Talanta 82 (2010) 296–303

Contents lists available at ScienceDirect

Talanta

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

Solid-phase microextraction followed by gas chromatography–mass spectrometry for the determination of ink photo-initiators in packed milk

N. Negreira, I. Rodríguez*, E. Rubí, R. Cela

Departamento de Química Analítica, Nutrición y Bromatología, Instituto de Investigación y Análisis Alimentario, Universidad de Santiago de Compostela, Av. Ciencias, Santiago de Compostela 15782, A CORUÑA, Spain

ARTICLE INFO

Article history:
Received 1 February 2010
Received in revised form 8 April 2010
Accepted 16 April 2010
Available online 24 April 2010

Keywords:
Ink photo-initiators
Packed food
Milk
Solid-phase microextraction
Gas chromatography
Mass spectrometry

ABSTRACT

A novel, single step method for the determination of seven ink photo-initiators in carton packed milk samples is described. Solid-phase microextraction (SPME) and gas chromatography (GC), combined with mass spectrometry (MS), were used as sample preparation and determination techniques, respectively. Parameters affecting the performance of the microextraction process were thoroughly evaluated using uni- and multivariate optimization strategies, based on the use of experimental factorial designs. The coating of the SPME fibre, together with the sampling mode and the temperature were the factors playing a major influence on the efficiency of the extraction. Under final conditions, 1.5 mL of milk and 8.5 mL of ultrapure water were poured in a glass vessel, which was closed and immersed in a water boiling bath. A poly(dimethylsiloxane)-divinylbenzene (PDMS–DVB) coated fibre was exposed directly to the diluted sample for 40 min. After that, the fibre was desorbed in the injector of the GC–MS system for 3 min. The optimized method provided limits of quantification (LOQs) between 0.2 and 1 $\mu g\,L^{-1}$ and a good linearity in the range between 1 and 250 $\mu g\,L^{-1}$. The inter-day precision remained below 15% for all compounds in spiked whole milk. The efficiency of the extraction changed for whole, semi-skimmed and skimmed milk; however, no differences were noticed among the relative recoveries achieved for milk samples, from different brands, with the same fat content.

1. Introduction

Photo-initiators are low molecular weight compounds added to some printing inks. Under ultraviolet (UV) irradiation these substances are decomposed generating reactive free radicals, which activate the polymerization of ink components allowing a fast drying of the printing film. Generally, photo-initiators present one or two aromatic rings in their molecules. Some of them, case of benzophenone and amino benzoate derivatives, show similar chemical structures to those compounds used as UV-filters in sunscreens and personal care products [1].

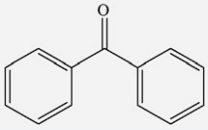
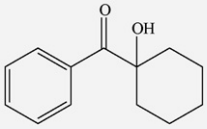
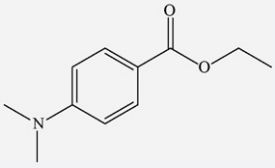
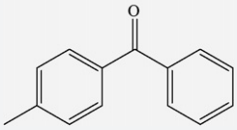
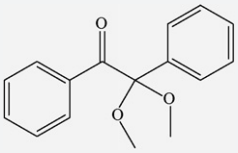
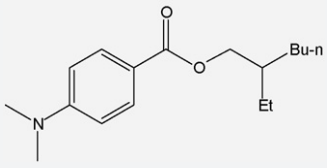
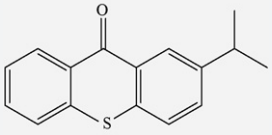
The use of ink photo-initiators in the external face of multilayered packaging cartons can lead to their occurrence in food. In 2005, the European Food Safety Authority (EFSA) reported the presence of 2-isopropylthioxanthone (ITX) in several liquid foods, particularly packed milk, and solid infant formula [2]. Further studies confirmed the presence of ITX not only in milk [3–5], but also in yoghurts [6], fruit juices [7] and even wine [8], at concentrations up to several hundreds of $\mu g\,L^{-1}$. In addition, other photo-initiators such as 2-ethylhexyl-4-dimethylaminobenzoate (EHPABA) [4,8] and benzophenone [8] have been also found in carton packed foods and beverages. Migration through multilayer materials and/or contamination of the inner face during storage of rolled bobbins of printing packages may lead to the presence of ink photo-initiators in food [4,9,10]. Potential long-term effects of photo-initiators exposure on human health remain unknown; however, they are considered as undesirable compounds, whose presence in packed foodstuff has to be controlled. Milk is a particularly concerning matrix, since it is considered a basic, worldwide consumed nourishment.

Gas chromatography (GC) and liquid chromatography (LC) based techniques are often applied to the determination of ink photo-initiators in packed food. In most cases, they are combined with single or tandem mass spectrometry (MS) detection [4,8,11]. Moreover, the complexity of foodstuff matrices makes necessary a previous step to isolate target compounds from the rest of constituents. Liquid–liquid extraction (LLE), using acetonitrile as extractant, is one of the most popular approaches for the extraction of photo-initiators (particularly ITX) from liquid and powdered milk, as well as from other fatty samples [4–6,9]. Acetonitrile is compatible with the use of LC, in the reversed-phase mode, as separation technique and provides extracts with a low level of lipids. N-hexane extraction followed by a further clean-up using a silica cartridge has also been reported [8]. Solid-phase extraction (SPE) of diluted milk samples [3], or of the primary LLE extract from

* Corresponding author. Tel.: +34 981 563100x14387; fax: +34 981 595012.
E-mail address: isaac.rodriguez@usc.es (I. Rodríguez).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.04.037

Table 1
Abbreviated names, CAS numbers, structures, log K_{ow} and vapour pressure (Pv) values of target species.

