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RECUPERACIÓN DE COMPUESTOS VOLÁTILES A PARTIR DE SUBPRODUCTOS DE MARISCO MEDIANTE EXTRACCIÓN CON FLUIDOS SUPERCRÍTICOS Y PERVAPORACIÓN

TESIS DOCTORAL

Rodrigo Martínez Velasco

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RECUPERACIÓN DE COMPUESTOS VOLÁTILES A PARTIR DE SUBPRODUCTOS DE MARISCO MEDIANTE EXTRACCIÓN CON FLUIDOS SUPERCRÍTICOS Y PERVAPORACIÓN.

Memoria que para optar al grado de Doctor por la Universidad de Burgos presenta el licenciado Rodrigo Martínez Velasco

Burgos, Julio de 2012.

UNIVERSIDAD DE BURGOS
FACULTAD DE CIENCIAS
DEPARTAMENTO DE BIOTECNOLOGÍA Y CIENCIA DE LOS ALIMENTOS
ÁREA DE INGENIERÍA QUÍMICA

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CERTIFICAN:

Que el licenciado D. RODRIGO MARTÍNEZ VELASCO ha realizado bajo su dirección el trabajo titulado "Recuperación de compuestos volátiles a partir de subproductos de marisco mediante fluidos supercríticos y pervaporación".

Considerando que dicho trabajo reúne los requisitos exigidos para ser presentado como Tesis Doctoral, expresan su conformidad con dicha presentación.

Y para que conste, firman el presente certificado en Burgos a 23 de Julio de 2012

Burgos 23 de Julio de 2012.

Fdo. Mª Teresa Sanz Diez

Fdo. Sagrario Beltrán Calvo

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DEPARTAMENTO DE BIOTECNOLOGÍA

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CERTIFICA:

Que la memoria titulada "RECUPERACIÓN DE COMPUESTOS VOLÁTILES A PARTIR DE SUBPRODUCTOS DE MARISCO MEDIANTE FLUIDOS SUPERCRÍTICOS Y PERVAPORACIÓN" presentada por el licenciado RODRIGO MARTÍNEZ VELASCO para optar al grado de Doctor por la Universidad de Burgos ha sido realizada en el Área de Ingeniería Química de este Departamento bajo la dirección de las doctoras Mª Teresa Sanz Diez y Sagrario Beltrán Calvo.

Burgos a 23 de Julio de 2012

Fdo. Pilar Muñiz Rodríguez

Directora del Departamento de Biotecnología y Ciencia de los Alimentos

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A mi familia y amigos porque siempre estáis conmigo.

ÍNDICE

Resumen de la Tesis.

La finalidad de esta Tesis doctoral es el estudio de la recuperación de los componentes responsables del aroma de sustancias naturales. Los concentrados aromáticos son muy utilizados actualmente en la Industria Alimentaria ya que el aroma en un factor determinante en la elección y la aceptación del alimento por parte de los consumidores. En este sentido, los consumidores demandan cada vez más aromas naturales, entendiendo como tales aquellos obtenidos mediante procedimientos físicos, enzimáticos o microbiológicos a partir de una materia prima de origen animal o vegetal.

Varios subproductos de la industria del marisco se emplean como fuente de aromas naturales: caparazones, pinzas, patas y demás subproductos no comestibles resultantes del aprovechamiento de la carne de buey de mar, así como los caldos de cocción del crustáceo ya mencionado.

Para llevar a cabo este propósito, existen varias tecnologías para la extracción de aromas naturales, entre ellas, la más comúnmente utilizada en la actualidad es la destilación por arrastre de vapor. No obstante, el consumo energético de este proceso es elevado y, en este sentido, es importante la búsqueda de procesos sostenibles que cumplan con los principios de la llamada “ingeniería verde”. Por tanto, se proponen dos procesos de separación alternativos, la pervaporación y la extracción con fluidos supercríticos, que en los últimos años han suscitado un enorme interés en la Industria Alimentaria debido a las ventajas tecnológicas que ofrecen.

Los **procesos de pervaporación** son de aplicación reciente comparados con procesos clásicos de membranas como la microfiltración o la ultrafiltración. La pervaporación con membranas hidrofóbicas se presenta en los últimos años como alternativa eficaz, frente a procesos clásicos de destilación o extracción con disolventes, para la separación y concentración de componentes orgánicos diluidos en medios acuosos. Desde el punto de vista industrial, la pervaporación con membranas hidrofóbicas permite alcanzar simultáneamente dos objetivos: medioambientalmente logra la reducción de compuestos orgánicos en las corrientes líquidas residuales en los procesos de producción y desde el punto de vista económico logra concentrados aromáticos potencialmente atractivos en la Industria Alimentaria. El empleo de membranas hidrofóbicas en procesos de pervaporación para procesar mezclas líquidas, que contienen compuestos volátiles de alto valor, conduce a la obtención de un permeado enriquecido en dichos compuestos. Durante el desarrollo de esta parte de la Tesis doctoral, se ha efectuado un estudio termodinámico previo de los componentes volátiles en disolución acuosa, encaminado al conocimiento de su comportamiento en el intervalo de dilución infinita. Posteriormente, se ha llevado a cabo el estudio y optimización de los parámetros del

proceso de pervaporación con membranas diferentes a partir de disoluciones sintéticas formadas por aromas que presentan una contribución importante en el aroma de buey de mar, Asimismo se ha realizado el estudio de estos parámetros con los caldos de cocción procedentes de la industria.

