T., & Dashiell, K. E. (2006). Tocopherol, phytosterol and phospholipid compositions of new high oleic peanut cultivars. *Journal of Food Composition and Analysis, 19*, 601–605.

Kalogeropoulos, N., Chiou, A., Ioannou, M., Karathanos, V. T., Hassapidou, M., & Andrikopoulos, N. K. (2010). Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. *Food Chemistry, 121*, 682–690.

Kornsteiner, M., Wagner, K. H., & Elmadfa, I. (2006). Tocopherols and total phenolics in 10 different nut types. *Food Chemistry, 98*, 381–387.

Laakso, P. (2005). Analysis of sterols from various food matrices. *European Journal of Lipid Science and Technology, 107*, 402–410.

Lagarda, M. J., García-Llatas, G., & Farré, R. (2006). Analysis of phytosterols in foods. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 1486–1496.

Lanina, S. A., Toledo, P., Sampels, S., Kamal-Eldin, A., & Jastrebova, J. A. (2007). Comparison of reversed-phase liquid chromatography–mass spectrometry with electrospray and atmospheric pressure chemical ionization for analysis of dietary tocopherols. *Journal of Chromatography A, 1157*, 159–170.

Lee, S. M., & Lee, J. (2006). Tocopherol and tocotrienol contents of vegetable oils, margarines, butters, and peanut butters consumed in the Korean diet. *Food Science and Biotechnology, 15*, 183–188.

Lee, Y. Y., Park, H. M., Lee, C. K., Kim, S. L., Hwang, T. Y., Choi, M. S., et al. (2012). Comparing extraction methods for the determination of tocopherols and tocotrienols in seeds and germinating seeds of soybean transformed with OsHGGT. *Journal of Food Composition and Analysis, 27*, 70–80.

López Ortíz, C. M., Prats Moya, M. S., & Berenguer Navarro, V. (2006). A rapid chromatographic method for simultaneous determination of β-sitosterol and tocopherol homologues in vegetable oils. *Journal of Food Composition and Analysis, 19*, 141–149.

López-Uriarte, P., Nogués, R., Saez, G., Bulló, M., Romeu, M., Masana, L., et al. (2010). Effect of nut consumption on oxidative stress and the endothelial function in metabolic syndrome. *Clinical Nutrition, 29*, 373–380.

Lu, B., Zhang, Y., Wu, X., & Shi, J. (2007). Separation and determination of diversiform phytosterols in food materials using supercritical carbon dioxide

extraction and ultraperformance liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry. *Analytica Chimica Acta, 588*, 50–63.
Martínez-González, M. A., & Bes-Rastrollo, M. (2011). Nut consumption, weight gain and obesity: Epidemiological evidence. *Nutrition Metabolism and Cardiovascular Diseases, 21*, S40–S45.
Mukuddem-Petersen, J., Oosthuizen, W., & Jerling, J. C. J. (2005). A systematic review of the effects of nuts on blood lipid profiles in humans. *Journal of Nutrition, 135*, 2082–2089.
Nyam, K. L., Tan, C. P., Lai, O. M., Long, K., & Che Man, Y. B. (2010). Optimization of supercritical fluid extraction of phytosterol from roselle seeds with a central composite design model. *Food and Bioproducts Processing, 88*, 239–246.
Pinheiro-Sant'Ana, H. M., Guinazi, M., da-Silva-Oliveira, D., Della-Lucia, C. M., de Lazzari-Reis, B., & Cardoso Brandão, S. C. (2011). Method for simultaneous analysis of eight vitamin E isomers in various foods by high performance liquid chromatography and fluorescence detection. *Journal of Chromatography A, 1218*, 8496–8502.
Rocco, A., & Fanali, S. (2009). Analysis of phytosterols in extra-virgin olive oil by nano-liquid chromatography. *Journal of Chromatography A, 1216*, 7173–7178.
San Andrés, M. P., Otero, J., & Vera, S. (2011). High performance liquid chromatography method for the simultaneous determination of α-, β- and γ-tocopherol in vegetable oils in presence of hexadecyltrimethylammonium bromide/n-propanol in mobile phase. *Food Chemistry, 126*, 1470–1474.
Santos, R., Limas, E., Sousa, M., Castilho, M. C., Ramos, F., & Noronha da Silveira, M. I. (2007). Optimization of analytical procedures for GC–MS determination of phytosterols and phytostanols in enriched milk and yoghurt. *Food Chemistry, 102*, 113–117.
Slavin, M., & Yu, L. (2012). A single extraction and HPLC procedure for simultaneous analysis of phytosterols, tocopherols and lutein in soybeans. *Food Chemistry, 135*, 2789–2795.
Urpi-Sarda, M., Casas, R., Chiva-Blanch, G., Romero-Mamani, E. S., Valderas-Martínez, P., Arranz, S., et al. (2012). Virgin olive oil and nuts as key foods of the Mediterranean diet effects on inflammatory biomarkers related to atherosclerosis. *Pharmaceutical Research, 65*, 577–583.
Vadivel, V., Kunyanga, C. N., & Biesalski, H. K. (2012). Health benefits of nut consumption with special reference to body weight control. *Nutrition, 28*, 1089–1097.
Vági, E., Simándi, B., Vásárhelyiné, K. P., Daood, H., Kéry, Á., Doleschall, F., et al. (2007). Supercritical carbon dioxide extraction of carotenoids, tocopherols and sitosterols from industrial tomato by-products. *The Journal of Supercritical Fluids, 40*, 218–226.
Wall, M. M. (2010). Functional lipid characteristics, oxidative stability, and antioxidant activity of macadamia nut (*Macadamia integrifolia*) cultivars. *Food Chemistry, 121*, 1103–1108.
Xiao, X. H., Yuan, Z. Q., & Li, G. K. (2013). Preparation of phytosterols and phytol from edible marine algae by microwave-assisted extraction and high-speed counter-current chromatography. *Separation and Purification Technology, 104*, 284–289.
Yang, J., Liu, R. H., & Halim, L. (2009). Antioxidant and antiproliferative activities of common edible nut seeds. *LWT – Food Science and Technology, 42*, 1–8.
Zarrouk, W., Carrasco-Pancorbo, A., Zarrouk, M., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2009). Multi-component analysis (sterols, tocopherols and triterpenic dialcohols) of the unsaponifiable fraction of vegetable oils by liquid chromatography–atmospheric pressure chemical ionization–ion trap mass spectrometry. *Talanta, 80*, 924–934.