

Preconcentration of volatile components of foods: optimization of the steam distillation–solvent extraction at normal pressure

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ABSTRACT

Optimization of the experimental conditions affecting the simultaneous steam distillation–solvent extraction of volatile components of foods was carried out by using the modified sequential simplex method. For this purpose, a new micro steam distillation–extraction device was constructed. The apparatus includes an enlarged surface condenser which contributes to preventing eventual losses of high-volatility components. Determination in the $\mu\text{g}/\text{l}$ range of compounds having different polarities and volatilities are accomplished with high recoveries. The construction of the apparatus is such that the use of extraction solvents having densities either higher or lower than that of the solvent sample is feasible with only one configuration.

INTRODUCTION

Numerous techniques for isolating and concentrating volatile chemicals prior to GC analysis have recently been published and several methods have been applied to obtain a representative concentrate of the sample to be analysed, *i.e.*, liquid–liquid extraction, headspace analysis and simultaneous steam distillation–solvent extraction (SDE). The last method, introduced by Likens and Nickerson [1] in 1964, has received great attention. Some modifications have been made to the original apparatus, including the incorporation of a vacuum jacket in the arm which conducts the solvent vapour to the extractor body [2] and the use of more efficient cooling surfaces [3,4].

Godefroot *et al.* [5] reported a micro SDE device which allows operation with a small volume of solvent without requiring a subsequent concentra-

tion of the extract by solvent evaporation. This configuration has been used in different investigations, mainly related to food flavour research [6–8]. Several studies of the effects of different process factors on recovery efficiencies [9] and minimum concentrations that can be efficiently extracted [10] by using the SDE procedure have already been reported, but as far as we know the optimization of the variables that influence the process has not been thoroughly investigated.

The optimization of analytical techniques usually leads to an enormous experimental effort as the number of variables involved increases. In that situation it is of the utmost importance to apply a suitable optimization procedure for simultaneous handling of experimental variables. The sequential simplex method [11] has been broadly recognized as a very efficient empirical optimization procedure [12,13] since it allows the optimized conditions affecting a process to be obtained with a moderate number of experimental runs. This method has been successfully used in chromatographic research, as reported in a previous paper [14].

The objective of this work was to optimize the

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simultaneous distillation–extraction procedure at normal pressure, in order to attain a minimum error in determinations of the extracted solutes. To this end, the modified sequential simplex method [15] was applied. A micro SDE apparatus was specially constructed for this study. The new design allows effective mixing of the solvent vapour with sample vapour and includes an enlarged surface water condenser.

EXPERIMENTAL

Steam distillation–solvent extraction

The test mixture included fourteen compounds, previously reported as food volatile components, which were selected by considering differences in polarity, volatility and water solubility (see Table III). The stock solution consisted of a pure mixture of about 7.14% per component and was stored at -30°C in the dark. Synthetic mixtures were prepared directly in the extraction flask by adding the appropriate volume of the stock solution to water purified in a Milli-Q system (Millipore) in order to obtain a concentration of 1 mg/l or 10 $\mu\text{g/l}$ of each compound. The reagents, all of GC grade, were purchased from Merck (Darmstadt, Germany) (iso-amyl acetate, ethyl hexanoate, ethyl octanoate, benzaldehyde, diethyl succinate, ethyl dodecanoate, 2-phenylethanol), Aldrich Chemie (Steinheim, Germany) (1-hexanol, linalool, α -terpineol, γ -decalactone) and Sigma (St. Louis, MO, USA) (β -ionone, ethyl tetradecanoate).

According to our previous experience [8], doubly distilled dichloromethane (Merck) was initially selected as the extraction solvent for the optimization study. Depending on the concentration range to be investigated, a 1- or 2-ml volume of the solvent was used. In all instances cleaned boiling chips were added to the sample and solvent flasks. The sample heating bath temperature, solvent heating bath temperature, coolant temperature and extraction time were varied according to the optimization procedure. Further concentration was not needed and a 2- μl volume of the extract was subsequently analysed by capillary GC.

Between successive runs the SDE apparatus was cleaned by rinsing with acetone (Merck) and Milli-Q-purified water.

