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*Graphical Abstract

Supercritical CO_2 extraction applied toward the production of a functional beverage from wine

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A two-step process using supercritical fluid extraction with CO_2 has been developed to produce a low-alcohol beverage from wine that maintains the aroma and the antioxidant activity similar to that of the original wine. First, the recovery of aroma from wine was attained in a countercurrent packed column (white and red wines were investigated) using very low CO_2/wine ratios. Then, the aroma-free wine recovered from the bottom of the extraction column was dealcoholized by applying different extraction conditions. The results obtained from these studies permit the design of a two-step countercurrent CO_2 extraction process at 9.5 MPa and 313 K, in which the different CO_2/wine ratios employed in each step lead to the recovery of aroma or the removal of ethanol. The two-step process was applied to rose wine and the low-alcohol beverage obtained proved to have similar antioxidant activity and similar aroma profile to that of the original wine. In the table, the antioxidant activities of a commercial rose wine, raffinate and the non-alcoholic beverage obtained are presented. The non-alcoholic functional beverage had similar DPPH and ORAC values than original wine, together with similar TPC.

	ABTS	DPPH	ORAC	TPC
Original wine	8.751 ± 0.055	1.499 ± 0.020	17.290 ± 0.593	429.860 ± 14.801
Raffinate	9.313 ± 0.181	1.666 ± 0.140	15.611 ± 0.550	444.513 ± 11.841
Non-alcoholic beverage	8.148 ± 0.046	1.542 ± 0.042	16.653 ± 0.834	423.587 ± 12. 617

Supercritical CO_2 extraction applied toward the production of a functional beverage from wine

Highlights

> We produce a low-alcohol beverage from wine based on a supercritical process. > The countercurrent-SFE process is a two-step process > First, recovery of aroma using very low CO_2/wine ratios > Second, dealcoholization of the aroma-free wine > Beverage obtained have similar antioxidant activities and aroma to that original wine

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Running title: Functional beverage from supercritical wine extracts.

Keywords: Supercritical CO_2 Extraction; Non-Alcoholic Beverages; Wine; Aroma; antioxidant

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Abstract

Supercritical CO_2 extraction has been proved to be a potential tool in the recovery of aroma compounds from different natural sources and in the removal of ethanol from aqueous solutions. In this work, both ideas are combined to develop a two-step process toward the production of a low-alcohol beverage from wine, but maintaining the aroma and the antioxidant activity similar to that of the original wine.

First, the recovery of aroma from wine was attained in a countercurrent packed column (white and red wines were investigated) using very low CO_2/wine ratios. Then, the aroma-free wine recovered from the bottom of the extraction column was dealcoholized by applying different extraction conditions.

The results obtained from these studies permit the design of a two-step countercurrent CO_2 extraction process at 9.5 MPa and 313 K, in which the different CO_2/wine ratios employed in each step lead to the recovery of aroma or the removal of ethanol. The two-step process was applied to rose wine and the low-alcohol beverage obtained proved to have similar antioxidant activity and similar aroma profile to that of the original wine.

Keywords: Supercritical CO_2 Extraction; Non-Alcoholic Beverages; Wine; Aroma.

1. Introduction

Several drinks with low ethanol content or without ethanol have been introduced on the market in recent years. The increasing public consciousness about the abuse of alcohol together with the severe control of alcohol consumption in drivers have led to more people to consume non-alcoholic drinks, and these drinks have gained significant sales percentages in the beverage industry.

Wine is one of the most complex alcoholic beverages; more than 800 volatile organic compounds (acids, esters, alcohols, aldehydes, lactones, terpenes, etc.) present in very low amounts were identified [1], which all together are responsible of each particular bouquet. Therefore, the production of an alcohol-free wine by removing ethanol while preserving the organoleptic properties of wine is a very complex and challenging problem.

In recent years, carbon dioxide (CO_2) extraction has been suggested as a promising alternative to the recovery of aroma compounds from natural matter [2-4]. On the other side, the removal of ethanol from aqueous solutions using high-pressure carbon dioxide has been comprehensively studied [5-7] and thus, supercritical fluid extraction has appear as a promising alternative to other conventional dealcoholization of beverages techniques [8-10], such as distillation [11, 12] or inverse osmosis [13-15]. All these techniques have the disadvantage of eliminating the beverage aromas together with ethanol, but still, among them, supercritical CO_2 extraction is particularly attractive because water, salts, proteins and carbohydrates are not substantially removed or denatured [9].

In a European patent for producing alcohol-free wine [16], a supercritical CO_2 extraction is at first employed to recover aroma compounds and then, the ethanol from

the raffinate is separated in a subsequent distillation column. Mixing the extracted aroma compounds into the bottom product of distillation, alcohol-free wine can be produced. Another European patent [17] describes a process in which the ethanol and aroma are removed in a first distillation step. Then, aroma compounds are extracted from the distillate using supercritical CO_2 and are recycled to the bottom product of the distillation to obtain an alcohol-free wine product.

In this work, supercritical CO_2 technology was employed to produce a low-ethanol content beverage from wine by combining to different countercurrent extraction steps. In the first step, the extraction and recovery of aroma from the original wine was the target, while in the second step the extraction was driven towards the dealcoholization of the aroma-free product obtained in the first step. The key factor to attain these two different objectives was the selection of an adequate ratio between the flow rates of solvent and wine employed.

2. Materials and methods

2.1 Samples and Reagents

The wines (white, red and rose) employed in this work were kindly supplied by a Spanish wine seller company (Bodegas Torres S.A., Vilafranca del Penedès, Catalonia, Spain). Ethanol content in wine was 9.5%, 10.5% and 11.3% v/v for white, red and rose wines, respectively.

Ethanol (GC-assay, 99.5% purity) and MilliQ-water were obtained from Panreac (Barcelona, Spain) and from Millipore (Millipore Iberica, Madrid, Spain), respectively.

CO_2, N48 (99.9998% purity), was supplied by AL Air Liquide España S.A. (Madrid, Spain).

2.2 Supercritical fluid extraction of ethanol

The supercritical fluid extraction (SFE) device (Thar Technologies) comprises a countercurrent packed column of 2.8 m height with two separator cells (S1 and S2), where a cascade decompression takes place. The liquid sample can be introduced into the column from two different points: the top (180 cm of effective packed height) and medium (120 cm of effective packed height) feed points. The solvent (CO_2) is feed into the column through the bottom and is heated up to the extraction temperature before be introduced into the packed column.

Once the operating pressure and temperature were reached, the wine was pumped from the top of the column at a constant flow rate of 200 ml/h during 1 h. The temperature of the extraction column was kept at 313 K in all experimental assays. Extraction pressure was varied from 9.5 to 18 MPa and thus, CO_2 densities varied from 692.3 kg/m^3 to 848.9 kg/m^3, maintaining an appropriate density difference between the solvent and the liquid sample (> 100 kg/m^3).

The CO_2 flow rate was varied from 1.8 to 6.0 kg/h in order to attain CO_2/wine ratios in the range of 9 - 30 kg/kg. The extracted material was decompressed up to 5 MPa in the first separator cell, while the second separator was maintained near ambient pressure. The temperature in both separator units was kept at 308 K in all experimental trials. Once the extraction was finished, CO_2 was pumped for another 20 min to extract the remaining liquid sample that could have been left inside the countercurrent column.

Three products were collected from each extraction assay: two ethanol enriched extracts were collected from S1 and S2, and a dealcoholized wine (raffinate) from the bottom of the column. Typically, 8-13 mL of extract was collected in S1 and amounts lower than 2 mL in S2. The mass balance closed in all experiments with accuracy greater than 85%.