La **extracción con fluidos en condiciones supercríticas** ha demostrado ser una alternativa real para la obtención de aceites esenciales y concentrados aromáticos. La mayoría de los compuestos responsables de los aromas de las sustancias naturales presentan una volatilidad elevada y se suelen extraer bien con dióxido de carbono supercrítico. El dióxido de carbono es el disolvente supercrítico más utilizado, ya que no es tóxico y permite operar a presiones moderadas y cerca de temperatura ambiente. Se comporta como un disolvente lipofílico que, a diferencia de los disolventes líquidos, tiene la ventaja de que su poder disolvente se puede ajustar simplemente cambiando sus condiciones de presión y temperatura. En el estudio del proceso de extracción de los componentes volátiles de buey de mar a partir de los subproductos sólidos ya mencionados, se ha determinado la influencia de las variables de operación (presión de extracción, temperatura de extracción y presión en el proceso de separación) sobre el rendimiento final del proceso, entendiendo como tal la cantidad y el número de componentes volátiles recogidos al final del proceso. Asimismo, la presión y temperatura de extracción son parámetros muy importantes ya que definen la capacidad disolvente del dióxido de carbono y, por tanto, limitan la posibilidad de co-extraer sustancias indeseadas que afectan directa o indirectamente a la calidad final del producto. La presión en el separador es un parámetro determinante en el rendimiento de la extracción debido a la elevada presión de vapor de estos compuestos y, por consiguiente, la elevada solubilidad que muestran por el dióxido de carbono en condiciones supercríticas, dificultando en gran medida el proceso de separación de las sustancias de interés.

Thesis abstract.

The scope of the Thesis project is the recovery of the chemical components responsible of the aroma of natural substances. Nowadays the aroma concentrates are widely employed in the Food Industry, since food aroma is a determinant factor in consumer´s food choice and acceptance. In this sense, the consumer demand for natural aromas is continuously increasing, defined as those substances obtained through physical, enzymatic and microbiological processes from an animal or vegetal material.

Some by-products of the shellfish processing industry were reused as a natural source of aroma compounds: carapaces, claws, viscera and other non-edible by-products resultant after brown crab meat processing and the juice obtained after the boiling process.

Several technological processes have been employed for the isolation of natural aromas, being widely used steam distillation. Nevertheless, the energy consumption is high and, therefore, it is important to develop new sustainable processes that comply the principles of *green engineering*. Thus, two separation processes, pervaporation and supercritical fluid extraction, that have aroused interest in the last years have been studied in this Thesis.

The **pervaporation process** is a recent and promising technology compared with those traditional membrane separation processes, such as microfiltration and ultrafiltration. Pervaporation through hydrophobic membranes is recently considered as an effective alternative to traditional processes, such as distillation and solvent extraction for the separation and concentration of organic, dilute components in aqueous solutions. From the industrial point of view, pervaporation makes possible to achieve two objectives simultaneously: reduction of organic compounds in the industrial liquid effluents and the collection of aroma concentrates that could be potentially useful for the Food Industry. The utilisation of hydrophobic membranes in pervaporation processes to separate liquid mixtures that contain high added-value aromatic compounds leads to a permeate rich in organic components. A previous thermodynamic study was carried out to know the behaviour of these molecules in aqueous solutions at the infinite dilution region. Subsequently, the study and optimization of the pervaporation process parameters from aqueous model dilute solutions.was carried out Finally, pervaporation was performed with the real effluent

The **supercritical fluid extraction** has demonstrated to be a real alternative for the extraction of essential oils and aroma concentrates. It can be considered as an technique between distillation and extraction, since the vapor pressure of the solute and its solubility in the supercritical fluid are important parameters influencing the performance and the selectivy of the process. Most of the volatile compounds responsible for the aroma of natural substances

show a high volatility and are easily extracted by supercritical carbon dioxide. Carbon dioxide is the supercritical fluid most commonly used, as it is not toxic and offers the possibility of working at moderate pressure and temperature. Additionally, its solvent capacity can be adjusted by changing the temperature and pressure conditions. The main drawback of supercritical fluid extraction, is the co-extraction of other substances contained in the food matrix, which could affect the quality of the final product. For this reason, it is necessary the study of the parameters involved in the process to maximize the quantity and number of volatile compounds extracted and to obtain the desired quality of the final product.

I. INTRODUCCIÓN

1.1 Importancia de los aromas en la Industria Alimentaria.

En los últimos años, la obtención de nuevos productos en la Industria Alimentaria está recibiendo un impulso considerable. Sin embargo, para conseguir la aceptación de este tipo de productos en el mercado, la calidad organoléptica de los mismos ha de ser elevada. En este sentido, el aroma es una de las características más importantes de un alimento y está directamente relacionado con su calidad y aceptación en el mercado (1, 2).