Capillary gas chromatography

The GC analyses were performed on a Perkin-Elmer Model 8500 gas chromatograph equipped with a PTV Perkin-Elmer injector and a flame ionization detector. The system was coupled to a Model 2600 chromatography software system (Nelson Analytical). A 50 m \times 0.22 mm I.D. fused-silica capillary column (SGE, Ringwood, Australia) coated with a 0.25- μm layer of cross-linked BP-21 (FFAP) was used. Helium at 276 kPa over atmospheric pressure served as the carrier gas. Injections were carried out in the cold split (1/10) mode by introducing the sample at 30°C and subsequently heating the body injector (at 14°C/s) to 250°C . The detector was operated at 250°C . The oven temperature was initially 70°C for 5 min, then programmed at 5°C/min to 180°C and maintained there for 15 min.

The SDE apparatus used (Fig. 1) was specially constructed for this study by modifying the device previously designed by Godefroot *et al.* [5]. In order to minimize losses of the most volatile compounds, the condenser surface has been enlarged by the introduction of a jacket water, concentric with the cold finger, which acts as a second cooling system. A chamber on the top of the device facilitates effective mixing between the sample and the extraction solvent vapour. The distillation solvent and distillation sample arms enter the mixing chamber at the same height, thereby allowing the use of both high- and low-density extraction solvents.

Simplex optimization

The effect of modifying the experimental conditions on the overall performance of the analytical method was evaluated in terms of the differences in concentrations of the compounds present in the extract, calculated by the internal standard method, and their corresponding expected values. In order to improve the discrimination between different experimental runs, errors affecting determinations were squared, thus magnifying small differences between their summations. Moreover, two different internal standards, methyl octanoate and methyl decanoate (Fluka, Buchs, Switzerland) were used and the concentrations obtained with each one of them were averaged. The response function (Y) can be expressed as:

$$Y = \sum (C_i - C_i^0)^2 \quad (1)$$

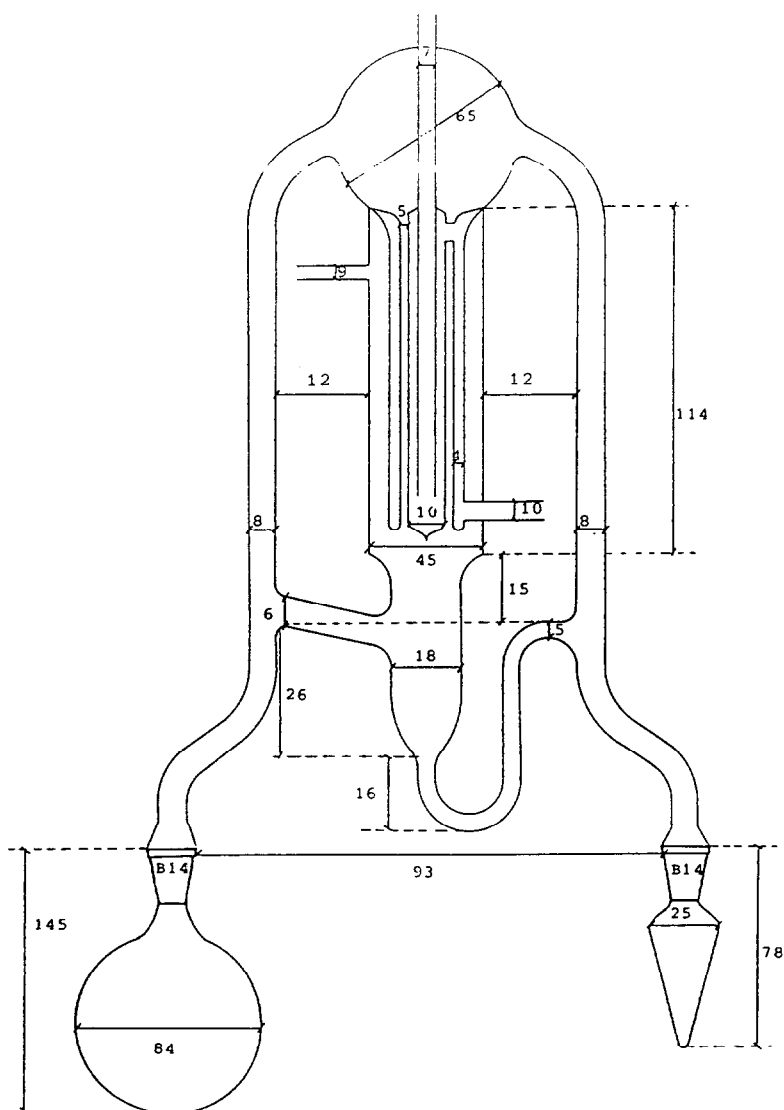


Fig. 1. Design of proposed simultaneous steam distillation– solvent extraction (SDE) device. Dimensions in mm.

