Supercritical CO_2 extraction applied toward the production of a functional beverage from 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 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 a previous contribution (Ruiz-Rodriguez et al., 2010) the authors developed a model to simulate the countercurrent supercritical CO_2 removal of ethanol from alcoholic beverages (brandy, wine, and cider) using the GC-EoS. The results obtained compared good with experimental data from the literature and thus, the model was used to estimate process conditions to achieve an ethanol content reduction from ca. 10 %wt to values lower than 1 %wt.

In this work, supercritical CO_2 technology was employed to produce a low-ethanol content beverage from wine by combining two 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) up to ethanol content lower than 1 %wt. 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

97 The wines (white, red and rose) employed in this work were kindly supplied by a
98 Spanish wine seller company (Bodegas Torres S.A., Vilafranca del Penedès, Catalonia,
99 Spain). Ethanol content in wine was 9.5%, 10.5% and 11.3% v/v for white, red and rose
100 wines, respectively.
101 Ethanol (GC-assay, 99.5% purity) and MilliQ-water were obtained from Panreac
102 (Barcelona, Spain) and from Millipore (Millipore Iberica, Madrid, Spain), respectively.
103 CO_2, N48 (99.9998% purity), was supplied by AL Air Liquide España S.A. (Madrid,
104 Spain).
105
106 **2.2 Supercritical fluid extraction of ethanol**
107
108 The supercritical fluid extraction (SFE) device (Thar Technologies) comprises a
109 countercurrent packed column of 2.8 m height with two separator cells (S1 and S2),
110 where a cascade decompression takes place. The liquid sample can be introduced into
111 the column from two different points: the top (180 cm of effective packed height) and
112 medium (120 cm of effective packed height) feed points. The solvent (CO_2) is fed into
113 the column through the bottom and is heated up to the extraction temperature before be
114 introduced into the packed column.
115 Once the operating pressure and temperature were reached, the wine was pumped from
116 the top of the column at a constant flow rate of 200 ml/h during 1 h. The temperature of
117 the extraction column was kept at 313 K in all experimental assays. Extraction pressure
118 was varied from 9.5 to 18 MPa and thus, CO_2 densities varied from 692.3 kg/m^3 to
119 848.9 kg/m^3, maintaining an appropriate density difference between the solvent and the
120 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/l. 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 to avoid dragging of the liquid sample, 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 is 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

169 The response used to evaluate the quality of the supercritical extracts was the
170 resemblance, based on a human olfaction test, of their aroma to that of their respective
171 starting wines. Aromatic extracts were evaluated with a panel of six experts panelist
172 (four females and two males, 25-50 year-old individuals) who judged the similarity of
173 the aromas. The scale used for sensorial evaluation was not structured [18] to mark the
174 similarity between the aroma of the extracts and that of the starting wines; that is, it only
175 had two extreme points, and the right end represented the aroma of the original wine.
176 Thus, the higher the score, the higher the similarity between the aroma of the
177 supercritical extracts and the aroma of the starting wines. The distance (in centimeters)
178 to the left end was considered for the statistical analysis of the data.
179
180 **2.6 Ethanol analysis**
181
182 A Perkin-Elmer Autosystem XL gas chromatograph (Perkin-Elmer, Norwalk CT)
183 equipped with a programmed split/splitless injector (PSS) and a flame ionization
184 detector (FID) was used to perform all the GC analysis. The system was coupled to a
185 Perkin-Elmer chromatography software system (Turbochrom). The column employed
186 was a 30 m x 0.25 mm i.d. fused silica capillary column (Quadrex Corp., New Haven,
187 CT) coated with a 0.25 μm layer of Carbowax 20M (polyethyleneglycol). To evaluate
188 the ethanol content of the raffinates obtained from red and white wines after
189 supercritical fluid extraction, a calibration curve was prepared using ethanol blank
190 solutions ranging from 1 to 20 % in ethanol content (v/v). The chromatographic
191 conditions were as follows: injector temperature, 210 ^{o}C; detector temperature, 280 ^{o}C,
192 Helium at 15 psig was used as a carrier gas. The split ratio was 1:20 and the volume
193 injected was 1 μL. The oven temperature program was as follows: starting at 39 ^{o}C

