Extraction of 2-Phenylethyl Alcohol

by Techniques such as Adsorption, Inclusion, Supercritical CO_2, Liquid-Liquid and Membrane Separations

By Cathy E. Fabre, Societé SAF-ISIS Z.A., Souston, France
Philippe J. Blanc, Alain Marty and Gérard Goma
Département de Génie Biochimique et Alimentaire, Institut National des Sciences Appliquées, Toulouse cedex, France
and Isabelle Souchon and Andrée Voilley,
Laboratoire de Génie des Procédés Alimentaires et Biotechnologiques ENSBANA, Université de Bourgogne, Dijon, France

Biotechnological processes to produce flavor compounds are in active development because of legislation that encourages production of natural components in the aroma industry. Also, the present consumer preference for natural instead of synthetic flavors will continue to rise. Therefore, we can expect to see increasingly more substitutions for previous sources of aroma. In fact, with respect to microorganisms able to produce flavor compounds, some have proved to be a rich source of enzymes and have the advantage of possessing complex enzyme systems able to carry out a series of successive conversions. However, some reports[1-2] on synthesis by microorganisms have shown that the production rate of a compound may decrease sharply when the product concentration is greater than a threshold value above which the growth and/or the production is inhibited. In such cases, the accumulation of compounds in the broth can be prevented by a continuous and selective removal of the fermentation-toxic products, in order to improve the productivity of the fermentation process.

Several extraction processes are known to recover such specific compounds. Organic liquid extractants and solid resinous adsorptive materials are widely used. In spite of their efficiency, the solvents have been cautiously used in the fermentations because of their toxic effects on the microorganisms.[3] However, in some cases, the extraction in situ by a biphasic system was effective. For example, the use of a mixture of water and hexane improved the bioconversion of benzyl alcohol into benzaldehyde by *Pichia pastoris*.[4] Bruce and Daugulis[5] confirmed the efficiency of a solvent mixture for compound extraction when *Zymomonas mobilis* was cultivated with 5% of heptanol and 95% of aldol. But, most of the time, extraction realized in situ led to toxic effects on the microorganisms. An alternative method of overcoming these problems might be to use an extractant that is both safe and effective. Previous experiments have indicated that some vegetable oils appeared to be suitable extractants for the removal of butanol, caffeine and carotenoid pigments, for example.[3]

On the other hand, polymeric resins have been shown to remove different classes of organic compounds selectively. The wide variations in functionality, surface area and porosity available for polymeric resins allowed the selective removal of specific organic solutes.[6] Some resins, such as Chromosorb 102, increased the growth inhibition of *Poria cocos* provoked by an accumulation of secondary products.[7] In the presence of Amberlite XAD-2, *Ceratocytis variospora* produced up to 1.9 g/L of terpen in fermentor; without an adsorbent, the concentration did not exceed 1.1 g/L.[8] With the same resin, Klingenberg and Hassen[9] improved the production of phenethyl acetate by *Kluyveromyces marxianus*. The works of Cheetman et al.[10] also revealed the efficiency of the resin XAD-2 in the extraction of the lactones synthetized by *Rhodotorula glutinis* and *Sporobolomyces odorus*. When the culture of *Streptomyces griseus* was coupled with an extraction on Amberlite XAD-2, the production of geosmin was significantly improved.[11] Moreover, Amberlite IRA-400 allowed salicylic acid produced by *Corynebacterium renale*[12] to be removed continuously. Finally, Gusler et al.[6] showed that the resin Amberlite XAD-12 was very effective for the adsorption of compounds with an aromatic nucleus.

The β-cyclodextrins, composed of several glucose units,

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were also used to extract numerous compounds by inclusion; they allowed us an increase in some production yields. The study by Bar,[13] carried out with yeast cultures, revealed the improvement of the bioconversion of aromatic alcohols into corresponding aldehydes in the presence of cyclodextrins. Szente and Szejtli[14] showed the stability of the benzaldehyde adsorbed on cyclodextrins. However, in some cases, the cyclodextrins performed less effectively than other adsorbents. In fact, Krings et al.[15] confirmed that the removal of 3-phenyl-1-propanol was more effective with adsorbents like zeolite than with Tenax or β-cyclodextrins.

Extraction with supercritical CO_2 is one of the newer techniques for recovering molecules (such as aroma chemical molecules) from dilute fermentation broths. The supercritical state, which has intermediary properties between those of liquids and gases, is also called a dense gas or an expanded liquid. Due to good solvent properties (high density) and good transport capacities (low viscosity and high diffusivity), this solvent appeared to hold promise for the extraction of compounds in a mixture.[16] Besides, it is nontoxic and nonflammable, and its natural character is well appreciated in the food industry. The separation is based on the principle of liquid extraction according to the volatility characteristics of the compounds; therefore, supercritical CO_2 seemed to be a good solvent for extracting a wide variety of organic compounds from water solutions.