where C_i is the concentration of the compound corresponding to the i th chromatographic peak and C_i^0 is the expected concentration for the same compound. Obviously, our objective was to minimize the value of the response function.

The initial experimental design was established according to Spendley *et al.* [16]. Physical values of factors were calculated from their mathematical coordinates using the equation

$$x_{\text{phys}} = x_0 + x_{\text{math}} \left(\frac{x_2 - x_1}{s} \right) \quad (2)$$

where x_{phys} is the physical value of the variable x , x_{math} the corresponding mathematical coordinate, x_0 its base level (starting physical value), x_1 and x_2 the lower and upper limits, respectively, of the range studied and s is the number of mathematical units into which the range has been divided.

The initial and successive simplexes were moved in the directions given by the rules of movement of the modified simplex method [15] and the corresponding responses were subsequently evaluated. Accordingly, different sets of variables were tested

until no further improvement was achievable. In all instances, at least two replicates of each analysis were carried out. The coordinates of every new vertex were calculated according to the expression

$$V_i^* = C + \alpha (C - V_i) \quad (3)$$

where V_i^* is the new vertex, C the centroid of the retained vertices in the movement, V_i the rejected vertex and α a factor with different values depending on whether a reflection ($\alpha = 1$), an expansion ($\alpha > 1$) or a contraction ($\alpha < 1$) was performed.

It should be pointed out that the self-directing nature of the optimization method makes possible a boundary violation (*i.e.*, a movement outside the experimental range initially considered). Then, the corresponding vertex must be rejected without experimentation and so the simplex is subsequently forced to move back inside the boundaries by applying a factor $\alpha = -0.5$ [17].

As far as this study is concerned, no variable except the sample heating bath temperature was considered to be object of upper boundary, as values higher than 150°C might eventually lead to artefact formation.

RESULTS AND DISCUSSION

The experimental variables to be optimized, their studied ranges and their starting values, for the 1 mg/l mixture, are given in Table I.

Table II summarizes the sets of experimental values tested throughout the optimization procedure. The base level of each variable (*i.e.*, the starting point) is also included (vertex no. 1). The s value (eqn. 2) was set to 3. Values obtained for the response function (eqn. 1) are included in the response column in Table II.

The optimization study was initiated by performing the first five experiments defined in Table II, which constitute the initial simplex. The assessment of the values obtained for the response function in each analysis allows the worst vertex (no. 5) to be rejected. Then, a new simplex was formed with the retained vertices and a new one resulting from the mirror image of the rejected vertex ($\alpha = 1$). The procedure must be repeated to move from one simplex into another by rejecting the worst observation and by selecting an adequate α value.

Vertex 23 proceeds from a boundary violation of the sample heating bath temperature occurring in vertex 22, which was rejected, as previously mentioned, without experimentation.

Searching was stopped after vertex 23 for a variety of reasons: (a) the differences between responses of vertices 15, 18, 19, 21 and 23 are small, thus indicating that a region had been already reached in which the influence of the variables in the response value was not important (see Fig. 2 and Table II), (b) no significant improvement was achieved in the last movements (Fig. 2) and (c) vertex 15 was maintained throughout the performance of seven successive simplices, which according to Spendley *et al.* [16] is indicative of the attainment of a true optimum, provided that four variables are involved in the optimization procedure. Bearing in mind the small differences occurring between experimental variables defining four vertices of the last simplex, we finally selected the experimental conditions corresponding to vertex 18, even though the best response value actually results from conditions corresponding to vertex 15, because the analysis is less time consuming. It should be emphasized that the optimum zone has been achieved by carrying out only 22 experimental runs.