2.3 Supercritical fluid recovery of aroma

The SFE device employed is the same equipment utilized for the ethanol removal. In this case, the wine was injected into the column from the middle point at a constant flow rate during 4-6 h. That is, a total amount of 1000-1500 mL of wine was feed to the extraction column in order to recover a significant amount of aroma in the separator cells. Extraction pressure was set to 9.5 MPa, the CO_2 flow employed was in the range 0.5-1.0 kg/h and the CO_2/wine ratio around 2-4 kg/l.

Again, temperature of the extraction column was kept at 313 K in all experiments. The extracted material was decompressed up to 5 MPa in the first separator cell, while the second separator was maintained near ambient pressure. Both separators were maintained at 308 K. Once the extraction finished, CO_2 was pumped for another 20 minutes to help extracting the remaining liquid sample that could have been left inside the countercurrent column.

Three products were obtained from each extraction assay: around 10-30 mL of extract was collected in S1, 1-5 mL of extract in S2, and a liquid raffinate sample was recovered from the bottom of the extraction column. The mass balance closed in all experiments with accuracy greater than 95%.

2.4 Aroma analysis

Characterization of the wine extracts was carried out by a GC-2010 (Shimadzu, Japan), equipped with a split/splitless injector, electronic pressure control, AOC-20i auto injector, GCMS-QP2010 Plus mass spectrometer detector, and a GCMS Solution software. The column used was a CW-20M (Carbowax) capillary column, 30 m x 0.32 mm I.D. and 0.25 µm phase thickness. Helium, 99.996% was used as a carrier gas at a flow of 58,2 mL/min. Oven temperature programming was as follows: 40 ºC isothermal for 1 min, increased to a final temperature of 150 ºC (held for 2 min) at 2 ºC/min. Sample injections (1 µL) were performed in split mode (1:30). Injector temperature was of 210 ºC and MS ion source and interface temperatures were 230 and 280 ºC, respectively. The mass spectrometer was used in TIC mode, and samples were scanned from 40 to 500 amu. Compounds were identified by comparison with the mass spectra from Wiley 229 library and by their linear retention indexes.

2.5 Sensory evaluation

The response used to evaluate the quality of the supercritical extracts was the resemblance, based on a human olfaction test, of their aroma to that of their respective starting wines. Aromatic extracts were evaluated with a panel of six experts panelist (four females and two males, 25-50 year-old individuals) who judged the similarity of the aromas. The scale used for sensorial evaluation was not structured [18] to mark the similarity between the aroma of the extracts and that of the starting wines; that is, it only had two extreme points, and the right end represented the aroma of the original wine. Thus, the higher the score, the higher the similarity between the aroma of the

supercritical extracts and the aroma of the starting wines. The distance (in centimeters) to the left end was considered for the statistical analysis of the data.

2.6 Ethanol analysis

A Perkin-Elmer Autosystem XL gas chromatograph (Perkin-Elmer, Norwalk CT) equipped with a programmed split/splitless injector (PSS) and a flame ionization detector (FID) was used to perform all the GC analysis. The system was coupled to a Perkin-Elmer chromatography software system (Turbochrom). The column employed was a 30 m x 0.25 mm i.d. fused silica capillary column (Quadrex Corp., New Haven, CT) coated with a 0.25 μm layer of Carbowax 20M (polyethyleneglycol). To evaluate the ethanol content of the raffinates obtained from red and white wines after supercritical fluid extraction, a calibration curve was prepared using ethanol blank solutions ranging from 1 to 20 % in ethanol content (v/v). The chromatographic conditions were as follows: injector temperature, 210 ^{o}C; detector temperature, 280 ^{o}C, Helium at 15 psig was used as a carrier gas. The split ratio was 1:20 and the volume injected was 1 μL. The oven temperature program was as follows: starting at 39 ^{o}C (held for 3 min), and then heating to 65 ^{o}C (held for 1 min) at 5 ^{o}C/min, and then heating to a final temperature of 200 ^{o}C (held for 1 min) at 40 ^{o}C/min.

2.7 Determination of antioxidant activity

2.7.1. ABTS assay

The TEAC (Trolox Equivalent Antioxidant Capacity) assay described by Re et al. [19] was used to measure the antioxidant activity of the wine samples. Briefly, $ABTS^{\bullet}$ radical cation was generated by reacting 7 mmol/l ABTS with 2.45 mmol/l potassium persulfate after incubation at room temperature for 16 h in the dark. The $ABTS^{\bullet}$ radical solution was diluted with PBS (pH 7.4) to an absorbance of 0.70 - 0.20 at 734 nm. 10 µl of wine (previously diluted) at five different concentrations extract was added to 0.990 ml of diluted $ABTS^{\bullet}$ radical solution. The reaction was measured until the absorbance reached a plateau. Trolox was used as reference standard, and results were expressed as TEAC values (mmol Trolox/g extract). All analyses were done, at least, in triplicate.

2.7.2. $DPPH^{\bullet}$ free radical-scavenging assay

The ability of wines to scavenge $DPPH^{\bullet}$ free radicals was determined according to the method proposed by Brand-Williams et al. [20]. Briefly, 25 µl of wine or standard (previously diluted) was added to 0.975 µl of a 6×10^{-5} M solution of $DPPH^{\bullet}$ in methanol. A control sample, containing the same volume of solvent in place of extact, was used to measure the maximum $DPPH^{\bullet}$ absorbance. The reaction was allowed to take place in the dark until the reaction reach a plateau. Trolox was used as reference standard, and results were expressed as TEAC values (mmol Trolox/g extract). All samples were assayed, at least, in triplicate.

2.7.3. Oxygen radical absorbance activity (ORAC)

The ORAC assay was performed essentially as described by Huang et al [21]. Briefly, AAPH was dissolved in 10 ml of 75 mM phosphate buffer (pH 7.4) to a final

concentration of 166 mM and made fresh daily. A fluorescein stock solution (8×10^{-4} mM) was made in 75 mM phosphate buffer and stored. The stock solution was diluted 1/10000 with phosphate buffer. To all experimental wells, 150 µl of working fluorescein solution were added. In addition, blank wells received 25 µl of 75 mM phosphate buffer, while standards received 25 µl of trolox dilution and samples 25 µl of wine (previously diluted). Reactions were initiated by the addition of 25 µl of AAPH solution. Results were expressed as trolox equivalent antioxidant capacity.

2.8. Total phenolic content (TPC)

Total phenolic content of wines was determined with Folin-Ciocaltea reagent by the Singleton et al. method [22] and the results were expressed as GAE (mg of gallic acid/L of wine). Briefly, 3 mL of distilled water was mixed with 50 µL of sample or standard. 250 µL of Folin-Ciocalteu reagen was added and the content of the tube was mixed thoroughly. After 3 min 0.75 mL of Na_2CO_3 (20% w/v) followed by 0.95 mL of water was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm. The TPC of the wines was expressed as GAE (mg of gallic acid equivalent per L of wine). All analyses were done in triplicate.

3. Results and discussion

3.1 Ethanol extraction

Table 1 shows the different extraction conditions (pressure and CO_2/wine ratios) applied at 313 K for the removal of ethanol from white (9.5 % v/v ethanol) and red (10.5 % v/v

ethanol) wines. Also given in the table are the corresponding ethanol content obtained in the raffinates. Certainly, for the same CO_2/wine ratio, CO_2 density defines the degree of dealcoholization achieved: the higher CO_2 density the lower ethanol content in raffinate (Ext. 1 and 4 in Table 1). Nevertheless, it can be clearly deduced from Table 1 that the significant variable in the dealcoholization process is the CO_2/wine ratio. This was previously observed by several authors [9, 10].

According to our experimental assays, CO_2/wine ratios of ca. 30 ensured almost a complete dealcoholization of the wines studied, under moderate temperature (313 K) and pressure (9.5 MPa) conditions. Results obtained when combining the highest CO_2 density with low CO_2/wine ratios (Ext. 1) were not better than those obtained when using the lower CO_2 density but high CO_2/wine ratios (Ext. 3).