Desde el punto de vista químico, el aroma se define como la mezcla de numerosas sustancias volátiles presentes en la matriz alimentaria cuya concentración total puede variar desde unas pocas partes por millón hasta aproximadamente 100 partes por millón, siendo la concentración de los componentes entre partes por billón y partes por trillón. Estas sustancias responsables del olor llegan directamente a la región olfativa e interaccionan con los receptores olfativos para producir un estímulo. Esta interacción depende su concentración y su límite de detección olfativo, definido como la concentración más baja a la que un compuesto aromático es percibido por el ser humano.

La Comisión del Codex Alimentarius define los aromatizantes como aquellas sustancias añadidas a un alimento capaces de impartir, modificar o realzar el aroma en un alimento. Estos compuestos se emplean en la Industria Alimentaria generalmente para mejorar las características organolépticas ya que pueden presentar diversas e interesantes funciones para este propósito, como las siguientes:

- Generar un sabor completamente nuevo. Este hecho no es habitual, no obstante cuando ha sido logrado ha cosechado gran éxito en el mercado (Coca Cola).
- Ingredientes que mejoren, amplíen o realcen el aroma que proporcionan los compuestos ya presentes en el alimento.
- Fortalecer o suplir los compuestos aromáticos perdidos durante el procesado de los alimentos.
- Emular aromas más costosos o reemplazar aquellos no disponibles.
- Enmascarar características organolépticas menos deseables o indeseables que se presentan de forma natural en algunos alimentos.

El estudio de la composición de la fracción volátil de un alimento es una tarea complicada debido al elevado número de compuestos y su baja concentración, la diversidad estructural y de propiedades físico-químicas que presentan los constituyentes del aroma de un alimento, así como de la complejidad estructural inherente a la matriz alimentaria. Afortunadamente, en

muchas ocasiones, la compleja composición que presenta la fracción volátil de un alimento puede simplificarse en un conjunto de compuestos cuyas características organolépticas tienen un gran impacto sobre el aroma final del alimento (3). Los aromas que se utilizan como aditivos alimentarios deben presentar las características organolépticas del aroma de origen y estar exentos de posibles compuestos químicos perjudiciales derivados del procesado (4). En este sentido el consumidor demanda cada vez más aromas de origen natural.

Los aromas se pueden clasificar en (5):

- Naturales, aquellos que se han obtenido a partir de procedimientos físicos, enzimáticos o microbiológicos a partir de materia prima de origen animal o vegetal.
- Idéntico al natural (I.N), obtenidos a parir de síntesis química, obteniendo una molécula idéntica a la sustancia natural en lo que respecta al sabor y estructura química.
- Artificial, con el fin de mejorar o reforzar el sabor se obtienen moléculas químicamente no idénticas a la natural pero manteniendo el aroma.

La demanda de sustancias aromatizantes ha experimentado un vasto crecimiento a lo largo de la última década, relacionado con la subida del volumen de ventas de las principales compañías comercializadoras de estos productos a nivel mundial (Figura 1.1).

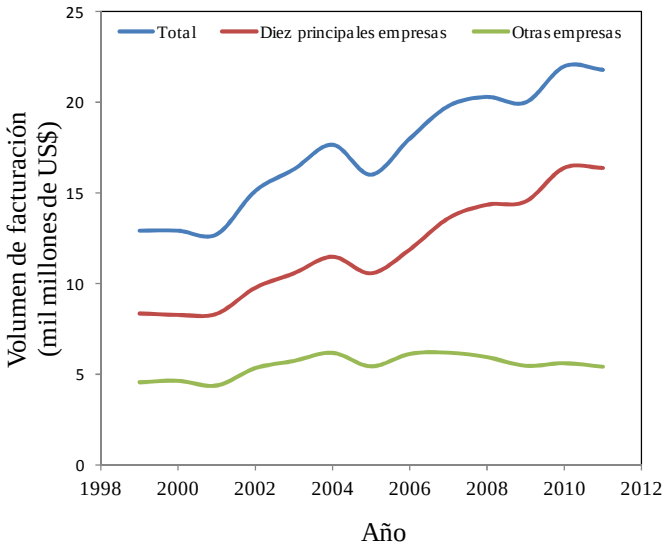


Figura 1.1. *Volumen de ventas global asociado al sector del aroma (Leffingwell and Associates).*

El mercado se encuentra monopolizado por varias compañías multinacionales que generan el setenta y cinco por ciento del volumen global de ventas de todo el mercado (Figura 1.2), siendo las principales compañías dos compañías suizas, *Givaudan* y *Firmenich*, y una

estadounidense, *International Flavor and Fragances*. No obstante numerosas empresas, adaptándose a la demanda del consumidor, se encuentran actualmente desarrollando productos cada vez más innovadores, medioambientalmente respetuosos y saludables. Por ello la investigación y desarrollo de nuevos productos es una parte crucial dentro de las políticas de estas empresas.