194 (held for 3 min), and then heating to 65 ^{o}C (held for 1 min) at 5 ^{o}C/min, and then
195 heating to a final temperature of 200 ^{o}C (held for 1 min) at 40 ^{o}C/min.
196
197 **2.7 Determination of antioxidant activity**
198
199 **2.7.1. ABTS assay**
200
201 The TEAC (Trolox Equivalent Antioxidant Capacity) assay described by Re et al. [19]
202 was used to measure the antioxidant activity of the wine samples. Briefly, ABTS$^{\bullet}$
203 radical cation was generated by reacting 7 mmol/l ABTS with 2.45 mmol/l potassium
204 persulfate after incubation at room temperature for 16 h in the dark. The ABTS$^{\bullet}$ radical
205 solution was diluted with PBS (pH 7.4) to an absorbance of 0.70 - 0.20 at 734 nm. 10 µl
206 of wine (previously diluted) at five different concentrations extract was added to 0.990
207 ml of diluted ABTS$^{\bullet}$ radical solution. The reaction was measured until the absorbance
208 reached a plateau. Trolox was used as reference standard, and results were expressed as
209 TEAC values (mmol Trolox/g extract). All analyses were done, at least, in triplicate.
210
211 **2.7.2. DPPH$^{\bullet}$ free radical-scavenging assay**
212
213 The ability of wines to scavenge DPPH$^{\bullet}$ free radicals was determined according to the
214 method proposed by Brand-Williams et al. [20]. Briefly, 25 µl of wine or standard
215 (previously diluted) was added to 0.975 µl of a 6×10^{-5} M solution of DPPH$^{\bullet}$ in
216 methanol. A control sample, containing the same volume of solvent in place of extact,
217 was used to measure the maximum DPPH$^{\bullet}$ absorbance. The reaction was allowed to
218 take place in the dark until the reaction reach a plateau. Trolox was used as reference

219 standard, and results were expressed as TEAC values (mmol Trolox/g extract). All
220 samples were assayed, at least, in triplicate.
221
222 **2.7.3. Oxygen radical absorbance activity (ORAC)**
223
224 The ORAC assay was performed essentially as described by Huang et al [21]. Briefly,
225 AAPH was dissolved in 10 ml of 75 mM phosphate buffer (pH 7.4) to a final
226 concentration of 166 mM and made fresh daily. A fluorescein stock solution (8×10^{-4}
227 mM) was made in 75 mM phosphate buffer and stored. The stock solution was diluted
228 1/10000 with phosphate buffer. To all experimental wells, 150 µl of working
229 fluorescein solution were added. In addition, blank wells received 25 µl of 75 mM
230 phosphate buffer, while standards received 25 µl of trolox dilution and samples 25 µl of
231 wine (previously diluted). Reactions were initiated by the addition of 25 µl of AAPH
232 solution. Results were expressed as trolox equivalent antioxidant capacity.
233
234 **2.8. Total phenolic content (TPC)**
235
236 Total phenolic content of wines was determined with Folin-Ciocaltea reagent by the
237 Singleton et al. method [22] and the results were expressed as GAE (mg of gallic acid/L
238 of wine). Briefly, 3 mL of distilled water was mixed with 50 µL of sample or standard.
239 250 µL of Folin-Ciocalteu reagen was added and the content of the tube was mixed
240 thoroughly. After 3 min 0.75 mL of Na_2CO_3 (20% w/v) followed by 0.95 mL of water
241 was added and the mixture was allowed to stand for 2 h. The absorbance was measured
242 at 760 nm. The TPC of the wines was expressed as GAE (mg of gallic acid equivalent
243 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 (Exp. 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 the results obtained using the simulation GC-EoS model (Ruiz-Rodriguez el al., 2010) S/F ratios greater than 30 are necessary at 308 K to achieve an ethanol reduction in wine from ca. 10 to 1 %wt. The same conclusion is driven from the 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 (Exp. 1) were not better than those obtained when using the lower CO_2 density but high CO_2/wine ratios (Exp. 3).

