ELSEVIER

Journal of Chromatography A, 954 (2002) 51–57

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Headspace solid-phase microextraction of higher fatty acid ethyl esters in white rum aroma

J. Pino[a], M.P. Martí[b], M. Mestres[b], J. Pérez[a], O. Busto[b], J. Guasch[b,*]

[a]*Instituto de Investigaciones para la Industria Alimenticia, Carretera del Guatao km 3½, 19200 La Habana, Cuba*

[b]*Departament de Química Analítica i Química Orgànica (Unitat d'Enologia, CeRTA), Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili, Avda. Ramón y Cajal 70, E-43005 Tarragona, Spain*

Received 12 September 2001; received in revised form 17 December 2001; accepted 14 February 2002

Abstract

Fatty acid ethyl esters are the main components of rum aroma and play an important sensorial impact in these distilled alcoholic beverages. Herein, a method for analysing these volatile compounds is described. It involves a separation and concentration step using headspace solid-phase microextraction and determination by capillary gas chromatography using flame ionisation detection. The influence of different parameters related to the isolation and concentration step, such as ethanol concentration, ionic strength, sample volume, time and temperature of extraction, was studied. The developed method enabled recoveries >91% for the analyzed compounds with limits of detection between 0.007 and 0.027 mg/l, all of them lower than the range of concentrations found in rum samples. The method was successfully applied to the analysis of fatty acid ethyl esters in different commercial white rums.

Keywords: Headspace analysis; Solid-phase microextraction; Aroma compounds; Fatty acid ethyl esters

1. Introduction

Rum is a fairly tasteless and neutral spirit mainly derived from the fermentation and distillation of sugar molasses. Once the alcohol has been obtained from the fermentation and distillation processes, it undergoes further processing such as passing through carbon filters, ageing in oak barrels and blending, which give rum its characteristic aroma.

Distilled alcoholic beverages have been distinguished by the presence of characteristic volatile components coming from the raw materials used in fermentation, distillation and ageing. These components are mainly fusel alcohols, ethyl acetate, acetic acid, fatty acids and esters [1–3]. Fusel alcohols, acetic acid and ethyl acetate are present in these beverages at relatively large amounts, generally between 10 and 4000 mg per l of ethanol, and they can be determined directly by gas chromatography (GC). However, fatty acid ethyl esters are usually present at concentrations below 10 mg per l of ethanol and their determination requires the use of a preconcentration technique.

Solid-phase microextraction (SPME) is a solvent-free extraction technique that enables the extraction and concentration steps to be carried out simultaneously [4–9]. It has been shown to be a very suitable technique for the analysis of volatile and

*Corresponding author.

E-mail address: qaenol@fe.urv.es (J. Guasch).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00167-X

semi-volatile compounds in alcoholic beverages [10–14].

In the SPME, a fused-silica microfiber coated with a stationary phase, which is immersed either directly into the liquid sample (DI-SPME) or into the headspace above it (HS-SPME), is used. Recently, direct sampling has been shown to be very efficient for extracting various esters in vodkas and white rums [12]. However, HS-SPME is preferable to DI-SPME to isolate the volatile compounds, because it allows shorter extraction times [5] and the lifetime of the fibre is longer.

Taking into account these observations, the purpose of this work is the development of a method for determining higher fatty acid ethyl esters in white rum aroma using HS-SPME as extraction technique and GC–FID as determination technique.

2. Experimental

2.1. Chemicals and reagents

The fatty acid ethyl esters studied were (CAS number in brackets): ethyl hexanoate [123-66-0], ethyl octanoate [106-32-1], ethyl decanoate [110-38-3] and ethyl dodecanoate [106-33-2]. Methyl octanoate [111-11-5] was used as internal standard (istd). They were supplied by Aldrich (Beerse, Belgium) and all had purity above 98%.

An individual standard solution of 5000 mg/l of each ester was prepared in HPLC-grade ethanol (Scharlau, Barcelona, Spain) and it was stored at 5 °C. A global standard solution containing all the esters was prepared with an aliquot of each individual standard solution and subsequent dilution with ethanol. Working solutions used in further studies were prepared by adding different amounts of the global standard solution to an ethanolic solution (12% v/v) that contained 2 mg/l of 3-methylbutanol (Aldrich, Beerse, Belgium) in order to obtain a matrix as similar as possible to a real rum.