Abbreviation	Name	CAS number	Structure	[a]log K_{ow}	[a]Pv (mTorr)
BP	Benzophenone	119-61-9		3.18	0.82
CPK	1-Hydroxycyclohexyl-phenylketone	947-19-3		2.34	0.037
EDMAB	Ethyl-4-dimethylaminobenzoate	10287-53-3		3.14	1.43
4-MBP	4-Methylbenzophenone	134-84-9		3.64	0.19
2,2-DMPA	2,2-Dimethoxy-2-phenylacetophenone	24650-42-8		4.76	0.011
EHPABA	2-Ethylhexyl-4-dimethylaminobenzoate	21245-02-3		6.15	0.0046
ITX	2-Isopropylthioxanthone	5495-84-1		5.33	0.0014

[a] Values obtained from SciFinder Scholar Database, http://www.cas.org/products/sfacad/.

the same matrix [7,12], allows a reduction in the consumption or organic solvents and a further improvement in the selectivity of the sample preparation process, respectively.

Solid-phase microextraction (SPME) is a valuable, solvent-free alternative for the extraction and concentration of organic compounds from different matrices. SPME integrates extraction and concentration in the same step; therefore, it competes with multi-step strategies in terms of cost and, many times, SPME provides a higher selectivity since it is based on equilibrium processes rather than in exhaustive extractions. Liquid foodstuffs, such as milk, constitute complex matrices, limiting the yield of SPME extractions in comparison with water samples. In spite of this, several authors have demonstrated the suitability of SPME for the determination of $\mu g\,L^{-1}$ levels of different organic compounds in milk [13–16]. When sample pre-treatment and/or headspace sampling are not possible, dilution of the matrix with water is a straightforward solution to preserve the integrity of the SPME coating and to limit the co-extraction of interfering compounds which might damage the GC column [15,16].

The aim of this study is to develop a single step sample preparation method, based on the SPME technique, for the determination of a group of seven ink photo-initiators in packed milk samples. To the best of our knowledge this is the first application of SPME to the determination of this family of compounds in milk. Parameters affecting the performance of the extraction were systematically evaluated using univariant and also experimental factorial designs studies. After extraction, fibres were thermally desorbed and compounds determined by GC–MS, in the selected ion monitoring (SIM) mode.

2. Experimental

2.1. Solvents, standards and SPME equipment

Methanol, acetonitrile (HPLC-grade) and ethyl acetate (trace analysis grade) were obtained from Merck (Darmstadt, Germany). Sodium chloride was provided by Aldrich (Milwaukee, WI, USA). Ultrapure water was obtained from a Milli-Q system

Table 2
Performance of the GC–MS quadrupole system for direct injection (2 μL) of photo-initiators standards prepared in ethyl acetate.

Compound	Retention time (min)	[a]Selected ions (*m*/*z*)	Linearity, R^2 (10–2000 μg L^{-1})	[b]Repeatability (RSDs, %), 50 μg L^{-1}	[c]Reproducibility (RSD, %), 50 μg L^{-1}	LOQs (μg L^{-1})
BP	14.98	105,77,182	0.9995	1.5	3.4	1.6
CPK	15.67	99,81	0.9995	0.6	5.4	2.3
EDMAB	15.86	148,193,164	0.9993	2.6	2.8	0.7
4-MBP	16.41	119,196	0.9993	5.3	3.4	1.9
2,2-DMPA	17.71	151,105	0.9999	2.0	1.6	0.7
EHPABA	21.24	165,277	0.9999	3.2	4.8	1.1
ITX	22.29	239,254	0.9997	2.8	3.6	3.5

[a] Underlined ions were used for quantification purposes.
[b] Data for $n = 3$ consecutive replicates.
[c] Data for $n = 9$ replicate injections in 3 different days.

(Millipore, Billerica, MA, USA). Standards of benzophenone (BP), 1-hydroxycyclohexyl-phenylketone (CPK), ethyl-4-dimethylaminobenzoate (EDMAB), 4-methylbenzophenone (4-MBP), 2,2-dimethoxy-2-phenylacetophenone (2,2-DMPA), EHPABA and ITX were acquired from Aldrich. Their chemical structures and some properties of relevance to predict their behaviour during extraction are summarized in Table 1. In general, target compounds show medium to low polarities and those with ionisable moieties (CPK, EDMAB and EHPABA) remain in the neutral form at the pH of milk (6.6–6.8 units). Individual solutions of each species were prepared in methanol, further dilutions and mixtures of them were also made in methanol, when used to fortify milk samples, and in ethyl acetate when considered to optimize GC–MS determination conditions.

A manual SPME holder and fibres coated with different polymers: poly(dimethylsiloxane) (PDMS, 100 μm film thickness), polyacrylate (PA, 85 μm film thickness), Carboxen–PDMS (CAR–PDMS, 75 μm film thickness), PDMS–divinylbenzene (PDMS–DVB, 65 μm film thickness) and DVB–CAR–PDMS (50/30 μm film thickness) were obtained from Supelco (Bellefonte, PA, USA). Before being used for first time, SPME fibres were thermally conditioned following conditions recommended by the supplier.

2.2. Samples and SPME procedure

The whole (3.6% fat), half-skimmed (1.55% fat) and skimmed (0.30% fat) milk samples were bought in local supermarkets. All samples were commercialized in multilayered *Tetra Pak* or *Combibloc* type carton packages. Optimization of SPME conditions was carried out with spiked aliquots (100 μg L^{-1}) of whole milk. The percentage of methanol in this matrix was maintained at 1%. In further experiments, samples with different fat contents were spiked at increased levels in the range between 1 and 250 μg L^{-1}. Spiked samples were thoroughly homogenized and stored overnight at 4 °C to simulate the interactions between analytes and matrix occurring in polluted samples.

