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RECOVERY OF AROMA COMPOUNDS FROM FERMENTATION BY PERVAPORATION

The paper pointed out feasibility of pervaporation for recovery of aroma compounds. Aroma compounds are widely used in food and beverages industry. They can be isolated from natural substrates or synthesised by means of chemical methods. The third most advantageous way is a production of flavours by means of microbial fermentation. The main problem in this case is separation of these very sensitive components in proper composition from fermentation broth. The separation process must fulfil several requirements to avoid deterioration of the flavour. The principles of mass transport in organophilic dense nonporous membranes are reviewed. The case study of the recovery of faithful aroma-profile from a muscatel wine-must is an example of the application of pervaporation. The data concerning fermentation of wine-must were reported including its properties and analytical methods. The experimental results of recovery of aroma compounds from a muscatel wine-must were analysed to prove full feasibility of the pervaporation in this case.

BACKGROUND

Aroma concentrates are widely used as food additives in order to enhance the overall flavour of foodstuff or to compensate for the loss of aromas during food processing. Aroma compounds formed by microbial de-novo synthesis, so-called bioflavours, are preferable to chemically synthesised ones due to their higher public acceptance (Unger, 1995). Law commonly labels chemically synthesised aromas 'artificial', whereas flavours from a microbial fermentation are classified as 'nature-identical' or 'natural', the latter being preferred by the consumer. In addition, bioflavours often exhibit a greater aromatic diversity than their chemically synthesised counterpart, as is the case for example with vanillin (Berger, 1995). Aromas that are to be used as food additives must represent the organoleptic characteristics of the aroma origin as well as be free of any harmful chemical contamination resulting from the aroma recovery process.

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Traditional aroma recovery processes such as distillation, adsorption and solvent extraction often are discouraged because they operate at an elevated temperature which deteriorates the aroma quality, are hardly environmentally friendly due to high energy consumption and the use of toxic solvents, or involve elaborate purification steps to remove solvent residues from the final food product (Fleming, 1992; Schreiber et al., 1997). As a consequence, in recent years, separation processes operating at a gentler temperature and avoiding harmful extraction aids have been investigated. These include steam distillation, air stripping (Le Thanh et al., 1993; Morin et al., 1994), the spinning cone column (Wright and Pyle, 1996), supercritical carbon dioxide extraction (Jolly, 1981) and membrane separation processes (Bengtsson et al., 1992).

Bio-flavours consist of aroma compounds of very differing physicochemical properties (Berger, 1995). Hence, the *in-situ* recovery of these aromas from microbial fermentation broth requires a process that is capable of extracting simultaneously aroma compounds of differing chemical nature. In addition, disturbing of or interfering with the ongoing fermentation should also be avoided. Thus, the recovery process should be efficient at the fermentation temperature, usually close to ambient, as well as recover, for example, low and high volatile compounds equally well. On the other hand, the fermenting biomass should not impair the process performance.

PERVAPORATION AS AN AROMA RECOVERY PROCESS

Pervaporation can be operated continuously, at low temperature, it does not require any extraction aid and does not exert high stress on the active biomass (Groot et al., 1992). It is a membrane separation process that has been extensively studied on the laboratory-scale for the recovery of flavor compounds (Baudot and Marin, 1997; Karlsson and Träghård, 1993). Membrane fouling is a minor problem in pervaporation because the membrane used is non-porous. In the following, the basic principles of pervaporation will be explained. Subsequently, the pervaporation coupled to a aroma-producing fermentation (case-study: a Muscatel wine-must fermentation) will be presented.

THEORETICAL ASPECTS

ORGANOPHILIC PERVAPORATION

In organophilic pervaporation a dense, hydrophobic, non-porous membrane separates the upstream (feed) side containing the feed solution from the downstream (permeate) side that contains the compounds (permeate) recovered (Fig. 1).

