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Optimization of indirect parameters which affect the extractability of volatile aroma compounds from Idiazábal cheese using analytical supercritical fluid extractions (SFE)

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Abstract

Various sample preparation techniques and collection procedures for a supercritical fluid extraction (SFE) system were tested and compared. Special attention was paid to parameters which influence the extraction results, but are not part of the extraction process *per se*. The application of SFE to the extraction of the aroma components from unsmoked ewe's milk Idiazábal cheese was undertaken for the first time. It was, therefore, necessary to optimize and validate the extraction conditions. The parameters which were optimized included sample preparation (lyophilization and sonication); sample to adsorbent ratio; reconstitution solvent; trap type and trapping temperature. The best extraction results were achieved for a comminuted cheese sample containing added alumina (1:1 ratio with cheese) as the adsorbent and *n*-hexane:acetone (2:1) as the reconstitution solvent, and a polar octadecylsilica (ODS) trap held at a temperature of −5°C to retain volatile compounds. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Supercritical fluid extraction (SFE) is a newer method for extracting the aroma components from foodstuffs than other, more commonly used, methods, such as simultaneous distillation extraction (SDE), static headspace, and dynamic headspace, etc. (Bosset and Gauch, 1993). The SFE system has been described widely by different authors (Luque de Castro et al., 1993; Chester et al., 1994). In fact, SFE has been employed in numerous applications in food and agriculture-related fields (Polesello et al., 1993; Fischer and Jefferies, 1995; Palmer and Ting, 1995), but to date it has not been used to extract the volatile fraction of unsmoked Idiazábal ewe's milk cheese, adding a topical interest to the present study. Some of the main advantages afforded by SFE, relative to other techniques, are retention of volatile substances at temperatures below 0°C using a CO_2 based cryogenic system and transfer of analytes from the extraction solvent (supercritical CO_2) to the reconstitution solvent without the need for aggressive solvents or treatments. Since the analytes are retained in a low-temperature trap; very different types of analytes can be extracted on successive runs using differing reconstitution solvents and small volumes of reconstituted extract can be collected, thereby reducing losses of volatiles due to concentration of volatile compounds.

Concern for improving extraction procedures themselves, sometimes causes more basic aspects to be overlooked, particularly parameters which influence the extraction results, but are not part of the extraction process itself. The identification and quantification of the volatile fraction of cheese involves the steps of sample preparation, analyte extraction, trapping of analytes, reconstitution of analytes in the extract, and gas chromatographic analysis. This paper presents the results of the optimization of the conditions exerting an indirect influence on the extraction process, namely: (a) sample preparation; (b) sample to adsorbent ratio; (c) reconstitution solvent; and (d) solid trap type and temperature. The optimization of parameters directly affecting the extraction process itself and the type of analytes extracted, such as extraction temperature, pressure (within the supercritical region), flow rate, static and dynamic extraction times, etc., will be considered in a subsequent paper. The study also comprised validation of the optimization results.

During the optimization of each individual parameter, the other conditions were held constant, to

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ensure that all the results would be comparable, so that the optimum value for each of the parameters considered could be selected. Triplicate analyses were performed for each parameter and the results were expressed as the means of the three replicate trials.

2. Materials and methods

2.1. Materials

All gas chromatography standards were obtained from either Sigma Chemical Co. (St. Louis, MO, USA) or Merck (Munich, Germany). The reconstitution solvents, *n*-pentane, *n*-hexane, acetone, and methanol were purchased from Panreac (Montcada i Reixat, Spain). Column chromatography grade alumina and cellite 545 (particle size 0.01–0.04 mm) were also obtained from Sigma. Carbon dioxide (99.9999% purity) was provided by BDP International Inc. (New York, USA).

2.2. Cheese manufacture

Unsmoked Idiazábal ewe's milk cheeses were manufactured at a single factory from a single milk batch (2000 litres) on the same day in April 1995 using a mixture of locally made and commercial ovine rennet (Boll, France) and added starter (Eurozime-Ezal, France) in accordance with the procedures approved by the Regulatory Board of the Idiazábal Cheese Appellation of Origin (Ministerio de Agricultura, Pesca y Alimentación, 1993). The cheeses were collected from the manufacturing plant after 120 days of ripening at 8–9°C.