3.2 Study of aroma recovery

The same wines employed in the dealcoholization experiments (white and red wines) were employed to study the recovery of aroma from wine using supercritical CO_2. The key idea to attain the target was utilizing a low CO_2/wine ratio. Considering the facilities of the available experimental device, the CO_2/wine ratio employed in this case was in the range 2-4 kg/l.

Certainly, low CO_2/wine ratios imply that the liquid sample is the continuous phase and the supercritical solvent is the disperse phase. Thus, the solvent phase would be saturated with the aroma compounds (which are present in wine in very low amounts) while reduced amounts of ethanol should be extracted. On the contrary, during the dealcoholization trials (CO_2/wine ratio = 9-30 kg/l), the supercritical CO_2 solvent is the

continuous phase and the wine is the disperse phase, and both aroma compounds and ethanol a readily extracted.

Table 2 shows the results obtained in the recovery of aroma from white and red wines. Ext. 1 and 2 in Table 2 are duplicates of the extraction accomplished for the white wine at 313 K and 9.5 MPa. By comparison of the amounts (ml) of extract obtained in each trial, it can be concluded that very good reproducibility is attained. Further, whilst the raffinate was colored and absolutely odorless, the samples obtained in both S1 and S2 separators were completely transparent and very aromatic. This was assessed by analyzing the scores given by the panelists to the different extracts obtained. It can easily be seen that the extracts obtained in S1 and S2 corresponding to extracts 1, 2 and 4 obtained a high score. This means that they had a high resemblance to the original aroma of the starting white and red wines. However, in the case of red wine, significantly lower amounts of extract were obtained when applying the same CO_2/wine ratio than in the case of white wine (Ext. 3 in Table 2). Additionally, the raffinate obtained in this experiment somewhat preserved the characteristic wine odor. Thus, the CO_2/wine ratio was slightly increased (Ext. 4 in Table 2) and then, also in this case, an odorless raffinate was obtained.

According to Table 2, around 14 ml per liter of wine sample was obtained in the separators (Ext. 1, 2 and 4); although in the case of white wine the amount of extract recovered in S2 was larger than in the case of red wine. Moreover, the amounts of extract recovered in these experiments are significantly lower than the amounts of extract obtained in the dealcoholization assays (50-75 ml of extract per liter of wine).

The GC-MS chromatograms for extracts corresponding to the white wine are shown in Figure 1. The figure shows a comparison between the chromatogram corresponding to the original (white) wine, the extracts recovered in the separators and the raffinate

obtained from the bottom of the extraction column. As can be qualitatively observed from the figures, the extracts are significantly concentrated in the aroma compounds while the raffinates contain reduced amounts of aroma compounds in comparison to the original wine. In the case of red wine the chromatograms followed the same pattern.

Figures 2 and 3 show the peak identification of the chromatograms corresponding to S1 extracts of experiments reported in Table 2. Figure 2 corresponds to the S1 extract recovered in Ext. 1 (white wine) while Figure 3 refers to the S1 extract of Ext. 4 (red wine). In qualitative terms, both extracts showed very similar chromatographic profile, being compounds such as 3-methyl-1-butanol, ethyl lactate, acetic acid, 2,3-butanediol and phenylethyl alcohol the ones who presented the highest chromatographic peak areas.

Further, Table 3 shows a comparison between the peak areas obtained for the different compounds identified in the original red wine and the corresponding extract (Ext. 4 in Table 2). All the injections were carried out following the same chromatographic method and conditions (see Materials and Methods section). Thus, peak areas in Table 3 were employed to estimate concentration factors (peak area in extract / peak area in original wine) of some aroma compounds observed in the samples. Concentration factors up to 50 could be calculated from the results of the GC-MS analysis. Nevertheless, it should be pointed out that several compounds that are present in very low concentration in the original red wine could only be identified in the extract. For example, several alcohols (n-butanol, 3-methyl-1-pentanol, 1-hexanol, 3-ethoxy-1-propanol, 3-hexen-1-ol, 3-methyl thiol propanol), acids (3-OH-ethyl ester -butanoic acid, 2-methyl-propanoic acid, isovaleric acid, 2-OH-ethyl-3-phenylpropionate, diethylhydroxybutanedioate, caprylic acid, 2-OH-diethyl-pentanedioate), esters (isoamyl acetate, ethyl hexanoate, ethyl octanoate), aldehides (2-furancarboxaldehyde),

and ethers (1-methoxy-3-methyl-butane) could only be detected in S1 extract and thus, it is expected that very high concentration factors (> 50) were attained for these substances.

3.3 Production of a non-alcoholic functional beverage from rose wine

On the basis of previous studies the manufacture of a non-alcoholic beverage from rose wine (11.3% v/v of ethanol) was accomplished. Two CO_2-SFE steps were carried out, both at 313 K and 9.5 MPa, but employing different CO_2/wine ratios in order to achieve (Step 1) the recovery of aroma and then (Step 2) the dealcoholization of the raffinate obtained in the first step. S1 separator was maintained at 5 MPa whereas in S2 the extract was depressurized up to 1 MPa. Temperature in both separators was kept at 308 K.

Step 1: recovery of aroma from rose wine. CO_2 flow rate was 0.9 kg/h and wine flow rate was 0.25 l/h (CO_2/wine ratio = 3.6). A total of 12 liters of wine were feed to the extraction column. Top and bottom products were collected during the continuous operation; 220 ml of extract were recovered in S1 and considerably lower amounts (30 ml) in S2 separator. The mass balance closed with accuracy greater than 97%.

The extract obtained in S1 (18.3 ml per liter of rose wine) was completely transparent and highly aromatic; the chromatogram obtained by GC-MS is shown in Figure 4. Additionally, Table 4 shows the chromatographic areas of the aromatic compounds identified in the original rose wine and in the S1 extract obtained. Again, high concentration factors could be calculated for some aromatic compounds, such as 14 for ethyl acetate, 36 for ethyl lactate, 47 for 3-methyl-1-butanol and 53 for phenyl ethyl alcohol, and higher concentration factors would be expected for those compounds which

could not be detected in the original red wine (2-methyl-1-propanol, isoamyl acetate, hexanoic acid, etc.).

The odorless raffinate obtained from the bottom of the extraction column contained 8.8% v/v of the ethanol.

Step 2: removal of ethanol from the raffinate obtained in step 1. The liquid sample collected from the bottom of the extraction column in Step 1 was utilized to completely remove the remained ethanol. In this case, the CO_2 flow rate was 4.8 kg/h and the liquid sample flow rate was 0.20 l/h (CO_2/liquid ratio = 24). The concentration of ethanol in the raffinate obtained in this case (850 ml per liter of original rose wine) was lower than 1%.

The non-alcoholic functional beverage from rose wine. 850 ml of the raffinate obtained from Step 2 (ethanol content < 1% v/v) was mixed with 18.3 ml of the extract produced in Step 1. This beverage (1.1% v/v ethanol) produced from rose wine contained several of the aromatic compounds detected in the original wine, as can be deduced from the GC-MS analysis given in Table 4. Some substances are present almost in the same concentration (3-methyl-1-butanol, acetic acid, 2,3-butanediol, 2-methyl-propanoic acid) although some other substances that were detected in the original wine, could not be detected in the non-alcoholic beverage (ethyl acetate, 3-hydroxy-2-butanoate, ethyl lactate, cis-5-hydroxy-2-methyl-1,3-dioxane).

As it is shown in Table 5 aroma removal from wine only caused slight modifications in its antioxidant activity and polyphenols content. ABTS and DPPH assays shown a very small increase in the antioxidant capacity according to the TPC increment. However ORAC value was slightly smaller in this odorless raffinate, maybe to the different mechanism of action of these methods. The non-alcoholic functional beverage had

similar DPPH and ORAC values than original wine, together with similar TPC. Only a smaller ABTS value was detected.