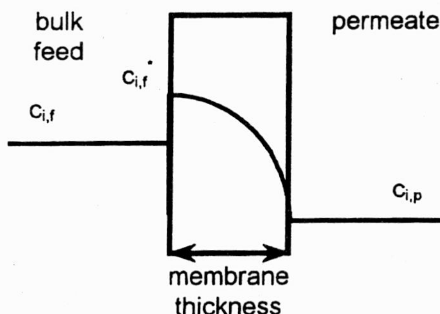

Fig. 1. Simplified scheme of the pervaporation of a compound *i* across the non-porous membrane

The separation is based on the gradient of the partial pressure of a compound *i* between the liquid feed and the vaporised permeate. The partial pressure of a compound *i* in the liquid feed is

$$P_i^f = x_i \gamma_i P_i^0, \tag{1}$$

and in the low pressure permeate vapour

$$P_i^p = y_i P. \tag{2}$$

Equation (1) is a modified Raoult's law where P_i^f is the partial pressure of a compound *i* in the feed (Pa); x_i the molar fraction and γ_i the activity coefficient of *i* in the feed. P_i^0 denotes the saturated vapour pressure of *i* (Pa). Equation (2) is Dalton's law where P_i^p is the partial pressure of *i* in the permeate (Pa); y_i the molar fraction of *i* and *P* the total downstream pressure (Pa).

For establishing the driving force, the partial pressure of compound *i* has to be lower in the permeate than in the feed, which can thus be expressed as (Bengtson and Böddeker, 1995)

$$\frac{P_i^f}{P_i^p} = \frac{x_i \gamma_i P_i^0}{y_i P} > 1. \tag{3}$$

Equation (3) can be rearranged to

$$\frac{\gamma_i P_i^0}{P} > \frac{y_i}{x_i} = \beta_i^{\mathrm{mol}} \tag{4}$$

with β_i^{mol} as the molar enrichment factor of *i*. If an enrichment of compound *i* is desired in the permeate, the right-hand side of Eq. (4) has to be bigger than unity, and so

has the left-hand side. Hence, an enrichment of compound i in the permeate is obtained by a low downstream pressure, a high saturated vapour pressure of compound i, or its high and positive activity coefficient in the feed solution, which illustrates the basic principle of pervaporation. Thus, even low-volatile compounds might be enriched at ambient temperature, provided that their activity coefficient in the feed solution is sufficiently high, as has been reported, for example, for vanillin (Böddeker et al., 1997).

It is common to use the enrichment factor β_i as the ratio of the mass concentration of i in the permeate, c_i^p (kg·m^{-3}), and the mass concentration of i in the feed, c_i^f (kg·m^{-3}), rather than the molar enrichment factor β_i^{mol}:

$$\frac{c_i^p}{c_i^f} = \beta_i . \tag{5}$$

The transport of solutes across the membrane can be described by a solution –diffusion (Mulder, 1996): The solute with a bulk feed concentration $c_{i,f}$ sorbs in the membrane according to its partition coefficient between the aqueous phase and the membrane phase where it possesses the equilibrium concentration $c_{i,f}^*$ (Fig. 1). The hydrodynamic conditions above the membrane can hereby influence significantly the partitioning of the compound i: If the compound has a very high affinity to the membrane it will be strongly sorbed and the adjacent liquid phase above the membrane will be depleted of compound i. Under turbulent hydrodynamic conditions, this depletion in compound i will be readily compensated by convective transport of i from the bulk to the membrane surface. Under laminar hydrodynamic conditions, however, compound i will reach the membrane surface from the bulk mainly by diffusion. If the diffusion of compound i in the feed is slower than its depletion at the membrane surface, the boundary layer above the membrane will have a concentration $c_{i,b}$ lower than $c_{i,f}$ (Fig. 2). As a result, the equilibrium concentration $c_{i,f}^*$ will also be lower and so will the flux across the membrane. Thus, the flux of compound i across the membrane will not only decrease but also be determined mainly by the resistance of the boundary layer and not the selective membrane. This phenomenon is commonly denoted as *concentration polarisation*. If it is not possible to eliminate the boundary layer resistance, as can be the case for compounds with a very high affinity to the membrane (Baker, 1997), a common approach is to increase the selective membrane thickness. This does not resolve the problem of lower fluxes but shifts the main transport resistance to the selective membrane. It should be noted that in processes involving porous membranes, concentration polarisation usually denotes an elevated concentration of the permeating species near the membrane surface whilst in pervaporation it mostly describes the phenomenon of compound depletion near the membrane surface.

Once the compound i is sorbed in the membrane surface, it diffuses through the membrane due to the driving force, evaporates due to its low partial pressure, leaves

the membrane downstream side as a vapour with a concentration $c_{i,p}$ and is subsequently condensed.