2.2. Headspace and SPME

The SPME holder, for manual sampling, and the polydimethylsiloxane fibres (100 μm) used in this study were purchased from Supelco (Bellefonte, USA). The fibres were conditioned by inserting them into the GC injector at 250 °C for 1 h and they were immediately used to prevent contamination.

In order to obtain optimal results, the experimental conditions were studied (Section 3.1). In the optimised procedure 10 ml of sample were placed in a 20-ml vial with 0.88 g of NaCl and were spiked with methyl octanoate as internal standard (0.5 mg/l). Then the vial was hermetically sealed with a silicone septum and shaken to obtain a homogeneous mixture. The HS-SPME of the sample was carried out at 30 °C in a thermostatic bath for 35 min with constant magnetic stirring (500 rpm). When the extraction step was finished, the SPME fibre was removed from the vial and inserted into the injection port of the GC for thermal desorption of the analytes at 250 °C, for 1 min, in splitless mode. For every sample, at least a duplicate analysis was carried out.

2.3. Chromatography

The analyses were carried out on a Hewlett-Packard 6890 gas chromatograph equipped with a flame ionisation detector (FID). The injection was made in splitless mode for 1 min using an inlet of 0.75 mm I.D., which improves the GC resolution. The temperature of the injector and detector was 250 °C. The separations were performed using a SPB-5 column (30 m×0.25 mm I.D., 0.25 μm) with an oven temperature program of 60 °C (2 min), 4 °C/min to 250 °C (20 min). The carrier gas was helium with a flow-rate of 1 ml/min.

To identify the esters and other rum volatiles, which were also extracted by the fibre, a Hewlett-Packard series 6890 (series II) gas chromatograph equipped with a HP-5973 mass-selective detector was used. The chromatographic conditions were the same as those described for GC–FID. The detector operated in electron impact mode (70 eV) at 230 °C. Detection was carried out in the scan mode between 30 and 400 amu.

3. Results and discussion

3.1. HS-SPME parameter optimisation

The parameters optimised were ethanol concen-

tration, sample ionic strength, sample volume, extraction time and temperature of the sample during extraction. In each experimental point, four samples were analyzed. The possible interference of the matrix was also taken into account.

The first parameter studied was the ethanol concentration. This alcohol is one of the major rum constituents and can compete with the other volatile components in the extraction process. In fact, some authors [15–18] have found that an increase in the ethanol content decreases the extraction efficiency. Furthermore, a high ethanol content can modify the nature of the matrix. Corner et al. showed that an amount of ethanol higher than 17% (v/v) increases the esters' solubility into the liquid sample and, therefore, the headspace concentration of these compounds is reduced [19]. To check these effects, samples of rum containing 40% v/v of ethanol were diluted to obtain different solutions with an alcohol content of 5, 12 and 20% v/v, which were subsequently analysed. In the extraction, 25 ml of sample were placed in a 50-ml vial with 6 g of NaCl. The process was carried out at 25 °C, for 30 min. The data obtained (Fig. 1) show that the higher the ethanol concentration, the lower the extraction efficiency; however, with 5% of ethanol there was also a great dilution of the other volatile components. Therefore 12% of ethanol was fixed for subsequent analysis.

Another consideration that had to be taken into account was that, in some cases, the ethanol contents of rums differ by ±2% v/v from the alleged 40% v/v. After determining how these changes could affect the fatty acid esters profile, we concluded that these small variations in ethanol content do not affect significantly the response of the compounds analysed provided that an internal standard is used.