Conclusion

Supercritical fluid CO_2 extraction was employed in a two-step process to produce a novel beverage from rose wine. Several aroma compounds were determined to be present both in the original rose wine and in the low-alcoholic beverage. Further, the new beverage maintains the antioxidant capacity of the original wine; it contains around 1% v/v ethanol, and thus might be potentially commercialized with a functional claim.

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Table 1. CO_2-SFE for the removal of ethanol from red and white wines at 313 K.

Ext.	P (MPa)	CO_2/wine ratio (kg/l)	% wt ethanol in raffinate
white wine			
1	18	9	3.5
2	13	12	2.1
3	9.5	29	< 1
4	9.5	9	5.5
red wine			
5	9.5	11	3.5
6	9.5	30	< 1

Table 2. CO_2-SFE for the recovery of aroma from red and white wines at 313 K and 9.5 MPa. Total extraction time = 4 h. Total amount of wine feed to the extraction column = 1000 ml.

	Ext. 1 white wine	Ext. 2 white wine	Ext. 3 red wine	Ext. 4 red wine
wine flow (l/h)	0.23	0.23	0.23	0.23
CO_2 flow (kg/h)	0.60	0.60	0.60	0.90
CO_2/wine ratio (kg/l)	2.6	2.6	2.6	3.8
S1 extract (ml)	11.0	10.8	5.2	13.5
Score	15.0	15.5	3.1	16.0
SD[a]	0.7	1.4	1.0	0.8
S2 extract (ml)	4.3	4.0	0.5	1.0
Score	17.3	19.1	2.4	17.0
SD[a]	0.7	0.7	0.8	1.4

[a] Standard Deviation

Table 3. Chromatographic areas obtained in the original red wine, S1 extract and raffinate (Ext. 4 in Table 2). NI: non identified compound.

compound	original red wine	S1 extract	concentration factor
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Isoamyl acetate		266518	
n-butanol		597800	
3-methyl-1-butanol	6561474	193130059	29.4
Ethyl hexanoate		210295	
2-butanone,3-hydroxy	139081	1782147	12.8
2-OH-propanoic acid,methyl ester		113465	
1-pentanol,3-methyl-		70898	
2-OH-isobutyric acid,methyl ester		106333	
Ethyl lactate	2632592	(*)	
1-hexanol		1159865	
3-ethoxy-1-propanol		141465	
3-hexen-1-ol		68231	
Ethyl octanoate		241426	
Tert-butoxymethoxy, methane		46473	
2-furancarboxaldehyde		52418	
Acetic acid	3957189	11090461	2.8
Butanoic acid,3-OH-ethyl ester		287263	
2,3 butanediol	7363015	7351706	1.0
Butane,1-methoxy-3-methyl		412724	
Ethanol,2-methoxyethanol	1990796	1210931	0.6
Propanoic acid,2methyl-		435945	
2(3H)-furanone,dihydro-	213612	2277658	10.7
NI-I		169072	
Butanedioic acid,diethyl ester	310726	15553593	50.1
Isovaleric acid		518754	
3-methyl thiol propanol		759264	
NI-II		624306	
N-(-3-methylbutyl)acetamide		774003	
NI-III		890390	
Phenylethyl alcohol	1339270	50154470	37.4
2-OH-ethyl-3-phenylpropionate		461626	
Diethylhydroxybutanedioate		289933	
Caprylic acid		1466425	
2-OH-diethyl-pentanedioate		1035159	

(*) Chromatographic area too high leading a saturated detector response.

Table 4. Chromatographic areas obtained in the original rose wine, S1 extract obtained from Step 1, raffinate obtained from Step 2 (dealcoholized wine) and non-alcoholic beverage produced. NI: non identified compound.

	original rose wine	S1 extract	dealcoholized wine	non-alcoholic beverage
Acetaldehyde		119166		
Ethyl acetate	194430	2894893		
2-methyl-1-propanol		2144850		
Isoamyl acetate		257327		
n-butanol		145410		
3-methyl-1-butanol	749848	34944236		674623
Ethyl hexanoate		172957		
3-hydroxy-2-butanoate	47548	561970		
Ethyl lactate	56900	2053307		
1-hexanol		474860		
Ethyl octanoate		203616		
2-furfural	309200		249722	210090
Acetic acid	1520309	7690182	1152546	1163573
Cis-5-hydroxy-2-methyl-1,3-dioxane	47770	132720	35001	
2,3-butanediol	3206841	4511741	3580614	3493937
5-methyl furfural			134611	
2-methyl-propanoic acid	964189	826606	1157857	1152847
1,2-propanediol			276019	245267
2-(3H)-dihydrofuranone	102998	288085	97772	64033
Butyric acid		322514		
NI-I			25156	
NI-II			84553	
Diethyl ester butanedioic acid		510897		
Hexanoic acid		3325559		
Phenyl ethyl alcohol	168806	9062757		106534
NI-III				505895
2-furancarboxaldehyde-5(hydroxymethyl)-				
NI-IV				2301994
Diethyl hydroxybutanedioate		804047		
Caprylic acid		6615062		
TOTAL	7090559	78062762	6793851	9918793

Table 5. Antioxidant activity of rose wine, raffinate and non-alcoholic beverage.

	ABTS[b]	DPPH[b]	ORAC[b]	TPC
Original wine	8.751 ± 0.055[b]	1.499 ± 0.020[b]	17.290 ± 0.593[a]	429.860 ± 14.801[b]
Raffinate	9.313 ± 0.181[a]	1.666 ± 0.140[a]	15.611 ± 0.550[b]	444,513 ± 11.841[a]
Non-alcoholic beverage	8.148 ± 0.046[c]	1.542 ± 0.042[b]	16.653 ± 0.834[a]	423, 587 ± 12. 617[b]

[a]Different superscript letters denotes statistically significant differences ($p<0.05$) among data in the same column

[b]Antioxidant activity was expressed as TEAC mmol of Trolox/g of extract.

[c]Total phenolic compounds was expressed as mg GAE/l)