3.2 Study of aroma recovery

268 The same wines employed in the dealcoholization experiments (white and red wines)
269 were employed to study the recovery of aroma from wine using supercritical CO_2. The
270 key idea to attain the target was utilizing a low CO_2/wine ratio. Considering the
271 facilities of the available experimental device, the CO_2/wine ratio employed in this case
272 was in the range 2-4 kg/l.
273 Certainly, low CO_2/wine ratios imply that the liquid sample is the continuous phase and
274 the supercritical solvent is the disperse phase. Thus, the solvent phase would be
275 saturated with the aroma compounds (which are present in wine in very low amounts)
276 while reduced amounts of ethanol should be extracted. On the contrary, during the
277 dealcoholization trials (CO_2/wine ratio = 9-30 kg/l), the supercritical CO_2 solvent is the
278 continuous phase and the wine is the disperse phase, and both aroma compounds and
279 ethanol a readily extracted.
280 Table 2 shows the results obtained in the recovery of aroma from white and red wines.
281 Ext. 1 and 2 in Table 2 are duplicates of the extraction accomplished for the white wine
282 at 313 K and 9.5 MPa. By comparison of the amounts (ml) of extract obtained in each
283 trial, it can be concluded that very good reproducibility is attained. Further, whilst the
284 raffinate was colored and absolutely odorless, the samples obtained in both S1 and S2
285 separators were completely transparent and very aromatic. This was assessed by
286 analyzing the scores given by the panelists to the different extracts obtained. It can
287 easily be seen that the extracts obtained in S1 and S2 corresponding to extracts 1, 2 and
288 4 obtained a high score. This means that they had a high resemblance to the original
289 aroma of the starting white and red wines. However, in the case of red wine,
290 significantly lower amounts of extract were obtained when applying the same CO_2/wine
291 ratio than in the case of white wine (Ext. 3 in Table 2). Additionally, the raffinate
292 obtained in this experiment somewhat preserved the characteristic wine odor. Thus, the

293 CO_2/wine ratio was slightly increased (Ext. 4 in Table 2) and then, also in this case, an
294 odorless raffinate was obtained.
295 According to Table 2, around 14 ml per liter of wine sample was obtained in the
296 separators (Ext. 1, 2 and 4); although in the case of white wine the amount of extract
297 recovered in S2 was larger than in the case of red wine. Moreover, the amounts of
298 extract recovered in these experiments are significantly lower than the amounts of
299 extract obtained in the dealcoholization assays (50-75 ml of extract per liter of wine).
300 The GC-MS chromatograms for extracts corresponding to the white wine are shown in
301 Figure 1. The figure shows a comparison between the chromatogram corresponding to
302 the original (white) wine, the extracts recovered in the separators and the raffinate
303 obtained from the bottom of the extraction column. As can be qualitatively observed
304 from the figures, the extracts are significantly concentrated in the aroma compounds
305 while the raffinates contain reduced amounts of aroma compounds in comparison to the
306 original wine. In the case of red wine the chromatograms followed the same pattern.
307 Figures 2 and 3 show the peak identification of the chromatograms corresponding to S1
308 extracts of experiments reported in Table 2. Figure 2 corresponds to the S1 extract
309 recovered in Ext. 1 (white wine) while Figure 3 refers to the S1 extract of Ext. 4 (red
310 wine). In qualitative terms, both extracts showed very similar chromatographic profile,
311 being compounds such as 3-methyl-1-butanol, ethyl lactate, acetic acid, 2,3-butanediol
312 and phenylethyl alcohol the ones who presented the highest chromatographic peak
313 areas.
314 Further, Table 3 shows a comparison between the peak areas obtained for the different
315 compounds identified in the original red wine and the corresponding extract (Ext. 4 in
316 Table 2). All the injections were carried out following the same chromatographic
317 method and conditions (see Materials and Methods section). Thus, peak areas in Table 3

318 were employed to estimate concentration factors (peak area in extract / peak area in
319 original wine) of some aroma compounds observed in the samples. Concentration
320 factors up to 50 could be calculated from the results of the GC-MS analysis.
321 Nevertheless, it should be pointed out that several compounds that are present in very
322 low concentration in the original red wine could only be identified in the extract. For
323 example, several alcohols (n-butanol, 3-methyl-1-pentanol, 1-hexanol, 3-ethoxy-1-
324 propanol, 3-hexen-1-ol, 3-methyl thiol propanol), acids (3-OH-ethyl ester -butanoic
325 acid, 2-methyl-propanoic acid, isovaleric acid, 2-OH-ethyl-3-phenylpropionate,
326 diethylhydroxybutanedioate, caprylic acid, 2-OH-diethyl-pentanedioate), esters
327 (isoamyl acetate, ethyl hexanoate, ethyl octanoate), aldehides (2-furancarboxaldehyde),
328 and ethers (1-methoxy-3-methyl-butane) could only be detected in S1 extract and thus,
329 it is expected that very high concentration factors (> 50) were attained for these
330 substances.
331
332 **3.3 Production of a non-alcoholic functional beverage from rose wine**
333
334 On the basis of previous studies the manufacture of a non-alcoholic beverage from rose
335 wine (11.3% v/v of ethanol) was accomplished. Two CO_2-SFE steps were carried out,
336 both at 313 K and 9.5 MPa, but employing different CO_2/wine ratios in order to achieve
337 (Step 1) the recovery of aroma and then (Step 2) the dealcoholization of the raffinate
338 obtained in the first step. S1 separator was maintained at 5 MPa whereas in S2 the
339 extract was depressurized up to 1 MPa. Temperature in both separators was kept at 308
340 K.
341 ***Step 1: recovery of aroma from rose wine.*** CO_2 flow rate was 0.9 kg/h and wine flow
342 rate was 0.25 l/h (CO_2/wine ratio = 3.6). A total of 12 liters of wine were fed to the