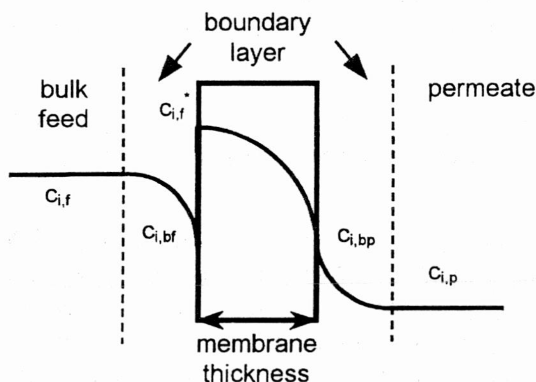

Fig. 2. Transport of a compound *i* across a non-porous membrane

Although being less frequent, boundary layer effects can also be observed on the downstream side of the membrane if the evaporation enthalpy of *i* is higher than the heat for evaporation available, or the downstream pressure is not low enough to remove the permeants from the membrane downstream side. In this case the compounds permeated will accumulate on the membrane downstream surface (or in the macro-porous support) and exhibit an additional transport resistance, as has been observed, for example, for phenol in PEBA-membranes (Stürken, 1994). These effects can be avoided by either supplying external heat, increasing the vacuum or even improving the design of the macro-porous support. It should be pointed out, however, that the upstream boundary layer resistance poses more often a problem than the downstream boundary layer.

The transport phenomena of pervaporation have been extensively studied and described in the literature (Néel, 1991; Rautenbach et al., 1991). Generally, the partial flux density J_i ($kg \cdot m^{-2} \cdot s^{-1}$) of a compound *i* across the membrane is described as a function of the permeability K_i ($kg \cdot m^{-2} \cdot s^{-1} \cdot Pa^{-1}$) and the driving force ΔP_i (Pa):

$$J_i = K_i \Delta P_i = P_i \left(P_i^f - P_i^p \right) \tag{6}$$

with the permeability K_i being the product of the solubility and the diffusivity of compound *i* in the polymer. Both the solubility and the diffusivity of *i* depend on the feed composition (Mulder, 1996). It should, moreover, be pointed out that in multi-component feed solutions the sorption and diffusion of a single compound might be affected by the presence of other components due to solute–solute interactions or strong solute–polymer interactions. This is accounted for, for example, the Flory–Huggins-theory (Mulder, 1991) or the Maxwell–Stefan-theory (Heintz and Stephan, 1994).

The membrane is not a mere barrier, but rather interacts with the solutes to a degree determined by the mutual chemical nature. Evidently, the right choice of the membrane polymer is thus crucial for the recovery of a complex aroma-profile such as that of a muscatel wine-must which will be the case study presented in the following.

CASE STUDY: RECOVERY OF A FAITHFUL AROMA-PROFILE FROM A MUSCATEL WINE-MUST

Wine-must fermentation is an anaerobic fermentation of *Saccharomyces cerevisiae* starting up with grape-juice. During the fermentation ethanol and carbon dioxide are formed from sugar as main metabolic products. Aromas such as esters, alcohols and aldehydes are produced as secondary metabolites in much smaller quantities (ppm-range). Hence, the aroma-profile of a wine-must changes daily until the fermentation ceases either due to depletion of sugar or product inhibition by ethanol.

A Portuguese winemaker is interested in obtaining aroma concentrates from a muscatel wine-must fermentation that is especially rich in aromas. The aim is to obtain two products: a muscatel aroma concentrate and the final wine-must that will then proceed in the vinification process. The aroma concentrate is to be used in the food industry.

On theses grounds the following demands of the aroma recovery process emerged:

- the aroma recovery process should be a 'cleaner technology' that neither contaminates the wine-must nor the aroma product obtained;
- the process should not impair the wine-must fermentation: it should not disturb the ongoing fermentation, nor alter the fermentation conditions such as the fermentation temperature;
- the process should recover the complete aroma-profile of the wine-must and not only groups of compounds;
- it should not deteriorate the aroma quality.

According to these demands all processes involving an elevated temperature or solvents were discouraged. Two rather recent technologies were considered: supercritical carbon dioxide extraction and pervaporation. Both processes do not apply an elevated temperature and do not leave any solvent contamination. Pervaporation, however, was the process of choice because it could be directly coupled to the fermentation whilst supercritical carbon dioxide extraction required a pre-treatment of the wine-must due to suspended matter.

