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Original Paper

Determination of haloanisoles in paper samples for food packaging by solid-phase microextraction and gas chromatography

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Abstract. A method was developed for the determination of trichloroanisole, tribromoanisole and pentachloroanisol by solid-phase microextraction and gas chromatography in paper samples (Kraft liner, Test liner and Miolo). Four commercial SPME fibers were evaluated: Polydimethylsiloxane (PDMS), Polyacrylate (PA), Carbowax/Divinylbenzene (CW/DVB) and Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS). DVB/CAR/PDMS gave the best results and was therefore selected. Other variables involved in the extraction procedure were studied and optimized, such as: sample volume, salting-out effect, temperature and extraction time, effect of organic solvent and previous sample preparation. Optimum conditions were obtained using 20 mL of sample with 5 mol L^{-1} NaCl in a 40 mL vial, extraction temperature of 70 °C and sonication and extraction time of 30 and 40 min, respectively. Detection limits ranged from 0.43 to 1.32 ng g^{-1} for all analytes. Recoveries between 70 and 100% were obtained and relative standard deviation was below 10% for all compounds.

Key words: Haloanisoles; SPME; gas chromatography; paper for food packaging.

Materials used in food packaging are the main contributors for food taint. In several cases, the cause has been the transfer of residual solvents used in package manufacturing, from machinery or even paints used in package printing. Traces of plastic monomers, such as, styrene have also caused package taint. The use of disinfectants and other chemicals must be rigorously controlled in an industrial process.

The production of paper and board for food contact used in Europe is 44% virgin pulp and 40% recycled. However, depending on the category of the material, the latter percentage can rise up to 90%. To ensure the safety of such materials, food-packaging regulations in Europe require that the packaging materials do not cause mass transfer (migration) of harmful substances to food. Studies on the virgin paper used for food packaging have shown that the levels of chemicals that can potentially migrate into food are very low [1].

One of the most common reasons that lead consumers to reject foodstuffs is its unacceptable odor. Every year food industries receive complaints from consumers due to unpleasant odor in fresh, processed or packaged foods [2]. This potent odorant can also produce sensorial alterations in other food products and its incidence in drinking water, coffee, raisins, chicken, etc. has been reported [3].

However, the most important contaminants associated with food packaging are haloanisoles, which arise from fungi-promoted methylation in trace amounts of halophenols. Halophenols are the active principles in many fungicides and algicides [2].

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Wood preservatives are based on halophenols and any material made of wood in contact with food or water supply could introduce these compounds in foodstuffs [4, 5].

Many fungi and other microorganisms convert halophenols to their corresponding haloanisoles, which have threshold odor many thousand times lower than halophenols [2].

The xenophilic fungi *Paecilomyces variotii* can convert quantitatively the fungicide 2,4,6-tribromophenol (TBP) to its odorous metabolite 2,4,6-tribromoanisole (TBA). In six weeks the conversion is complete. Tribromophenol is readily formed in water containing chlorine and bromine. Industrially, synthetic derivatives of bromophenols, in particular TBP, were widely recommended as anti-flame agents in laminated resins and fungicides for use in leathers, textiles, paints, plastics, celulosic pulp and paper [6].

Other studies also affirm that TBP to TBA conversion is possible for a fungus present in paperboard [2, 7].

Some fungi found on the ground of containers are capable of methylating 2,4,6-triclorophenol (TCP) to form 2,4,6-trichloroanisole (TCA). This compound was also found in wines and Brazilian coffees. Other studies demonstrate that the incubation of some fungi in synthetic media containing sodium hypochloride yields considerable amounts of 2,4,6-trichloroanisole [8].

A relatively large number of papers have been published regarding the determination of haloanisoles in wines and water samples [9–16]. On the other hand, there was only one paper found in literature relating to the determination of haloanisoles in paper samples, employing solvent extraction [17].

Solid-phase microextraction (SPME) offers a quick, accurate, and inexpensive tool for analysing volatile components, including TCA [10]. The first SPME device was developed by Arthur and Pawliszyn (1990) as a preconcentration technique for the analysis of water pollutants. Within recent years, however, SPME has become a very popular tool and has extended its application to numerous other fields [18].

