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Optimisation of solid-phase microextraction coupled to gas chromatography for determination of phenolic compounds in smoked herring

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Abstract

Solid-phase microextraction coupled to gas chromatography was evaluated for analysis of phenolic compounds in herring fillet samples, using a polyacrylate fibre (film thickness: 85 μm). An experimental design approach determined that time and temperature, the main parameters affecting the extraction process, were optimal at approximately 55 min and 50 °C. The effects of different amounts of fillet on percent recovery of phenolic compounds were then studied. Finally, the method developed was used to determine phenolic compound content in smoked herring fillets. The results showed good correlation ($R^2 = 0.96$) with those obtained by simultaneous distillation extraction.

Keywords: Gas chromatography; Herring; Phenolic compounds; Solid-phase microextraction; Smoking

1. Introduction

The smoking of fish, a traditional preservation process still used in modern industrial applications, is also responsible for significant modifications of the organoleptic properties of fishmeal. It is generally agreed that phenolic compounds are the major agents involved in the preservation and organoleptic properties of smoked products (Kjällstrand & Petersson, 2001) The relative concentrations of phenolic compounds in products depend on the nature of the wood used for smoking, the method of smoke generation, pyrolysis temperature, and the smoking process (Cardinal, Berdagué, Dinel, Knockaert, & Vallet, 1997). The chemical features of meat smoking have been investigated (Borys, 1995; Girard, Talon, & Sirami, 1982) and the composition of dry or liquid smoke has been assessed in several studies (Guillen & Ibargoitia, 1998; Guillen & Manzanos, 1996; Kjällstrand & Petersson, 2001). However, the composition of phenolic compounds in smoked fishmeal has not often been studied (Mc Gill, Murray, & Hardy, 1985).

Analysis of phenolic compounds is currently based on liquid–liquid extraction or steam distillation followed by gas chromatography. These methods are time-consuming and require the use of organic solvents, which are dangerous for health and the environment. In recent years, a new analytic technique known as solid-phase microextraction (SPME) has been developed by Pawliszyn (1997). SPME coupled to gas chromatography has been applied for the analysis of many organic compounds, particularly phenolic compounds in water or soil (Baciocchi, Attinà, Lombardi, & Boni, 2001; Barták & Lubomír, 1997; González-Toledo, Prat, & Alpendurada, 2001; Ohlenbusch, Kumke, & Frimmel, 2000). However, no studies of the extraction and quantification of phenolic compounds in a complex matrix such as fish muscle have been reported.

The purpose of the present study was to assess the use of SPME coupled to gas chromatography for extraction and determination of the main phenolic compounds in smoked herring fillets. The SPME method was first optimised by using an experimental design approach on parameters such as extraction temperature and time. Time desorption of fibre in the injection port and fish muscle mass were also optimised. SPME determinations of the phenolic

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compound content of smoked herring were performed using an internal standard to overcome matrix problems. The results were compared with those obtained by simultaneous distillation extraction.

2. Experimental

2.1. Instrumentation and reagents

Phenol compounds (phenol, *p*-cresol, *o*-cresol, guaiacol, 4-methyl guaiacol, 4-ethyl guaiacol, syringol, eugenol, 4-propyl guaiacol, isoeugenol and 2-chlorophenol, purity < 99%) were purchased from Sigma-Aldrich (l'Ile d'Abeau, France). Stock standard solutions of phenolic compounds were prepared by dissolution in HPLC grade methanol from Carlo Erba (Val de Reuil, France). All solutions were stored in brown glass bottles at −20 °C.

2.2. Apparatus

Gas chromatography analysis was performed on a Trace GC 2000 apparatus (from ThermoFinnigan, Les Ulis, France) equipped with a split/splitless injector and FID detector. A capillary column (DB1, J&W Scientific, Folsom, CA, USA; 30 m×0.25 mm I.D. $d_f = 0.25$ μm) was used to separate phenolic compounds. Injector and detector temperatures were 280 °C, the flow rate of carrier gas (helium) was 1 ml/min, and oven temperature was programmed from 50 °C (1 min) to 80 °C (1 min) at 4 °C/min, then to 150 °C at 2 °C/min, and finally to 280 °C (10 min) at 10 °C/min. Chromatographic data acquisition and processing were carried out using ChromCard software version 1.07 (Thermoquest, Milan, Italy).