SPME experiments were carried out in 10 and 22 mL glass vials furnished with a PTFE-faced septum and an aluminium crimp cap. A given volume of milk (from 1.5 to 3 mL) and the corresponding amount of ultrapure water were poured in the vessels, which contained a magnetic stir bar (10 mm × 4 mm). After being closed, vessels were stirred for 5 min and then stabilized at the selected temperature for the same period. Then, a SPME fibre was exposed to the headspace (HS) of the vial or dipped directly into the liquid matrix for a pre-established period. In some experiments, sodium chloride (NaCl) was also added to the SPME vessel in order to assess the effect of the ionic strength on the yield of the extraction. Under optimized conditions, extractions were carried out in 10 mL (nominal volume) vessels containing 1.5 mL of milk and 8.5 mL of ultrapure water, without addition of NaCl. A PDMS–DVB fibre was exposed directly to the stirred sample (700 rpm), previously thermostated at 100 °C, for 40 min. After this time, the fibre was retracted into the SPME holder. Drops of sample attached to the outlet surface of the metallic needle were removed with a soft paper tissue and the fibre was desorbed for 3 min at 270 °C in the injector of the GC–MS system.

2.3. Carton packages

Additionally to the optimization of SPME conditions for milk samples, ink photo-initiators were also investigated in a limited number of carton packages. Samples were extracted using the method previously developed and validated by Sanches-Silva et al. [17]. In brief, packages were opened, the internal side was rinsed with ultrapure water and then, they were cut in small pieces (around 2 mm × 2 mm). One gram of the above matrix was accurately weighed and extracted with 10 mL of acetonitrile, at 70 °C for 24 h. After filtration, the supernatant solution was concentrated to 2 mL, using a gentle stream of nitrogen, and injected directly in the GC–MS system.

2.4. Determination

Analytes were determined using a GC–MS system consisting of an Agilent (Wilmington, DE, USA) 7890A gas chromatograph connected to a quadrupole type mass spectrometer (Agilent MS 5975C), furnished with an electron-impact (EI) ionization source. The mass analyzer was operated in the selected ion monitoring (SIM) mode. Separations were carried out in a HP-5ms type capillary column (30 m × 0.25 mm i.d., d_f: 0.25 μm) supplied by Agilent. Helium (99.999%) was used as carrier gas at a constant flow of 1.0 mL min^{-1}. The GC oven was programmed as follows: 70 °C (held for 3 min), at 10 °C min^{-1} to 280 °C (held for 10 min). Ionisation source, mass analyzer and transfer line temperatures were set at 230, 150 and 290 °C, respectively. Standards prepared in ethyl acetate were injected in the splitless mode (splitless time 3 min), with the injector port at 280 °C. SPME fibres were desorbed at 270 °C, case of PDMS–DVB, PDMS and DVB–CAR–PDMS, or 290 °C for PA and CAR–PDMS. A desorption step of 3 min, maintaining the injector in the splitless mode during this time, was used in all cases. Retention times and *m*/*z* ratios of ions used to monitor the signal of each compound are summarized in Table 2.

An ion-trap type Varian (Walnut Creek, CA, USA) 240 mass spectrometer (MS), furnished with an EI source and connected to 450 model GC instrument, from the same supplier, was also used to confirm the presence of photo-initiators in some milk cartons. The system was also equipped with a Factor Four (Varian) BP-5 type capillary column (30 m × 0.25 mm i.d., d_f: 0.25 μm). Injector, transfer line temperatures and rest of chromatographic conditions were the same as those reported in the above paragraph for the quadrupole GC–MS instrument; however, the splitless time was

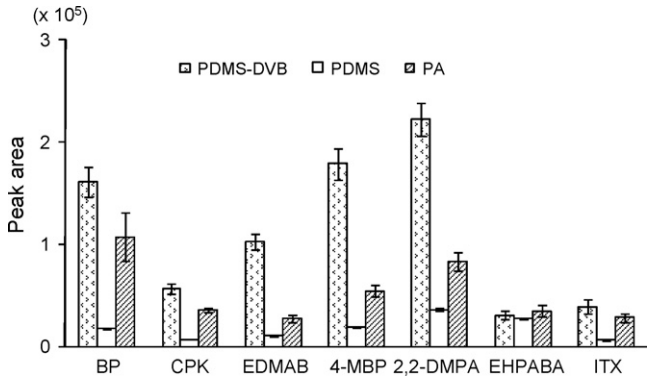


Fig. 1. Responses obtained with different fibres for spiked whole milk samples, $n = 3$ replicates. Direct sampling at 100 °C for 25 min.

reduced to 1 min. Source and trap temperatures were set at 200 and 150 °C, respectively. MS spectra were recorded in the range from 50 to 400 m/z.

3. Results and discussion

3.1. GC–MS determination conditions

Table 2 summarizes some relevant features of the GC–MS (quadrupole) system for the determination of ink photo-initiators. Under conditions reported in Section 2, all compounds were baseline separated showing retention times comprised between 15 and 23 min. The plots of peak area, corresponding to quantification ions (see Table 2), versus concentration fitted a linear model with determination coefficients (R^2) higher than 0.999, within the interval between 10 and 2000 $\mu g\,L^{-1}$. Limits of quantification (LOQs), defined as the concentration of each species producing a signal 10 times higher than the baseline noise, remained between 0.7 and 3.5 $ng\,mL^{-1}$. Relative standard deviations (RSDs) corresponding to injections performed within the same day and in consecutives days stayed below 6%.