Numerous studies revealed the efficiency of supercritical CO_2 for removing terpenes,[17] limonene, eugenol and thymol[18] from plants. It was also possible to obtain aromatic extracts from lilac and rose with this supercritical fluid.[19] Aromas from vanilla, ginger, rosemary,[20] sage, wild rose and clove[21] were also recovered by this process. These studies insisted on the conservation of organoleptic properties of extracts. Other works[22,23] also revealed that supercritical CO_2 could be used to extract inhibitor compounds such as ethyl alcohol from fermentation broth. Another great advantage of using supercritical CO_2 lies in the option to couple extraction with downstream fractionation. Indeed, due to a variable solvent power of this fluid versus pressure and temperature, it is possible to fractionate extracted compounds at the outflow of the extraction vessel by means of a cascade of depressurization steps.[24]

Finally, it was possible to use membrane processes like pervaporation, perstraction or "extraction based on membrane" in order to remove compounds continuously and selectively from an aqueous solution. Such recent techniques were used for three purposes: as a sensor to monitor the metabolism of microorganisms, to remove inhibiting compounds in a batch or continuous fermentation and to recover specific metabolites.[25] The main advantage of these processes was to allow an extraction under gentle operating conditions. Pervaporation offered many potential food industry applications, such as wine or beer dealcoholization, juice concentration, production of spirits and extraction of volatile organic compounds from fermentation broths or from evaporation condensates of fruit juices.[26] Lamer and Voilley[25] confirmed the efficiency of this technique for removing butanol, acetone and ethyl alcohol produced by *Clostridium acetobutylicum* or *Saccharomyces cerevisiae*. Pervaporation also facilitated the extraction of 1-octen-3-ol, ethyl acetate, ethyl butanoate and octanal.[26] Benzaldehyde production by *Bjerkandera adusta* improved when culture and pervaporation were combined.[27] Also, Bengson et al.[28] showed that 6-pentyl-α-pyrone could be recovered by pervaporation from a culture broth of *Trichoderma viride*.

The pervaporation technique could be modified in some cases to recover compounds characterized by a low vapor pressure. This particular pervaporation, called "perstraction," consisted of a diffusion of molecules from a liquid phase toward an organic solvent through a dense membrane. The first industrial applications appeared in 1992 with a study concerning the recovery of heavy metals in water treatment.[29] Later, perstraction was applied to aroma extraction such as decalactones produced by microorganisms.[30]

In addition to pervaporation and perstraction, which is characterized by diffusion of solutes through a dense membrane, a third process called "extraction based on membrane" is known today. The only difference between this technique and perstraction was that the membrane was microporous instead of dense. These microporous membranes, hydrophobic or hydrophilic, were used in a wide variety of systems including organic pollutants, pharmaceu-

tical products, aromatic compounds and molecules produced by fermentation. This process has been tested in the continuous removal of ethyl alcohol produced by *Saccharomyces cerevisiae*.[31]

The purpose of this paper is to discuss these different techniques as they apply to the removal of 2-phenylethyl alcohol, rose-like aroma, from an aqueous solution. This compound, selected because of its potential use in the food and fragrance industries, may be obtained by fermentation[32] or by extraction from natural sources.[33] However, when it was produced by microbial synthesis, it led to toxic effects toward the microorganisms. In our work, the first method tested corresponds to the classical liquid-liquid extraction with solvents or vegetable oils. The second is the extraction by adsorption on resins or by inclusion in cyclodextrins. The third process is based on the abilities of supercritical CO_2 to separate selectively 2-phenylethyl alcohol. The last techniques correspond to three membrane processes: pervaporation, perstraction and extraction based on membrane. Thus, the work presented here shows the efficiency of each method in removing 2-phenylethyl alcohol from an aqueous solution.

Materials

In our study we used the materials and suppliers listed in Figure 1.

Figure 1. Materials and suppliers

Substrate
2-phenylethyl alcohol (Sigma)

Substrate properties
Formula: $C_8H_{10}O_2$ phenylethyl alcohol
Molecular weight: 122.17 g/mol
Boiling point: 220-222°C
Density: 1.02 at 25°C
Solubility in water: 1-60 (19 g/l)
Characteristic: rose-like odor
CAS: 60-12-8, FEMA/GRAS: 2858

Solvents
methyl-propanol-1 (Normapur, Prolabo, France)
ethyl acetate (Normapur, Prolabo, France)
diethyl ether (Normapur, Prolabo, France)
ethyl alcohol (Normapur, Prolabo, France)
butanol-1 (Normapur, Prolabo, France)
butanol-2 (Normapur, Prolabo, France)
butyl acetate (Normapur, Prolabo, France)
n-hexane (Normapur, Prolabo, France)

Vegetable oils
peanut oil (Lesieur, France)
canola oil (France)
corn oil (Epi d'or, France)
olive oil (Puget, France)
pips of grape oil (Prouvenço, France)
soybean oil (Salador, France)
sunflower oil (Lesieur, France)

Supercritical CO_2 (Alphagaz)
pressure: 49.5 bar (15°C)
contains less than 7 ppm water

Dense membranes
The membrane used for pervaporation and perstraction experiments was an organophilic composite membrane produced by GFT (Le Carbone Lorraine). The active layer was a PDMS (polydimethylsiloxane) layer with a thickness of 10 µm. The exchange area varied for the two methods: 39.6 $10^{-4}m^2$ in the case of pervaporation and 16.4 $10^{-4}m^2$ in the case of perstraction.