SPME was performed using a commercially available polyacrylate fibre (film thickness: 85 μm) housed in a manual holder (Supelco l'Ile d'Abeau, France). Before initial use, fibres were conditioned in the injection port of the gas chromatograph at 300 °C for 2 h.

2.3. Fish sample

Frozen herring fillets were obtained from a local fish and seafood wholesaler (Nantes, France). Two techniques were used for smoking the fillets: a traditional method using smoke production by pyrolysis (450 °C) of sawdust from beech wood, and a method using atomisation of liquid smoke (Lutetia, France). In both cases, fillets were hand-salted with refined salt (Salins du Midi, France), left for 6 h at 12 °C, and then rapidly rinsed with water (15 °C) before storage in a cold room at 2 °C until smoking. Smoking was performed in an HMI Thirode (PC90 Model) apparatus (Thirode, France) set at 32 °C.

2.4. Solid-phase microextraction procedure

2.4.1. Optimisation of extraction time and temperature

The optimal conditions for extraction of phenolic compounds from herring fillets were obtained by means of a central composite experimental design (Box & Draper, 1998), including $2^2 = 4$ factorial experiments and 6 axial points. Repetition of the central points allowed the error of repeatability to be calculated. Each experiment was performed in duplicate.

The intervals of factor variation (time and temperature) were determined in preliminary tests. The time interval was between 30 and 70 min, and the temperature interval between 30 and 70 °C. The amounts of extracted phenolic compounds were determined by comparison with a calibration curve.

Fillet homogenates were prepared by homogenising 10 g of herring fillet in 50 ml of NaCl (30%) solution, acidified to pH 2 (Alpendurada, 2000; González-Toledo et al., 2001; Helaleh, Fujii, & Korenaga, 2001). Twenty-five microlitres of phenolic compound mixtures (0.4, 0.8, 1.2, 2.0 mg/ml) containing 2.0 mg/ml of internal standard, prepared from a stock mixture (4 mg/ml), were added to the homogenate. An experimental design was performed for each amount of phenolic compound (10, 20, 30 and 50 μg/10 g of fish muscle). After the content was mixed, vials were placed in a water bath (temperature within the interval of variation) under constant stirring and allowed to equilibrate for 15 min. For each experiment, the fibre was introduced into the vial headspace and held for different time periods within the interval of variation. The fibre was then removed from the headspace and desorbed at the GC injection port (splitless mode) for 3 min.

2.4.2. Optimisation of desorption time

After removal from the headspace, the fibre was placed in the GC injection port, and the splitless mode was maintained for 2, 3, or 5 min.

2.4.3. Optimisation of fish fillet amounts

Various amounts of fish muscle (2, 5 and 10 g) were homogenised in NaCl solution. The homogenates were spiked with phenolic compound mixtures in the range of 30–5000 μg/100 g of muscle. These concentrations were chosen on the basis of previous results for phenolic compound content in smoked herring obtained by simultaneous distillation extraction (unpublished results). Phenolic compounds were extracted in the optimal conditions given by the experimental design. Yield and linearity of extraction were assessed for the three amounts of muscle.

2.5. Statistical analysis

All statistical tests (ANOVA, experimental design) were performed using Statgraphics plus software, version 4.0 (Manugistics, Rockville, MD, USA).

3. Results and discussion

3.1. Optimisation of extraction time and temperature

Variance analysis made it possible to distinguish two models. For the first model, the influence of time and temperature among the linear terms, the quadratic effects of time and temperature, and the interaction between time and temperature were statistically significant (P-value < 0.05). This model was fitted to the extraction of *p*-cresol, *o*-cresol, guaiacol, and syringol. For the second model, which was fitted to the extraction of 4-ethyl guaiacol, 4-methylguaiacol, eugenol, and phenol, only the quadratic effect of times and the linear time effect were statistically significant. The results for the Pareto chart show that extraction time for the first model had the greatest effect on phenolic compound extraction, followed in decreasing order of importance by the time quadratic effect, the temperature effect, the temperature quadratic effect, and interaction between time and temperature. For the second model, the quadratic effect of time had the greatest influence on phenolic compound extraction. The coefficient of determination of the two models for extraction of all studied compounds was highly significant (> 0.95), indicating that the models fit the data correctly. Therefore, these models were used as prediction tools to optimise the conditions of phenolic compound extraction with SPME.