Pervaporation experiments. In preliminary studies, laboratory experiments were carried out using the set-up shown in Fig. 3.

The pervaporation module and the membrane were obtained from the GKSS Research Center, Germany. The membrane used is a modified silicon rubber composite membrane, polyoctylmethylsiloxane (POMS) on polyetherimide (Fritsch et al., 1992) with an active layer thickness of 15–17 μm and an effective membrane area of 100

cm^2. In previous studies, this membrane had been found most suitable for the recovery of the target aromas. The feed volume was seven liters and chosen sufficiently large for the depletion of aroma compounds being negligible over the duration of each experiment. Experiments were run at 18°C, a downstream pressure of 40 Pa and a mean Reynolds-number over the membrane of 900. The permeate, condensed by liquid nitrogen, consisted of two phases and care was taken to recover a representative sample

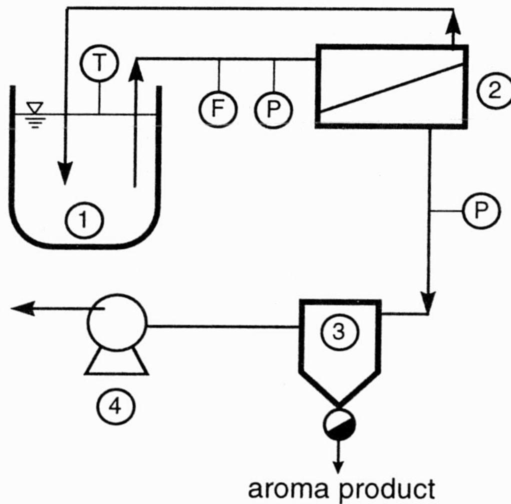

Fig. 3. Laboratory-scale pervaporation unit:
1 – feed tank, 2 – pervaporation module,
3 – condenser, 4 – vacuum pump, T – temperature
control, F – rotameter, P – Pirani gauge

for the analysis. Since the wine-must is in a state of active fermentation with constantly changing aroma-profile, a three-hour purging of the system with part of the wine-must was performed before each experiment for the conditioning of the membrane. The feed solution was then discarded and replaced by fresh wine-must. The role of membrane conditioning in pervaporation has been described elsewhere (Marin et al., 1992).

Wine-must fermentation. Samples of ten liters were taken daily from two independent, but initially organoleptically similar industrial wine-must fermentations with a volume of about 15000 liters each (José Maria da Fonseca Sccrs., Portugal). The samples served for the characterization of the wine-must fermentation as well as the feed for the pervaporation experiments. The yeast was a commercially available strain of *Saccharomyces cerevisiae* applied in the wine-industry for inoculation. The fermentations were controlled at a temperature of about 18 °C, and the only characteristic parameter measured was the density of the wine-must, ranging from 1080 to 990 g·l^{-1} owing to the formation of ethanol. The samples were processed immediately

or stored for not more than three hours at 4°C in order to avoid any change or loss in the aroma-profile.

Analytical. Samples from the feed, the retentate and the permeate were analyzed by a gas chromatograph HP 5890, Series II, connected to an automatic static head-space sampler 19395A (all Hewlett Packard, USA) and equipped with an FFAP-capillary column (Chrompack, The Netherlands) of 25 m length and with an internal diameter of 0.25 mm. The analytical conditions were as follows: injector temperature 200 °C, detector temperature 230 °C, initial column temperature 35 °C (held for 5 minutes) with an oven temperature rise rate of 10 °C $\cdot min^{-1}$ until 220 °C; the carrier gas used was nitrogen, the column head pressure 45 kPa, and the split-flux 20 ml$\cdot min^{-1}$. For increased sensitivity, 1g of Na_2SO_4 (Merck) was added to the wine-must samples of 2.5 ml in 10 ml-vials. The effect of ethanol was quantified and considered during wine-must analysis. The sample volume for analysis of the permeates was 0.5 ml. After a preliminary analysis, permeates were diluted to an ethanol content of 10 vol.% in order to standardize the sample matrix. The analytical error for both the must and the permeates was less than 5%. 48 compounds were identified in the permeates ob-