Microporous membrances
The microporous membrane used was a hydrophobic membrane made up of polypropylene (Hoechst Celanese) with an effective area equal to 0.23 m^2 (diameter of fiber 240 µm, size of pores 0.05 µm).

Resin polymerous adsorbent materials
Amberlite IR 35 (Rohm & Haas)
Amberlite IR 45 (Rohm & Haas)
Amberlite IR 120 (Rohm & Haas)
Amberlite IRA 400 (Rohm & Haas)
Amberlite XAD-2 non-polar (Rohm & Haas)
Amberlite XAD-16 non-polar (Rohm & Haas)
Amberlite XAD-761 (Rohm & Haas)
Amberlite XAD-4 non-polar (Rohm & Haas)
Amberlite XAD-7 (Rohm & Haas)
Anion Cellex Pab (Biorad)
Anion Cellex PEI (Biorad)
Cation Cellex CM (Biorad)
Cation Cellex P (Biorad)
Cation Cellex SE (Biorad)
Chromosorb 101 (Interchim)
Chromosorb 45/60 (Johns Manville)
Chromosorb 80/100 (Intersmat)
Chromosorb 102 (Chrompack)
Chromosorb P.R. NAW (Girdel)
Duolite C2/2560 (Duolite International)
Hayesep Q (Chrompack)
PHS alumine (Girdel)
Porapak Q 80/100 mesh (Interchim)
Porapak R 80/100 mesh (Interchim)
Primisil K0143 AT (Johns Manville)
Sup chromosorb GAW DMCS (Girdel)
Sup chromosorb WHMDS (Girdel)
Sup Fluoropak (Girdel)

Cyclodextrins
α-cyclodextrin (Sigma)
β-cyclodextrin (Sigma)

Liquid-Liquid Extraction Procedure

Extractions were performed by layering a volume of aqueous solution containing 1 g/L of 2-phenylethyl alcohol over an appropriate volume of extractant, then mixing with a bar magnet for 15 minutes. After a quiescent period, a second mixing was followed by a second quiescent period. Such experimental conditions resulted in a stable chemical equilibrium for the solute between the aqueous phase and the extractant (solvent or oil). Both the aqueous solution and extractant phase were then sampled to analyze the concentration of 2-phenylethyl alcohol. The aroma that passed into the extractant phase was evaluated by headspace gas-phase chromatography (GPC) and the residual solute in the aqueous phase was quantified by high performance liquid chromatography (HPLC). Data analyses were then used to calculate the distribution coefficient Kd for the partitioning of the 2-phenylethyl alcohol between the two phases. Kd was defined as the ratio of the concentration of the dissolved substance in the extract to that in the aqueous phase:

$$Kd = \frac{[E]}{[R]} = \frac{[\text{extract}]}{[\text{purified phase}]_{\text{equilibrium}}}$$

in which E corresponded to the concentration in the solvent (extract) and R corresponded to the residual concentration of the solute in the aqueous phase when equilibrium was reached.

Extraction Using Adsorption and Inclusion Procedures

First, resins and cyclodextrins were washed with ethyl alcohol and filtered. Then, they were rinsed thoroughly with distilled water, filtered again and dried at 50°C overnight. The sorption and inclusion experiments consisted of placing 300 mg of clean dry support in 10 ml of aqueous solution of 2-phenylethyl alcohol. The samples were then rotated for 10 minutes at a rate of 500 rpm. After a quiescent period, the samples were shaken again, under the same conditions, in order to reach sorption or inclusion equilibrium. Then, the aqueous solution was sampled and analyzed by HPLC to determine the amount of recovered solute and to calculate the sorption or inclusion capacity Q of each support for the 2-phenylethyl alcohol (each set of experiments was carried out twice at a room temperature of 23°C). The parameter Q was determined from a solute mass balance on the sorption or inclusion vials giving the initial bulk solution concentration and the final bulk solution concentration when equilibrium was reached. The formula is presented below.[6]

$$Q = \frac{(C_o - C_e)\,V}{m}$$

in which C_o was the initial concentration of 2-phenylethyl alcohol being in contact with the support (mg), C_e the final equilibrium concentration in the liquid phase (mg), V the volume of aqueous solution of 2-phenylethyl alcohol, m the

mass support (g) and Q the amount extracted (mg/g).

Extraction Using Supercritical CO_2

The CO_2 installation we used (Separex, Nancy, France) was described by Marty et al.[24] The solution containing the compound to be extracted was put in contact with the supercritical fluid at the temperature and pressure leading to its solubilization. After extraction, the solvent/aqueous mixture passed through different separators in series where the temperature and pressure conditions were different. Thus, this was a multi-stage process. The solubilized products in the supercritical fluid were then fractionated by modifying the temperature and pressure of the fluid. The CO_2 extracted part of the aroma from the aqueous solution that was then passed through the separators. At the entrance to each separator, the pressure fall decreases the solvent power of the fluid, precipitating the aroma part that is no longer soluble. The separators were emptied and samples were taken at two times—after 2 hours and at the end of the experiment. Then, by HPLC analyses of the samples, we determined the concentration of 2-phenylethyl alcohol in the separator. With the formula given earlier, we calculated the distribution coefficient Kd of this aroma in CO_2.