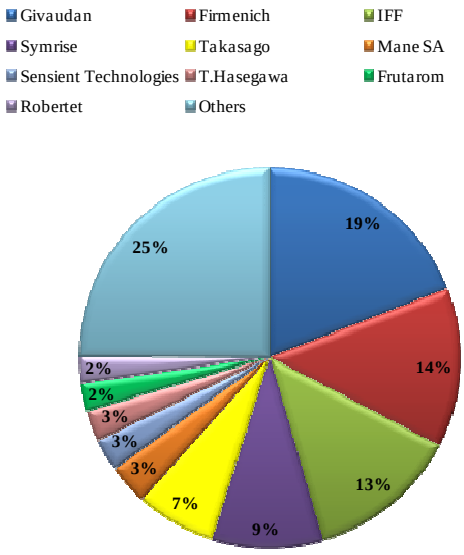


Figura 1.2. *Porcentaje de ventas de las principales compañías comercializadoras de sustancias aromatizantes.*

La metodología analítica de la fracción volátil de una matriz alimentaria ha sufrido un gran desarrollo en las últimas décadas (6). Al comienzo de la década de los setenta únicamente mil quinientos compuestos químicos habían sido identificados en los diferentes alimentos, frente a los más de siete mil que se conocen en la actualidad. A medida que los métodos tradicionales de separación (extracción con disolventes orgánicos, destilación o destilación-extracción simultánea) dejaron paso en los últimos años a las técnicas de análisis en espacio de cabeza, un mayor número de compuestos, con un límite de detección olfativa menor, pudieron ser identificados. Estas técnicas son sencillas, rápidas, versátiles y eficientes, requieren poca cantidad de muestra y previenen la formación de compuestos y la contaminación de la muestra. Posteriormente, el desarrollo de técnicas en espacio de cabeza con elevada capacidad de concentrar, basadas tanto en la acumulación estática como dinámica de volátiles sobre polímeros (adsorción y absorción) o menos frecuente sobre disolventes, tuvieron un éxito inesperado e inmediato debido principalmente a que eran tan simples, rápidas, fáciles, automáticas y fiables como las técnicas en espacio de cabeza estáticas, pero, al mismo tiempo,

su capacidad de concentrar al analito era comparable a la que ofrecían las técnicas en espacio de cabeza dinámicas.

Tradicionalmente, la recuperación de aromas naturales se ha llevado a cabo a través de numerosas técnicas:

- Adsorción sobre distintos adsorbente como carbón activo
- Extracción con disolventes
- Destilación por arrastre de vapor.

Con frecuencia este tipo de procedimientos presenta un consumo energético elevado ya que durante el proceso se llegan a alcanzar temperaturas elevadas que además pueden afectar a la calidad de los aromas (3). Otras desventajas que derivan de estos procesos son el uso de disolventes tóxicos, las posibles etapas posteriores necesarias para la purificación de los compuestos volátiles y el limitado intervalo de aplicación (7-9). En este sentido, es importante la búsqueda de procesos sostenibles que cumplan con los principios de la llamada "ingeniería verde".

En la tesis realizada se ha considerado el empleo de "**tecnologías limpias**" para la obtención de concentrados aromáticos a partir de distintos subproductos generados en la Industria Alimentaria, en concreto de subproductos generados en el procesado de buey de mar. Las dos tecnologías que se han abordado en el desarrollo de la Tesis doctoral han sido la extracción con fluidos supercríticos y los procesos de pervaporación. Este tipo de tecnologías limpias se presentan como más eficaces desde el punto de vista energético que los procesos convencionales de recuperación de aromas.

El procesado de los productos provenientes del mar genera grandes cantidades de residuos, derivadas de los procesos de cocción y pelado de las partes no comestibles de los mismos. Estos subproductos consisten fundamentalmente en caparazones, cabeza y vísceras, y representan un gran problema medioambiental ya que únicamente entre un veinticinco y un cincuenta por ciento del material de partida de los productos del mar es transformado en productos primarios. Estos subproductos han generado gran interés en los últimos años debido a la presencia de un numeroso grupo de sustancias de interés en la Industria Alimentaria entre los que se encuentran polisacáridos (como la quitina), colorantes (como la astaxantina), antioxidantes, sustancias responsables del sabor y aroma de marisco y ácidos grasos poliinsaturados.

En este sentido, se han identificado gran cantidad de compuestos volátiles en los subproductos del procesado de marisco, como la jaiba (10), la cigala (11) o la gamba (12). Por lo que estos subproductos pueden suponer una fuente importante en la recuperación de los compuestos responsables del aroma de estas especies. El aroma de marisco fresco ha sido definido como dulce, con inconfundibles notas vegetales, a menudo acompañadas de una nota metálica y un

olor a pescado de intensidad variable (13), englobando una gran cantidad de compuestos de diversa naturaleza química. Los principales compuestos responsables del aroma de marisco fresco son aldehídos y alcoholes insaturados. Por otra parte, el aroma de marisco cocinado se caracteriza por la presencia de numerosos compuestos de características químicas muy diferentes, como alcoholes, aldehídos, cetonas, furanos, pirazinas, hidrocarburos alifáticos saturados e insaturados, hidrocarburos aromáticos, compuestos organoazufrados, compuestos nitrogenados, ésteres y terpenos, en proporciones determinadas.