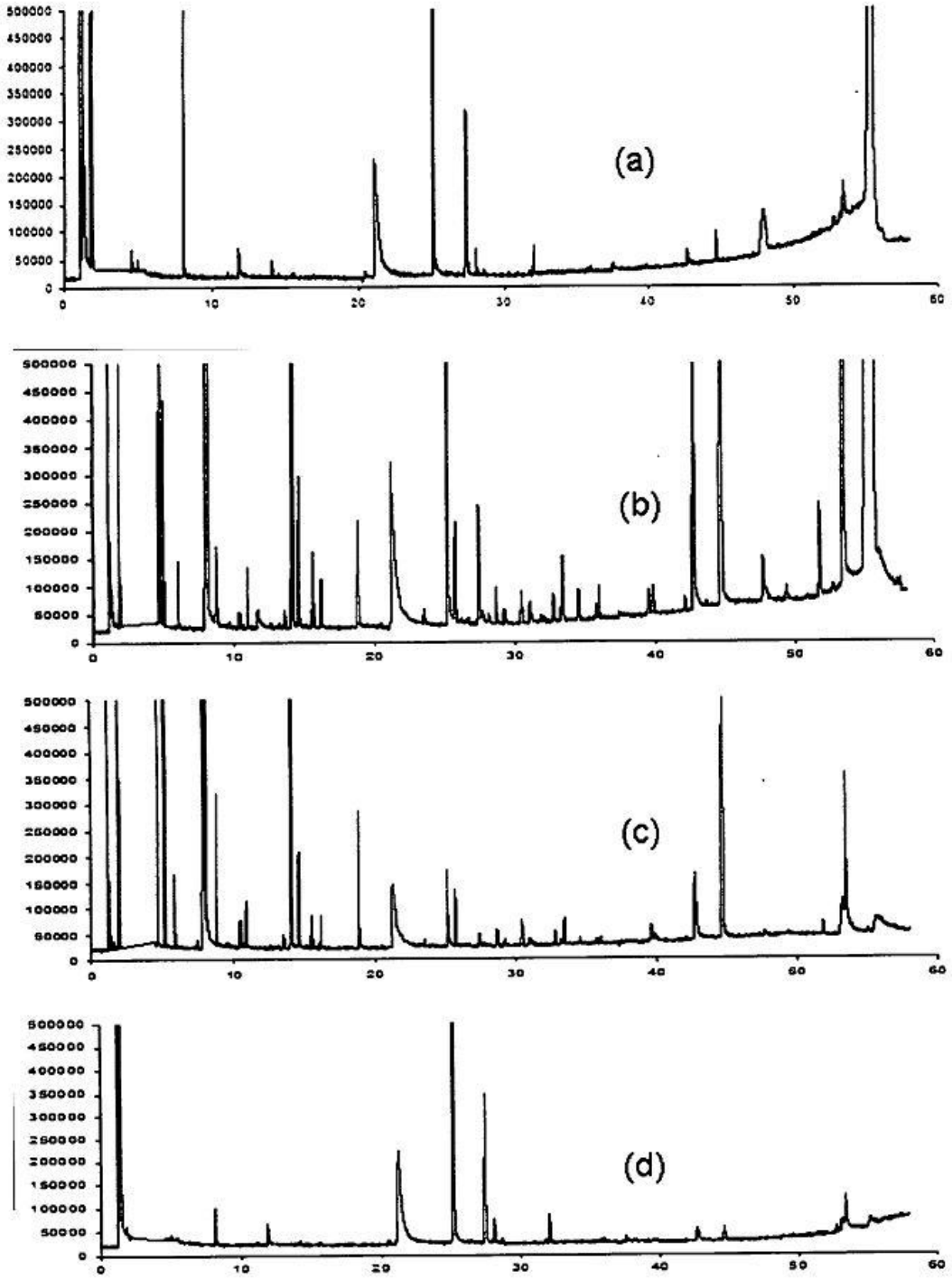

Figure 1. Aroma recovery from white wine (Ext. 1 in Table 2): comparison between the GC-MS chromatograms obtained for (a) the original wine; (b) S1 extract; (c) S2 extract; (d) raffinate.

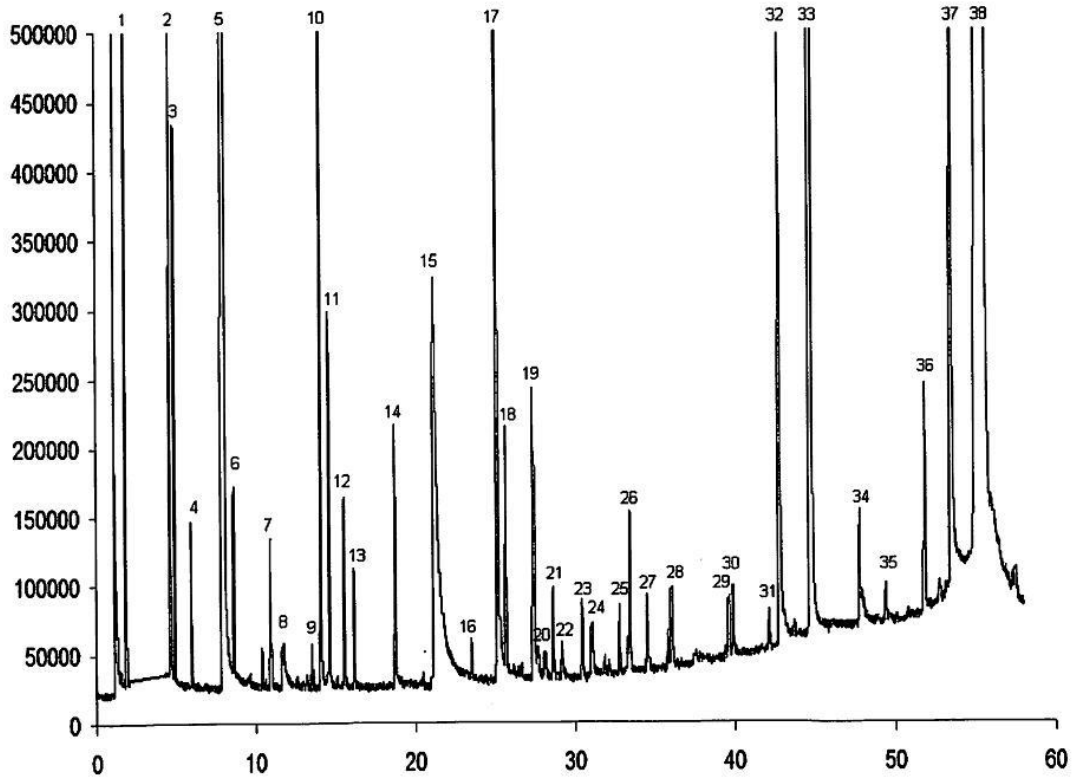

Figure 2. Chromatogram corresponding to the extract recovered from white wine in S1 separator (Ext. 1 in Table 2).

1) ethyl acetate, 2) 2-methyl-1-propanol, 3) isoamyl acetate, 4) n-butanol, 5) 3-methyl,1-butanol, 6) ethyl hexanoate, 7) hexyl acetate, 8) 2-butanone,3-hydroxy-, 9) 2-hydroxy-isobutyric acid,methyl ester, 10) ethyl lactate, 11) 1-hexanol, 12) 3 ethoxy-1-propanol, 13) 3-hexen-1-ol, 14) ethyl octanoate, 15) acetic acid, 16) butanoic acid, 3-hydroxy-ethyl ester, 17) 2,3-butanediol, 18) linalool, 19) etanol, 2-methoxyethanol, 20) 1,2 propanediol, 21) 2(3H)-furanone, dihydro-, 22) Ho-trienol, 23) NI-I, 24) butanoic acid, 25) butanedioic acid, dietil ester, 26) isovaleric acid, 27) 3-methyl thiol propanol, 28) 1,3 propanediol, diacetate, 29) Acetic acid, 2-phenylethyl ester, 30) NI-II, 31) Nerol, 32) N-(3-methylbutyl)acetamide, 33) phenylethyl alcohol, 34) ethyl-2-hydroxy-3-phenylpropionate, 35) 3,7-dimethyloct-1-en-3,7-diol, 36) diethylhydroxybutanedioate, 37) caprylic acid, 38) glycerol. NI: non identified compound.