343 extraction column. Top and bottom products were collected during the continuous
344 operation; 220 ml of extract were recovered in S1 and considerably lower amounts (30
345 ml) in S2 separator. The mass balance closed with accuracy greater than 97%.
346 The extract obtained in S1 (18.3 ml per liter of rose wine) was completely transparent
347 and highly aromatic; the chromatogram obtained by GC-MS is shown in Figure 4.
348 Additionally, Table 4 shows the chromatographic areas of the aromatic compounds
349 identified in the original rose wine and in the S1 extract obtained. Again, high
350 concentration factors could be calculated for some aromatic compounds, such as 14 for
351 ethyl acetate, 36 for ethyl lactate, 47 for 3-methyl-1-butanol and 53 for phenyl ethyl
352 alcohol, and higher concentration factors would be expected for those compounds which
353 could not be detected in the original red wine (2-methyl-1-propanol, isoamyl acetate,
354 hexanoic acid, etc.).
355 The odorless raffinate obtained from the bottom of the extraction column contained
356 8.8% v/v of the ethanol.
357 ***Step 2: removal of ethanol from the raffinate obtained in step 1.*** The liquid sample
358 collected from the bottom of the extraction column in Step 1 was utilized to completely
359 remove the remained ethanol. In this case, the CO_2 flow rate was 4.8 kg/h and the liquid
360 sample flow rate was 0.20 l/h (CO_2/liquid ratio = 24). The concentration of ethanol in
361 the raffinate obtained in this case (850 ml per liter of original rose wine) was lower than
362 1%.
363 ***The non-alcoholic functional beverage from rose wine.*** 850 ml of the raffinate
364 obtained from Step 2 (ethanol content < 1% v/v) was mixed with 18.3 ml of the extract
365 produced in Step 1. This beverage (1.1% v/v ethanol) produced from rose wine
366 contained several of the aromatic compounds detected in the original wine, as can be
367 deduced from the GC-MS analysis given in Table 4. Some substances are present

368 almost in the same concentration (3-methyl-1-butanol, acetic acid, 2,3-butanediol, 2-
369 methyl-propanoic acid) although some other substances that were detected in the
370 original wine, could not be detected in the non-alcoholic beverage (ethyl acetate, 3-
371 hydroxy-2-butanoate, ethyl lactate, cis-5-hydroxy-2-methyl-1,3-dioxane).
372 As it is shown in Table 5 aroma removal from wine only caused slight modifications in
373 its antioxidant activity and polyphenols content. ABTS and DPPH assays shown a very
374 small increase in the antioxidant capacity according to the TPC increment. However
375 ORAC value was slightly smaller in this odorless raffinate, maybe to the different
376 mechanism of action of these methods. The non-alcoholic functional beverage had
377 similar DPPH and ORAC values than original wine, together with similar TPC. Only a
378 smaller ABTS value was detected.
379

380 **Conclusion**

381 Supercritical fluid CO_2 extraction was employed in a two-step process to produce a
382 novel beverage from rose wine. Several aroma compounds were determined to be
383 present both in the original rose wine and in the low-alcoholic beverage. Further, the
384 new beverage maintains the antioxidant capacity of the original wine; it contains around
385 1% v/v ethanol, and thus might be potentially commercialized with a functional claim.
386

387 **Acknowledges**

388 The authors gratefully acknowledge the financial support from the Comunidad
389 Autónoma de Madrid (ALIBIRD, project number S-505/AGR-0153) the Ministerio de
390 Ciencia e Innovación (project AGL2007-64198/ALI), Spain and Miguel Torres S.A.
391 (Proyect CENIT HIGEA CEN-20072003).
392
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453

