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Migration of monomers and plasticizers from packed foods and heated microwave foods using QuEChERS sample preparation and gas chromatography/mass spectrometry

Evelina Fasano [a, *], Teresa Cirillo [a], Francesco Esposito [a], Silvia Lacorte [b]

[a] Department of Agricultural Sciences, University of Naples "Federico II", via Università 100, 80055 Portici, Naples, Italy
[b] Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Catalonia, Spain

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ABSTRACT

The objective of this study was to evaluate the migration of monomers and plastic additives in microwave heated homemade courses and in packed liquid food. Compounds studied were 3 phthalates, 4-tert-octylphenol (OP), 4-nonylphenol (NP), bisphenol A (BPA) and di(2-ethylhexyl)adipate (DEHA). A QuEChERS based method was optimized for the analysis of these compounds in solid or liquid packed and retailed food. Using gas chromatography coupled to mass spectrometry, recoveries ranged from 49 ± 16% (OP) to 130 ± 16% (BPA) and from 63 ± 22% (OP) to 127 ± 29% (NP) in solid and liquid foods, with limits of detection below 14.37 ng g^{-1} and 2.25 ng mL^{-1}, respectively. NP showed the highest mean level in homemade foods (1064 ± 363 ng g^{-1} wet weight) and reheating did not produce an increase in the levels detected. Phthalates and DEHA were detected at low concentrations. Among liquid foods, meat broth mean concentrations of NP were of 9.61 ± 1.93 ng mL^{-1} and of 9.68 ± 1.75 ng mL^{-1} for butylbenzylphthalate in fish broth, while in wine, the most abundant compound was di-n-butylphthalate. Overall, the developed QuEChERS method permitted to determine the presence of investigated chemicals in packed food allowing the evaluation of compounds that can affect food quality.

1. Introduction

The migration of chemical compounds such as monomers or additives from plastic food packaging containers to food can have severe health implications. Different polymers are employed during plastic production depending on the specific characteristics of food products, storage conditions or shelf life. Retail or cooked packed food and beverages are the main source of plasticizers and additives for humans (Fromme et al., 2007; Goulas, Zygoura, Karatapanis, Georgantelis, & Kontominas, 2007; Loyo-Rosales, Rosales-Rivera, Lynch, Rice, & Torrents, 2004; Vandenberg, Hauser, Marcus, Olea, & Welshons, 2007) and can contribute to the total daily intake of xenobiotics. The major parameters that can influence the migration of plasticizers or additives to food are the chemical properties of the compound, the contact surface, the plastic material, the type of food, the packaging temperatures, heat or sterilization treatment, and storage time of the product (Arvanitoyannis & Bosnea, 2004). Contact time and temperature are most relevant, and the Commission Regulation 10/2011/EU on plastic materials and articles intended to come into contact with food consider tests at specific time/temperature conditions. In this Regulation, Specific Migration Limits (SMLs) for single contaminants or group of contaminants and Overall Migration Limit (OML) of 10 mg of all compounds in one dm^2 of contact surface between food and packaging are established. Among others, phthalates (PAEs), alkylphenols (APs), 2,2-bis(4-hydroxiphenyl)propane or bisphenol A (BPA) and di(2-ethylhexyl) adipate (DEHA) have received special attention since they are considered as "Endocrine Disruptors" (ED) or suspected to be carcinogenic (Ferrara, Fabietti, Delisea, & Funari, 2005; Fromme et al., 2007; Ghisari & Bonefeld-Jorgensen, 2009; Vom Saal & Hughes, 2005) and therefore studies on contamination sources and ingestion intake need attention.

One of the most common way to cook or reheat food is based on the use of microwave oven, a very widespread practice because of the short time and ease of use, provided specific containers which can withstand heating cycles are used. Most commonly these containers are made out of glass, ceramic or plastic (polypropylene

* Corresponding author.
E-mail address: evelina.fasano@unina.it (E. Fasano).

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or polycarbonate). When using plastic containers, PAEs, APs, BPA and DEHA can be transferred to the food fostered by the high temperatures of the microwave radiations. However, little is known on the migration of these compounds during the so common microwave heating of home prepared foods.