Extraction Using Membrane Processes

Pervaporation: Pervaporation, first described by Kahlenberg in 1906, is a method of separation using a membrane. The method calls for partially spraying a liquid mixture through a thin dense layer, leading to the diffusion of compounds from a liquid phase toward a gas phase. This diffusion occurs because of a low partial pressure of the species in the gas phase. Then, the permeate is continuously removed from inside the tubing by a carrier gas flowing axially.

In our experiments, we used a dense composite membrane. The aqueous mixture, containing 2.9 g/L of 2-phenylethyl alcohol was put in a 2 L thermostated storage tank at 30°C. The feed solution, stirred at a rate of 300 rpm, was recirculated between the tank and the upper part of the pervaporation cell using a PTFE pump. Permeate was removed from the downstream side of the membrane by continuous pumping and collected in either of the parallel cold traps at –196°C. Using high performance liquid chromatography on liquid samples taken from the tank after 2 hours and at the end of the experiment (3.5 hours), we estimated the residual concentration of 2-phenylethyl alcohol in the aqueous solution. Cold traps were weighed and the trapped liquid was analyzed to determine the mass of water and aroma compound passing through the polymer. From this data we were able to calculate the pervaporation flux J and the selectivity β, two parameters which described the transport through the membrane. J represented the quantity of aroma compound passing through one unit area during one unit of time ($g/m^2/hr$). β, the selectivity or enrichment factor of a membrane, corresponded to a nondimensional ratio β-C'/C where C' and C were the mass fraction of the component in the pervaporate and in the aqueous phase, respectively. This parameter characterized the separation ability of a membrane.

Perstraction and extraction based on membrane: These two processes were carried out with the "liquid cell," a system supplied by Hoechst Celanese. The organic solvent used here corresponded to butyl acetate, characterized by a low solubility in water (0.68 g/L). For each method, experiments were carried out with 500 ml of aroma solution and 250 ml of extractant. The initial concentration of 2-phenylethyl alcohol was 2.8 g/L for perstraction and 2.7 g/L for liquid-liquid extraction based on membrane.

Circulation of both phases, carried out by pump, allowed them to recycle in order to concentrate the 2-phenylethyl alcohol in butyl acetate. Our tests were performed under ambient temperature (22°C). The aqueous and organic flows were 415.8 ml/min and 307.6 ml/min, respectively. The upstream pressure (waterside) was fixed at 0.1 bar for perstraction experiments and at 0.3 bar (entry) and 0.2 bar (exit) for extraction based on membrane experiments (because this process required a pressure gradient to immobilize the interface between the two phases).

For the perstraction experiments, samples were taken from the organic phase every 5 minutes during the first hour, then every 10 minutes from 1 hour to the end of the

assay (2.17 hours or 130 minutes). For the extraction based on membrane, samples were taken from butyl acetate every 5 minutes for 45 minutes. In both cases, thie composition of feed and permeate at the end of the experiments and the measuring of all samples (by CPG) allowed us to calculate the aroma flux J and the selectivity β.

Analytical Methods

HPLC: Using HPLC, we were able to monitor the variation in 2-phenylethyl alcohol concentration in the aqueous mixture. After suitable dilutions and filtration of each sample, chromatography was performed using a Waters 740 liquid chromatograph with a Nucleosil C18 reversed-phase column packed with PEG, particle size 5 µm. A guard column containing the same packing was used to protect the analytical column. Resolution of 2-phenylethyl alcohol was obtained at room temperature with 40% acetonitrile in water. In such conditions, retention time of the 2-phenylethyl alcohol was 8 minutes. The eluents, degazed with helium, were monitored by a Waters automated controller, with a flow rate fixed at 0.8 ml/min. The detection was realized by a Waters 481 UV spectrophotometer at 216 nm. The injection valve was equipped with a 20 µl sample loop.

Headspace GPC or indirect GPC: The quantity of rose-like aroma contained in both the organic phase and oil was estimated by gas chromatography. These analyses were performed on a Perkin-Elmer 8500, equipped with a FID detector and a BP 20 superox column (i.d. 0.32 µm, 50 cm length, packed with PEG). Nitrogen was used as the carrier gas. Initial column temperature was maintained at 60°C for 6 minutes, then it was raised to 200°C at a rate of 20°C/min. This temperature was maintained for 2 minutes, then was increased to 240°C at a rate of 20°C/min.

GPC: The GPC method (gas phase chromatography) was used to measure the 2-phenylethyl alcohol passed through butyl acetate in the membrane processes. These analyses were performed on a Perkin-Elmer 8500, equipped with a FID detector and a Supercowax-10 column (i.d. 1 µm, 30 cm length, packed with Carbowax 20M). Helium was used as the carrier gas. Initial column temperature was maintained at 65°C for 2 minutes, then it was raised to 210°C at a rate of 20°C/min. This temperature was maintained for 4 minutes.