2.3. Instrumentation

2.3.1. Lyophilization

Lyophilization was performed on frozen cheese samples (20 g) using a CHRIST lyophilization apparatus (Braun Biotech Internat, Melsungen, Germany). The equipment was equilibrated at −50°C, and the sample lyophilization procedure took 6 h.

2.3.2. Sonication

Samples (weight) were homogenized and placed in screw-capped extraction cells. The cells were then placed in an ULTRASONS bath (P-Selecta, Barcelona, Spain) and sonicated for 3 min. All cells were held in crushed ice during sonication to maintain low sample temperatures.

2.4. SFE

All SFE trials were performed using a model 7680 A supercritical fluid extractor (Hewlett-Packard, Wilmington, DE, USA). The instrument was equipped with a variable restrictor that allowed independent control of the flow rate and pressure and reduced plugging during large sample throughputs.

The frozen cheese samples (1 g) were comminuted, homogenized, and weighed out to a precision of 1 mg, after which they were mixed with differing proportions (2:1, 1:1 and 1:2 ratios) of adsorbent, namely, activated alumina. A modifier, methanol (0.5 ml), was added to the mixture, which was then rehomogenized. Static addition of the modifier to the sample took place outside the extraction cell, to enhance distribution of the modifier in the homogenized sample and thereby improve system reproducibility. The preparation (cheese/absorbent/modifier) was then placed in a 7 ml extraction cell, the bottom of which was lined with a double layer of cellulose filter paper and a thin layer of cellite. The sample was covered with a thin layer of cellite and a double layer of cellulose filter paper. The extract was collected in 0.3 ml of reconstitution solvent.

2.5. Analytical gas chromatography

A Hewlett-Packard model 5890 gas chromatograph (Hewlett-Packard Co., Avondale, PA, USA) equipped with an HP Innowax cross-linked polyethylene glycol fused silica-gel capillary column (30 m × 0.32 mm I.D.; film thickness 0.25 μm) and a flame ionization detector was employed, using helium (flow rate: $2\,\mathrm{ml\,min^{-1}}$) as the carrier gas. The sample was injected in the splitless mode. The injector and detector were operated at 200 and 250°C, respectively. The signal-to-noise ratio was 1.

Gas chromatographic analysis of the Idiazábal cheese (1 μl) extracts was performed using the following temperature programme: analysis was started at 35°C for 1 min, after which the temperature was raised to 170°C at $3^\circ\mathrm{C\,min^{-1}}$ and then to 200°C at $4^\circ\mathrm{C\,min^{-1}}$, where the temperature was then held constant for 20 min. Chromatograms were recorded using two-level attenuation. Semi-quantification was achieved using camphor as the internal standard (0.035 mg of camphor per ml of methanol).

3. Results and discussion

3.1. Sample preparation

Lyophilized and non-lyophilized samples were compared, because some reports have mentioned possible competition between the water in the matrix and the supercritical carbon dioxide (Luque de Castro et al., 1993), which is immiscible in water. All the other preparation and system condition parameters were the same for the extractions of both the non-lyophilized and lyophilized cheese samples. The results (Fig. 1) indicated significant differences between the two preparation procedures. Extraction of the volatile fraction was more complete using the non-lyophilized (Fig. 1(a)) cheese sample. This may be because part of the volatile fraction is lost during lyophilization of the sample.

The use of ultrasound during sample preparation, either before or after the addition of the modifier, has also been recommended (Luque de Castro et al., 1993). In this trial, lower quantities of the volatile fraction were extracted using a previously sonicated sample, possibly because ultrasonic waves may drive off part of the volatile fraction.

3.2. Sample to adsorbent ratio

Many SFE studies have reported on the need to use adsorbents (Fujimoto et al., 1987; Saito et al., 1991; Lack and Seidlitz, 1993) because of their ability to immobilize interfering species and water in different matrices. Activated alumina was used as the adsorbent in the present experiment, because it is capable of binding sample fat and preventing it from interfering with extraction of the volatile fraction. The alumina was activated at 200°C, a known quantity was added to the cheese sample, and the mixture was homogenized completely. Three cheese to alumina ratios were tested, 2:1, 1:1, and 1:2. The best results were obtained using a ratio of 1:1 (Fig. 2(b)). The levels of most volatile compounds in the extraction trial using a ratio value of 2:1 (Fig. 2(a)) were distinctly lower than those for other trials, perhaps due to the high amount of sample, the adsorbent is not capable of trapping the fatty acids. This may result in a trap supersaturation with a subsequent loss of volatile compounds. At a ratio value of 1:2, the excess of alumina not only bound the fat present in the sample, but also interfered with extraction of the volatile fraction in the cheese, particularly the high molecular weight aroma components, namely, the free fatty acids and the phenolic compounds, which are less volatile than the rest of the extracted compounds (Fig. 2(c)).