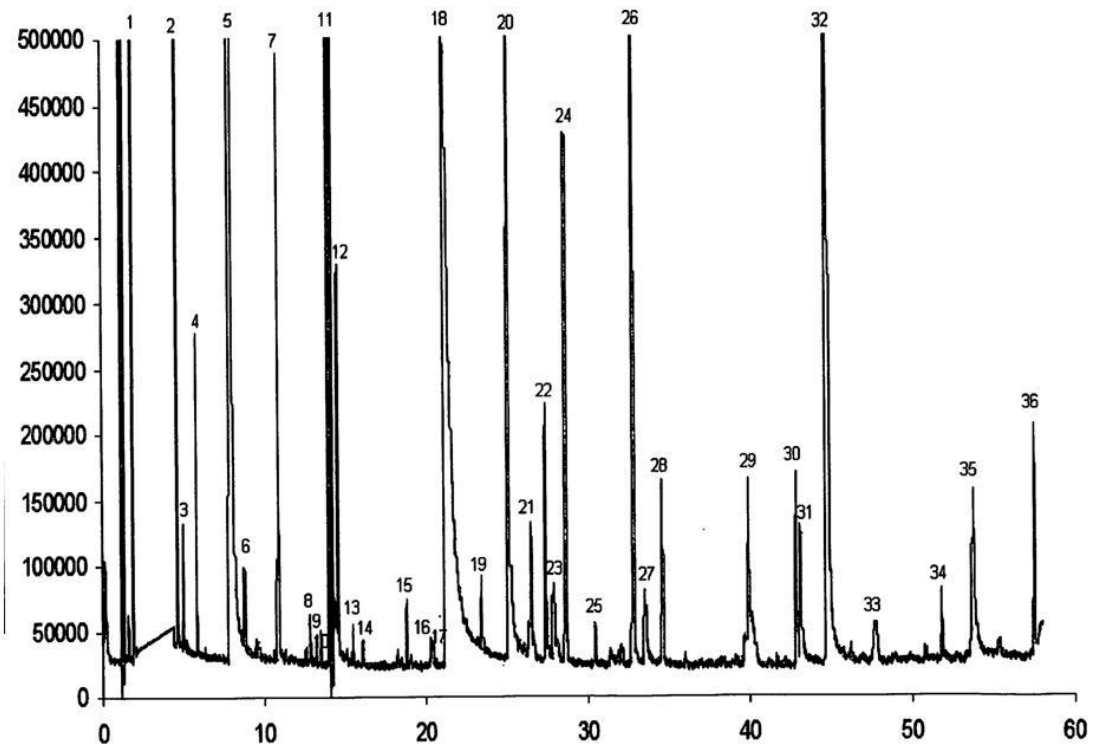

Figure 3. Chromatogram corresponding to the extract recovered from red wine in S1 separator (Ext. 4 in Table 2).

1) ethyl acetate, 2) 2-methyl-1-propanol, 3) isoamyl acetate, 4) n-butanol, 5) 3-methyl,1-butanol, 6) ethyl hexanoate, 7) 2-butanone,3-hydroxy-, 8) propanoic acid, 2-hydroxy-, methyl ester, 9) 1-pentanol, 3-methyl-, 10) 2-hydroxy-isobutyric acid, methyl ester, 11) ethyl lactate, 12) 1-hexanol, 13) 3 ethoxy-1-propanol, 14) 3-hexen-1-ol, 15) ethyl octanoate, 16) tert-butoxymethoxy, methane, 17) 2-furancarboxaldehyde, 18) acetic acid, 19) butanoic acid, 3-hydroxy-ethyl ester, 20) 2,3-butanediol, 21) butane,1-methoxy-3-methyl-, 22) etanol, 2-methoxyethanol, 23) propanoic acid, 2-methyl, 24) 2(3H)-furanone, dihydro-, 25)NI-I, 26) butanedioic acid, dietil ester, 27) isovaleric acid, 28) 3-methyl thiol propanol, 29) NI-II, 30) N-(3-methylbutyl)acetamide, 31) NI-III, 32) phenylethyl alcohol, 33) ethyl-2-hydroxy-3-phenylpropionate, 34) diethylhydroxybutanedioate, 35) caprylic acid, 36) dietil-2-hydroxy-pentanedioate. NI: non identified compound.

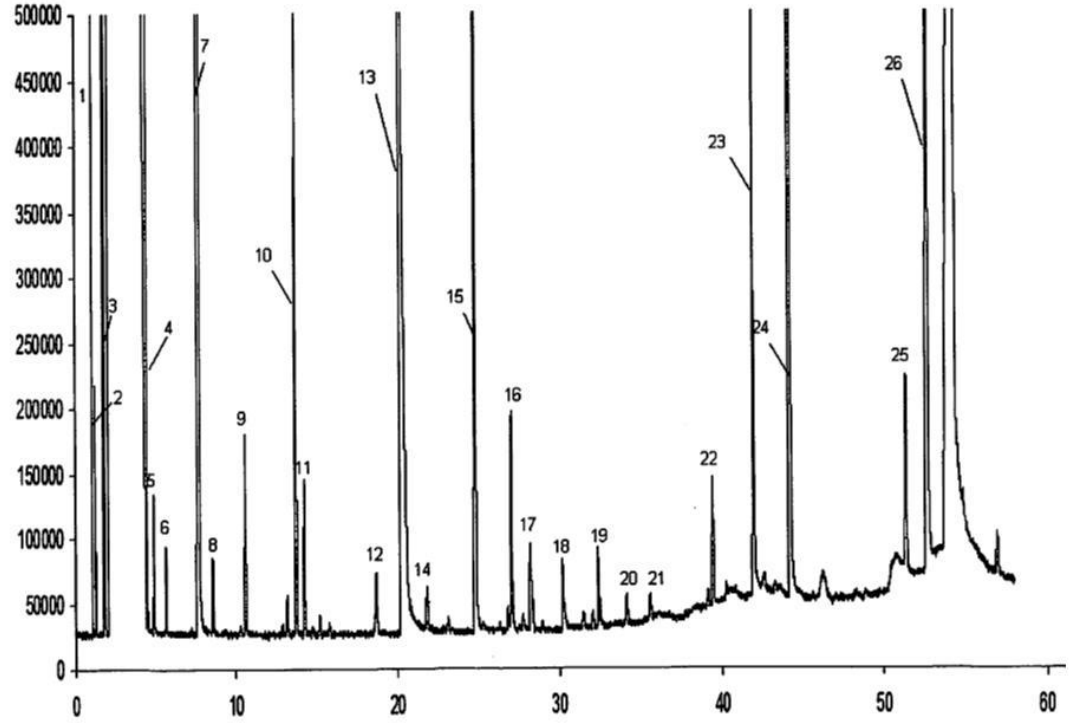

Figura 4. Chromatogram corresponding to the extract recovered from rose wine (S1 separator).

1: carbon dioxide, 2: acetaldehyde, 3: ethyl acetate, 4: 2-methyl-1-propanol, 5: isoamyl acetate, 6: n-butanol, 7: 3-methyl-1-butanol, 8: ethyl-hexanoate, 9: 3-hydroxy-2-butanoate, 10: ethyl lactate, 11: 1-hexanol, 12: ethyl-octanoate, 13: acetic acid, 14: cis-5-hydroxy-2-methyl-1,3-dioxane, 15: 2,3-butanediol, 16: 2-methyl-propanoic acid, 17: 2(3H)-dihydro-furanone, 18: butyric acid, 19: dietil succinate, 20: 3-methyl-mercapto-1-propanol, 21: metil-2-acetylhydroxy-palmitate, 22: butanedioic acid, dietil ester, 23: hexanoic acid, 24: phenyl ethyl alcohol, 25: diethyl hydroxybutanedioate, 26: caprylic acid.