Table I. Kd obtained for each solvent (20°C, with an aqueous solution containing 1 g/L of 2-phenylethyl alcohol)

Solvent	Kd
ethyl acetate0.95	
butyl acetate	34.00
butanol-1	11.00
butanol-2	10.00
diethyl ether	12.00
hexane	0.50
methyl-propanol-1	22.00

Table II. Kd obtained for each oil (20°C, with an aqueous solution containing 1 g/L of 2-phenylethyl alcohol)

Oil	Kd
sunflower	2.2
corn	2.7
pips of grape	2.7
olive	2.2
soybean	2.7
peanut	2.2
canola	2.2

Results with Liquid-liquid Extraction

Solvent extraction: The first process studied here concerned classical liquid-liquid extraction with organic solvents. Because of European Economic Community (EEC) regulations with regard to permitted solvents for the extraction of ingredients, only seven extractants were tested. The results obtained by HPLC analyses enabled us to estimate the distribution coefficient Kd for the partitioning of the 2-phenylethyl alcohol between the solvent and the aqueous phase. The estimates for each solvent are shown in Table I.

The results reveal that the 2-phenylethyl alcohol was poorly removed (Kd<1) from the aqueous phase with ethylacetate and hexane, while the other solvents presented a better affinity for this aroma (Kd>10). Butyl acetate was the most effective extractant, characterized by a Kd equal to 34. Such a solvent could be used to recover 2-phenylethyl alcohol from aqueous solutions or natural substrates. However, it was not used to remove 2-phenylethyl alcohol continuously from fermentation broth because of its toxic effects on numerous microorganisms. Some veg-

Table III. Sorption of the 2-phenylethyl alcohol on 11 resins

Resin	% of extraction	Sorption capacity Q mg/g
Amerlite IR 45	46	15
Chromosorb 101	27	9
Hayesep Q	85	28
Amberlite XAD-2	40	13
Amberlite XAD-16	77	26
Amberlite XAD-761	53	18
Amberlite XAD-7	84	27
Amberlite XAD-4	75	25
Chromosorb 102	55	18
Porapak R	84	28
Porapak Q	73	24

etable oils may be suitable extractants for removing organic compounds in such cases.[3] We therefore tested seven vegetable oils and compared the relative extraction of 2-phenylethyl alcohol.

Vegetable oil extraction: By using vegetable oils as extractants, some difficulties associated with the use of conventional liquid extractants could be overcome. Table II summarizes the results obtained for each oil.

Similar distribution coefficients were obtained for the extraction of 2-phenylethyl alcohol with all the oils tested, and the values of Kd did not exceed 3 in any case. Changing the type of vegetable oil appeared to have limited effect on the extraction. These results (Table II) were in agreement with a study by Welsh and Williams[3] revealing a similar efficiency of vegetable oils in the recovery of vanillin. However, in their work, the values of Kd obtained for the removal of other aromatic compounds such as benzaldehyde were higher, and these results revealed the importance of the choice of the oil. In fact, extraction performance depended not only on the solubility and the polarity of the compound, but also on the properties of the oil.

Although vegetable oils presented a low distribution coefficient for the 2-phenylethyl alcohol, they could be considered as potential extractants because of their biocompatibility with microorganisms.

Results with Sorption on Several Resins

To recover the 2-phenylethyl alcohol from an aqueous solution (1 g/L of 2-phenylethyl alcohol), we tested sorption on several resinous polymers. Among 28 resins used, only 11 showed a relative affinity for this rose-like aroma (Table III).

Less than 7% of 2-phenylethyl alcohol was removed by the resinous polymers not mentioned here. Among

Table IV. Aroma concentration obtained in the purified phase

Time (minutes)	Reactor pressure bars	Aroma g/L	% of extraction
0-33	200	1.20	94
33-75	170-200	1.33	93
75-105	165-195	1.50	92
105-200	160-175	1.76	91

Table V. Analyses carried out on the three separators

	Phase	Removed volume (m/L)	Concentration (g/L)
separator 1	only 1	1.6	3.6
separator 2	upper phase	2.4	18
	lower phase	1	872
separator 3	upper phase	7	18
	lower phase	9	926

the 11 polymers presented in Table III, only eight enabled more than 50% of the aroma contained in the aqueous solution to be extracted. This observation revealed that the choice of the polymer was very important because of the chemical characteristics of 2-phenylethyl alcohol (polar compound).

The best results were observed with the Hayesep Q, the Amberlite XAD-7 and the Porapak R characterized by a sorption capacity of 27-28 mg/g for the 2-phenylethyl alcohol. Such resinous polymers could be used in our case, but new investigations were required to improve the sorption conditions. Moreover, this technique required an additional operation to remove the adsorbed aroma with an appropriate organic solvent.