3.3. Reconstitution solvent

The reconstitution solvent used initially was *n*-pentane, which has been used to collect the aroma components in SDE. However, this solvent gives rise to serious difficulties in SFE, because *n*-pentane is highly volatile and quickly evaporates in the solvent flow circuit, making it necessary to prime the solvent pump continuously and constantly monitor the presence of solvent in the feed line. Therefore, *n*-pentane was replaced as the reconstitution solvent by *n*-hexane, which is less volatile than *n*-pentane because of its higher molecular weight,

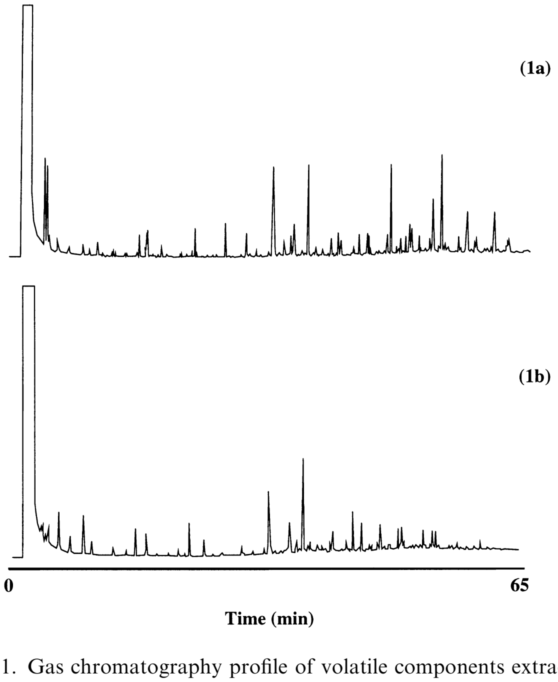

Fig. 1. Gas chromatography profile of volatile components extracted from non-lyophilized (a) and lyophilized (b) unsmoked Idiazábal cheese by supercritical fluid extraction using an octadecylsilica trap cooled to −5°C and collected in *n*-hexane.

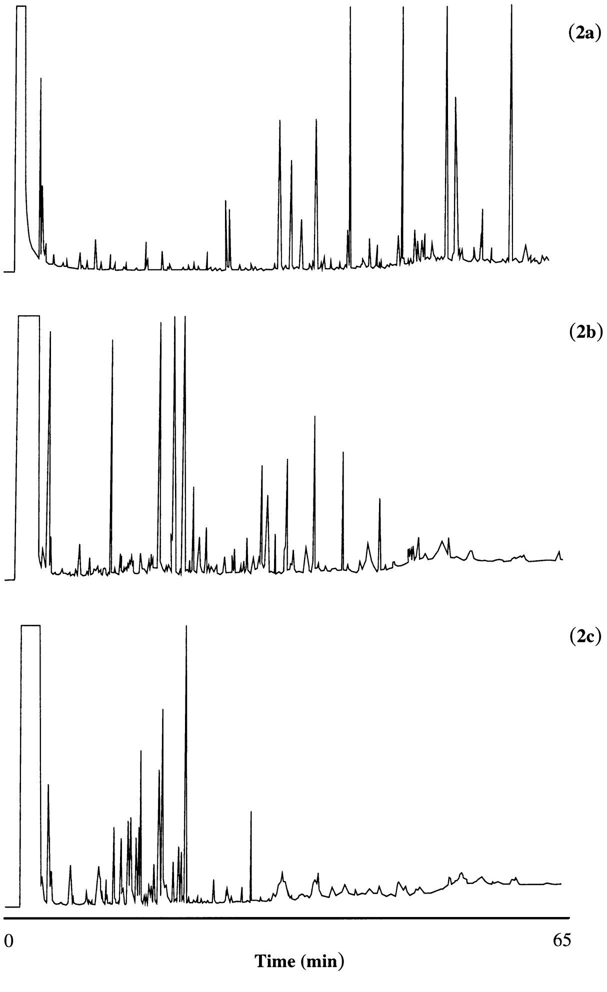

Fig. 2. Gas chromatography profile of volatile components extracted from unsmoked Idiazábal cheese by supercritical fluid extraction using an octadecylsilica trap cooled to −5°C at a sample to adsorbent ratio of 2:1 (a), 1:2 (b) and 1:2 (c).