The analysis of plasticizers and additives intended to migrate from food containers is very difficult due to the complexity of the food matrix and the low concentration levels expected. Extraction methods are based on liquid–liquid (Catalina, Dalluge, Vreuls, & Brinkman, 2000; Koesukwiwat, Lehotay, Miao, & Leepipatpiboon, 2010) or liquid–solid extraction (Casajuana & Lacorte, 2004; Tsumura, Ishimitsu, Saito, Kobayashi, & Tonogai, 2001) followed by a clean-up step using Florisil (Cirillo, Fasano, Castaldi, Montuori, & Amodio Cocchieri, 2011). However, poor recoveries or matrix interferences may intricate the proper quantification of target compounds. Taking into account these problems, the QuEChERS method (acronym of quick, easy, cheap, effective, rugged and safe) (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003) has been applied to a large variety of contaminants and foods. It usually consists in acetonitrile extraction and then liquid–liquid partitioning by anhydrous magnesium sulphate. Then, an aliquot of organic layer is cleaned up by solid phase extraction (SPE) with primary secondary amine (PSA) to remove organic acids and anhydrous magnesium sulphate to remove water from the extract. Other sorbents, such as graphitized carbon black (GCB) can be used to remove pigments as chlorophyll, carotenoids and sterol or C18 to eliminate lipids (Cunha et al., 2007; Mastovska, Dorweiler, Lehotay, Wegscheid, Szpylka, 2010). In this way, it is possible to eliminate chromatographic interferences and to increase the sensitivity of the detection by gas and liquid chromatography coupled to mass spectrometry (Lehotay et al., 2010). QuEChERS method is highly efficient for the detection of target compounds, requires short time of application, permits high sample throughput and uses low quantities of organic solvent. Usually the QuEChERS concept has been used for the simultaneous extraction of polar or non polar pesticides from fruits and vegetables (Koesukwiwat et al., 2010; Padilla-Sánchez et al., 2010) or for acrylamide, clinical, veterinary drug residues, perfluorinated compounds, polycyclic aromatic hydrocarbons, alkaloids and mycotoxin detection (Lehotay et al., 2010) or neonicotinoid insecticide residues in soils (Dankyi, Gordon, Carboo, Fomsgaard, 2014). This method has also been applied for the extraction of PAEs in fruit jellies (Yuwei, Yuki, Feng, & Jin-Ming, 2010) and BPA and bisphenol B in canned seafood (Cunha, Cunha, Ferreira, & Fernandes, 2012).

Considering the difficulty of evaluation of PAEs, alkylphenols, bisphenol A and di(2-ethylhexyl) adipate in complex food matrix and especially applying long methods that require many steps, the principal aim of this study was to determine the levels of plasticizers and additives in retailed foods (packed grated cheese, broths and wine packed in a tetrapack) and to study their migration in homemade cooked foodstuff placed in plastic containers before and after microwave heating. To do so, we optimized a QuEChERS method for the detection of di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl)phthalate (DEHP); 4-tert-octylphenol (OP) and technical nonylphenol (NP); BPA and DEHA in both solid and liquid foods.

2. Materials and methods

2.1. Sampling

Samples analyzed according to typology were packed foods used in the preparation of homemade courses: (i) polyethylene packed grated cheese; (ii) meat ($n = 2$), fish ($n = 2$) and vegetables ($n = 2$) broths and, white ($n = 2$) and red ($n = 2$) wine, all packed in aseptic plastic laminate paperboard carton (tetrapack). This packed food was purchased in a supermarket of Barcelona (Spain). In a second step, 11 homemade courses, made of pasta with tomato sauce and cheese (pasta, $n = 4$), rice with seafood and vegetables (rice, $n = 4$) and chicken and vegetables ($n = 3$) were prepared. Foods were cooked, cooled and placed in container suitable for microwave made of polypropylene and polycarbonate plastic. Before microwave heating, a portion of each dish was sampled and placed in aluminium foil. Then, the food was heated in the conventional way by inserting the plastic container in the microwave for an average time of 3 min at 800 W. After heating, another portion was collected, and wrapped in aluminium foil. Samples were weighed, homogenized, codified, then lyophilized and aliquoted (1 g).

2.2. Chemicals and reagents

Chromatography grade acetonitrile, ethyl acetate and HPLC water were from Merck (Darmstadt, Germany). Deuterated surrogate standards were NP D8 (100 ng μL^{-1}), DPP D4 and BPA D16, purchased as solids from Dr. Ehrenstorfer (Augsburg, Germany) and anthracene D10, used as internal standard, was purchased at a concentration of 10 ng μL^{-1} (Supelco Bellefonte, PA, USA). Phthalate Esters Mix was purchased at a concentration of 500 ng μL^{-1} in methanol (Supelco, Bellefonte, PA, USA) and contained DBP, BBP and DEHP, DEHA; NP was purchased as a solid technical mixture of isomers (Riedel-de Haën, Seelze, Germany); OP and BPA were purchased as solids from Supelco (Bellefonte, PA, USA) and Dr. Ehrenstorfer (Augsburg, Germany), respectively. A working solution containing all chemicals was prepared at a concentration of 10 ng μL^{-1}.