Inclusion in cyclodextrins was also tested as a method of removing 2-phenylethyl alcohol, but the results revealed that such a technique was not efficient in our case. So, to achieve direct extraction of 2-phenylethyl alcohol from an aqueous solution, we tested the efficiency of supercritical CO_2 used as the extractant.

Results with Supercritical CO_2 Extraction

Preliminary study concerning the extraction of 2-phenylethyl alcohol by supercritical CO_2 operated in a discontinuous way, revealed that 35°C was the optimal temperature, allowing an extraction of 78% of 2-phenylethyl alcohol from an aqueous solution (2.4 g/L of 2-phenylethyl alcohol) after 90 minutes. Moreover, an increase in the pressure from 100 to 200 bars led to an increase in the aroma solubility in the CO_2.

Further experiments were carried out, under the following experimental conditions, in order to remove aroma continuously from a solution containing 20 g/L of 2-phenylethyl alcohol:

- 2-phenylethyl alcohol = 20 g/L
- CO_2 flow: 2.4 liters/hour
- aqueous phase flow: 192 ml/h
- reactor temperature: 35°C
- reactor pressure: 160-200 bars
- separator pressure
  - first: 100 bars
  - second: 75 bars
  - third: 40 bars
- separator temperature: 35-40°C

Analyses carried out by HPLC on the several samples, taken between 0 and 200 minutes, allowed us to quantify the residual concentration of the rose-like aroma in the reactor. The data showed that the supercritical CO_2 extracted continuously 91-94% of the aroma from the initial aqueous solution (Table IV). Under such experimental conditions, the technique of extraction with supercritical CO_2 gave promising results for the recovery of the 2-phenylethyl alcohol.

This study was completed by the analyses concerning the three separators (Table V). Separators 2 and 3 were characterized by two distinct phases: an upper phase corresponding to water saturated with 2-phenylethyl alcohol (maximal solubility = 19.7 g/L) and a lower phase corresponding to the pure aroma saturated with water.

This process allowed a good percentage of extraction to be obtained. The global recovery of the 2-phenylethyl alcohol in separators 2 and 3 corresponded to 77% of the aroma introduced initially. Thus, supercritical CO_2, characterized by low cost, nontoxicity and nonflammability, appeared also to be an effective solvent for the removal and purification of the rose-like aroma.

Table VI. Pervaporation experiments with an aqueous solution of 2-phenylethyl alcohol

	Aqueous Phase	Permeate				
Time (hours)	Aroma (g/L)	Total flux (g/m²/hr)	Water flux (g/m²/hr)	Aroma flux J (g/m²/hr)	Selectivity β	Aroma (g/L)
0.0	2.90	-	-	-	-	-
2.0	2.89	238.4	234.5	3.79	5.50	15.9
3.5	2.86	182.3	179.4	3.00	5.73	16.4

Table VII. Extraction based on membrane with an aqueous solution of 2-phenylethyl alcohol

	Aqueous	Solvent		
Time (minutes)	Aroma (g/L)	Aroma (g/L)	Aroma flux J (g/m²/hr)	Selectivity β
0	2.700	-	-	-
45	0.147	4.8	57	32

Results with Membrane Processes

As a final step in determining the most appropriate process for the removal of the 2-phenylethyl alcohol from an aqueous mixture, we tested three new techniques using selective membranes: pervaporation, perstraction and liquid-liquid extraction based on a membrane. Our main interest in these methods lay in the feasibility of using them to carry out continuous extraction.

First, pervaporation experiments were carried out with a solution initially containing 2.9 g/L of 2-phenylethyl alcohol. After 2 and 3.5 hours, cold traps were weighed in order to estimate total flux, water flux and aroma flux J. The results are summarized in Table VI. The slight decrease in the 2-phenylethyl alcohol concentration (from 2.9 to 2.86 g/L) corresponded to the transfer of a part

of the solute through the dense membrane. That the aroma flux and selectivity kept approximately the same value for the samples taken after 2 and 3.5 hours meant that an equilibrium state had been reached after 2 hours of mixture circulation. The value of aroma flux, approximately 3.5 g/m^2/hr, was not negligible: 2-phenylethyl alcohol seemed to be characterized by a sufficient polarity to pass through the selected dense membrane. However, the measurement of the membrane selectivity revealed a low value around 5.6. Thus, while pervaporation seemed to be suitable for extracting and concentrating the rose-like aroma, this method was selective not only toward 2-phenylethyl alcohol, but also toward water. For this reason, it could be interesting to test another membrane—a homogeneous membrane, for example, exclusively composed of polymethylsiloxane (PDMS)—to attempt to decrease the water flux.

For the next step, perstraction experiments made use of the conditions described previously. Based on the relative efficiencies of organic solvents tested at the beginning of this study, we selected butyl acetate to treat an aqueous solution containing 2.8 g/L of 2-phenylethyl alcohol. Samples taken during a period of 130 minutes allowed us to track the enrichment of the organic phase in 2-phenylethyl alcohol through analyses by CPG. After 130 minutes, with the aroma flux reaching a value of 43 g/m^2/hr, this method appeared to be a promising technique for the recovery of 2-phenylethyl alcohol. However, compared with the pervaporation method, which allowed concentrated rose-like aroma to be recovered, this technique required an additional stage to extract pure aroma from the solvent.