In this work, an analytical methodology was developed for the determination of haloanisoles in Test liner, Kraft liner and Miolo paper samples by SPME-GC. The following parameters were studied and optimized: fiber coating, headspace volume, salting-out effect, extraction temperature, extraction time and sample preparation before SPME extraction.

Experimental

Instrumentation and chromatographic conditions

All analyses were carried out in a Shimadzu (www.shimadzu.com) GC-14B gas chromatograph, equipped with a split/splitless injector and electron capture detector (ECD). An OV-5 capillary column (30 m × 0.25 mm × 0.25 μm) from OV Specialty Chemical (Marietta, USA, www.ovsc.com) was used for chromatographic separation. Carrier gas and make-up gas was ultra pure nitrogen (AGA, São Paulo, Brazil) with a flow rate of 1.0 and 35 mL min^{-1}, respectively. Injector and detector temperatures were set at 270 and 280 °C, respectively. Oven temperature program was 80 °C (1 min), 4 °C min^{-1} up to 200 °C (0 min), 20 °C min^{-1} up to 300 °C (4 min).

Water bath (Nova Técnica, São Paulo, Brazil, www.novatecnica.com.br) and magnetic stirrer (Speedlab, São Paulo, Brazil) were also used for both temperature stabilization and sample stirring.

For sample preparation before SPME procedure an USC 1450 ultrasonic stirrer (Unique, São Paulo, Brazil, www.unique.ind.br) was used.

Standars and reagents

Standard stock solutions of the target analytes 2,4,6-trichloroanisole and pentachloroanisole (Sigma-Aldrich, Milwaukee, USA, www.sigmaaldrich.com) and 2,4,6-tribromoanisole (Fluka, Buchs, Switzerland) were prepared in HPLC-grade acetone (Tedia, Fairfield, USA, www.tedia.com). Sodium chloride (Merck, Darmstadt, Germany, www.merck.com) was used for ionic strength modification.

All glassware was left to soak in neutral detergent Extran® (Merck, Darmstadt, Germany) for 24 hours. After being washed with deionized water and immersed in nitric acid aqueous solution 10% for 24 hours, it was then sonicated for 1 hour. The final washing step was rinsing thoroughly with deionized water and drying at room temperature.

SPME procedure

Four commercial SPME fibers (Supelco, Bellefonte, USA) were conditioned following the manufacturer's instructions and were then tested: Polydimethylsiloxane 100 μm (PDMS), Polyacrilate 85 μm (PA), Carbowax/Divinylbenzene 65 μm (CW/DVB) and Divinylbenzene/Carboxen/Polydimethylsiloxane 50/30 μm (DVB/CAR/PDMS). Fiber coating optimization was performed with a triplicate headspace extraction of a sample solution containing 50.0 ng L^{-1} of the target analytes.

Sample preparation before SPME procedure

Three different lots of paper samples Test Liner, Kraft Liner e Miolo were analyzed, each in triplicate. The samples were cut into small pieces of (3 × 3) mm. The optimized sonication procedure was obtained as follows: approximately 500 mg of samples were weighed directly in a 4 mL amber vial, in which 3.0 mL of acetone HPLC-grade (Tedia, Fairfield, USA) was added. This sample was then sonicated for an optimized preset time. Immediately after sonication, 500 μL of the extract was collected by means of a micropipette and transferred to a 40 mL vial and submitted to the optimized SPME extraction procedure. For optimization of sonication time, a paper sample was spiked with a stock solution in acetone with the target analytes to reach a concentration of 10 ng g^{-1}. The samples were aged for one week before analysis. This same procedure

was carried out to obtain the spiked samples for recovery tests. Sonication times were tested at a range of 5–60 min.

Results and discussion

Optimization of variables affecting SPME procedure

Optimization of fiber coating

Four commercially available fibers were tested. This study was carried out under the following conditions: the concentration of target analyses was 50 ng L^{-1}, sample was magnetically stirred (stirring bar of 30 mm × 8 mm), no salt was added, sample volume was 25 mL in a 40 mL vial, temperature was 25 °C, extraction time of 30 min was used and headspace extraction mode was preferred in the whole work to save the fiber from possible damaging substances that might be present in the real samples. As can be seen in Fig. 1, the fiber DVB/CAR/PDMS showed the best extraction efficiency for all the target analyses. Malleret et al. [15] suggested that π interactions between target compounds and divinylbenzene present in this fiber are responsible for the higher extraction efficiency. Therefore, DVB/CAR/PDMS fiber was used in the subsequent studies.