3.2. Optimization of SPME parameters

3.2.1. Preliminary experiments

In the initial steps of this study, microextraction experiments were carried out in 10 mL volume vessels, which contained 2 mL of spiked (100 $\mu g\,L^{-1}$) whole milk plus 8 mL of ultrapure water. Vessels were equilibrated at 100 °C and fibres were exposed directed to the diluted samples for 25 min. After that, they were desorbed using conditions provided in Section 2. Fig. 1 depicts the responses (peak areas) obtained for triplicate assays using three SPME coatings. With the only exception of EHPABA, the most hydrophobic of the considered species, the PDMS fibre provided much lower responses than PDMS–DVB and PA ones. The latter two coatings showed similar extraction efficiencies for EHPABA and ITX, whereas PDMS–DVB was preferred for the rest of photo-initiators. CAR–PDMS and DVB–CAR–PDMS fibres showed a very low affinity for ITX, as well as a poor repeatability for the rest of species (data not shown). Thus, PDMS–DVB and PA were selected for additional experiments. Carry-over effects were evaluated by desorbing each fibre twice at 270 °C (PDMS–DVB) and 290 °C (PA). Relative responses in the second desorption remained below 0.2% of those observed in the first one. In order to eliminate any risk of cross-contamination, they were additionally desorbed (3 min), at the above temperatures, in the split injector of a non-operative GC instrument under a nitrogen stream of 30 $mL\,min^{-1}$.

Table 3
Experimental domain of the 2^{5-1} fractional design.

Factor	Level	
	Low (−)	High (+)
Fibre	PDMS–DVB	PA
Temperature (°C)	55	100
Sampling mode	Direct	HS
NaCl (%)	0	15
Dilution factor	1:5	1:10

3.2.2. Multifactor optimization of SPME conditions

Efficiency of SPME methods is affected by a considerable number of factors, which are sometimes correlated. A strategy based on the use of experimental factorial designs was adopted to identify those parameters playing a major effect on the performance of the SPME process, and to achieve optimal conditions with a minimum effort and cost.

Initially, a two levels 2^{5-1} type fractional factorial design was used to assess the effects of temperature, sampling mode, ionic strength, dilution factor and fibre coating on the efficiency of the extraction. Low and high values for each of these parameters are given in Table 3. Previous assays showed poor efficiencies operating at room temperature; therefore, the domain of this factor was established between 55 and 100 °C. The dilution ratio was chosen according to the information reported in a previously work, dealing with the application of SPME to the determination of pesticides in cow milk [16]. The volume of milk used in each experiment was 1.5 or 3 mL, depending on the dilution ratio, and the total volume in the SPME vessel was made up to 15 mL with ultrapure water in all cases. Extractions were performed in 22 mL vials to allow working in direct and HS modes, depending on the conditions defined by the experimental design. Peaks areas obtained for each compound in the 16 extractions involved in the above design were used as variable responses. Standardized values for main effects corresponding to each factor were calculated with the Statgraphics Centurion XV software (Manugistics, Rockville, MD, USA), data not shown. Similar responses were attained for EHPABA and ITX with both fibres, whereas for the rest of species the PDMS–DVB coating provided higher yields. Direct exposure of the fibre to the diluted samples was preferred to HS sampling and

Table 4
Experimental domain, standardized main effects and relevant interactions of factors involved in the $3^1 \times 2^2$ experimental design.

Factor	Code	Level			Standardized value						
		Low	Medium	High	BP	CPK	EDMAB	4-MBP	2,2-DMPA	EHPABA	ITX
Temperature (°C)	A	60	80	100	6.3*	4.6*	6.7*	8.2*	6.6*	18*	21*
NaCl (%)	B	0	–	10	−2.2	0.26	−2.3	−2.6*	−2.3	−10*	−9.0*
Time (min)	C	20	–	40	1.5	1.0	1.8	1.5	1.7	3.4*	4.5*
	AA	–		–	−0.32	−0.55	0.20	1.24	1.23	7.86*	8.81*
	AB	–		–	1.52	3.11*	1.58	0.69	0.77	−8.02*	−5.96*

* Significant factors at the 95% confidence level.

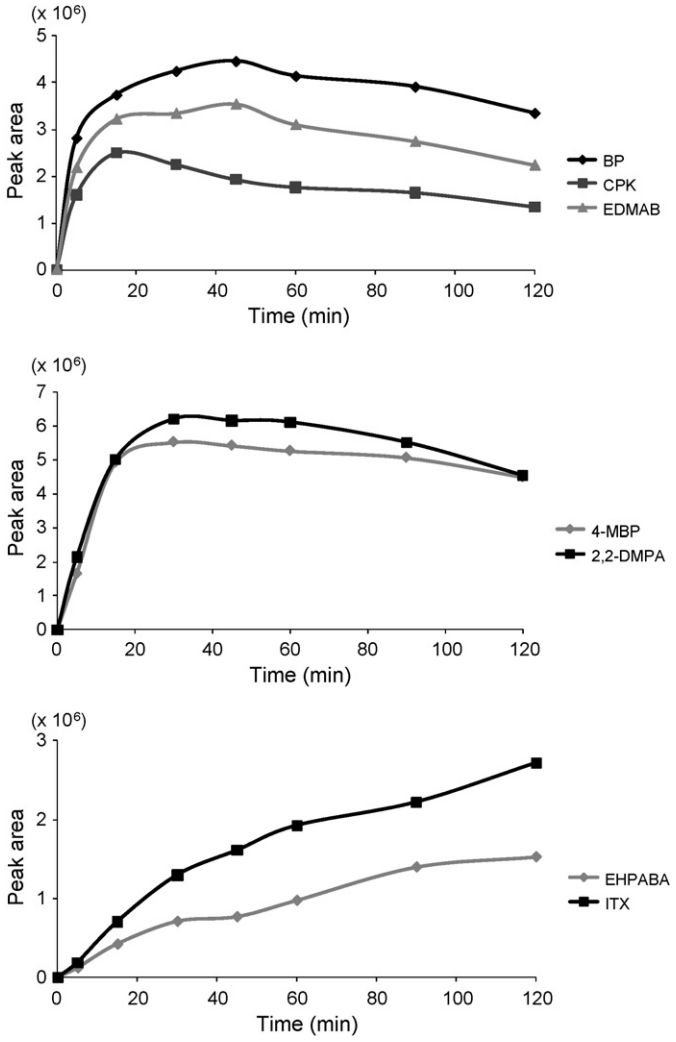


Fig. 2. Kinetics of the SPME for whole milk. Direct sampling at 100 °C using a PDMS–DVB fibre.