For the QuEChERS extraction and purification, two reactive products were used, labeled as Reactive 1 and Reactive 2 (Agilent, Santa Clara, California). In particular, Reactive 1, was constituted by a solid multilayer pouches containing sodium citrate, sodium hydrogen citrate, magnesium sulfate and sodium chloride; Reactive 2 consisted in 15 mL of primary secondary amine (PSA), C18 EC and magnesium sulfate for the analysis of fatty samples.

2.3. Optimization of the method

Method optimization was performed using packed grated cheese to simulate a fatty food, HPLC water to simulate liquid foods and a course (rice and eggs), to simulate homemade foods. These 3 types of foods were tested to determine the effectiveness of QuEChERS method. One g of solid samples was hydrated with 9 mL of HPLC water and stabilized for 2 h. Then, the sample was spiked with 100 μL of standard solution at a concentration of 6 ng μL^{-1} (600 ng) and 500 ng of surrogate (100 μL of a solution of 5 ng μL^{-1}). 10 mL of HPLC water was directly spiked with no hydratation step. 10 mL of acetonitrile were added in each sample, then the sample was shaken with a vortex for 1 min and Reactive 1 was added and the solution was shaken and centrifuged for 5 min at 4000 rpm. The acetonitrile layer was dosed in a centrifuge tube and Reactive 2 was added, shaken with the vortex for 1 min and the sample was centrifuged again for 5 min at 4000 rpm. The extracts were dried in a TurboVap under a current of nitrogen and reconstituted with 480 μL of ethyl acetate and 20 μL of anthracene D10 (10 ng μL^{-1}), as internal standard (Fig. 1).

All analysis were performed in triplicate. Besides during the study, blanks constituted by 10 mL of HPLC water, spiked with surrogate standards were processed in the same way of samples in order to investigate the background contamination. Besides blanks were used to calculate the Detection Limit (LOD), as mean blank