Sample volume

To achieve the best sample volume, a 40 mL vial was used in all experiments and volumes of working solution (50.0 ng L^{-1}) ranging from 15 to 25 mL were tested. Headspace extraction was performed with no salt addition, at 25 °C with continuous magnetic stirring. Since higher sample volumes do not compromise sample stirring and consequently mass transfer process of the analytes from sample solution to headspace, it could be expected that a higher sample volume could enhance the amount extracted by the fiber, provided that a higher concentration of the analytes in the sample headspace is reached by lowering headspace volume. Since there was no significant difference between the peak areas obtained by the 20 and 25 mL sample volumes, 20 mL of sample solution was chosen to carry on optimization.

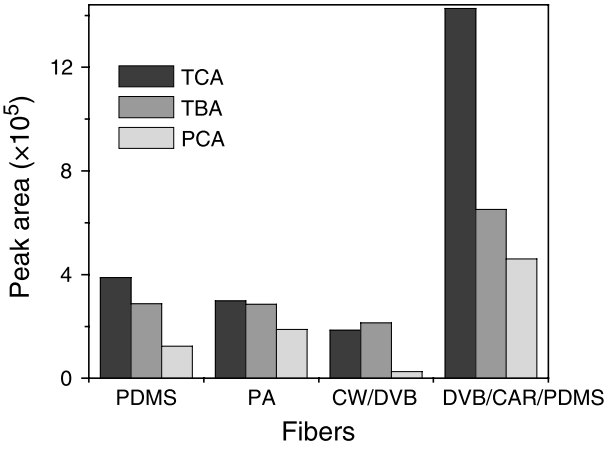

Fig. 1. Evaluation of different commercial SPME fibers on extraction efficiency of haloanisoles

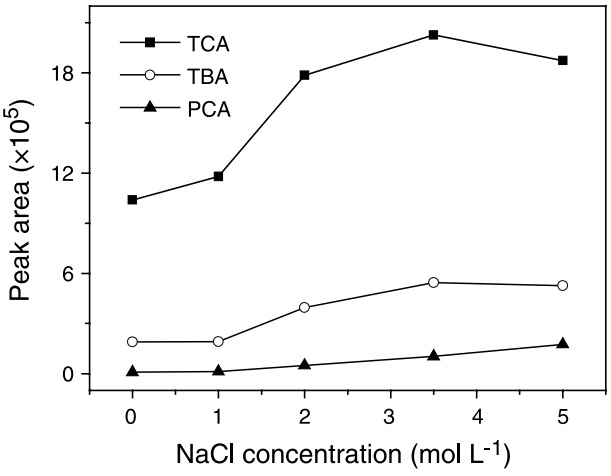

Fig. 2. Influence of salt addition (NaCl) on SPME extraction procedure

Salting-out effect

The salting-out effect was accomplished by adding NaCl, being very soluble in water, it does not change water pH significantly and can be obtained easily and relatively free of impurities. Examining Fig. 2, it can be observed that there is an enhancement in the extraction efficiency for all the analytes when the NaCl concentration was raised up to 5 mol L^{-1}. The increased ionic strength tends to increase Henry's constant for the analytes, lowering their solubilities in aqueous media, forcing them to occupy the sample headspace. Then, for subsequent experiments, the samples were adjusted to 5 mol L^{-1} with sodium chloride.

Extraction temperature

Optimization of extraction temperature was carried out at a range of 25–80 °C. Extraction temperature plays a very important role in the extraction procedure, as it affects both the partition coefficient of the analytes between fiber coating and sample matrix and the rate constant of the mass transfer process