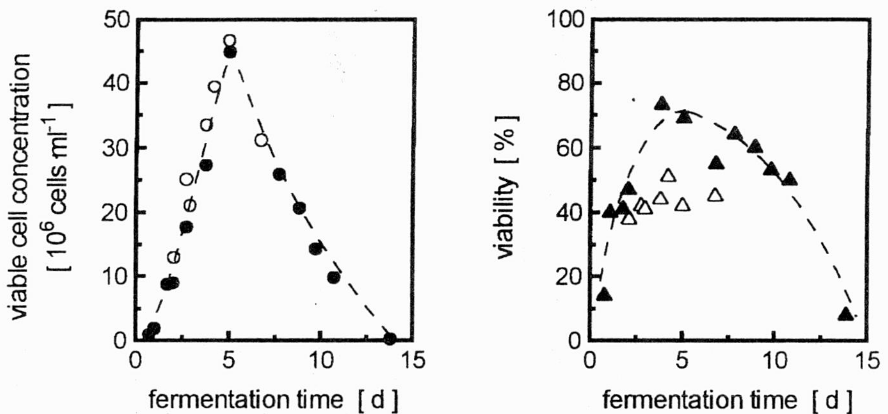

Fig. 4. Comparison of the viable cell concentration and the viability of a wine-must fermentation coupled to a pervaporation (open symbols) with a reference fermentation (filled symbols)

tained by gas chromatography-mass spectroscopy (Finnigan, USA) and subsequent comparison with the relative retention times. Data on the density of the wine-must fermentation were provided by the wine-maker. The viability was determined by staining wine-must samples with methylene blue and counting the cells using a hema-cytometer. According to the aim of this work an olfactometric evaluation of the per-meates was indispensable and carried out by a sensory panel (José Maria da Fonseca Sccrs., Portugal).

COMPATIBILITY OF THE PERVAPORATION WITH THE WINE-MUST FERMENTATION

The viability of a reference fermentation was compared with that of a fermentation that was coupled to the pervaporation throughout five days. As can be seen in Fig. 4, the viable cell concentrations in both fermentations are identical, indicating that the pervaporation did not impair the yeast growth at all. The viability was found to be lower in the fermentation coupled to the pervaporation probably owing to mixing effects of the non-agitated fermentation rather than increased cell-death. All in all, it was concluded that pervaporation does not affect the wine-must fermentation under well-defined experimental conditions.

RECOVERING THE MUSCATEL AROMA-PROFILE

The aroma produced by yeast during wine-must fermentation comprises up to eight hundred aroma compounds (Rapp, 1990) which makes it a challenge to be recovered faithfully to its origin. It should be noted that an aroma-profile is a delicate balance between numerous compounds in varying concentrations and of varying physicochemical properties as is demonstrated by Table 1. It is therefore not evident that a process based on complex solute–polymer interactions and possible solute –solute interactions is capable of recovering an aroma-profile without altering its organoleptic properties.

Pervaporation experiments were carried out in regular intervals during two wine-must fermentations, and both the aroma extracts (permeates) as well as the wine-must from which they were obtained were analyzed by gas chromatography. Complete quantification of all aroma compounds in both the must and the permeate is extremely time-consuming and was beyond the scope of this work. Therefore, out of 48 compounds identified in the permeates obtained, eight compounds of the two wine-must fermentations of an initially similar organoleptic quality were selected and analysed quantitatively (Table 1). The compounds quantified were chosen either due to their relatively high concentration, or their organoleptic significance, or both. Table 2 summarises the organoleptic properties and the contribution to the overall muscatel wine-must aroma of the aroma compounds chosen. Compounds of limited significance contribute to a desirable overall aroma only within a narrow concentration range above which they turn into off-odours. Compounds of high significance are desirable within a wider concentration range because of their fruity aroma or their role as a character impact compound, such as linalool. At concentrations too high they do not turn into off-odours but may rather result in an unbalanced wine-must aroma.

Recovering the aroma-profile from the wine-must theoretically requires the extraction of each aroma compound to the same degree because the profile might otherwise be altered. Since the aroma-profile is determined by a certain ratio of up to one hundred compounds generating a complex response in the human nose, the simplest and maybe most reliable way of evaluating an individual aroma product recovered is by a sensory panel. With regard to the compounds quantified in this study a distinc-

tion can be made between compounds of limited organoleptic value such as ethyl acetate, isobutyl alcohol, isoamyl alcohol and 1-hexanol, and compounds of high organoleptic value such as ethyl hexanoate, isoamyl acetate and linalool. Having grouped these compounds, the ratio between compounds of limited and higher organoleptic value was calculated for the must and the permeate, respectively, and is depicted in Fig. 5 as a function of the wine-must density.