Fig. 1A, which provides a three-dimensional representation of the polynomial within the experimental interval, visualises the response surface for *p*-cresol (corresponding to the first model). This extraction model shows an increase in the amount of extract with time, which reaches equilibrium at about 55–60 min. A different behaviour, corresponding to the second model, was observed for the extraction of compounds such as 4-ethyl guaiacol, 4-methylguaiacol, eugenol, and phenol (Fig. 1B). The amount extracted increased with time until a maximum was reached at about 55 min. A further time increment reduced the extracted amount of these compounds. This pattern can be attributed to the strong quadratic effect of time. Similar results were obtained by Sukola, Koziel, Augusto, and Pawliszyn, (2001) for SPME of aromatic hydrocarbon. The first compounds (*p*-cresol, *o*-cresol, guaiacol, and syringol) might have higher affinities for the adsorptive coating of fibre and tend to displace the second compounds (4-ethyl guaiacol, 4-methylguaiacol, eugenol, and phenol), which are less strongly adsorbed. As extraction time increased, more of the second compounds was desorbed. Competition with endogenous volatile compounds of fish muscle might also have occurred. Temperature curves for both models reached a maximum indicative of optimal efficiency at about 50 °C. This pattern may have been due to an increase of analytes in the gaseous phase as extraction temperature increased. Once maximum adsorption on fibre was attained, a further increment of temperature might have caused a decrease in the distribution coefficient (Luan, Li, & Zhang, 2000). On the basis of these results, subsequent SPME of phenolic compounds was performed under the following conditions: a vial containing fish muscle homogenate was placed in a water bath at 50 °C and allowed to equilibrate for 15 min. The fibre was then introduced into the vial headspace and held for 55 min before being removed and desorbed at the GC injection port (splitless mode).

3.2. Effect of desorption time and determination of precision

Ten grams of fish homogenate were spiked with 25 µl of phenolic compound mixture (1.2 mg/ml). SPME was then performed under the optimal conditions described above. Optimal desorption time was determined by varying the desorption time of polyacrylate fibre from 2.0 min to 5.0 min in the GC injector. The results showed that the desorbed amount of phenolic compounds was the same, regardless of desorption time, indicating that they were easily desorbed. Thus, a period of 3 min was chosen for subsequent experiments.

The precision of SPME analysis was determined by performing seven replications under the previously described conditions. The relative standard deviation (RSD) ranged between 5.45 and 8.70% for the studied compounds. Guaiacol and 4-methylguaiacol had the lowest RSD values (5.65 and 5.45%, respectively), whereas the highest values were obtained with syringol and isoeugenol (8.70 and 8.25, respectively). The internal standard (2-chlorophenol) was detected with a good RSD value (7.7%). Thus, the precision of SPME for the extraction of the studied phenolic compounds was considered acceptable.

3.3. Optimisation of fish muscle mass in homogenate

The effect of fish fillet mass in homogenate on percent recovery and linearity of extraction was investigated under the optimal conditions described above.

The results (Fig. 2) show that the amount of phenolic compounds extracted by SPME increased when the amount in the sample increased (i.e. when the concentration of phenolic compounds in the homogenate increased), or when there was an increase in homogenate mass for a given concentration. However, when the amount of extracted compounds was linear ($R^2 = 0.999$) for 10 and 5 g of fish muscle, a non-linear curve was obtained for a 2-g mass. Moreover, a negative relation was obtained when an increasing mass (2, 5, 10 g) of fish was spiked with a constant amount of analyte (Fig. 3). These observations were valid for all studied phenolic compounds.