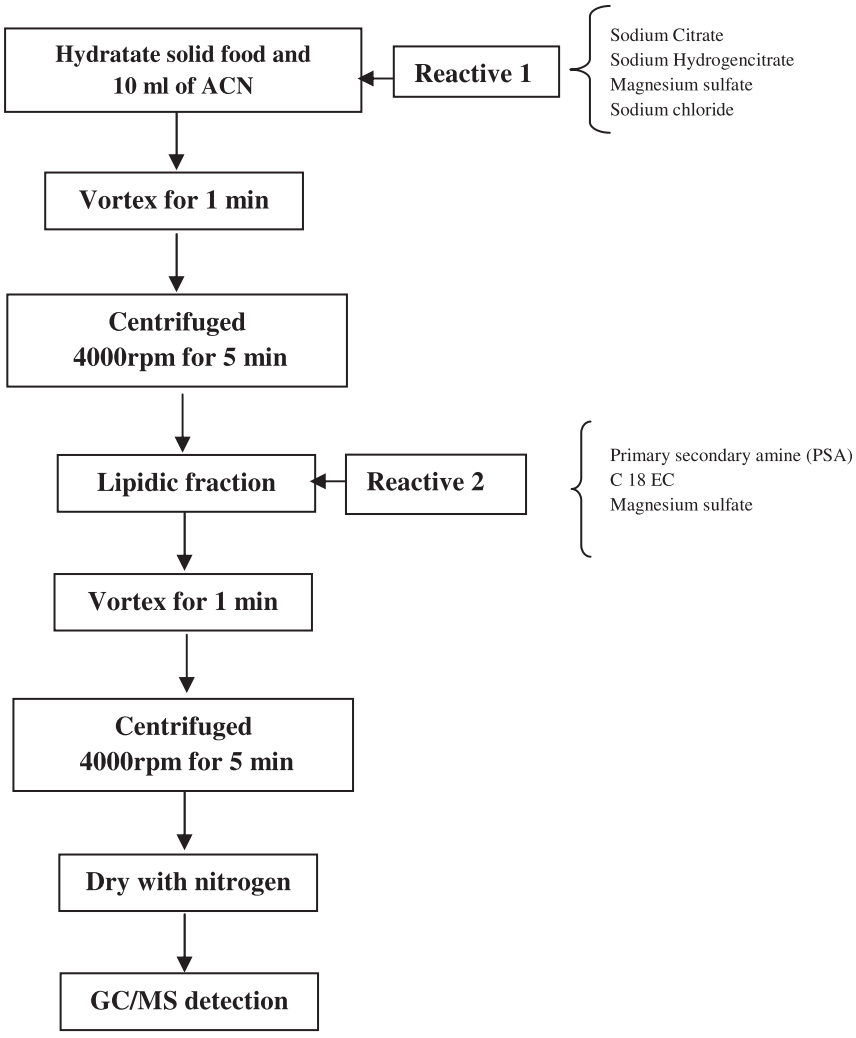

Fig. 1. Scheme of application of QuEChERS method.

value plus 3 times the standard deviation of the blank contribution of each compound.

2.4. Instrumental analysis

Analysis was carried out by gas chromatography coupled to a quadrupole mass spectrometer (Agilent 6890 series GC System connected with a HP 5973 MS). The separation was achieved with a 30 m × 0.25 mm I.D. DB-5MS column (J&W Scientific, Folsom, CA, USA) coated with 5% phenyl-95% dimethylpolysiloxane (film thickness 0.25 μm). The oven temperature was programmed from 65°C (holding time 2 min) to 160°C at 15°C min^{-1}, to 170°C at 3°C min^{-1} and finally to 310°C at 10°C min^{-1}, keeping this condition for 10 min. Two μL were injected in the splitless mode, keeping the split valve closed for 1 min. The carrier gas used was Helium at a flow rate of 1.2 mL min^{-1} and the inlet temperature was 290°C. The calibration curves were made in a range of 0.01–1 ng μL^{-1} (0.01, 0.05, 0.1, 0.25, 0.5, 0.75 and 1 ng μL^{-1}) with coefficient of regression (R^2) > 0.99. Peak detection and integration were carried out using MSD ChemStation software. Acquisition was performed in Selected Ion Monitoring (SIM) using 3 ions per compound (Fasano, Bono-Blay, Cirillo, Montuori, & Lacorte, 2012) (Table 1). External standard quantification was used and surrogate standards were used to determine the extraction efficiency of each sample.

Table 1
Molecular weight (g/mol), retention time (min) and ions (*m*/*z*) of di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl)phthalate (DEHP), 4-tert-octylphenol (OP), nonylphenol (NP), 2,2-bis(4-hydroxiphenyl)propane or bisphenol A (BPA) and di(2-ethylhexyl) adipate (DEHA).

Compound	Molecular weight (g/mol)	Retention time (min)	Ions (*m*/*z*)
DBP	278.3	16.29	149
			150
			223
			149
BBP	312.4	20.13	91
			206
DEHP	390.6	21.63	149
			167
			279
			135
OP	206.3	11.83	107
NP	220.4	13.89–14.54	135
			149
			107
			213
BPA	228.3	18.92	228
			119
DEHA	370.6	20.44	129
			112
			147

2.5. Statistical analysis

To evaluate if the heating process of courses, through microwaves oven, can influence the migration of investigated chemicals, statistical analysis by SPSS 19.0 was carried out between the same food category before and after microwave reheating. When contaminants were not detected, the LOD calculated from the blanks analyses were considered. Analysis of variance was carried out by ANOVA. The level of significance was set at $p < 0.05$.

3. Results and discussion

3.1. Method performance

The recoveries of target compounds in spiked cheese, cooked food and liquid food (HPLC water), using QuEChERS extraction, are indicated in Table 2. For spiked grated cheese at 600 ng g^{-1}, the recoveries ranged from 36 ± 3% to 106 ± 12%, with lower extraction efficiency for OP (36 ± 3%) and for DEHA (41 ± 5%). Similar trend was observed for spiked rice and eggs course, except for DEHP which was not quantified due to a peak interference. Therefore, DEHP detection was not considered for the analysis of courses. Finally for liquid foods, good extraction efficiency was observed. From the analysis of blanks (n = 6), LODs for solid foods ranged from 0.20 (DEHA) to 14.37 ng g^{-1} wet weight (NP). As for the liquid foods, the LODs ranged from 0.02 (DEHA) to 2.25 ng mL^{-1} (DEHP) (Table 2). An example of standard and sample injections were reported in Fig. 2.