Finally, the last method tested to complete this study corresponded to the extraction based on membrane. The experiments were carried out on an aqueous solution containing 2.7 g/L of 2-phenylethyl alcohol. Samples taken regularly on the solvent (butyl acetate) between 0 and 45 minutes allowed us to track the enrichment of the extractant phase in rose-like aroma. Then, with GPC analyses, the aroma flux J and selectivity β were calculated (Table VII). The slight residual concentration of rose-like aroma in the aqueous phase revealed the efficiency of this method. In fact, both the kind of microporous membrane and the choice of organic solvent seemed to be very appropriate for the recovery of 2-phenylethyl alcohol. The data obtained for the aroma flux and the selectivity gave interesting information. For instance, the selectivity equal to 32 confirmed the value of the distribution coefficient Kd equal to 34 for the butyl acetate toward the 2-phenylethyl alcohol (see earlier results with solvent extraction). Moreover, the material balance (the solute concentration in the organic phase plus the residual solute concentration in the aqueous phase compared to the initial solute concentration in the aqueous phase) accounted for 100%, indicating that all the solute which passed through the membrane was concentrated in the solvent.

Finally, with regard to aroma flux and selectivity, it was not possible to determine strictly the most effective method among the three membrane processes tested because of the different hydrodynamic conditions applied in each case. However, all the results revealed that these new methods seemed to be very promising for the recovery of specific compounds such as the rose-like aroma contained in a dilute aqueous solution.

Conclusion

The recovery of the 2-phenylethyl alcohol, rose-like aroma, from an aqueous solution was first tested by a classical liquid-liquid extraction with organic extractants. Among the seven solvents permitted by the EEC for the removal of ingredients, only butyl acetate showed a high distribution coefficient Kd, equal to 34; of the seven vegetable oils tested, no Kd exceeded a value of 2.7. Sorption experiments carried out with 28 resins revealed that only eight polymers allowed more than 50% of 2-phenylethyl alcohol to be extracted from a dilute aqueous solution, and only three polymers showed a sorption capacity greater than 25 mg/g. Inclusion of this aroma in cyclodextrins was not efficient. Supercritical CO_2 appeared to be an effective solvent that allowed 77% of 2-phenylethyl alcohol to be removed continuously from an aqueous solution. Finally, among the new membrane processes tested, it was difficult to compare the results because of the different hydrody-

namic conditions applied in each case. Although pervaporation presented only a slight aroma flux in our work, this method was characterized by the ability to remove aroma in a concentrated state. Perstraction allowed a suitable aroma flux to be achieved, but the use of organic solvents required an additional stage to remove pure compounds. Finally, extraction based on membrane, the third process tested, appeared to be a very promising method: aroma flux reached 53 g/m^2/hr and the selectivity reached a value of 32. The use of a microporous membrane in association with butyl acetate seemed to be very appropriate to the extraction of rose-like aroma.

The principal aim of this paper consisted in presenting new methods for the extraction of an aroma such as 2-phenylethyl alcohol. Because of the different experimental conditions used for each method, it was difficult to compare the results of one method with the results of the other methods. So, this study did not allow us to select the most effective technique for the removal of this compound. In order to determine the most efficient technique for the removal of 2-phenylethyl alcohol, new experiments will be required to determine the optimal experimental conditions for each method of extraction.

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References

Address correspondence to Cathy E. Fabre, Societé SAF-ISIS Z.A., 40140 Souston, France.

1. WD Elnicke, B Gläser and R Schöller, In situ recovery of ethanol from fermentation broth by hydrophobic adsorbents, *Acta Biotechnol* **11** 353-358 (1991)
2. TK Murphy, HW Blanch and CR Wilke, Water recycling in extractive fermentation, *Process Biochem* **17** 6-9,40 (1982)
3. FW Welsh and RE Williams, The use of vegetable oils to recover compounds from aqueous solutions, *J Chem Tech Biotechnol* **46** 169-178 (1989)
4. SJB Duff and WD Murray, Oxidation of benzyl alcohol by whole cells of *Pichia pastoris* and by alcohol oxidase in aqueous and nonaqueous reaction media, *Biotechnol Bioeng* **34** 153-159 (1989)
5. LJ Bruce and AJ Daugulis, Extractive fermentation by *Zymomonas mobilis* and the use of solvent mixtures, *Biotech Lett* **14**(1) 71-76 (1992)
6. GM Gusler, TE Browne and Y Cohen, Sorption of organics from aqueous solution onto polymeric resins, *Ind Eng Res* **32** 2727-2735 (1993)
7. RG Berger, K Neuhauser and F Drawert, Characterization of the odour principles of some basidiomycetes: *Bjerkandera adusta, Poria aurea* and *Tyromyces sambuceus*, *Flav Frag J* **1** 181-185 (1986)
8. J Schindler and RD Schmid, Fragrance or aroma chemicals - Microbial synthesis and enzymatic transformation, *Process Biochem* **9** 2-8 (1982)
9. A Klingenberg and HP Hanssen, Enhanced production of volatile flavour compounds from yeasts by adsorber techniques - Model investigations, *Chem Biochem Eng* **2**(4) 222-224 (1988)
10. Pat 0 258 993, Production of lactones, PSJ Cheetman, KA Maune and JF De Rooij (1987)
11. NN Gerber and HA Lechevalier, Production of geosmin in fermentors and extraction with anion-exchange resin, *Appl Environ Microbiol* **34**(6) 857-858 (1977)
12. SK Tangnu and TK Ghose, Environmental manipulations in