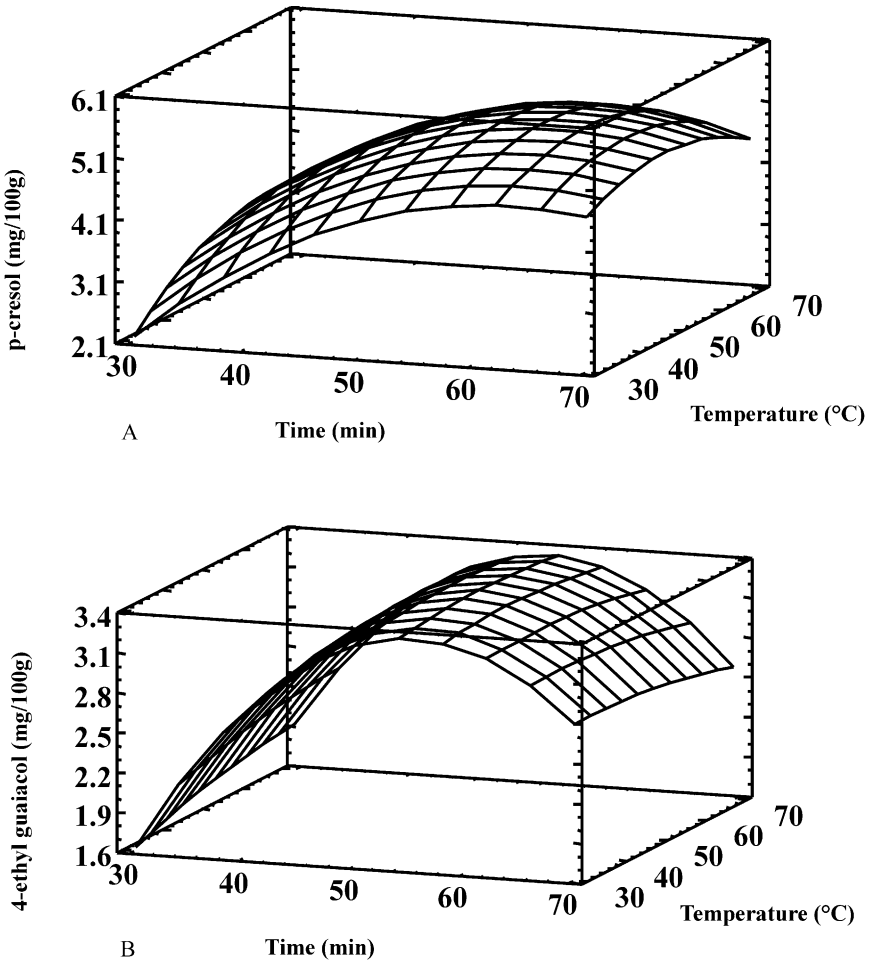

Fig. 1. Response surface for the effect of time and temperature on solid-phase microextraction of *p*-cresol (A) and of 4-ethyl guaiacol (B).

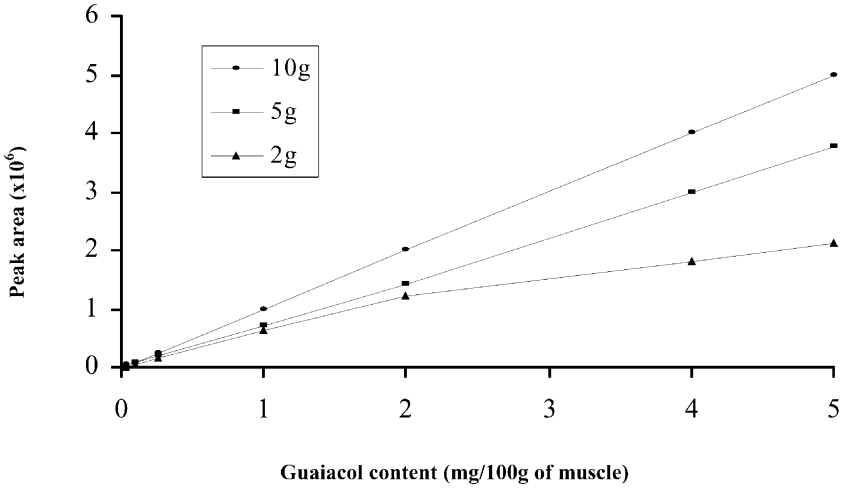

Fig. 2. Extraction of guaiacol in relation to its content in homogenate. Three amounts of muscle were studied (2, 5 and 10 g).