but still capable of carrying and solubilizing the components of the volatile fraction in the cheese (Fig. 3(a)). Polarity is one of the most critical properties of a reconstitution solvent (Langenfeld et al., 1992; Porter et al., 1992; Lack and Seidlitz, 1993); it plays a direct role in achieving complete collection of the extract retained in the trap and, thus, affects system efficacy. For that reason, a more highly polar mixture, *n*-hexane:acetone (2:1) was subsequently tested (Fig. 3(b)). In fact, mixtures with higher acetone concentrations were tested, but an overlap phenomenon appeared with some volatile compounds researched. Table 1 presents the quantitative results achieved for the trials in which the analytes extracted were eluted from the trap with either 0.3 ml of *n*-hexane or 0.3 ml of the *n*-hexane:acetone (2:1) mixture. Most likely solubilizing most polar components with the acetone additions.

3.4. Trap type and temperature

The trap employed in the optimization and validation trials was a solid Hypersil octadecylsilica (ODS) trap (Shandon Scientific, Runcorn, Cheshire, UK). The polarity and selectivity attributes make this packing ideal for retaining the volatile components of cheese, which are polar. Even so, the suitability of this type of trap in retaining the volatile components of the cheese was also tested by extracting the volatile fraction using a solid steel trap. The results (Fig. 4) showed the low effi-

Table 1
Effect of reconstitution solvent on the recovery (ppm) of the components in the volatile fraction in Idiazábal cheese using supercritical fluid extraction

Component		Reconstitution solvent	
		n-Hexane (ppm)	*n*-Hexane:acetone (ppm)
1	2-Hexanone	0.058	0.234
2	2-Heptanone	0.105	0.221
3	Ethyl hexanoate	0.385	1.538
4	2-Heptanol	0.291	0.166
5	Ethyl heptanoate	0.228	1.240
6	2-Nonanone	0.143	0.634
7	1-Octen-3-ol		1.748
9	Benzaldehyde	0.049	0.116
10	Methyl decanoate	0.196	0.782
11	Butyric acid	0.485	8.188
12	Acetophenone	0.048	0.431
13	Ethyl decanoate	0.184	0.844
14	Isovaleric acid	0.148	2.458
15	Caproic acid	4.174	23.165
16	Ethyl dodecanoate	0.157	0.816
17	Heptanoic acid		0.926
18	Phenol	0.032	0.086
19	Caprylic acid	1.459	7.523
20	Ethyl tetradecanoate	0.130	0.734
21	4-Methyl octanoic acid	0.408	1.066
22	4-Ethyl phenol		0.135
23	3-Ethyl phenol	0.055	0.263
24	3,4-Dimethyl phenol	0.041	0.151
25	Capric acid	4.391	21.517
26	Lauric acid	2.880	6.550
27	Myristic acid	0.290	4.921