The addition of salt to the sample (salting out

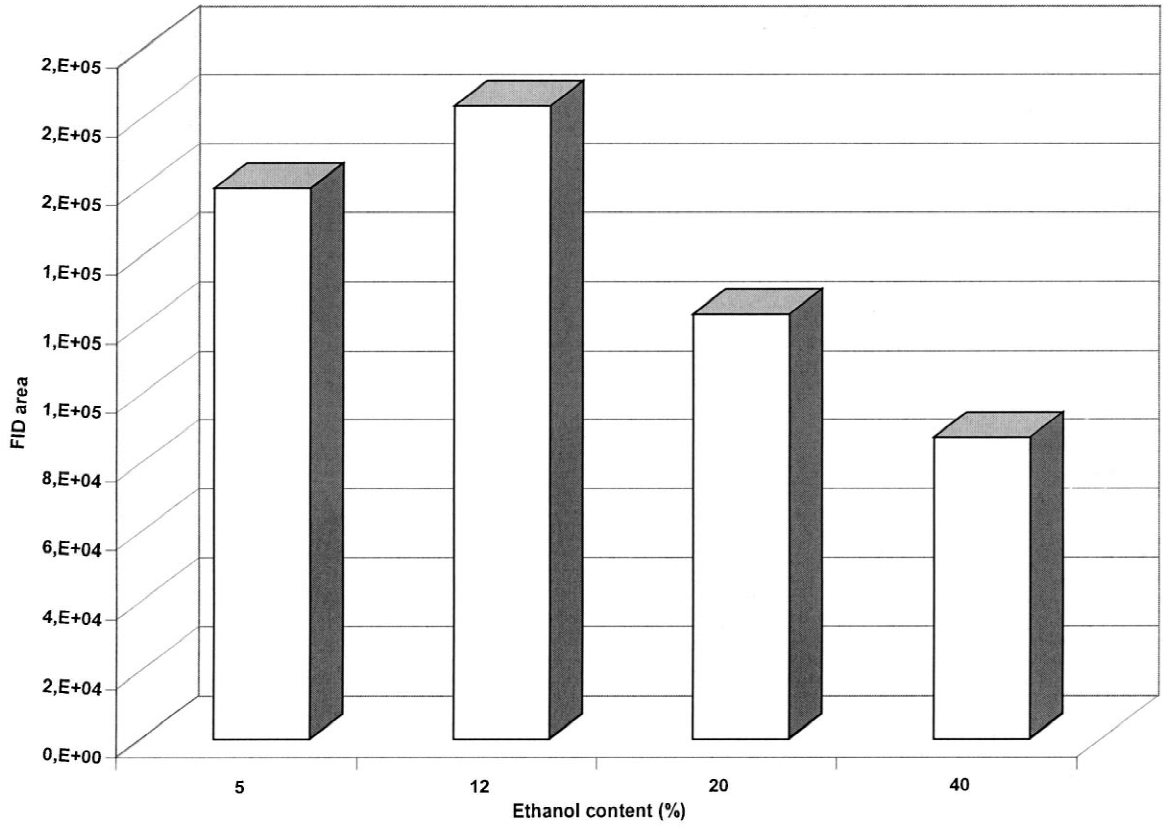

Fig. 1. Effect of ethanol content on the responses of analytes (summary of all the areas of the ethyl esters).

effect) can modify the extraction efficiency. It seems that the nature of the matrix can be modified by adding a salt because this addition can affect the liquid–gas partition coefficients of the analytes [6]. Therefore, the effect of the salt was investigated and several experiments were made with the same extraction conditions as in the previous study, but different amounts of sodium chloride (0–5 *M*) were added to a hydroalcoholic matrix (12% ethanol). As it is shown in Fig. 2, there are two types of behaviour: (a) for ethanol, adsorption increases when the salt concentration also increases; (b) for 3-methylbutanol, ethyloctanoate and ethyldecanoate, adsorption increases initially and then levels off at higher salt concentration. Since ethanol and 3-methylbutanol are major rum components and they can be measured by direct GC analysis without a concentration step, 3 *M* concentration was selected in all the HS-SPME experiences in order to increase ester extraction but not the alcohol extraction.

Once the salt concentration was fixed, the influence of the sample volume was also tested by working at a constant ratio of liquid–gas phases (1:1) with 20- and 50-ml vials. Absorption times of 30 min at 25 °C in 20-ml vials gave higher extractions than those obtained with 50-ml vials. This fact can be due to the reduction of the equilibration time when a smaller headspace is used because the analytes would take less time to diffuse through the headspace of the fibre [20]. Thus, a sample volume of 10 ml in a 20-ml vial was selected.

The SPME is strongly influenced by the sample temperature during extraction because the partition coefficients are temperature-dependent and the extraction of the analytes by the fibre is an exothermic process [5]. Furthermore, the sample temperature is closely related to the extraction time, so both parameters were studied simultaneously. Periods of time of 10, 35 and 60 min were tested at 10, 30 and 50 °C. In these extractions the already ionic strength, sample volume and vial volume conditions were used. By way of example, the results for ethanol, ethyl octanoate and ethyl decanoate are shown in Fig. 3. The best results were obtained in 35 min at 30 °C, since at this experimental point there is a high extraction of ethyl esters, while the ethanol extraction is minimized.