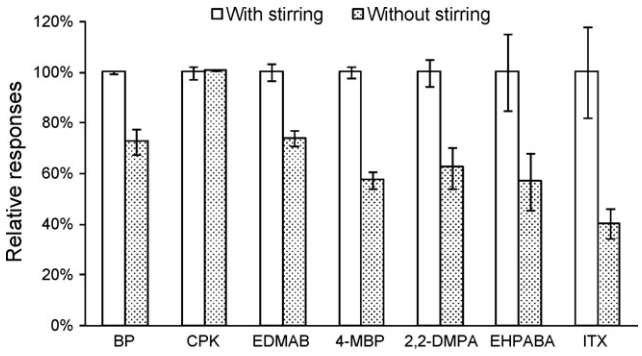


Fig. 3. Effect of stirring (700 rpm) on the relative efficiency of the SPME. Sampling time 40 min, $n = 3$ replicates.

higher extraction efficiencies were attained at 100 °C versus 55 °C. The effect of the ionic strength was compound dependent and the dilution factor affected negatively to the obtained responses. On the basis of these comments, direct sampling using a PDMS–DVB fibre was fixed for further experiments. Extractions were carried out in 10 mL vessels containing 1.5 mL of milk plus 8.5 mL of water.

Optimal values corresponding to temperature, sodium chloride and extraction time were evaluated with more detail using a $3^1 \times 2^2$ type factorial design, with three replicates of the central point, Table 4. The temperature was the most important of the considered factors, with a positive and statistically significant effect (95% confidence level) in the efficiency of the extraction for all compounds. In the case of ITX and EHPABA, the quadratic term associated to this factor (AA) was also significant, Table 4. The corresponding main effect plot suggested an exponential increase in the efficiency of the SPME with the temperature of the sample for both species, figure not shown. The sampling time also affected positively to the extraction process, although it overpass the level of statistically significance only for EHPABA and ITX. The interaction temperature-salt (AB) was also statistically significant for CPK, EHPABA and ITX. The corresponding interaction plots (figure not given) demonstrated that, for EHPABA and ITX, the negative effect of salt was more relevant at 100 than at 60 °C. Taking these comments into account, further extractions were performed at 100 °C, without addition of sodium chloride to the SPME vessel.

A detailed study of the extraction kinetics showed that EHPABA and ITX required more than 2 h of direct sampling at 100 °C to achieve equilibrium conditions, Fig. 2. For the rest of species, the efficiency of the extraction normally reached a maximum between 30 and 45 min and then it decreased slightly, Fig. 2. This trend might be the result of competitive adsorption processes on the surface of the PDMS–DVB coating. An exposure time of 40 min was adopted.

3.2.3. Stirring and methanol addition

Agitation is expected to increase the kinetics of the extraction, improving the transport of the compounds between the liquid sample and the interface with the SPME coating. On the other hand, PTFE covered stirrers are a potential source of cross-contamination problems. Experiment data (Fig. 3) demonstrated that stirring improved significantly the yield of the extraction for all compounds except CPK. As previously commented, this is the most polar of the species involved in this study; therefore, it is expected to be that showing the higher diffusion rates towards the interface between the solution and the SPME fibre and thus the less affected by stirring. This type of dependence between the efficiency of stirring and the polarity of analytes has been previously reported in the literature [18]. In order to avoid the risk of cross-contamination problems, stir bars were wrapped with PTFE tape, which was removed after each extraction.

Addition of an organic solvent to the SPME vessel contributes to reduce competitive adsorptions of hydrophobic species on the walls of glass vessels, improving the efficiency of microextraction for these compounds. On the other hand, the yield of the process decreases for hydrophilic species, which turn out more soluble in the sample. During optimization of SPME conditions, fortified aliquots of milk containing a 1% of methanol were employed. After dilution (1.5–10 mL), the percentage of methanol in the sample was 0.15%. A series of extractions was performed using samples containing also 1% and 3% of methanol. Except in the case of EHPABA and ITX, the efficiency of the extraction was negatively affected by the addition of methanol, figure not shown. To limit the contribution of this parameter to the variability of the extraction, the percentage of methanol in the sampling vessel was always maintained below 0.5%.

3.2.4. Fat content

In general, the performance of microextraction techniques is affected by the characteristics of the sample. As a general rule, the higher is the complexity of the matrix, the lower the yield of the extraction. The complexity of milk samples is mainly related with their lipidic content. Fig. 4 depicts the responses obtained for samples of whole, half-skimmed and skimmed cow milk spiked

Table 5
Linearity, repeatability ($n = 3$ replicates), reproducibility ($n = 9$ replicates in 3 different days) and LOQs of the proposed method for full-fat milk samples.

Compound	Linearity (R^2) 1–250 μg L^{-1} [a]	Repeatability (RSDs, %)			Reproducibility (RSDs, %), 16 μg L^{-1} [a]	LOQs μg L^{-1}
		5 μg L^{-1} [a]	25 μg L^{-1} [a]	50 μg L^{-1} [a]		
BP	0.9962	7.0	5.5	5.1	8.2	0.2
CPK	0.9966	4.7	5.3	1.3	8.3	0.6
EDMAB	0.9964	0.6	4.8	4.4	9.5	1
4-MBP	0.9969	5.0	3.5	3.3	11.9	0.4
2,2-DMPA	0.9977	6.5	1.9	4.2	15.1	0.3
EHPABA	0.9931	1.0	4.1	3.4	9.6	0.8
ITX	0.9969	5.4	4.7	2.4	9.4	0.4

[a] Added concentration.

with target photo-initiators at 50 μg L^{-1}. Their declared fat percentages were 3.6%, 1.55% and 0.3%, respectively. The yield of the extraction was inversely proportional to the fat content in the sample.