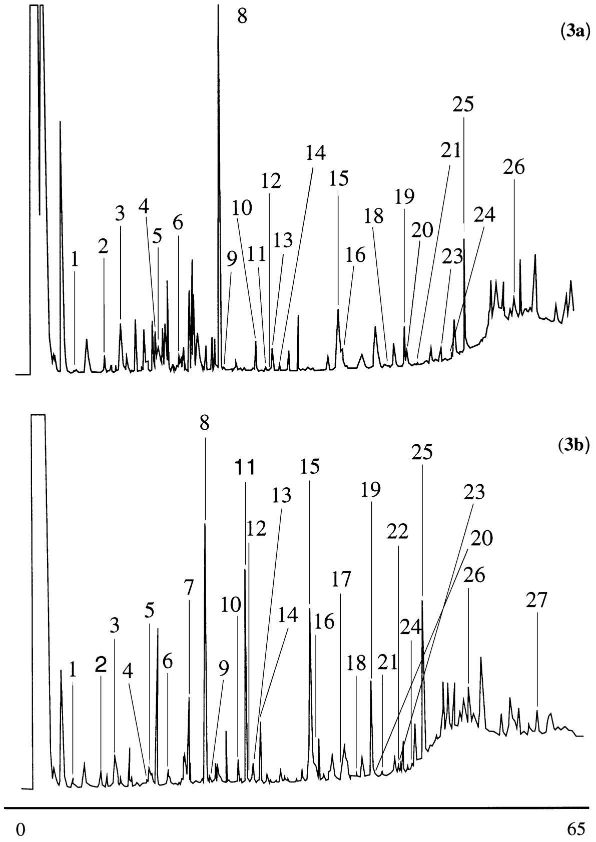

Fig. 3. Gas chromatography profile of volatile components extracted from unsmoked Idiazábal cheese by supercritical fluid extraction using an octadecylsilica trap cooled to −5°C at a sample to adsorbent ratio of 1:1, with collection in 0.3 ml *n*-hexane (a) and 0.3 ml of *n*-hexane:acetone (2:1) (b). (8: Camphor as an internal standard).

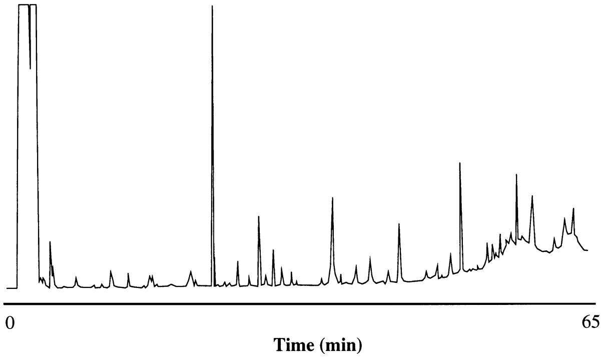

Fig. 4. Gas chromatography profile of volatile components extracted from unsmoked Idiazábal cheese by supercritical fluid extraction using a stainless steel trap cooled to −5°C at a sample to adsorbent ratio of 1:1, with collection in 0.3 ml of *n*-hexane:acetone (2:1).

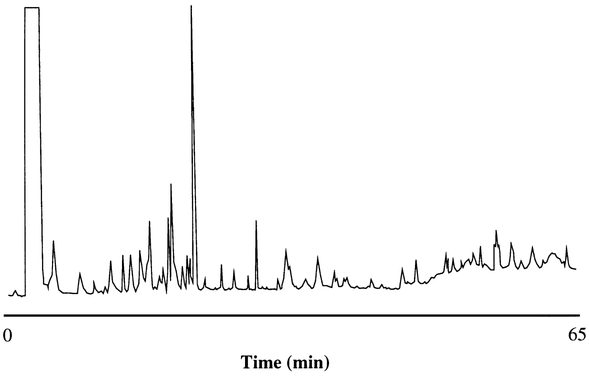

Fig. 5. Gas chromatography profile of volatile components extracted from Idiazábal cheese by supercritical fluid extraction using an octadecylsilica trap cooled to 0°C at a sample to adsorbent ratio of 1:1, with collection in 0.3 ml of *n*-hexane:acetone (2:1).

cacy of the stainless steel trap in retaining the volatile components of the cheese, especially the most volatile components, due to the low specificity of that type of trap. A high polarity, cianure (CN^-) trap, was not considered, because it required solvents with high polarity which produced an overlap phenomenon.

The optimum working temperature for an ODS trap is −5°C for the extraction of volatile substances (Fig. 3). This system parameter has the greatest effect on operating costs, because the lower the temperature of the solid ODS trap, the higher the cryogenic CO_2 consumption and, hence, increased operating costs. A working temperature of 0°C was, therefore, tested (Fig. 5). However, this 5°C increase in temperature adversely affected extraction yield, both qualitatively (fewer compounds) and quantitatively (lower concentration). The reason for that decrease in operating yield, is that at a temperature of 0°C or higher, the chemical packing in the trap does not retain the volatile components and allows the components dissolved in the supercritical CO_2 to be carried away and flushed out through the solvent outlet circuit.

4. Conclusion

In conclusion, based on the results discussed above, the best SFE extraction of the volatile fraction of the cheese was achieved using comminuted sample with added alumina as adsorbent at a cheese to adsorbent ratio of 1:1, a mixture of *n*-hexane:acetone (2:1) as the reconstitution solvent, and a polar ODS trap at a temperature of −5°C to retain the extracted volatile components. The different trials carried out yielded considerable differences in the results achieved, underlining the significance and repercussion of proper sample preparation. Accordingly, while new extraction methods simplify sample handling, close attention to preparation remains extremely important in ensuring reproducibility and reliability of results.

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