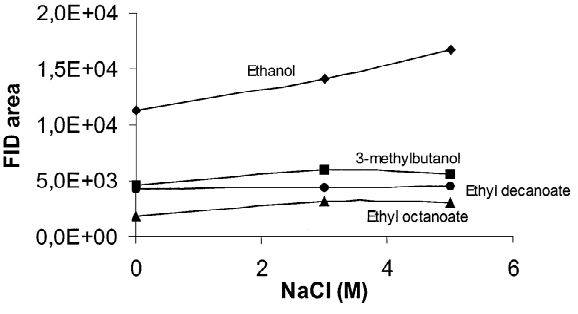

Fig. 2. Effect of salt concentration on the responses of the analytes.

3.2. Method validation

To check the matrix interference on the extraction process, a hydroalcoholic solution 12% (v/v) of ethanol spiked with different concentrations of fatty acid esters, and different rums diluted to 12% (v/v) of ethanol and also spiked with the same analytes at different concentration levels, were analysed under the optimal conditions of the extraction procedure. The calibration curves obtained from the analyses of the different ethanolic solutions and of the diluted rums were compared with the computer programme ULC (Univariate Linear Calibration) [21]. No differences at 95% confidence were observed between slopes of both curves for fatty acid ethyl esters. So, for determining the ester concentration in real samples, the calibration curves constructed with ethanolic solutions were used. Table 1 shows that the calibration curves were linear over the selected concentration ranges, with high determination coefficients ($r^2 > 0.99$). The limits of detection (LODs) ranged between 0.015 and 0.070 mg/l.

In order to calculate the recovery of the method, the matched matrix standard method was used. This parameter was determined as the quotient between the amount of analyte calculated from the calibration curves and the real amount of the analyte in the sample. This real concentration corresponds to initial concentration, which is determined using the standard addition technique, plus the quantity of analyte added. To calculate the recovery the analytes were added to a rum sample at three different concentrations (lower, middle and higher level) of the calibration range specified in Table 1. Table 2 shows the average recoveries with their relative standard