salicylic acid fermentation, *Process Biochem* **8** 24-26 (1981)
13. R Bar, Cyclodextrin-aided bioconversions and fermentations, *Tichtech* **7** 2-4 (1989)
14. L Szente and J Szenjtli, Stabilization of flavors by cyclodextrins, in *Flavor Encapsulation*, SJ Rish and GA Reineccius, eds (1988) pp 148-157
15. U Krings, M Kelch and RG Berger, Adsorbents for the recovery of aroma compounds in fermentation processes, *J Chem Tech Biotech* **58** 293-299 (1993)
16. L Peyron, Le dioxyde de carbone liquide et supercritique - solvant d'extraction des produits naturels, *Parfums, cosmetiques, arômes* **55** 47-54 (1984)
17. H Richard, A Loo and Ph Morin, L'extraction d'arômes par le dioxide de carbone, *IAA* 383-386 (1989)
18. K Schultz, E Martinelli and GA Mansoori, Supercritical fluid extraction and retrograde condensation applications in biotechnology, in *Supercritical Fluid Technology*, J Penninger et al, eds, Amsterdam: Elsevier (1985) pp 415-478
19. JP Vidal and H Richard, Extraction d'arômes de lilas et de roses au CO_2 supercritique, *Colloque sur les fluides supercritiques. Caractéristiques et applications. Pont à Mousson, France* (1987) pp 277-286
20. P Langley-Danysz, Les nouveaux arômes naturels sont arrivés, *RIA* **486** 48-52 (1992)
21. R Peplonski, J Kwiatkowski, J Malgorzata and Z Lisicki, Extraction of flavours using carbon dioxide under subcritical and supercritical conditions, in *12th International Congress of Flavours, Fragrances and Essential Oils, Vienna, Austria, 4-5 Oct 1992* (1992)
22. Th Darde and RM Vissier, Extraction d'éthanol de fermentation par du dioxyde de carbone supercritique, *Colloque sur les fluides supercritiques. Caractéristiques et applications. Pont à Mousson, France, 1987* (1987) pp 265-266
23. JM Wong and KP Johnston, Solubilization of biomolecules in carbon dioxide based supercritical fluids, *Biotech Progress* **2**(1) 29-38 (1986)
24. A Marty, D Combes and JS Condoret, Continuous reaction-separation process for enzymatic esterification in supercritical carbon dioxide, *Biotech Bioeng* **43** 497-503 (1994)
25. T Lamer and A Voilley, Influence of different parameters on the pervaporation of aroma compounds, in *Proceedings of the 5th International Conference on Pervaporation Processes in Chemical Industry*, R Bakish, ed, Englewood: Bakish Materials Corp (1991) pp 308-318
26. T Lamer, A Voilley, D Beaumelle and M Martin, Extraction of aroma compounds by pervaporation: Comparison of the performances of a laboratory cell and a pilot module, in *Récents progrès en génie des procédés* **6**(21) 419-424 (1992)
27. T Lamer, Extraction de composés d'arôme par pervaporation: Relation entre les propriétés physicochimiques de substances d'arôme et leurs transferts à travers des membranes à base de polydiméthylsiloxane, Thèse, Université de Bourgogne, Dijon, France (1993)
28. G Bengtson, KW Boddeker, HP Hanssen and I Urbasch, Recovery of 6-pentyl-α-pyrone from *Trichoderma viride* culture medium by pervaporation, *Biotech Tech* **6** 23-26 (1992)
29. R Prasad and KK Sirkar, Membrane-based solvent extraction, in *Membrane Handbook*, Ho WS Winston and KK Sirkar, eds, New York: Van Nostrand Reinhold (1992) pp 727-732
30. FR Pat 53 939 C, Microorganismes producteurs de lactones, en particulier de R-γ-décalactones et procédé de production et d'extraction des dites lactones, HE Spinnler, L Dufosse, L Souchon, A Latrasse, C Piffaut and A Voilley (1994)
31. GT Frank and KK Sirkar, An integrated bioreactor-separator: in situ recovery of fermentation products by a novel dispersion-free solvent extraction technique, *Biotech Bioeng* **17** 303-316 (1986)
32. E Albertazzi, R Cardillo, S Servi and G Zucchi, Biogeneration of 2-phenylethanol and 2-phenylethylacetate important aroma components, *Biotech Lett* **16**(5) 491-496 (1992)
33. OM Sahai, in *Bioprocess Production of Flavor and Color Ingredients*, A Gabelman, ed, New York: Wiley-Interscience (1994) p 266