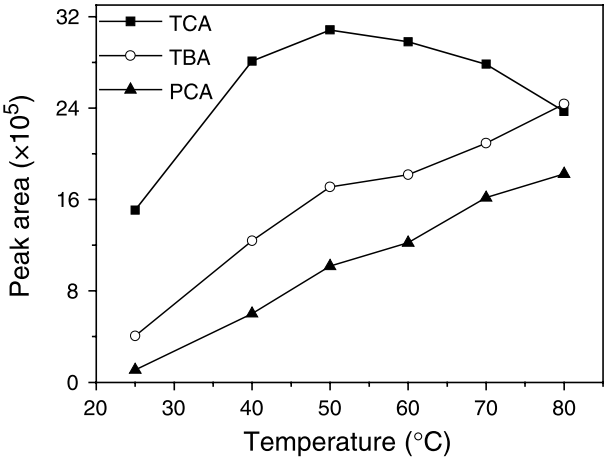

Fig. 3. Optimization of extraction temperature for SPME procedure

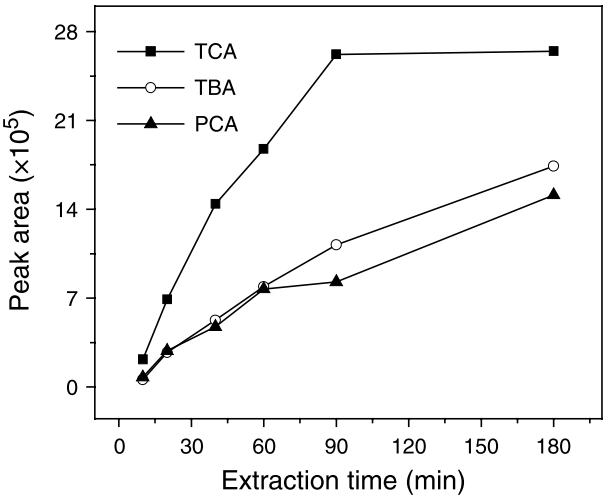

Fig. 4. Extraction time profile for TCA, TBA and PCA

of the analytes in the three phases existing in the system: condensed, gaseous and extracting phases. In Fig. 3, an increase of up to 50 °C is seen in the amount extracted by the fiber for TCA, most certainly because of increase in mass transfer coefficients. Above this temperature, the analytical signal falls, probably due to a large decrease in the partition coefficient. For TBA and PCA the analytical signal increases up to 80 degree. Therefore, as a compromise, 70 degree was selected as extraction temperature.

Effect of organic solvent content

The solvent acetone was chosen as co-solvent for its complete miscibility in water and high solubility of the analytes. It was tested in concentrations from 0 up to 10% in volume to check if this solvent may interfere with extraction efficiency. It was observed that a concentration of up to 5% does not change the amount extracted by the fiber for the three compounds. Besides this, a small decrease in peak areas was observed. In order to reduce the amount of organic solvents used and consequently the amount of organic rejects produced, we use a concentration of only 2.5% in volume for extraction procedure.

Extraction time

An extraction time profile was studied at a range of 10–180 min. As can be seen in Fig. 4, only TCA reaches equilibrium at about 90 min. TBA and PCA do not reach equilibrium even when extraction time of 180 min is used. As a compromise between analytical frequency and sensitivity, 40 min were set up as extraction time for the analytical methodology.

Optimization of sample preparation

Several amounts of spiked samples (500–1000 mg) were put into the vial and submitted to the optimized SPME procedure. However, poor recoveries between 30 and 45% were obtained. Comparatively to the standard calibration solutions, a rough change in sample agitation is easily noted when 500 or 1000 mg of sample are used. A similar procedure for direct determination of pentachlorophenol (PCP) in paper samples was described by Domeño et al. [19]. The results were compared with conventional extraction methods, resulting in recoveries between 57 and 84%. This study demonstrated that there is a strong interaction between analytes and paper sample matrix. Thus, for an accurate determination of PCP, the standard addition procedure should be performed. In order to avoid this procedure, which is tedious and time-consuming, we tried to sonicate the sample (about 500 mg) with 20 mL of NaCl 5 mol L^{-1} and acetone 2% (v/v) at 25 °C for different times (between 10 and 60 min) before carried it out to the SPME procedure (40 min of extraction at 70 °C). However, poor recoveries between 38 and 49% were obtained, indicating that the analytes is still strongly linked to the solid sample and/or the presence of paper particles suspended in the vial might be interfering in releasing kinetics of the analytes from matrix to the sample headspace. With that in mind, we decided to include a previous sample preparation step before SPME for extraction