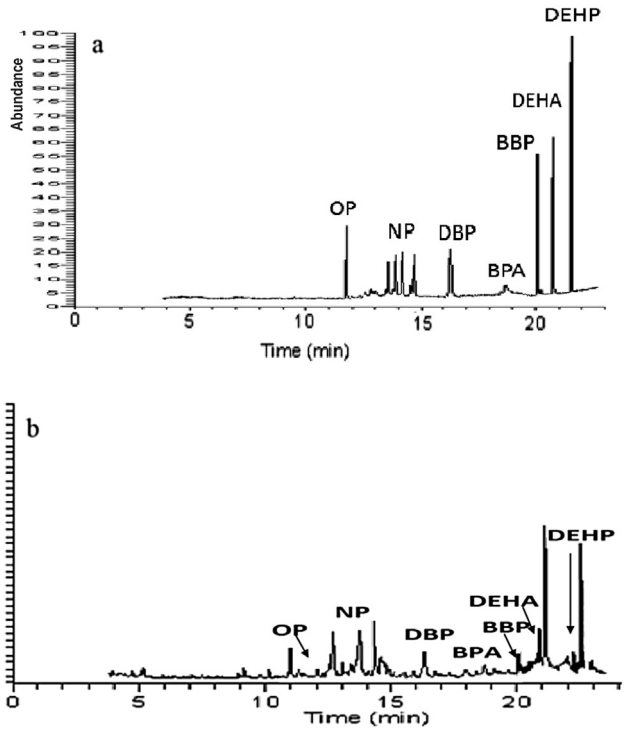

Fig. 2. Example of chromatograms of di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl)phthalate (DEHP), 4-tert-octylphenol (OP), technical nonylphenol (NP), 2,2-bis(4-hydroxiphenyl)propane or bisphenol A (BPA) and di(2-ethylhexyl) adipate (DEHA) in standard mix (a) and sample (b) injection.

3.2. Plasticizer and additives in packed liquid food

Table 3 indicates the levels of monomers and additives in meat broth, fish and vegetable broths and wines. Levels detected varied according to each category of food depending on the specific composition of each matrix, such as lipidic content (Tsumura et al., 2003), which was higher for meat > fish > vegetable broths, the alcohol content in red and white wines or to manipulation during food processing. In fact, meat, fish and vegetable broths contained all investigated compounds, whereas in white and red wine categories NP and DEHP levels were below the LOD and BPA only detected in white wine. The values found in white wine were always higher than in red wine, except for DBP. The highest mean value was of 9.68 ± 1.75 ng mL^{-1} for BBP in fish broth, followed by NP (mean of 9.61 ± 1.93 ng mL^{-1}) and DEHA (7.30 ± 0.95 ng mL^{-1}) in meat broth. BPA and OP were the compounds with the lowest mean values in both broth and wine categories probably for the composition of the packaging.

Regarding the meat broth, the trend was NP > BBP > DEHP > DEHA > DBP > BPA > OP with a mean range of 0.37 ± 0.03 ng mL^{-1} to 9.61 ± 1.93 ng mL^{-1}. Instead, in fish broth it was BBP > DEHA > NP > DEHP > DBP > BPA > OP with a mean concentrations that ranged from 0.40 ± 0.04 ng mL^{-1} to 9.68 ± 1.75 ng mL^{-1} and in vegetable it was DEHA > NP > BBP > DBP > BPA > OP > DEHP and the mean concentrations varied from <LOD to 7.30 ± 0.95 ng mL^{-1}.

Wormuth, Scheringer, Vollenweider, and Hungerbuhler (2006) that studied PAE contamination in different edibles (i.e. pasta, rice, vegetables, meat etc) and drinks, found BBP levels (2.0 ng mL^{-1}) similar to our results and DBP mean was higher (148 ng mL^{-1}) than values obtained in the present study; whereas DEHP showed a mean level of 9.0 ng mL^{-1} always higher than our results. Carrillo, Martınez, Tena, (2008) optimized a method to detect PAE levels in different tetrapack wines, it was found that BBP was always not detected, while DBP levels were lower than our results for white and red wines; however, DEHP, not detected in our study, was identified in Carillo's study. As reported in Table 3 our results for white and red wines showed DBP highest mean levels, followed by BBP and DEHA. This trend was confirmed in Russo, Notardonato, Cinelli, and Avino (2012) research for DBP and BBP (DBP > BBP); while for DEHP was observed a different result in fact in our study it was always not detected.