TABLE I
VARIABLES INCLUDED IN THE 1 mg/l MIXTURE OPTIMIZATION STUDY

Variable	Minimum value	Maximum value	Base level
Sample heating bath temperature, T (°C)	100	150	125
Solvent heating bath temperature, T_s (°C)	40	70	55
Coolant temperature, T_c (°C)	-5	17	6
Extraction time, t (min)	15	180	97.5

TABLE II

EXPERIMENTAL RUNS AND RESULTS FOR THE SIMPLEX OPTIMIZATION OF THE SDE PROCEDURE WITH THE 1 mg/l MIXTURE

Vertex No.	Simplex No.	Retained vertices	Experimental variable levels				Response Y
			T (°C)	T_s (°C)	T_c (°C)	t (min)	
1	1	—	125.0	55.0	6.0	97.5	2572
2	1	—	140.4	57.2	7.6	109.5	2656
3	1	—	128.6	64.3	7.6	109.5	1652, 1887 ^a
4	1	—	128.6	57.2	12.8	109.5	2896
5	1	—	128.6	57.2	7.6	148.4	4804
6	2	1, 2, 3, 4	132.7	59.6	9.4	64.6	2353, 2298 ^a
7	3	1, 2, 3, 6	134.7	60.8	2.5	81.1	1291, 1461 ^a
8 ^b	3	1, 2, 3, 6	137.8	62.7	-2.6	66.8	5782
9	4	1, 3, 6, 7	120.1	62.7	5.1	66.8	2590
10 ^c	4	1, 3, 6, 7	125.2	61.3	5.8	77.5	2089
11	5	3, 6, 7, 10	135.6	68.0	6.6	68.8	1018, 1103 ^a
12 ^b	5	3, 6, 7, 10	141.0	74.5	6.9	54.5	1707
13	6	3, 7, 10, 11	129.4	67.6	1.9	103.8	2330
14 ^d	6	3, 7, 10, 11	131.9	61.6	7.5	74.4	2072
15	7	3, 7, 11, 14	140.3	66.1	6.4	89.4	947, 828 ^a
16 ^e	7	3, 7, 11, 14	144.0	67.2	6.5	92.4	1570
17	8	3, 7, 11, 15	137.8	68.0	4.0	100.0	1389
18	9	7, 11, 15, 17	148.7	66.8	3.4	56.3	956, 927 ^a
19	10	11, 15, 17, 18	146.4	73.6	7.7	76.2	1047
20	11	11, 15, 18, 19	147.8	69.3	8.0	45.4	1246
21 ^c	11	11, 15, 18, 19	145.3	69.0	7.0	59.1	1042
22	12	15, 18, 19, 21	154.7	69.7	5.6	71.7	—
23 ^d	12	15, 18, 19, 21	140.4	68.4	6.4	69.6	893

^a The second response value was obtained by performing a new run to check a vertex maintained in $k + 1$ movements.

^b Obtained from expansion $\alpha = 2$.

^c Obtained from contraction $\alpha = 0.5$.

^d Obtained from contraction $\alpha = -0.5$.

^e Obtained from expansion $\alpha = 1.5$.

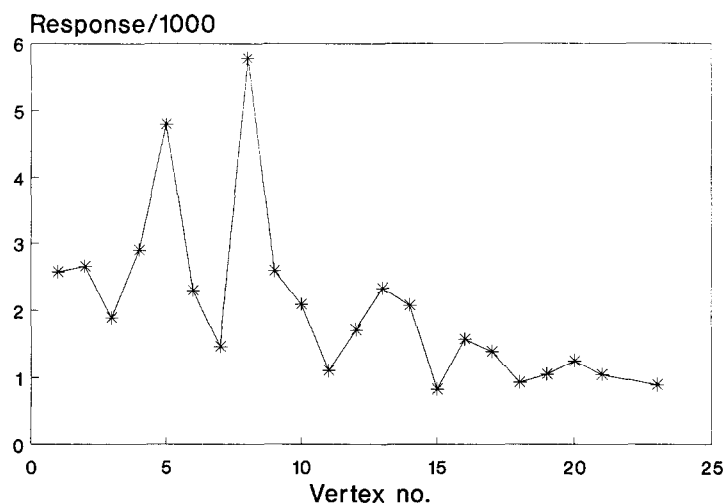


Fig. 2. Evolution of the response values obtained for the simplex optimization of the SDE procedure.