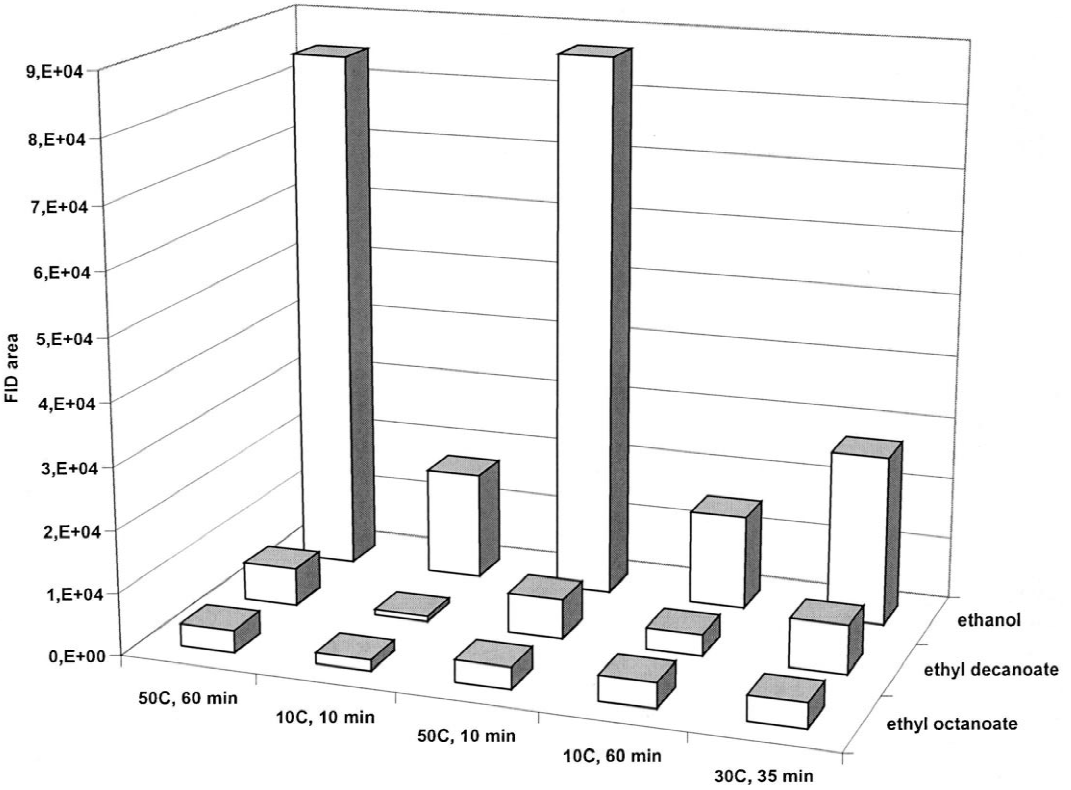

Fig. 3. Effect of time and sample temperature on the responses of the analytes.

Table 1
Parameters of the calibration curves and limits of detection of the method (LOD)

Compound	Range (mg/l)	Slope	Intercept	R^2	LOD (mg/l)
Ethyl hexanoate	0.05–2	0.3476	0.0140	0.998	0.007
Ethyl octanoate	0.05–1	5.2501	−0.0085	0.996	0.018
Ethyl decanoate	0.2–1	16.8630	−0.0142	0.998	0.015
Ethyl dodecanoate	0.1–2	3.2138	−0.0842	0.995	0.027

Table 2
Recovery percentages and relative standard deviations (in parentheses)

Compound	Low level	Middle level	High level
Ethyl hexanoate	99.6 (17.2)	96.8 (8.7)	99.7 (12.9)
Ethyl octanoate	100 (3.7)	100.6 (0.9)	99.6 (0.6)
Ethyl decanoate	100 (12.9)	105.6 (8.0)	100 (5.8)
Ethyl dodecanoate	98.7 (5.5)	91.1 (11.8)	99.2 (1.2)

deviations (RSDs). It can be seen that the recoveries ranged between 91 and 105% and their RSDs were lower than 20%.

The repeatability of the response ratios (analyte/internal standard) was determined using hydroalcoholic solutions (12% ethanol) with addition of different concentrations of analytes. The measurements ($n=4$) were found to be repeatable with 2–8% RSD, although for some analytes the precision deteriorated at low concentrations.

The reproducibility of the method was determined

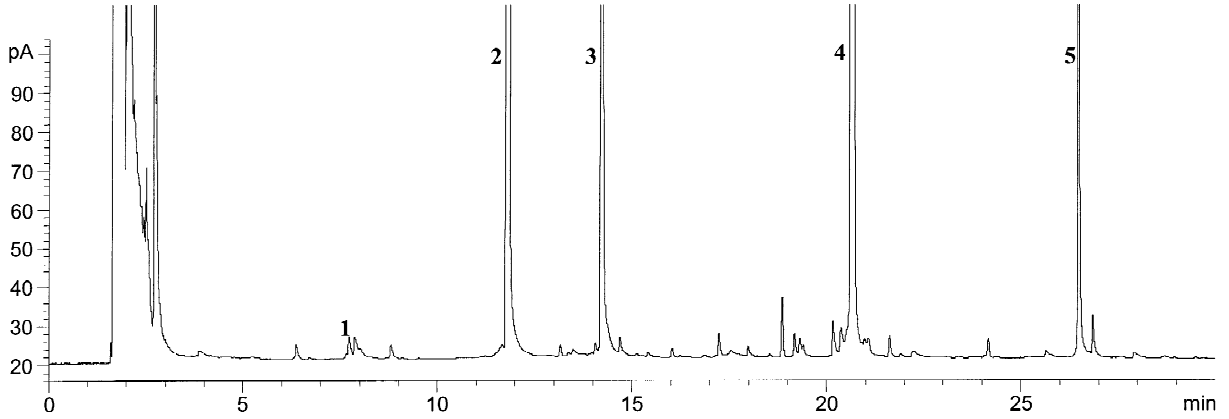

Fig. 4. Typical HS-SPME–GC analysis of a white rum (Cuban rum, 3 years old) using the proposed procedure. 1, Ethyl hexanoate; 2, methyl octanoate (istd); 3, ethyl octanoate; 4, ethyl decanoate; 5, ethyl dodecanoate.

using several rum samples which contain low and high levels of esters. The samples were analysed by duplicate, one analysis per week on two consecutive weeks. The determination of the analyte concentration was generally reproducible within 2–10% RSD.