Figure(s)

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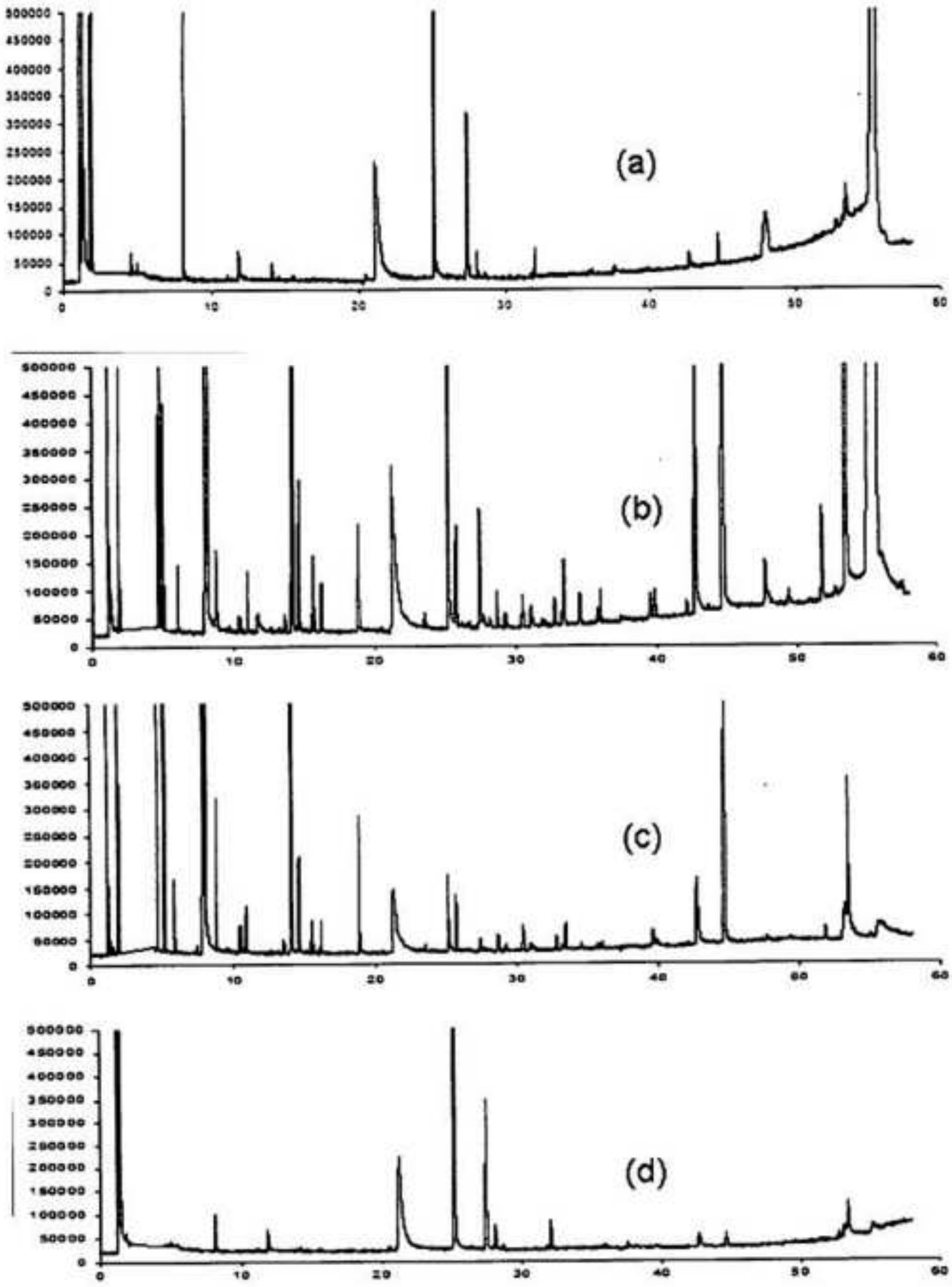

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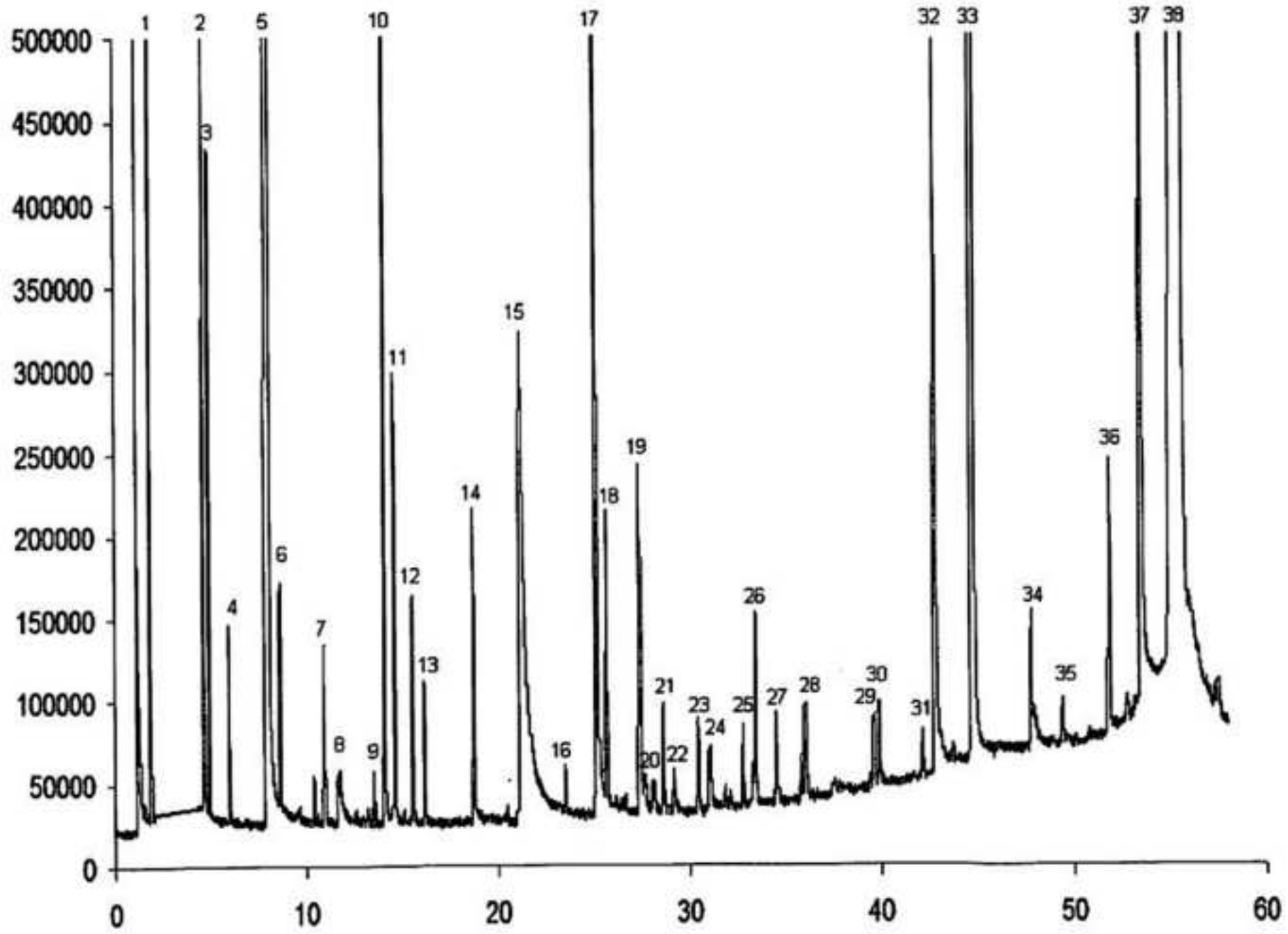