and separation of the analytes from the solid paper matrix. A fixed amount of sample (500 mg) was weighted directly in a 4 mL amber vial in which was added 3 mL of acetone and immediately sonicated for an optimized preset time. The optimization of sonication time of a spiked sample at 10.0 ng g^{-1} level revealed that 30 min of incubation were adequate for TCA, TBA, and PCA extraction. Longer sonication did not improve the result and – in case of TCA and TBA – even led to a somewhat less efficient extraction. After this time, 500 µL of this extract was collected and submitted to the optimized SPME procedure. Satisfactory recoveries between 70 and 100% were obtained. The direct injection of 1.5 µL of the extract was performed for comparison with SPME of 500 µL of the extract. Peak areas for TCA, TBA and PCA were, respectively 15, 24 and 36 times higher when used SPME instead of direct injection of the extract. This is a very important result that confirms the necessity of the SPME step for the high sensitivity and good accuracy of the proposed methodology.

Analytical figures of Merit

The analytical figures of merit for the proposed methodology were evaluated and are presented in Table 1. Quantification and detection limits were obtained as 10 and 3 s/S, respectively, where s is the standard deviation estimation of the linear coefficient from the calibration curve and S is the slope. Using the proposed methodology (500 mg of paper sample, 3 mL of acetone and collecting 500 µL of the extract and the optimized SPME extraction procedure) the detection limits for the methodology are 0.43 ng g^{-1}, 1.32 ng g^{-1} e 0.79 ng g^{-1}, respectively for TCA, TBA and PCA, which are suitable limits for detection of these compounds in paper samples. The analytical curve has an excellent correlation for all the target analytes ($R > 0.999$) in the range of concentration studied. Repeatability of the methodology, evaluated as relative standard deviation, was satisfactory for all compounds (RSD <10%, 20 ng L^{-1}, $n = 5$).

Table 1. Analytical figures of merit of the HS-SPME methodology

Compounds	LD (ng L^{-1})[a]	LQ (ng L^{-1})[b]	Linear range (ng L^{-1})[c]	R[d]	RSD (%)[e]
TCA	1.8	6.0	1.0–50	0.9998	7.8
TBA	5.5	18.4	1.0–50	0.9999	6.5
PCA	3.3	10.9	1.0–50	0.9999	5.3

[a] Detection limit calculated from the standard deviation estimation of the linear coefficient of calibration curve (3 s/S); [b] quantification limit (10 s/S); [c] studied linear range; [d] correlation coefficient; [e] relative standard deviation, 20 ng L^{-1}, $n = 5$.

Table 2. Recoveries obtained for the three paper samples

Compounds	Recovery (% ± SD)		
	Kraft liner	Test liner	Miolo
TCA	85.7 ± 9.3	93.9 ± 9.1	93.2 ± 9.6
TBA	100.6 ± 11.7	69.8 ± 6.3	73.8 ± 7.0
PCA	100.5 ± 14.4	85.8 ± 9.1	72.6 ± 6.3

In literature, only one article was found concerning the determination of haloanisoles (2,4,6-trichloroanisole, 2,3,4,6-tetrachloroanisole and pentachloroanisole) in paper for food packaging. The sample preparation presented by Whitfield et al. was steam distillation solvent extraction (SDE), in which was used 25 g of the paper sample, 1.5 L of water and 30 mL of a pentane/ether (9:1) solution. The extract of distillation was concentrated by evaporation of solvent under N_2 stream. The concentrated extract was then injected in the GC-MS for separation and quantification. The detection limits were evaluated in 0.01 µg kg^{-1} in the linear range of 0.1–100 µg kg^{-1}. Even with this methodology presenting lower detection limits than those proposed in our work, this methodology uses a large amount of sample, besides employing high amounts of hazardous, expensive and difficult discarding solvents.

Three lots of paper samples Kraft liner, Test liner and Miolo were analysed each in three replicates. TCA, TBA and PCA concentration in all paper samples were below detection limit of the analytical methodology. In order to check the trueness of the methodology, recovery tests were performed. The samples were spiked at 10 ng g^{-1} level of each target analyte with a standard stock solution in acetone, were aged one for week before analysis and then analysed in triplicate. The results are presented in Table 2.

Satisfactory recoveries from about 70 to 100% for all compounds were obtained, taking into account the difficulties of treating solid samples for gas chromatographic determination.

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