The results are of particular interest in terms of percent recovery. Firstly, even though percent recoveries were very low for all phenolic compounds, the results show clear differences: *o*-cresol and *p*-cresol were extracted with a percent recovery close to 0.045% (5 g of fish muscle), while percent recovery of 4-ethylguaiacol and phenol was close to 0.03% and that of guaiacol at about 0.02%. Secondly, similar values were observed when the same fillet mass was spiked with different amounts of phenolic compounds (Fig. 4). However

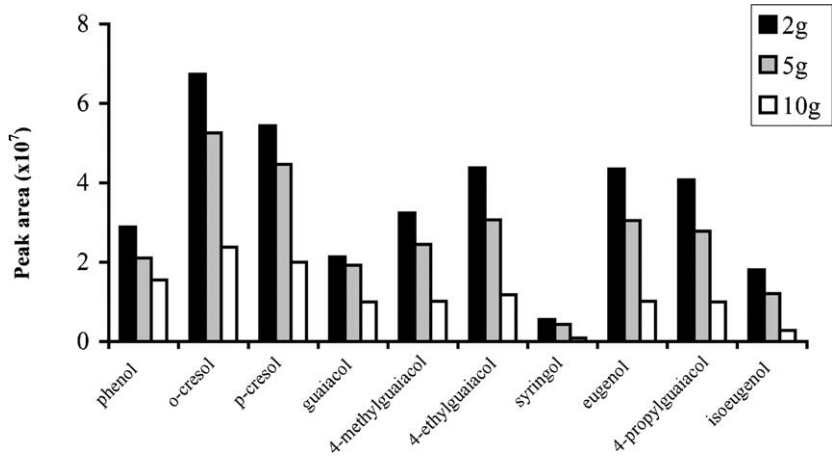

Fig. 3. Influence of the amount of herring muscle on solid-phase micro extraction of compounds (in this case the homogenate was spiked with 100 μg of each phenolic compound).

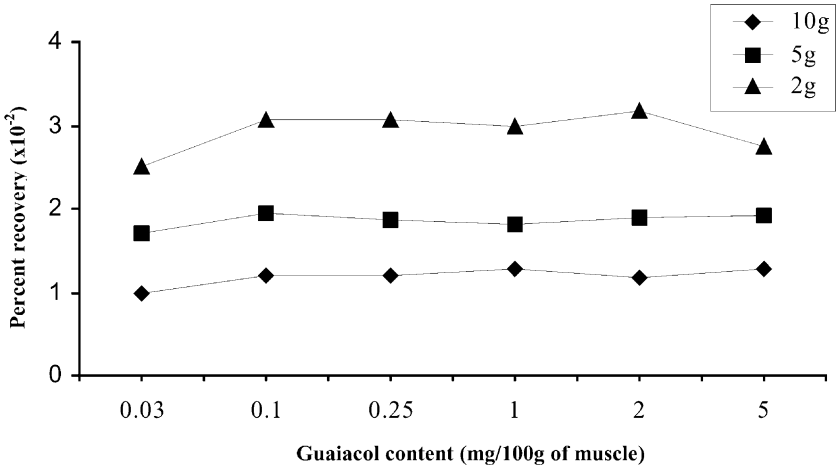

Fig. 4. Influence of guaiacol concentration on its percent recovery for three amounts of herring muscle in homogenate.