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

455

Exp.	P (MPa)	CO_2 density (g/cm^3)	CO_2/wine ratio (kg/l)	% wt ethanol in raffinate
white wine				
1	18	0.820	9	3.5
2	13	0.742	12	2.1
3	9.5	0.516	29	< 1
4	9.5	0.516	9	5.5
red wine				
5	9.5	0.516	11	3.5
6	9.5	0.516	30	< 1

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460 **Table 2.** CO_2-SFE for the recovery of aroma from red and white wines at 313 K and 9.5
461 MPa. Total extraction time = 4 h. Total amount of wine feed to the extraction column =
462 1000 ml.
463

	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

464 [a] Standard Deviation
465
466
467
468

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

471

compound	original red wine	S1 extract	concentration factor
Ethyl acetate		14467940	
2-methyl-1-propanol	975555	28864100	29.6
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	

472 (*) Chromatographic area too high leading a saturated detector response.
473
474

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

478

	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

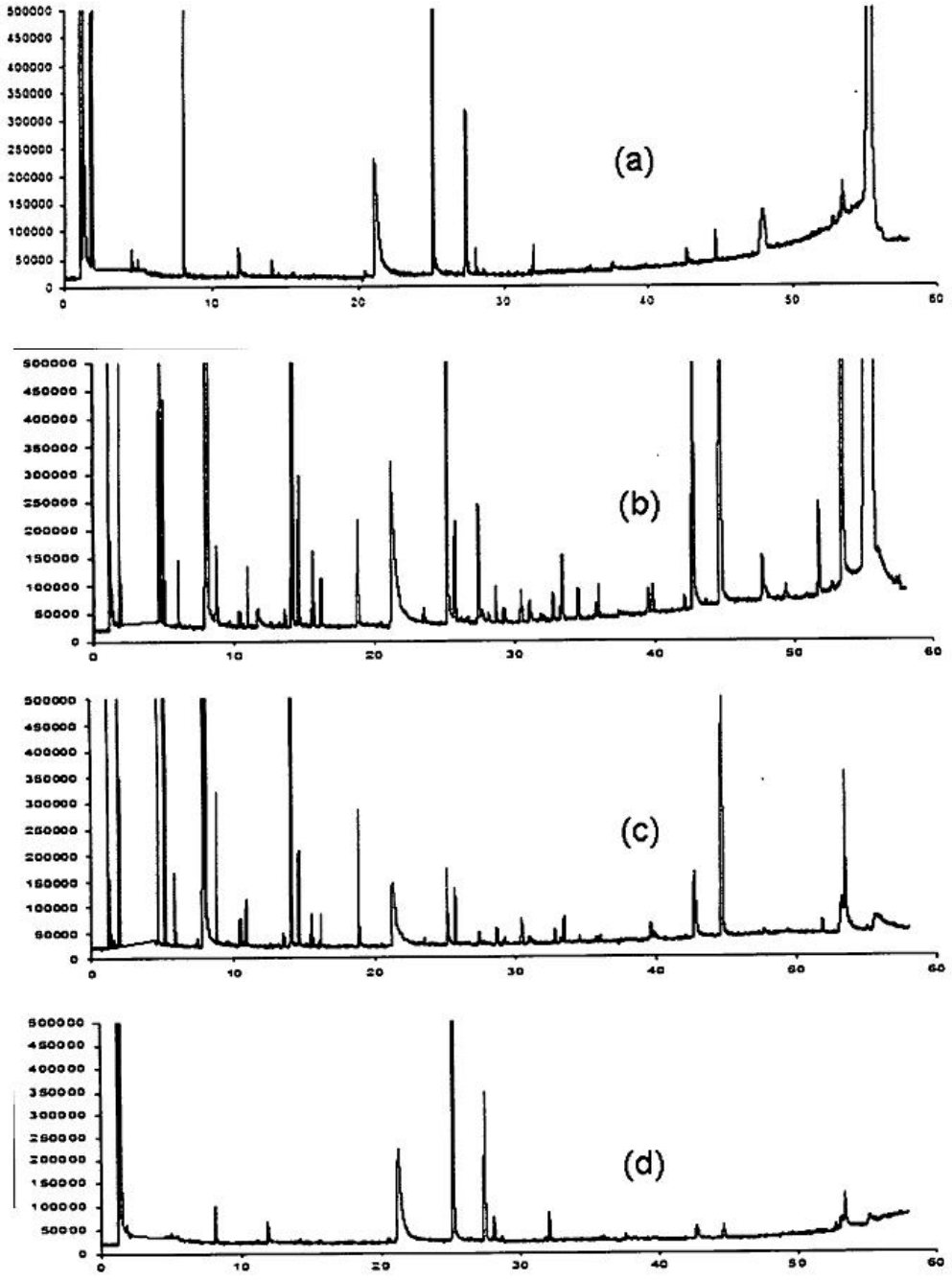
479

480

481 **Table 5**. Antioxidant activity of rose wine, raffinate and non-alcoholic beverage.
482

	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]

483 [a]Different superscript letters denotes statistically significant differences ($p<0.05$) among data in the same
484 column
485 [b]Antioxidant activity was expressed as TEAC mmol of Trolox/g of extract.
486 [c]Total phenolic compounds was expressed as mg GAE/l)
487



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489

490 **Figure 1.** Aroma recovery from white wine (Ext. 1 in Table 2): comparison between the

491 GC-MS chromatograms obtained for (a) the original wine; (b) S1 extract; (c) S2 extract;

492 (d) raffinate.

493

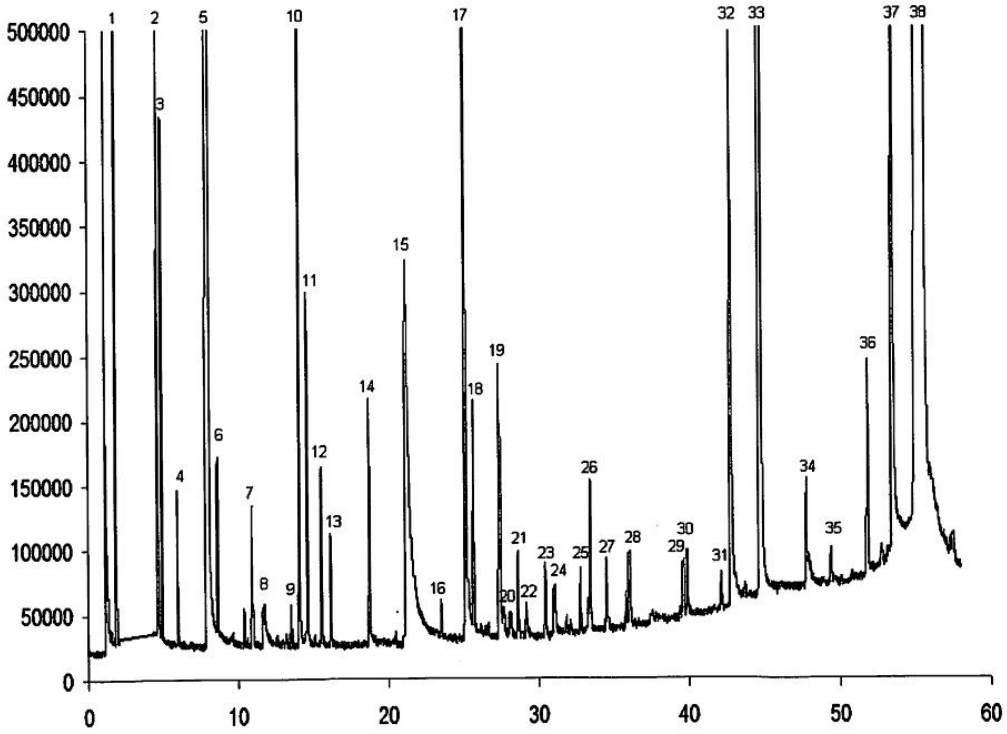

Figure 2. Chromatogram corresponding to the extract recovered from white wine in S1 separator (Ext. 1 in Table 2).

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.