Table I

Formula, boiling point T_b (at atmospheric pressure) and saturated vapour pressure P_i^0 (at 298.15 K) of the compounds selected for analysis

Compound	Formula	T_b (K)	P_i^0 (298.15), [kPa]
Ethanol	C_2H_6O	351.45	7.87
Ethyl acetate	$C_4H_8O_2$	350.26	12.6
Isobutyl alcohol	$C_4H_{10}O$	381.04	1.53
Isoamyl alcohol	$C_5H_{12}O$	401.83	0.42
1-Hexanol	$C_6H_{14}O$	430.65	0.11
Isoamyl acetate	$C_7H_{14}O_2$	417.15*	1.27§
Ethyl hexanoate	$C_8H_{16}O_2$	441.15*	0.23§
Linalool	$C_{10}H_{18}O$	473.15*	0.01§

All data from CRC Handbook of Chemistry and Physics (1997), except from Baudot A. and Marin,M. (1997); § from Beilstein-Online Database.

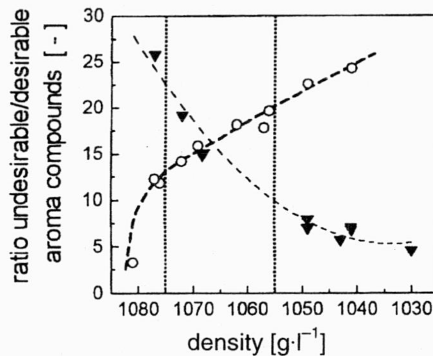

Fig. 5. The ratio between the concentration of aroma compounds of limited and higher organoleptic values in the must (open circles) and the permeate (down triangle)

At the early stage of the fermentation, until 1075 $g \cdot l^{-1}$ few aroma compounds are formed by the yeast-metabolism and the aroma-profile is consequently poor in aroma. With advancing fermentation the discrepancy between the ratios depicted increased dramatically and, at densities lower than 1040 $g \cdot l^{-1}$, the aroma-profile recovered and that of the wine-must would hardly be alike.

Table II

Organoleptic properties of the aroma compounds selected and their contribution to the aroma of the muscatel wine-must

Compound	Organoleptic property	Organoleptic value for the wine-must aroma
Ethyl acetate	nail-polish pungent	limited
Isobutyl alcohol	pungent	limited
Isoamyl alcohol	pungent	limited
1-hexanol	alcohol	limited
Isoamyl acetate	banana	high
Ethyl hexanoate	pleasant, fruity	high
Linalool	lilies of the valley	very high

From these considerations the optimum aroma product should be obtained where there are already aromas formed during the fermentation and where the ratio between compounds of limited and higher organoleptic value in the must and the permeate still coincides. This range is depicted in Fig. 5 by dotted lines.

Table III

Results from the sensory panel evaluation of aroma products obtained by pervaporation along the wine-must fermentation

Wine-must density ($g \cdot l^{-1}$)	Sensory evaluation
1082	no muscatel aroma, clean
1080	some muscatel aroma, but very dilute
1075	muscatel aroma stronger, clean
1067	muscatel aroma
1055	very aromatic, a lot of finesse, very sweet
1040	perfume with few solvent
1030	muscatel with solvent

The sensory evaluation of the aroma products extracted along the fermentation is summarised in Table 3. Permeates obtained at an early stage of the fermentation were, as expected, very dilute in aromas. With advancing fermentation their quality increased and the best quality was obtained in the vicinity of 1067 $g \cdot l^{-1}$, thus confirming the previous results from analytical gas chromatography. Permeates from later stages of the fermentation were very concentrated in compounds of higher organoleptic quality but rejected due to strong solvent-like smells. At that stage some aroma compounds, particularly the esters, appeared in such high concentrations that the delicacy of the aroma-profile was lost.

To conclude, it could be shown that organophilic pervaporation is capable of recovering faithfully a complex aroma-profile, such as muscatel wine aroma, under well-defined conditions.

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