Las tecnologías estudiadas en esta Tesis no sólo son "tecnologías limpias", sino que resultan rentables desde el punto de vista económico. Así, aromas como el del melocotón (γ-decalactona) adquiere un valor en el mercado de 1400$/kg como extracto natural y sólo 75$/kg el sintético. Otro ejemplo lo tenemos en el aroma de frambuesa que alcanza valores de 3000 $/kg como extracto natural y 58$/kg cuando se obtiene de forma sintética (Aldrich, 2000). Por lo que los procesos de separación con membranas como la pervaporación o la extracción con fluidos supercríticos pueden ser una buena opción no solo por ser un proceso "verde", sino por los beneficios económicos.

En el procesado de los alimentos, se generan normalmente una gran cantidad de aguas residuales que tradicionalmente han sido como un serio problema. Sin embargo, estas aguas también pueden ser consideradas como una fuente natural rica en biomoléculas de alto valor añadido, ya que la mayoría contienen notables cantidades de proteínas, lípidos, polisacáridos y sustancias aromatizantes y saborizantes (14). Por tanto el primer gran objetivo de esta Tesis doctoral consiste en la **recuperación de compuestos orgánicos volátiles** responsables del perfil aromático de los caldos de cocción generados en el procesado de buey de mar mediante procesos de membrana, en concreto mediante procesos de **pervaporación**.

En segundo lugar se ha estudiado la obtención de **concentrados aromáticos a partir de los residuos sólidos** generados en el procesado del buey de mar empleando procesos de **extracción mediante carbono dióxido supercrítico**. Estos residuos sólidos están formados fundamentalmente por caparazones, cabezas, etc.

1.2 Antecedentes en la obtención de concentrados de aroma de marisco.

En las últimas décadas, se han propuesto varios procesos para la obtención de concentrados del aroma y sabor del marisco. El término marisco, o fruto del mar, hace referencia a los animales marinos invertebrados entre los que se incluyen los crustáceos y moluscos entre otros. En 1970, Gray (15) patentó un proceso partiendo de caparazones y material fibroso del que se retira previamente la carne de marisco. El proceso requiere la limpieza de los caparazones, eliminando las vísceras residuales presentes, y la molienda húmeda de los subproductos para la obtención de una pasta que posteriormente se acidifica y se somete a secado por pulverización para obtener partículas con el aroma y sabor deseados. El principal objetivo fue la obtención económicamente viable de un concentrado que presentara la mínima cantidad de humedad posible, estable durante largos periodos de almacenamiento, y con buenas características organolépticas. No obstante, en 1979, Henneberry e Idziak (16) encontraron que aquel producto presentaba escasa aceptabilidad debido a la presencia de cáscaras residuales y a su elevado contenido en calcio. Por ello, propusieron otro método basado en la adición de enzimas proteolíticas sobre una pasta previamente filtrada para obtener extractos solubles en agua que posteriormente se secan para obtener un extracto en polvo con el aroma, sabor y color deseados.

En 1984, Noda y col. (17) prepararon un aderezo libre de olor a pescado mediante la descomposición enzimática de diferentes tipos de marisco y la adición de sustancias (e.g.: 2,6-dimetoxifenol, guayacol o 3-metilguayacol) que proporcionan notas ahumadas al producto final.

Por otro lado, Oonishi y Satou (18) se centraron en la obtención de un material pulverulento con el aroma y sabor de marisco, mezclando un extracto acuoso de marisco con dextrinas solubles en agua y posterior liofilización o secado por pulverización. Este tratamiento previene la absorción de humedad, aglomeración, decoloración y desarrollo de olores manteniendo el delicado aroma del marisco.

En 1984, Nishikawa (19) obtuvo un aderezo natural, con un fuerte poder aromático, mediante un proceso consistente en moler el marisco fresco hasta obtener partículas finas, añadir agua y sal, ajustar el pH de la mezcla (opcionalmente se pueden añadir protesasas), descomponer los lípidos y las proteínas mediante un proceso de autodigestión enzimática, dejar reaccionar la mezcla durante un periodo de 7-30 días, extraer con agua caliente y desalar y concentrar mediante ósmosis inversa.

Este mismo autor, en 1985 (20), obtuvo un concentrado de bajo coste, con bajo contenido en sal y con aroma y sabor mejorados. Este proceso está basado en la descomposición enzimática de una sopa de marisco de la que se han retirado los sólidos no solubles y los aceites, para obtener sustancias de bajo peso molecular, como aminoácidos, etc. Por otra parte, las sustancias responsables de la formación de coloración marrón en el extracto, los componentes amargos tales como Mg, Ca, etc., y los componentes iónicos de bajo peso molecular se eliminan mediante electrodiálisis, de manera que se incrementa la proporción de componentes responsables del aroma y el sabor. Posteriormente, la sopa se concentra mediante un proceso de ósmosis inversa y evaporación a vacío del disolvente.

Por su parte, Okomura y Suzuki (21) obtuvieron un concentrado de marisco con unas excelentes características organolépticas cociendo el marisco, pelándolo y posteriormente tratando el caldo obtenido con un material adsorbente, como carbón activo en polvo, calentando con agitación, con enfriamiento posterior y separando el sólido adsorbente mediante filtración. El filtrado lo concentraron por evaporación a vacío obteniendo un líquido concentrado con un contenido en sólidos del 65%. Este producto final posee una excelente palatabilidad y destaca la ausencia de malos olores como el olor a mar y sabores desagradables como amargor o astringencia que presenta el caldo de cocción original.