Table 2
Recoveries (%) of di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl)phthalate (DEHP), 4-tert-octylphenol (OP), nonylphenol (NP), 2,2-bis(4-hydroxiphenyl)propane or bisphenol A (BPA) and di(2-ethylhexyl) adipate (DEHA) in spiked foods and LODs for cheese, solid food and liquid ones.

Compound	Recovery (%) mean ± sd			LOD	
	Cheese (n = 3)	Solid food (n = 3)	Liquid food (n = 2)	Cheese/Solid food (ng g^{-1} wet weight)	Liquid food (ng mL^{-1})
DBP	77 ± 33	77 ± 33	123 ± 1	2.75	2.08
BBP	60 ± 4	67 ± 10	112 ± 20	0.75	0.08
DEHP	94 ± 8	Not recovered	104 ± 9	–	2.25
OP	36 ± 3	49 ± 16	63 ± 22	3.50	0.35
NP	84 ± 29	109 ± 32	127 ± 29	14.37	1.44
BPA	106 ± 12	130 ± 16	107 ± 2	7.10	0.71
DEHA	41 ± 5	68 ± 19	106 ± 17	0.20	0.02

Table 3
Di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl)phthalate (DEHP), 4-tert-octylphenol (OP), nonylphenol (NP), 2,2-bis(4-hydroxiphenyl)propane or bisphenol A (BPA) and di(2-ethylhexyl) adipate (DEHA) levels on ng ml^{-1} (mean ± sd and range) in broth and wine samples analyzed in this study.

Compound	Broth						Wines			
	Meat (*n=2*)		Fish (*n=2*)		Vegetable (*n=2*)		White (*n=2*)		Red (*n=2*)	
	mean ± sd	Min–max	mean ± sd	Min–max	mean ± sd	Min–max	mean ± sd	Min–max	mean ± sd	Min–max
DBP	1.59 ± 0.16	1.48–1.70	1.35 ± 0.30	1.15–1.54	1.12 ± 0.31	0.90–1.34	6.67 ± 1.16	5.86–7.49	8.72 ± 1.41	7.73–9.72
BBP	5.76 ± 0.59	5.35–6.18	9.68 ± 1.75	5.35–6.18	5.05 ± 0.49	4.70–5.40	3.08 ± 0.89	2.45–3.70	1.69 ± 0.01	1.69–1.70
DEHP	3.76 ± 0.05	3.72–3.80	3.55 ± 1.63	2.73–4.37	<LOD		<LOD		<LOD	
OP	0.37 ± 0.03	0.35–0.39	0.40 ± 0.04	0.37–0.43	0.48 ± 0.02	0.46–0.49	0.83 ± 0.42	0.54–1.14	0.62 ± 0.32	0.42–0.88
NP	9.61 ± 1.93	8.25–10.98	4.87 ± 3.96	2.07–7.68	6.14 ± 2.81	4.16–8.13	<LOD		<LOD	
BPA	0.87 ± 0.15	0.76–0.97	1.10 ± 0.32	0.87–1.32	0.77 ± 0.07	0.72–0.82	0.89 ± 0.17	0.77–1.02	<LOD	
DEHA	2.97 ± 0.09	2.91–303	5.67 ± 1.07	4.92–6.43	7.30 ± 0.95	6.63–7.97	2.39 ± 0.42	2.09–2.68	1.31 ± 0.04	1.28–1.34

However Russo et al. analyzed different packed, commercial and home wine and, DBP and DEHP were detected in tetrapack white wine at 10.0 and 16.0 ng mL^{-1}, respectively, higher than the ones detected in the present study (6.67 ± 1.16 ng mL^{-1} and not detected, respectively for DBP and DEHP); while BBP levels were lower (1.0 ng mL^{-1}) than in our study (3.08 ± 0.89 ng mL^{-1}).

3.3. Migration of plasticizers and additives in microwave heated courses

Homemade courses were analyzed before and after microwave heating to determine the migration of plasticizers and additives from microwave plastic containers to food. In Table 4 the concentrations of target compounds in the three course categories ("Pasta", "Rice" and "Chicken and vegetables") are reported.

The courses showed a diffuse contamination of the chemicals as suggested in literature data that regarded the evaluation of these compounds in different food matrices (Fromme et al., 2007; Goulas et al. 2007; Guenther et al., 2002; Loyo-Rosales et al., 2004; Tsumura et al. 2001a, b; Vandenberg et al., 2007; Wormuth et al., 2006). In fact, OP, NP, BPA and DEHA were detected in all samples both before and after microwave heating, followed by DBP (91%) and BBP (82%). NP was characterized by the highest mean values.