3.3. Analytical figures of merit

The developed method was characterized in terms of linearity, repeatability and reproducibility using aliquots of whole milk fortified at different concentrations in the range between 1 and 250 μg L^{-1}. Plots of peak areas versus added concentrations fitted a linear model with determination coefficients higher than 0.993, Table 5. RSDs of extractions carried out in the same day (repeatability study) for samples spiked at three different concentrations (between 5 and 50 μg L^{-1}), remained below 7%, only slightly higher than the repeatability of the GC–MS system for direct injection of standards in ethyl acetate, see Table 2. RSDs for extractions performed during 3 consecutive days varied between 8 and 15%, Table 5. LOQs of the proposed method, defined as the concentration of each specie providing a chromatographic peak with a signal to noise ratio (S/N) of 10, were estimated from the lowest addition level in the linearity study. Obtained values varied between 0.2 μg L^{-1} for BP and 1 μg L^{-1} for EDMAB, Table 5. In the case of ITX, the photo-initiator more often investigated in packed milk, a LOQ of 0.4 μg L^{-1} was achieved. Globally, these values are similar to those attained using LLE, with additional SPE purification, followed by GC–MS [8] or LC–MS/MS [12]; they are also equivalent to LOQs reported for ITX using SPE with GC–MS/MS determination [3], and LLE followed by LC–MS/MS [6]. Gallart-Ayala et al. [11] achieved a 100-fold reduction in the LOQ of ITX at the expense of using a last generation LC–MS/MS instrument providing accurate mass measurements [11]. Taking into account the trend depicted in Fig. 4, LOQs reported in Table 5 can be further reduced for semi-skimmed and skimmed milk. The only exception was BP. For this compound the lower attainable LOQ was limited by the presence of this specie in procedural blanks corresponding to the extraction of ultrapure water.

Accuracy is a major issue during the validation of any analytical procedure. Microextraction methodologies are prone to variations in their efficiency depending on the characteristics of the matrix as it has been already proved for whole, semi-skimmed and skimmed milk samples, Fig. 4. A further series of assays was carried out to investigate whether the yield of the SPME method varies also among samples, with the same fat content, from different brands, or not. Aliquots corresponding to specimens of whole, semi-skimmed and skimmed milk, from three different suppliers, were fortified at concentrations in the range from 10 to 20 μg L^{-1}. Non-spiked aliquots of each specimen were also processed, Fig. 5. One of the specimens was used as reference and the responses measured for the other two normalized to the first. Significant differences were not observed among milk samples with the same fat content, Table 6. Thus, levels of ink photo-initiators in unknown samples can be quantified by external calibration, using matrix-matched standards. The only requirement is that both, unknown samples and calibration standards, present the same fat content.

3.4. Real samples

The presence of ink photo-initiators was investigated in a total of 20 samples corresponding to whole, semi-skimmed and skimmed milk. Around 60% of them were distributed in *Tetra Pak* cartons and the rest in *Combibloc* ones. All species remained under the LOQs of the method, although BP was detected, at concentrations below the LOQ of this species, in some of them. In order to establish if these results indicate the phase-out of ink photo-initiators in milk cartons, or simply the lack of significant migration problems, six packages (*Tetra Pak* and *Combibloc* types) were extracted under

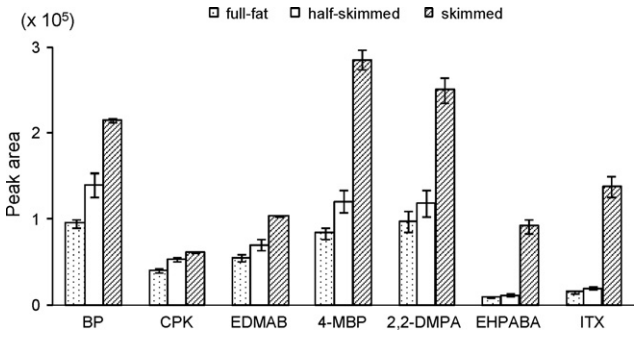


Fig. 4. Comparison of peak areas for spiked (50 μg L^{-1}) milk samples with different fat contents, $n = 3$ replicates.

Table 6
Relative recoveries provided by the SPME method for milk samples with different fat contents, $n = 3$ replicates.

Compound	Relative recoveries (%) ± SD					
	[a]Skimmed		[a]Semi-skimmed		[b]Whole	
	Brand 1	Brand 2	Brand 1	Brand 2	Brand 1	Brand 2
BP	101 ± 1	101 ± 12	102 ± 4	98 ± 7	103 ± 2	94 ± 9
CPK	94 ± 1	95 ± 11	97 ± 1	94 ± 3	101 ± 1	98 ± 3
EDMAB	100 ± 4	94 ± 8	106 ± 3	94 ± 7	101 ± 5	99 ± 6
4-MBP	100 ± 3	104 ± 2	97 ± 2	93 ± 7	101 ± 5	100 ± 13
2,2-DMPA	101 ± 3	98 ± 4	100 ± 2	98 ± 4	105 ± 3	98 ± 14
EHPABA	108 ± 3	100 ± 0.2	106 ± 9	101 ± 6	89 ± 5	92 ± 4
ITX	101 ± 6	106 ± 7	100 ± 8	97 ± 3	98 ± 2	96 ± 1

[a] Added concentration 10 μg L^{-1}.
[b] Added concentration 20 μg L^{-1}.