En 1987 Yoshikawa (22) diseñó un proceso de fermentación para la obtención de un concentrado aromático. El proceso consiste en hacer reaccionar un extracto de marisco con bacterias pertenecientes al género *Lactobacillus*, *Streptococcus* o *Pediococcus*, o añadiendo además levaduras pertenecientes al género *Saccharomyces* y/o moho de Koji perteneciente al género Aspergillus.

En 1988, Ishii (23) obtuvo un concentrado estable y con excelentes características organolépticas, sometiendo una sopa de marisco a una etapa de concentración seguida de ultrafiltración o viceversa, con una etapa final de intercambio iónico en ambos casos.

En 1989, Usui (24) observó que un extracto convencional de marisco, en concreto de ostras, obtenido mediante concentración del caldo residual en la industria de procesado del mismo (enlatado), contiene por lo general los componentes responsables del aroma y sabor de marisco (e.g.: ácido glutámico) o componentes potenciadores del aroma (e.g.: glucógeno), pero también diversas sustancias de coloración negra y marrón que proporcionan al extracto, además de un color no aceptable en muchos productos, olores amargos, irritantes y picantes y un sabor pesado y graso. Estos compuestos de coloración marrón y negra son productos de la reacción de Maillard, producida por compuestos que contienen el grupo amino, por lo general aminoácidos, y grupos carbonilo, generalmente azúcares. Las reacciones de Maillard en las que intervienen azúcares reductores provocan en el caldo una reducción del valor nutritivo del caldo y deterioro de los componentes del sabor. Por tanto, estos autores propusieron utilizar adsorbentes, formados fundamentalmente por un compuesto inorgánico de magnesio de gran área superficial, para eliminar de los caldos las sustancias precursoras de estos compuestos de

coloración negra y marrón, sin reducir de manera sustancial los compuestos responsables del aroma y el sabor y nutritivos presentes en el caldo. Posteriormente se concentra el líquido refinado en atmósfera no oxidante.

En 1994, Sakamoto (25) preparó un aderezo consistente en un aceite de color rojo-anaranjado obtenido a partir de los subproductos de marisco, sometiendo los materiales a un proceso de fritura, cocción, separación de los aceites y las grasas, adición de un agente antioxidante y purificación del mismo. El resultado fue un aceite con un olor menos intenso que los aderezos comerciales, pero más próximo al natural de los crustáceos y con la posibilidad de variar sus características organolépticas en función de las condiciones de fritura.

En 2003, Kuroda y Nishimura (26) obtuvieron un extracto de marisco sin olor a pescado y con sabor mejorado. El método desarrollado consiste en ajustar el pH del extracto entre 3,5 y 4,8, eliminar los productos insolubles mediante filtración o centrifugación, ajustar el pH del filtrado entre 5 y 7, añadir y mezclar 5 – 20 % de Kieselguhr y filtrar la mezcla y/o añadir un azúcar y disolverlo agitando y posteriormente calentar la mezcla entre 100 y 150 ºC durante 1 a 60 minutos.

En 2004, Sato y Yoshikawa (27) propusieron un método sencillo consistente en calentar una mezcla de extracto de crustáceo o marisco, aminoácidos y azúcares, seguida de una destilación del producto obtenido. El aroma y sabor obtenidos tenían características organolépticas que recordaban al marisco asado.

En 2005, Kawasaki (28) llevó a cabo la formulación del aroma asociado a los crustáceos empleando para ello materiales sintéticos de perfumería de diferente naturaleza química para crear un producto con el aroma y sabor naturales de los crustáceos.

Hayakawa et. al (29) y Hayashi y col. (30) obtuvieron un aroma de pescado y marisco ahumado y a la parrilla, mediante extracción con dióxido de carbono en condiciones subcríticas y supercríticas mezclado con cosolvente (mezcla de agua y alcohol), a partir de distintos pescados y mariscos que en la mayor parte de los casos habían sido previamente secados, asados a la parrilla o en el horno, o ahumados. Los autores afirman que esta técnica evita los problemas que presentan los concentrados obtenidos mediante métodos tradicionales como la destilación por arrastre con vapor o la extracción con disolventes orgánicos, como son la carencia de ciertas características organolépticas presentes en el pescado o marisco a la parrilla, baja intensidad, corta durabilidad y baja palatabilidad. El concentrado obtenido fue mezclado con una sustancia adsorbente, homogeneizado y posteriormente sometido a secado por pulverización o liofilización para obtener un polvo con el aroma deseado.

1.3 Procesos de pervaporación.

En los últimos años los procesos de separación por membrana han logrado un importante desarrollo tecnológico y comercial en la Industria Alimentaria. Algunas de las ventajas del empleo de la tecnología de membranas en la Industria Alimentaria son (31):

- Menor consumo energético que las operaciones convencionales de concentración.
- Menor daño térmico de los componentes presentes en el alimento.
- Reducción de la pérdida de aromas durante el proceso.
- Permite la eliminación de microorganismos.