Among courses collected before microwave heating, the "Pasta" category contained the highest NP mean values (mean 946 ± 390 ng g^{-1} wet weight (ww) followed by OP (mean values of 217 ± 92 ng g^{-1} ww) and BPA (mean values of 133 ± 56 ng g^{-1} ww). DEHA and DBP showed similar mean values (44 ± 15 and 32 ± 12 ng g^{-1} ww, respectively) while the lowest levels were found for BBP (3.2 ± 0.9 ng g^{-1} ww). After microwave heating of this "Pasta" course, the NP levels were quite similar to those obtained before reheating with means of 1064 ± 363 ng g^{-1} ww, followed by OP (mean 155 ± 36 ng g^{-1} ww) and BPA (mean 132 ± 53 ng g^{-1} ww). Similar to samples collected before heating, BBP presented the lowest levels (7.0 ± 3.1 ng g^{-1} ww).

In "Rice", NP was detected at a mean level of 442 ± 198 ng g^{-1} ww, followed by OP with a mean of 140 ± 26 ng g^{-1} ww; DBP and BBP showed similar mean values. After microwave reheating, NP mean concentration was 408 ± 104 ng g^{-1} ww and for OP was 155 ± 26 ng g^{-1} ww so similar to those found before heating; BBP was characterized by the lowest mean value of this category (28 ± 10 ng g^{-1} ww). Other compound showed similar mean values.

"Chicken and vegetables" was characterized by the same trend of "Pasta" with a mean of 503 ± 52 ng g^{-1} ww for NP, of 104 ± 30 ng g^{-1} ww for OP and of 1.7 ± 0.5 ng g^{-1} ww for BBP, the least detected compound. After, reheating, NP increased to 623 ± 244 ng g^{-1} ww whereas OP remained at similar concentration (194 ± 60 ng g^{-1} ww). DEHA and DBP showed similar mean levels (47 ± 19 and 44 ± 9 ng g^{-1} ww, respectively) after reheating.

No statistical differences were found comparing samples collected before and after heating, except for DEHA in "Rice" category ($p = 0.039$) and BBP in "Chicken and vegetables" ($p = 0.01$). In general, the mean values and minimum and maximum concentrations before heating and after heating were quite similar for most compounds in all courses, and this indicates that the microwave heating of the food, independent of the type of container, has little effect in the migration of plasticizers and additives in food, and rather the cooking process or the initial contact of food in the plastic container may be the responsible of the levels detected. There is little information on the presence of PAE in cooked courses, and in particular in microwave food heating (Cirillo et al., 2011; Fasano et al., 2012; Tsumura et al., 2001) and therefore it is difficult to compare the levels obtained and define the potential risk this may pose.

3.4. Tolerable daily intake

The Tolerable Daily Intake TDI is an estimate of the amount of a substance in food that can be taken in daily over a lifetime without appreciable health risk. TDIs are calculated on the basis of

Table 4
Di-n-butylphthalate (DBP), butylbenzylphthalate (BBP), di(2-ethylhexyl)phthalate (DEHP), 4-tert-octylphenol (OP), nonylphenol (NP), 2,2-bis(4-hydroxiphenyl)propane or bisphenol A (BPA) and di(2-ethylhexyl) adipate (DEHA) concentrations on ng g^{-1} wet weight (mean ± sd and range) in "Pasta", "Rice" and "Chicken and vegetables" samples analyzed in the study before and after microwave heating.

Compound	"Pasta" (*n=4*)				"Rice" (*n=4*)				"Chicken and vegetables" (*n=3*)			
	Before heating		After heating		Before heating		After heating		Before heating		After heating	
	mean ± sd	Min–max	mean ± sd	Min–max	mean ± sd	Min–max	mean ± sd	Min–max	mean ± sd	Min–max	mean ± sd	Min–max
DBP	32 ± 12	19–41	52 ± 30	25.1–83.6	25 ± 10	14–33	43 ± 19	29–65	27 ± 9.1	21–37	44 ± 9	36–53
BBP	3.2 ± 0.9	2.2–4.0	7.0 ± 3.1	4–10	24 ± 7.0	18–32	28 ± 10	21–39	1.7 ± 0.5[a]	1.1–2.1	13±6[a]	12–24
OP	217 ± 92	136–298	155 ± 36	105–199	140 ± 26	115–167	155 ± 26	128–180	104 ± 30	76–135	194 ± 60	134–255
NP	946 ± 390	421–1360	1064 ± 363	435–1908	442 ± 198	210–667	408 ± 104	265–493	503 ± 52	452–555	623 ± 244	414–891
BPA	133 ± 56	65–185	132 ± 53	68–197	47 ± 29	20–77	47 ± 18	26–57	82 ± 18	61–94	116 ± 89	57–218
DEHA	44 ± 15	15–61	42 ± 12	26–55	11 ± 5.1[a]	7.0–17	48 ± 31[a]	13–79	47 ± 29	24–80	47 ± 19	34–98

[a] Statistical differences was found.