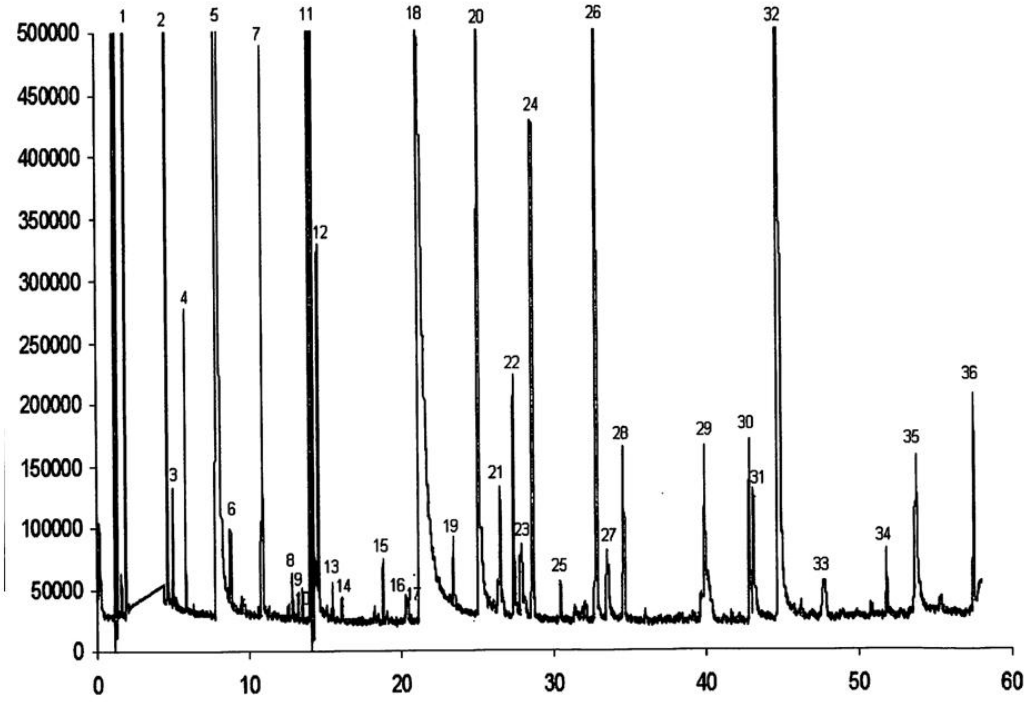

Figure 3. Chromatogram corresponding to the extract recovered from red wine in S1 separator (Ext. 4 in Table 2).

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.

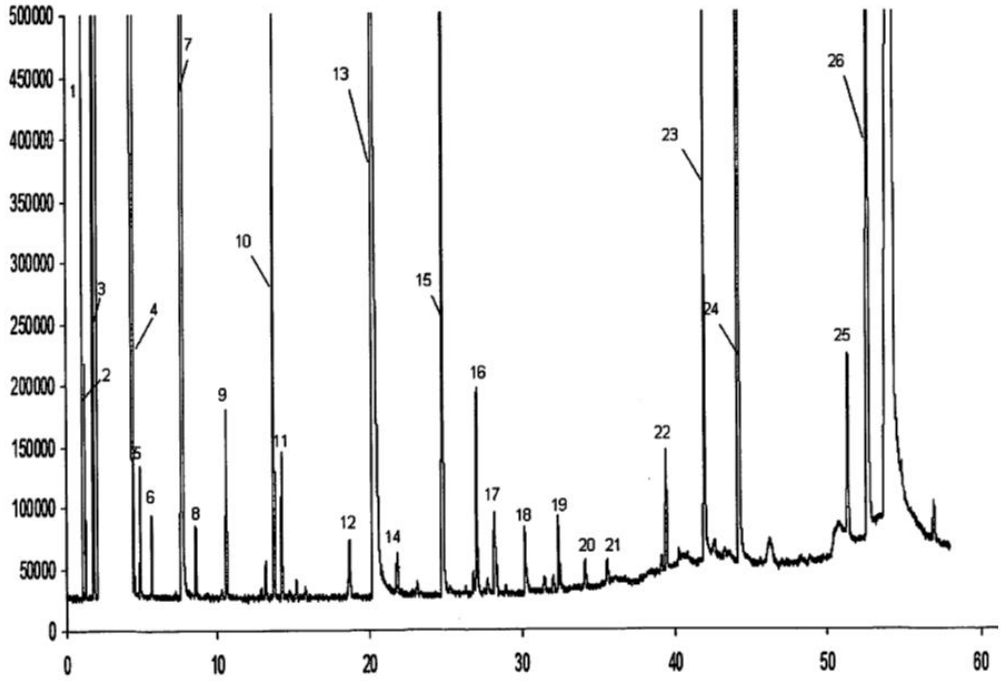

Figura 4. Chromatogram corresponding to the extract recovered from rose wine (S1 separator).

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.