when the homogenate contained 2 g of fish fillet, percent recoveries were decreased for both high and low concentrations. This confirms the lack of linearity observed previously. Moreover, percent recoveries decreased when the fish mass in homogenate increased, regardless of the phenolic compound. The mechanism of headspace SPME is based on equilibrium between analyte concentration in the headspace and the solid-phase fibre coating. The amount of analytes in the headspace results from equilibrium between the analyte concentration in the sample and that in the gas phase. Extraction time and temperature determine the diffusion of analytes in headspace and thus their trapping on the fibre, which is maximal under equilibrium conditions. For the same concentration (g of phenolic compounds/100 g of muscle), the amount of analytes in headspace increased when the amount of fish fillet in homogenate was increased, which caused an increase in the concentration of analytes in the solid-phase fibre coating. The negative relationship between percent recoveries and fillet mass in homogenate for a given concentration confirms one of the major features of the SPME, i.e. the volume of the fibre coating is extremely small. Thus the amount of analyte extracted by coating is insignificant compared to the amount of analyte in headspace (Zhang & Pawliszyn, 1993). Therefore a low amount of analyte in the headspace (low amount of analyte in the sample) results in a higher ratio of analyte absorbed by coating/analyte in gas phase than a high amount of analyte in headspace (high amount of analyte in the sample).

In a previous study, the recovery of phenolic compounds was close to 100% when analytes were extracted from an aqueous system (Clark & Bunch, 1997). However, Wilkes et al. (2000) found that partitioning into the gas phase was strongly reduced by the presence of a food matrix. The low recoveries obtained in our study and the negative effect of fish mass in homogenate tend to confirm this observation. In fact, strong interactions between phenolic compounds and fish muscle components could occur in these conditions.

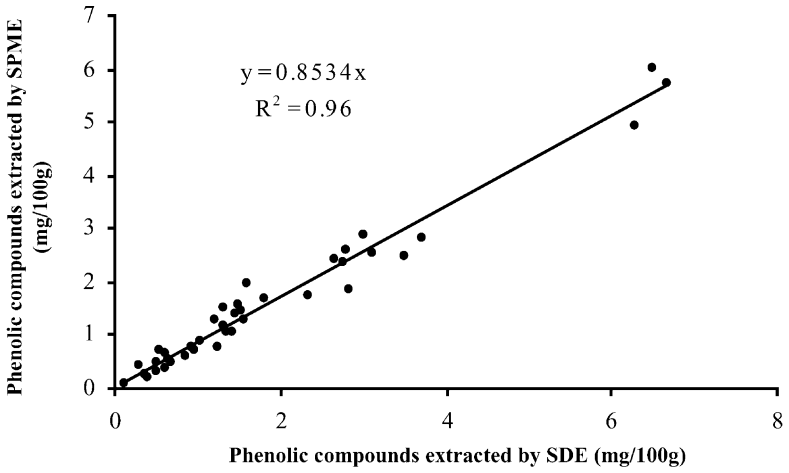

Fig. 5. Comparison of solid-phase microextraction and simultaneous distillation extraction for the extraction of phenolic compounds from smoked herring fillets.

3.4. Validation and application of the method: determination of phenolic compounds in smoked herring

SPME under the optimal conditions described above was used to assess phenolic compounds in smoked herring fillets. The content of the ten phenolic compounds studied was determined by SPME and SDE in herring fillets smoked for 1, 2, 3, and 4 h by the traditional process and for 4 h by the liquid smoke atomisation process. SPME was performed on 5 and 10 g of smoked herring fillet. Phenolic compound content determination was improved by using an internal standard (2-chlorophenol), and calibration curves, constructed by spiking 5 or 10 g of non-smoked herring muscle with phenolic compound standard mixtures. The accuracy of the SPME method was tested by comparing the phenolic compound contents obtained with those obtained with SDE. The results for SPME relative to 5 and 10 g of muscle were highly correlated ($R^2 = 0.99$), which confirmed the direct linearity between analyte concentration in the sample and in the solid-phase fibre coating. However, this result was obtained only when the matrix used to elaborate the calibration curve was the same as that used for analyte determination (same mass and same fish species muscle). The correlation between the results obtained by SPME and SDE was acceptable ($R^2 = 0.96$, 0.95), regardless of fish muscle mass (Fig. 5). SPME tended to underestimate phenolic compound content, particularly when a phenolic compound concentration was high in the sample.

4. Conclusion

This work shows that HS-SPME using polyacrylate fibre is a suitable method for determining phenolic compound content in smoked herring. A careful choice of conditions by means of an experimental design procedure allows quantification of phenolic compounds in a complex matrix such as fish muscle, despite low percent recovery of analytes. Precision and linearity were good when optimal extraction time and temperature were determined.

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