Table 5
Mean Meal intake on mg kg^{-1} bw day^{-1} for "Pasta", "Rice" and "Chicken and vegetables" categories and white and red wine and comparison with EFSA TDI and NOEL usually adopted.

Compound[a]	Mean meal intake (mg kg^{-1} bw day^{-1})					EFSA TDI (mg kg^{-1} bw day^{-1})	NOEL (mg kg^{-1} bw day^{-1})
	Pasta	Rice	Chicken/vegetables	White wine	Red wine		
DBP	1.85×10^{-4}	0.15×10^{-4}	0.16×10^{-4}	1.91×10^{-6}	0.25×10^{-6}	0.01	50 (male reproductive system)
BBP	0.25×10^{-4}	0.10×10^{-4}	0.04×10^{-4}	0.88×10^{-6}	0.05×10^{-6}	0.05	20 (male reproductive system)
DEHP	–	–	–	0.07×10^{-6}	–	0.05	4.8 (reproductive/developmental effects)
OP	5.52×10^{-4}	0.55×10^{-4}	0.45×10^{-4}	0.24×10^{-6}	0.02×10^{-6}	–	10 (reproductive effects) (Tyl et al., 1999)
NP	38×10^{-4}	2.23×10^{-4}	1.48×10^{-4}	–	–	0.005	15 (reproductive effects)
BPA	4.72×10^{-4}	0.17×10^{-4}	0.26×10^{-4}	0.25×10^{-6}	0.02×10^{-6}	0.005	5 (reduction in body weight)
DEHA	1.50×10^{-4}	0.17×10^{-4}	0.13×10^{-4}	0.68×10^{-6}	0.04×10^{-6}	0.3	30 (developmental effects)

[a] DBP: di-n-butylphthalate; BBP: butylbenzylphthalate, DEHP: di(2-ethylhexyl)phthalate; OP: 4-tert-octylphenol; NP: nonylphenol; BPA: 2,2-bis(4-hydroxiphenyl)propane or bisphenol A; DEHA: di(2-ethylhexyl) adipate.

laboratory toxicity data to which uncertainty factors are applied. TDIs are used for foreign chemicals not used intentionally in food production. Instead NOEL is acronym of Lowest No Observed Effect Level and it is evaluated through toxicological studies. In our specific case, calculations were performed according to 1 microwave heated meal consumed per day (250 g) and one glass of wine (200 mL). We considered the evaluation of mean daily exposure, related to ingestion of only a one portion of 250 g of food of each category. In this way concentration of each compound, expressed on ng g^{-1} of ww was multiplied for 250 g of food and divided by a body weight of 70 kg.

In Table 5 were reported the mean intake for a meal composed by a portion of food and a glass of white or red wine in order to compare these mean values with TDI and NOEL usually adopted. The results showed mean concentrations usually lower (10^4 times) than the TDI.

4. Conclusion

In this study we have developed a method based on QuEChERS for the determination of plastic monomers and additives in solid and liquid food matrices. PAEs, NP, BPA and DEHA could be recovered in several foods with good sensitivity and selectivity. In particular a rapid and cheap method was performed with acceptable recoveries even if complex matrices were evaluated. Besides carrying out this method it was possible simultaneous detection of different compounds usually find in food in contact with plastic materials. Plasticizers and additives were detected in cheese and broth, which are common ingredients used in cooking and that contributed to the presence in homemade meals. Finally, there was no statistical significant difference between the levels of monomers and additives before and after microwave heating of homemade courses, indicated that heating is not the main cause of food contamination and rather foods might be contaminated during cooking or when placed in the PC or PP container. Microwave heating produces water heating in the core of the food, so probably the food contact surface does not reach temperatures that favor the migration of contaminants from the plastic container, independently from food composition.

However considering the intake rates for each compound, it was estimated that food or wine should be ingested, at least, 10^4 more. So the consumption of food heated in microwave does not represent a risk for consumer even if a diffuse contamination by different chemicals was found in different packaged and homemade meals.

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