Finally, the method was successfully applied to determine the fatty acid esters in different commercial white rums. A typical HS-SPME–GC profile of white rum is shown in Fig. 4. Table 3 shows the fatty acid ethyl ester concentration found in each rum analysed. Several distinct profiles were observed in the commercial rum brands.

4. Conclusions

Headspace solid-phase microextraction is a very simple and fast technique for determining fatty acid ethyl esters in rums and shows very good reproducibility and recovery. A synthetic matrix was used for quantitative analysis because no differences to real sample behaviour were observed. However, the high ethanol content in distilled alcoholic beverages is a problem when SPME is used in the analyses of this kind of drinks. The interference of this major rum volatile constituent was minimised by diluting the sample at 12% v/v of ethanol.

Acknowledgements

The authors wish to thank the Ministerio de Educación, Cultura y Deporte of Spain (Scientific Co-operation Program with Latin America) for the financial support given and the CICYT (project ALI97-0765) for providing Mrs. Martí's doctoral fellowship.

Table 3
Fatty acid ethyl esters (mg per l of ethanol) in commercial white rums (40% v/v ethanol)

Samples	Ethyl hexanoate	Ethyl octanoate	Ethyl decanoate	Ethyl dodecanoate
Cuban rum, 3 years old	0.15	6.80	39.17	0.75
Cuban rum, 7 years old	0.04	5.05	23.40	0.35
Cuban rum, aged	0.03	2.41	19.87	0.27
Bahamian rum	0.06	4.40	19.48	0.09
Spanish rum 1, golden	n.d.	0.65	3.79	0.03
Spanish rum 2, golden	0.04	14.14	103.33	1.59

n.d., not detected.

References

[1] H.M. Liebich, W.A. Koenig, E. Bayer, J. Chromatogr. Sci. 8 (1970) 527.
[2] G.E. Martin, R.H. Dyer, P.C. Buschemi, J. AOAC 57 (1974) 610.
[3] J. Pino, Alimentaria 269 (1996) 79.
[4] Z. Zhang, J. Pawliszyn, Anal. Chem. 65 (1993) 1843.
[5] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844.
[6] J. Pawliszyn, Solid Phase Microextraction. Theory and Practice, Wiley–VCH, New York, 1997.
[7] A.D. Harmon, Solid phase microextraction for the analysis of flavors, in: R. Marsili (Ed.), Techniques for Analyzing Food Aroma, Marcel Dekker, New York, 1997, p. 81.
[8] R. Eisert, J. Pawliszyn, Crit. Rev. Anal. Chem. 27 (2) (1997) 103.
[9] H. Kataoka, H.L. Lord, J. Pawliszyn, J. Chromatogr. A 880 (2000) 35.
[10] L. Ng, M. Hupé, J. Harnois, D. Moccia, J. Sci. Food Agric. 70 (1996) 380.
[11] D. De la Calle, M. Reichenbächer, K. Danzer, J. High Resolut. Chromatogr. 20 (1997) 665.
[12] L. Ng, in: J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, Cambridge, UK, 1999, p. 393.
[13] S.E. Ebeler, M.B. Terrien, C.E. Butzke, J. Sci. Food Agric. 80 (2000) 625.
[14] M. Mestres, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 881 (2000) 583.
[15] L. Urruty, M. Montury, J. Agric. Food Chem. 644 (1996) 3871.
[16] C. Fischer, U. Fischer, J. Agric. Food Chem. 45 (1997) 1995.
[17] M. Mestres, O. Busto, J. Guasch, J. Chromatogr. A 808 (1998) 211.
[18] M. Mestres, C. Sala, M.P. Martí, O. Busto, J. Guasch, J. Chromatogr. A 835 (1999) 137.
[19] J.M. Corner, L. Birkmyre, A. Paterson, J.R. Piggott, J. Sci. Food Agric. 77 (1998) 121.
[20] X. Yang, T. Peppard, J. Agric. Food Chem. 42 (1994) 1925.
[21] R. Boqué, F.X. Rius, J. Chem. Educ. 71 (1994) 230.