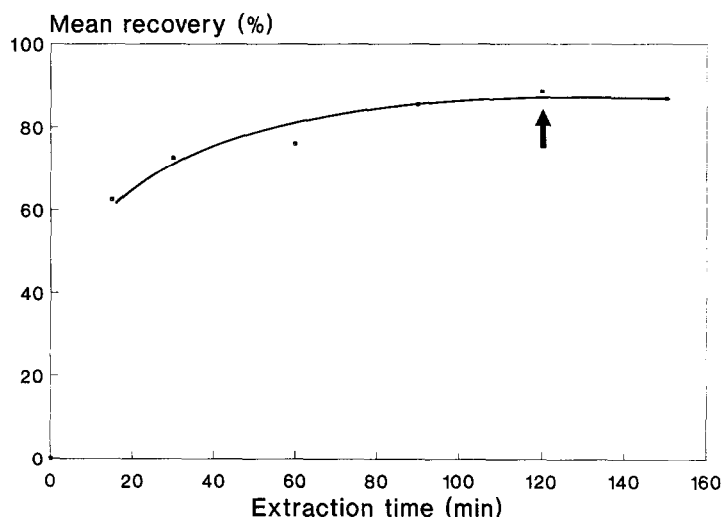


Fig. 3. Recovery of components by SDE at a concentration of 10 $\mu\text{g/l}$ for each test compound as a function of the extraction time.

Fig. 3 depicts the effect of the extraction time, using dichloromethane as solvent, on the recoveries of the investigated compounds occurring at a concentration of 10 $\mu\text{g/l}$. From these data, a 2-h extraction time was considered to be the best option to determine components in the $\mu\text{g/l}$ range. Other experimental conditions were fixed at the values previously optimized by applying the simplex procedure.

Tables III and IV show for concentrations of 1 mg/l or 10 $\mu\text{g/l}$ for each test compound, respectively, the recoveries obtained by SDE, using dichloromethane as solvent, under the experimental conditions corresponding to the base level and under the optimized conditions. As can be seen, near 100% values were obtained after optimization, except for 2-phenylethanol, probably owing to its high solubility in water. However, it is noteworthy that the optimization method proposed also improves the recovery obtained for this compound. In order to check the possibility of using the modified SDE apparatus with solvents lighter than water, Table III also includes recoveries obtained by using *n*-pentane as solvent. In all instances, relative standard deviations, calculated from a minimum of six replicates, of less than 10% were obtained.

Fig. 4 shows the chromatogram obtained from the aroma extract resulting from a Muscat grape juice by using the SDE procedure under the optimized conditions for determining compounds in the $\mu\text{g/l}$

range. Tentative peak identification of several compounds characteristic of juice was carried out by matching retention times with those of reference solutes.

TABLE III

RECOVERIES OBTAINED FOR THE COMPOUNDS SELECTED IN THE mg/l RANGE, UNDER THE EXPERIMENTAL CONDITIONS CORRESPONDING TO THE BASE LEVEL OF THE SIMPLEX OPTIMIZATION AND THE OPTIMUM CONDITIONS SELECTED FOR DICHLOROMETHANE OR *n*-PENTANE

Recovery as percentage of initial amount. Mean value of a minimum of six replicates.

Compound	Base level CH_2Cl_2	Optimum CH_2Cl_2	Optimum <i>n</i> -pentane
Isoamyl acetate	94.24	100.86	89.50
Ethyl hexanoate	93.76	102.66	90.68
Terpinolene	76.96	98.84	85.36
1-Hexanol	102.34	101.32	73.54
Ethyl octanoate	83.96	100.46	87.56
Benzaldehyde	105.96	102.46	87.18
Linalool	105.40	101.74	90.12
Diethyl succinate	80.56	97.00	75.04
α -Terpineol	105.04	102.84	91.42
Ethyl dodecanoate	68.18	99.10	94.58
2-Phenylethanol	18.36	55.28	11.26
β -Ionone	98.82	99.20	89.62
Ethyl tetradecanoate	74.82	101.48	75.20
γ -Decalactone	73.94	98.62	85.42

TABLE IV

RECOVERIES OBTAINED BY SDE FOR THE COMPOUNDS SELECTED AT A CONCENTRATION OF 10 $\mu\text{g/l}$

Recovery as a percentage of initial amount. Mean value of a minimum of six replicates.