Este desarrollo se ha producido fundamentalmente en tecnologías bien establecidas como son la ósmosis inversa y los procesos de ultrafiltración. Sin embargo, existen otros procesos de membranas emergentes, como los procesos de pervaporación, aún en fase de desarrollo, cuyas potenciales aplicaciones a nivel industrial son de importancia creciente.

La pervaporación (PV) es una tecnología de membranas utilizada para separar mezclas líquidas. El término "pervaporación" es una contracción de los términos permeación y evaporación, ya que se trata de un proceso de separación en el que una mezcla líquida se pone en contacto con una membrana selectiva y uno de los componentes de la mezcla se transporta mediante permeación preferencial a través de la membrana, saliendo en fase vapor al otro lado de la membrana

El transporte a través de la membrana se consigue manteniendo la presión parcial de vapor en el lado del permeado inferior a la presión parcial en el lado de la alimentación. Esta diferencia en las presiones parciales se puede conseguir mediante la aplicación de vacío en el lado del permeado y/o empleando un gas portador. El permeado condensa, mientras que el retenido se enriquece así en el componente que no permea de forma preferente. Debido a que sólo es necesario evaporar una fracción de la alimentación, el consumo energético es menor que en destilación.

La pervaporación ofrece un gran potencial en campos como la obtención y recuperación de compuestos aromáticos a partir de sus fuentes naturales. En comparación con procesos tradicionales de recuperación de aromas, la pervaporación presenta las siguientes ventajas:

- No se producen daños en compuestos volátiles termosensibles
- Bajo consumo energético
- No se requiere una etapa posterior de separación de disolventes o adsorbentes añadidos

- Pérdida mínima de aromas.

Aunque el mecanismo exacto por el que se produce la pervaporación no se conoce, el modelo de disolución-difusión es el que está más ampliamente aceptado para describir el proceso de pervaporación en membranas poliméricas.

Este mecanismo fue descrito por primera vez por Graham (32) basándose en experiencias realizadas en la permeación de gases a través de membranas homogéneas. Este modelo divide el proceso de separación en tres etapas consecutivas (Figura 1.4):

- Primera etapa: adsorción de las moléculas en la superficie de la membrana
- Segunda etapa: difusión de los componentes a través de la membrana. De acuerdo con el modelo de disolución-difusión, la membrana de pervaporación es no porosa por lo que la difusión es el único mecanismo de transporte posible.
- Tercero etapa: desorción de los componentes en el lado del permeado en forma de vapor.

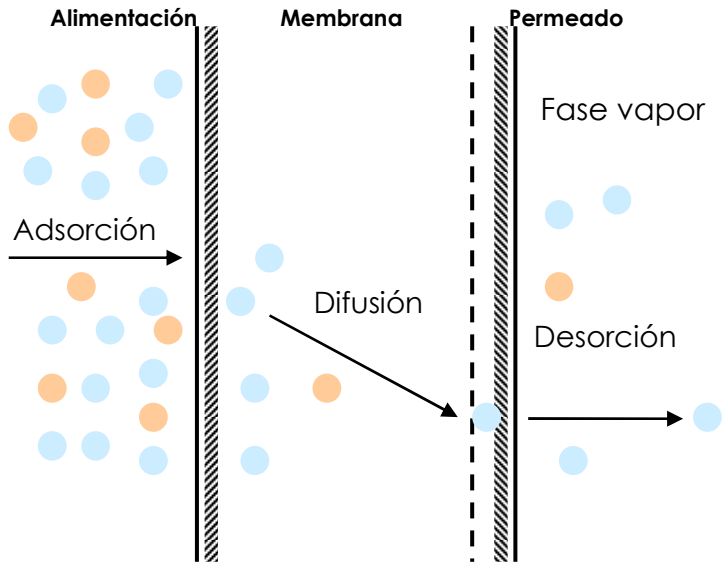


Figura 1.3. *Esquema básico de funcionamiento de una membrana de pervaporación*

La fuerza impulsora del proceso se basa en un gradiente del potencial químico a ambos lados de la membrana, que como ya se ha descrito, se suele obtener aplicando vacío en el lado del permeado. El permeado, en estado vapor, se recupera mediante condensación:

$$J_i = Q_{OV,i}\left(x_i \gamma_i p_i^s - y_i p_p\right) \qquad [1.1]$$

El tipo de membranas que se utilizan en los procesos de pervaporación determina la selectividad del proceso. Así, las membranas hidrofílicas (polímeros de polivinilalcohol o acetato de celulosa) son selectivas hacia compuestos polares como el agua y se utilizan con éxito en la deshidratación de compuestos orgánicos. La pervaporación con membranas hidrofóbicas se presenta en los últimos años como alternativa eficaz, frente a procesos clásicos de destilación o extracción con disolventes, para la separación y concentración de componentes orgánicos diluidos en medios acuosos.

Algunas de las aplicaciones más importantes de los procesos de pervaporación son:

- Deshidratación de disolventes orgánicos como alcoholes, éteres, ésteres y ácidos.
- Eliminación de compuestos orgánicos diluidos en disoluciones acuosas.
- Separación de mezclas de compuestos orgánicos.