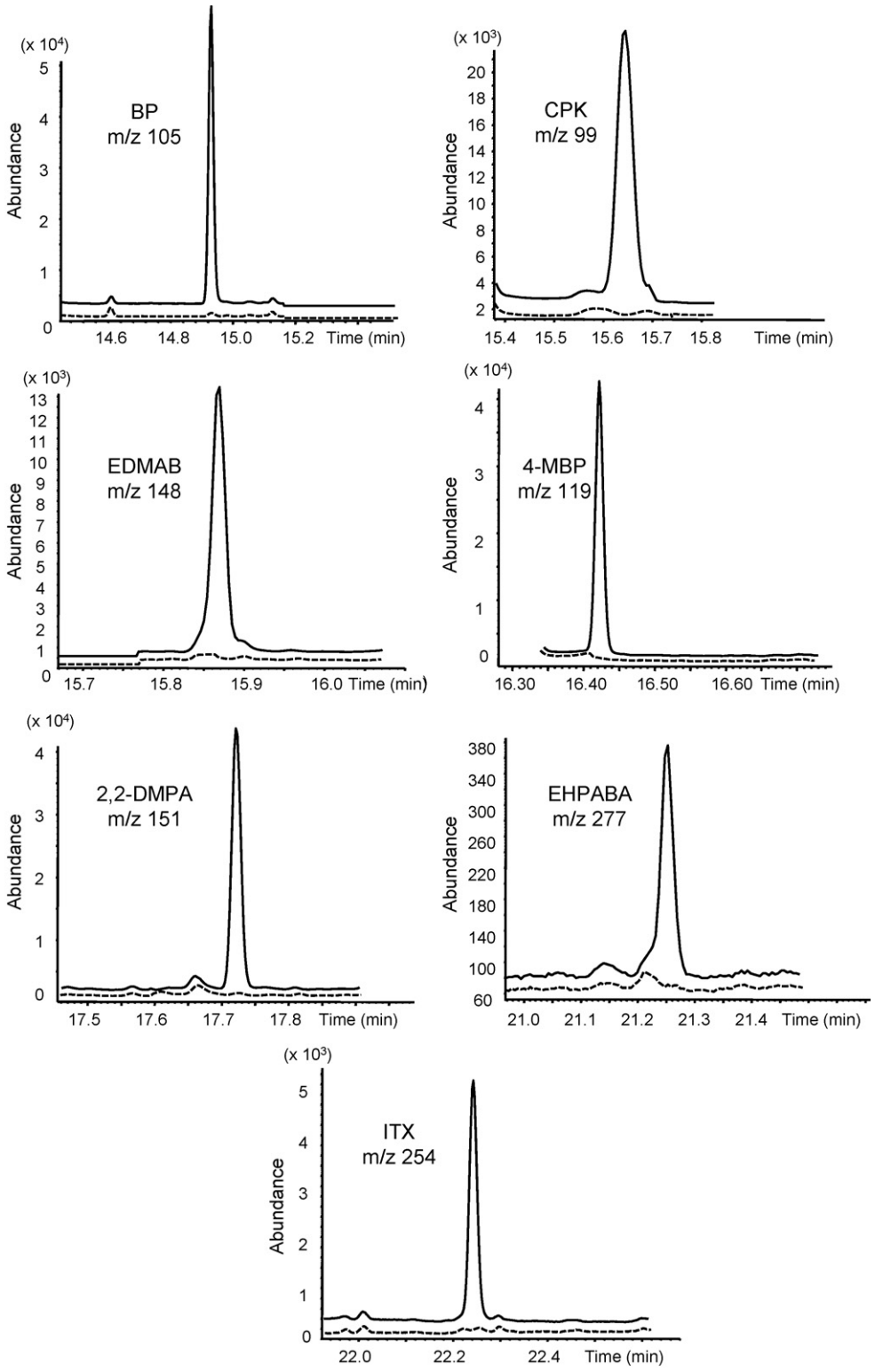


Fig. 5. GC–MS chromatograms for half-skimmed milk. Dotted line, un-spiked sample. Solid line, same matrix fortified at 10 $\mu g\,L^{-1}$.

conditions reported in Section 2. A peak at the retention time of BP was found in the chromatograms provided by the quadrupole GC–MS instrument for the six samples, whereas the rest of photoinitiators were not noticed. The identity of this compound was confirmed with the ion-trap GC–MS system, Fig. 6. A detailed quantification of BP levels in milk packages was beyond the scope of this research. A rough estimation points out to concentrations below 1 $\mu g\,g^{-1}$ in the six processed samples.