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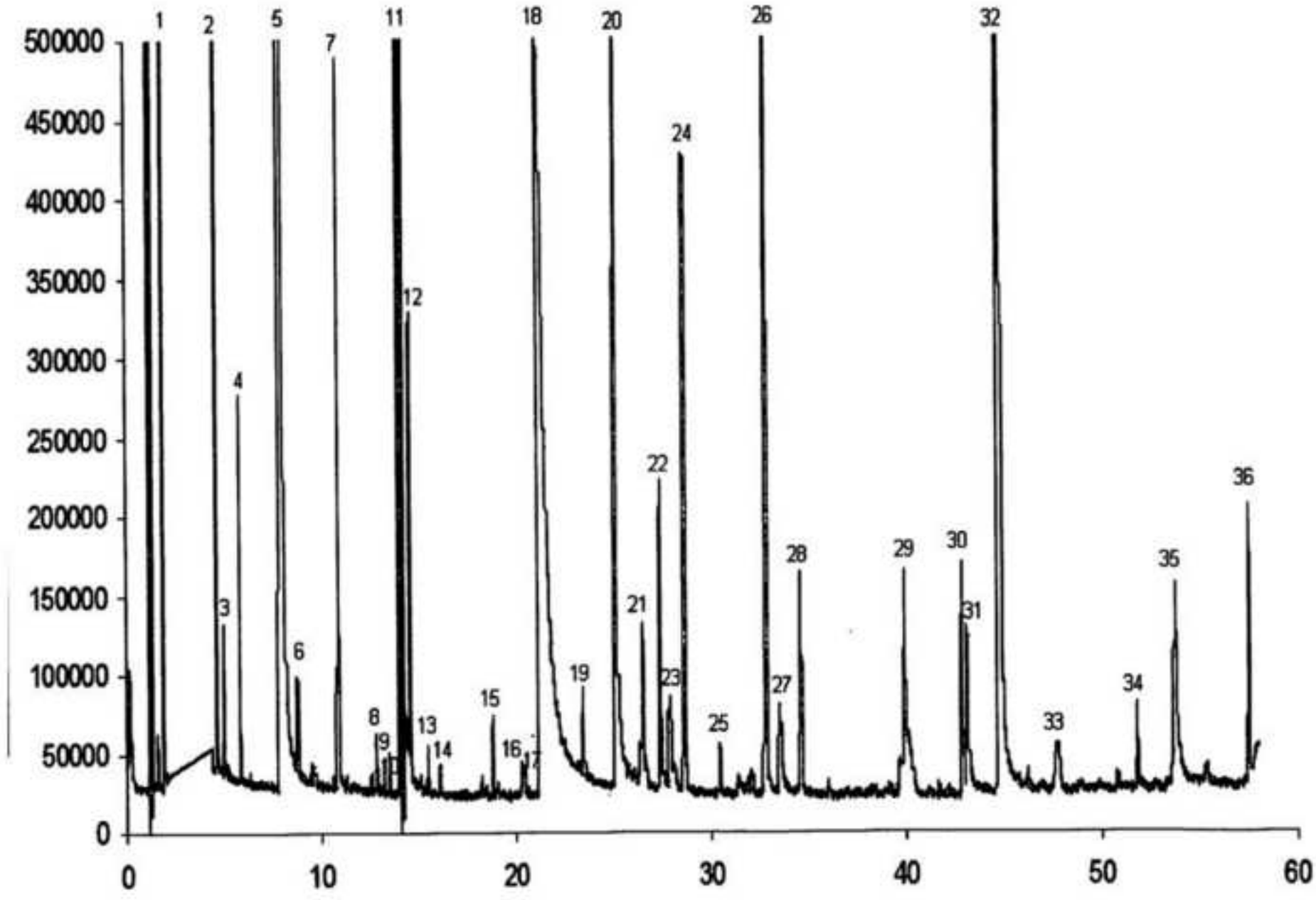

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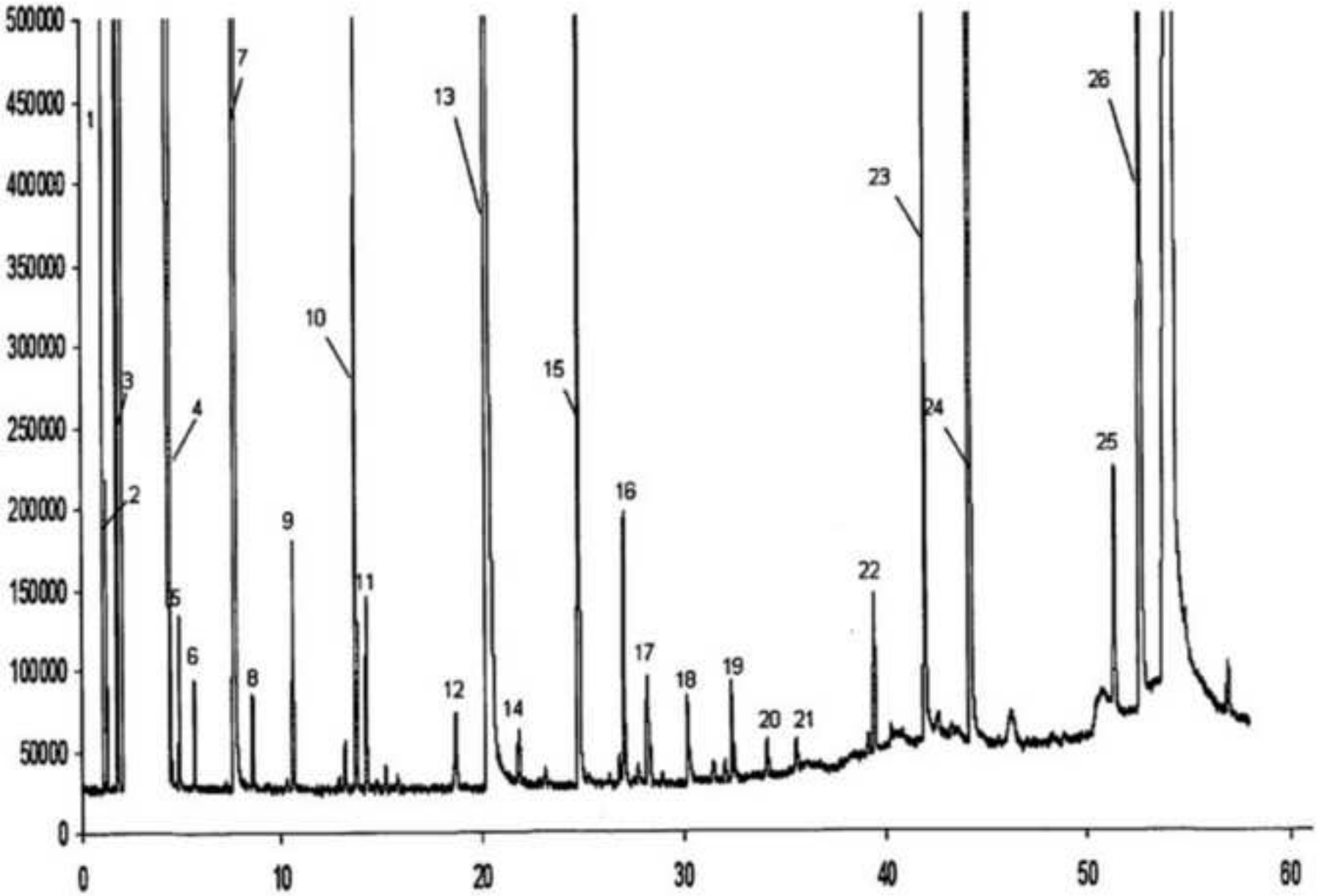

Table(s)

Table 1. CO_2-SFE for the removal of ethanol from red and white wines at 313 K.

Ext.	P (MPa)	CO_2/wine ratio (kg/l)	% wt ethanol in raffinate
white wine			
1	18	9	3.5
2	13	12	2.1
3	9.5	29	< 1
4	9.5	9	5.5
red wine			
5	9.5	11	3.5
6	9.5	30	< 1

Table 2. CO_2-SFE for the recovery of aroma from red and white wines at 313 K and 9.5 MPa. Total extraction time = 4 h. Total amount of wine feed to the extraction column = 1000 mL.

	Ext. 1 white wine	Ext. 2 white wine	Ext. 3 red wine	Ext. 4 red wine
wine flow (L/h)	0.23	0.23	0.23	0.23
CO_2 flow (kg/h)	0.60	0.60	0.60	0.90
CO_2/wine ratio (kg/L)	2.6	2.6	2.6	3.8
S1 extract (mL)	11.0	10.8	5.2	13.5
Score	15.0	15.5	3.1	16.0
SD[a]	0.7	1.4	1.0	0.8
S2 extract (mL)	4.3	4.0	0.5	1.0
Score	17.3	19.1	2.4	17.0
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