Compound	Base level CH_2Cl_2	Optimum CH_2Cl_2
Isoamyl acetate	67.33	98.00
Ethyl hexanoate	86.50	97.50
Terpinolene	95.50	97.25
1-Hexanol	55.83	94.50
Ethyl octanoate	95.00	94.50
Benzaldehyde	48.33	98.50
Linalool	78.83	97.00
Diethyl succinate	17.33	79.00
α -Terpineol	55.00	97.75
Ethyl dodecanoate	96.33	85.00
2-Phenylethanol	16.50	26.00
β -Ionone	60.83	82.00
Ethyl tetradecanoate	83.50	100.90
γ -Decalactone	17.66	87.50

CONCLUSIONS

The use of the sequential simplex method allows the performance of the SDE procedure to be significantly improved. The proposed modified apparatus operated under the optimized conditions renders, in general, excellent recovery efficiencies for the investigated compounds in the mg/l or $\mu\text{g/l}$ range. A notable advantage is the fact that the use of solvents denser or lighter than the solvent sample is possible with only one configuration. Moreover, the proposed micro SDE apparatus may work efficiently in different approaches including operation at normal pressure, at reduced pressure and concentration of the dynamic headspace from the sample. These aspects will be the topics of forthcoming papers.

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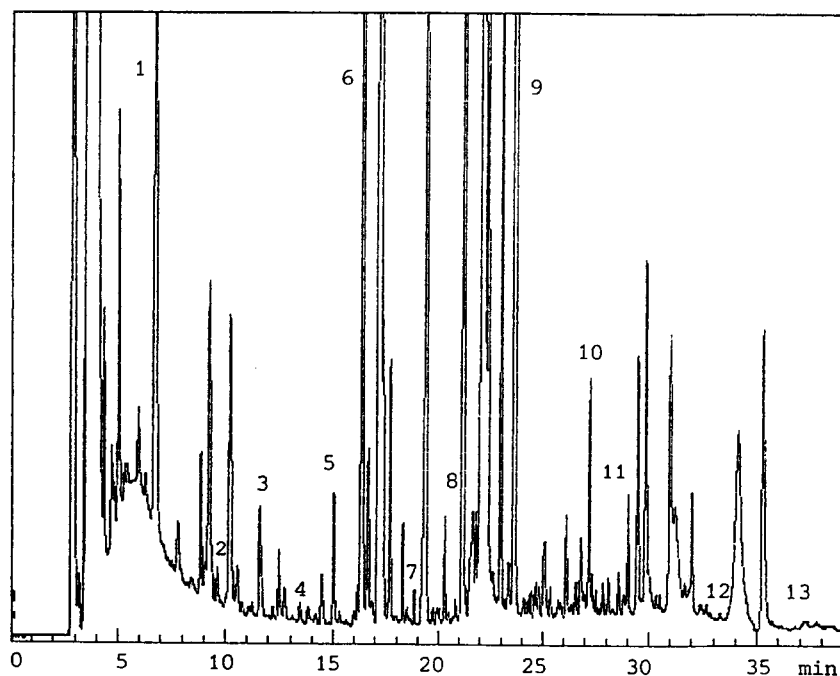


Fig. 4. Chromatogram of the aroma concentrate obtained from simultaneous steam distillation–solvent extraction (SDE) of a Muscat grape juice by using dichloromethane. Experimental conditions as optimized for determining components occurring in the $\mu\text{g/l}$ range. Peaks: 1 = isoamyl acetate; 2 = ethyl hexanoate; 3 = terpinolene; 4 = 1-hexanol; 5 = methyl octanoate; 6 = ethyl octanoate; 7 = benzaldehyde; 8 = methyl decanoate; 9 = α -terpineol; 10 = ethyl dodecanoate; 11 = 2-phenylethanol; 12 = ethyl tetradecanoate; 13 = γ -decalactone. See text for further details.

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