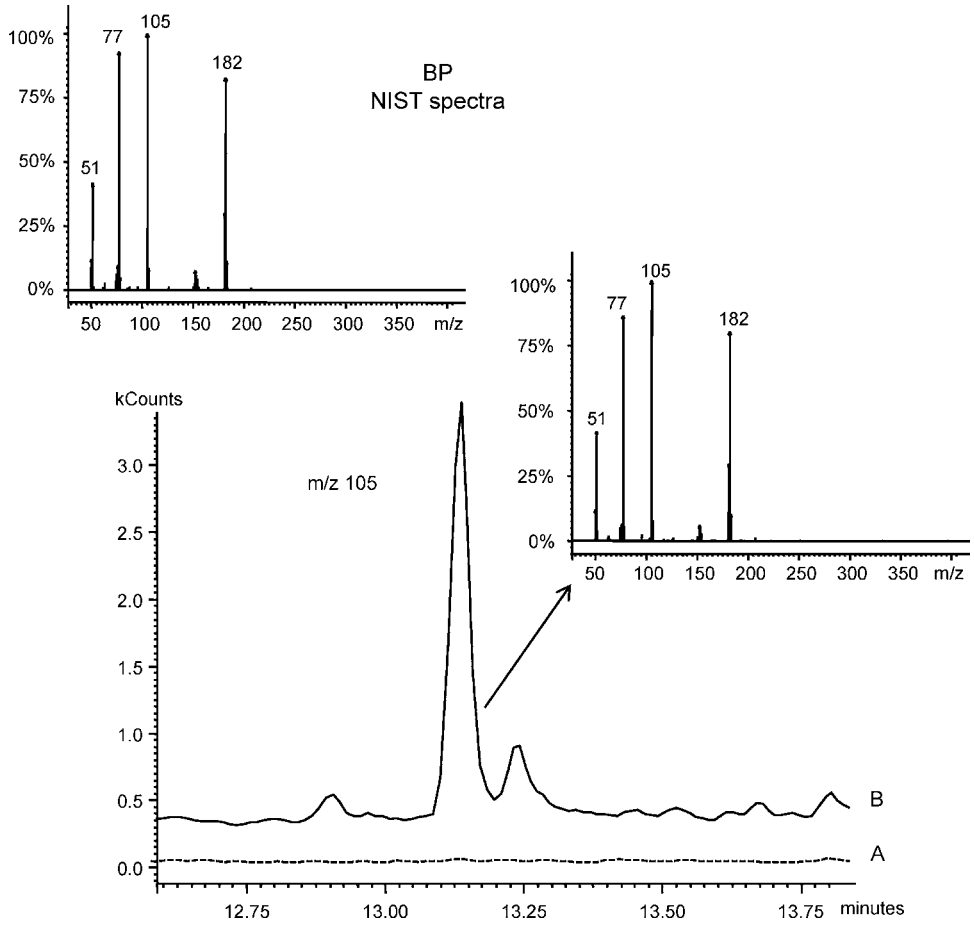


Fig. 6. GC–MS (ion-trap) chromatograms and spectra showing the signal of BP in the extract from a *Tetra Pak* package. (A) Procedural blank and (B) carton extract.

4. Conclusions

The suitability of SPME for the extraction of seven ink photo-initiators in packed milk has been demonstrated for first time. Its major advantages over previously published methods are integration of extraction and concentration in the same step and null consumption of organic solvents. Although the yield of the microextraction is conditioned by the fat content in the sample, no differences were noticed among milk specimens from different brands, with the same nominal fat content. When combined with GC–MS detection, the developed procedure provides adequate figures of merit to screen the potential contamination of milk with photo-initiators used during the printing of packaging materials. Although this possibility has not been explored in this study, it is expected that the proposed method can be also adapted to other liquid foodstuffs, such as fruit juices and wine. Target compounds could not be quantified in any of the processed milk samples; however, BP was found in carton packages.

Acknowledgements

This study has been supported by the Spanish Government and E.U. FEDER funds (project CTQ2009-08377). N.N. thanks a FPU contract to the Spanish Ministry of Science and Innovation.

References

[1] N.A. Shaath, The Encyclopedia of Ultraviolet Filters, Allured, IL, 2007.
[2] R. Anton, S. Barlow, D. Boskou, L. Castle, R. Crebelli, W. Dekant, K.H. Engel, S. Forsythe, W. Grunow, M. Heinonen, J.C. Larsen, C. Leclercq, W. Mennes, M.R. Milana, I. Pratt, I. Rietjens, K. Svensson, P. Tobback, F. Toldrá, EFSA J. 293 (2005) 1.
[3] G. Allegrone, I. Tamaro, S. Spinardi, G. Grosa, J. Chromatogr. A 1214 (2008) 128.
[4] A. Gil-Vergara, C. Blasco, Y. Picó, Anal. Bioanal. Chem. 389 (2007) 605.
[5] R. Bagnati, G. Bianchi, E. Marangon, E. Zuccato, R. Fanelli, E. Davoli, Rapid Commun. Mass Spectrom. 21 (2007) 1998.
[6] C. Benetti, R. Angeletti, G. Binato, A. Biancardi, G. Biancotto, Anal. Chim. Acta 617 (2008) 132.
[7] C. Sun, S.H. Chan, D. Lu, H.M.W. Lee, B.C. Bloodworth, J. Chromatogr. A 1143 (2007) 162.
[8] G. Sagratini, G. Caprioli, G. Cristialli, D. Giardiná, M. Ricciutelli, R. Volpini, Y. Zuo, S. Vittori, J. Chromatogr. A 1194 (2008) 213.
[9] A. Sanches-Silva, S. Pastorelli, J.M. Cruz, C. Simoneau, I. Castanheira, P. Paseiro-Losada, J. Agric. Food Chem. 56 (2008) 2722.
[10] G. Morlock, W. Schwack, Anal. Bioanal. Chem. 385 (2006) 586.
[11] H. Gallart-Ayala, E. Moyano, M.T. Galceran, J. Chromatogr. A 1208 (2008) 182.
[12] D. Shen, H. Lian, T. Ding, J. Xu, C. Shen, Anal. Bioanal. Chem. 395 (2009) 2359.
[13] M.J. González Rodríguez, F.J. Arrebola Liébanas, A. Garrido Frenich, J.L. Martínez Vidal, F.J. Sánchez López, Anal. Bioanal. Chem. 382 (2005) 164.
[14] C. Mardones, D. Von Baer, J. Silva, M.J. Retamal, J. Chromatogr. A 1215 (2008) 1.
[15] N. Aguinaga, N. Campillo, P. Viñas, M. Hernández-Córdoba, Anal. Chim. Acta 596 (2007) 285.
[16] M. Fernandez-Alvarez, M. Llompart, J.P. Lamas, M. Lores, C. García-Jares, R. Cela, T. Dagnac, Anal. Chim. Acta 617 (2008) 37.
[17] A. Sanches-Silva, S. Pastorelli, J.M. Cruz, C. Simoneau, P. Paseiro-Losada, J. Food Sci. 73 (2008) C92.
[18] P. Canosa, I. Rodríguez, E. Rubí, M.H. Bollaín, R. Cela, J. Chromatogr. A 1124 (2006) 3.