De todas las aplicaciones de los procesos de pervaporación, es la deshidratación de disolventes orgánicos, mediante el empleo de membranas de pervaporación hidrofílicas, la más desarrollada. Así, la primera planta a nivel industrial se instaló en Brasil en 1982 para la producción de etanol anhidro (33). Sin embargo, los procesos de pervaporación con membranas organofílicas han experimentado un notable desarrollo en los últimos 20 años en el campo de la concentración de aromas a partir de disoluciones acuosas diluidas (34).

Desde el punto de vista industrial, la pervaporación permite alcanzar simultáneamente dos objetivos: medioambientalmente logra la reducción de compuestos orgánicos en las corrientes líquidas residuales en los procesos de producción y desde el punto de vista económico logra concentrados aromáticos potencialmente atractivos en la Industria Alimentaria (35).

La mayoría de los trabajos desarrollados en la recuperación pervaporativa de compuestos volátiles aromáticos suelen ir enfocados a su recuperación de zumos de frutas, ya que es uno de los problemas en el procesado de los mismos (36). De esta forma se han llegado a obtener permeados enriquecidos en compuestos aromáticos algunos de los cuales no eran detectables en la alimentación de partida (35). Así, por ejemplo en el estudio de la concentración de zumo de manzana mediante pervaporación (34) se han obtenido factores de enriquecimiento elevados para ésteres y aldehídos, siendo más bajos para alcoholes.

Los experimentos de pervaporación se llevaron a cabo en una planta semi-piloto diseñada y construida por el Área de Ingeniería Química de la Universidad de Burgos. La planta de pervaporación consta de los siguientes elementos (Figura 1.4):

- Un tanque de reacción con agitación de cinco litros de capacidad.
- Un módulo de pervaporación para membranas planas con un área efectiva de 170 cm2.

- Un sistema de condensación consistente en uno o dos vasos Dewar en los que se introduce una sustancia refrigerante (nitrógeno líquido o etanol enfriado).
- Una bomba de vacío, que permita alcanzar la presión adecuada en la zona de recogida del permeado, para que tenga lugar la separación de los componentes de interés.
- Medidores de temperatura y de presión, que ayuden a registrar la temperatura de operación y la presión en el lado del permeado.

Para poder analizar correctamente los resultados obtenidos en los procesos de pervaporación, el conocimiento de las propiedades termodinámicas en el intervalo de dilución infinita cobra especial importancia debido a la baja concentración de los compuestos aromáticos en la que se encuentran en la materia prima de partida. En esta tesis doctoral se han determinado los coeficientes de actividad a dilución infinita de algunos de los principales compuestos aromáticos presentes los caldos de cocción del buey de mar. Para la determinación de estos coeficientes se ha utilizado la técnica de espacio de cabeza acoplada directamente a un cromatógrafo de gases. En la Figura 1.5 se recoge un diagrama del equipo que se ha utilizado.

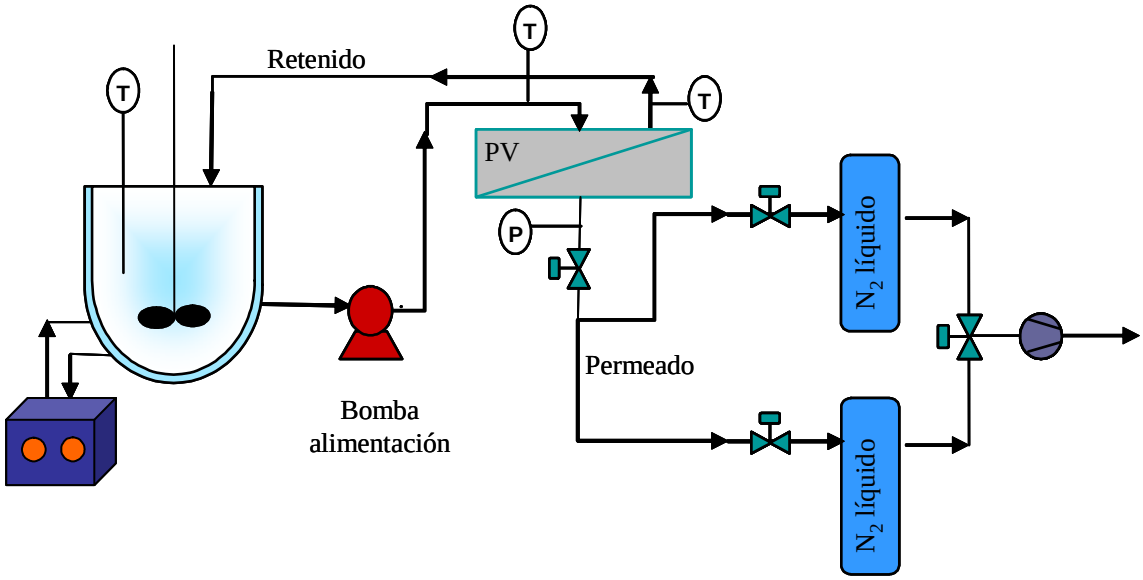


Figura 1.4. *Esquema del equipo de pervaporación*

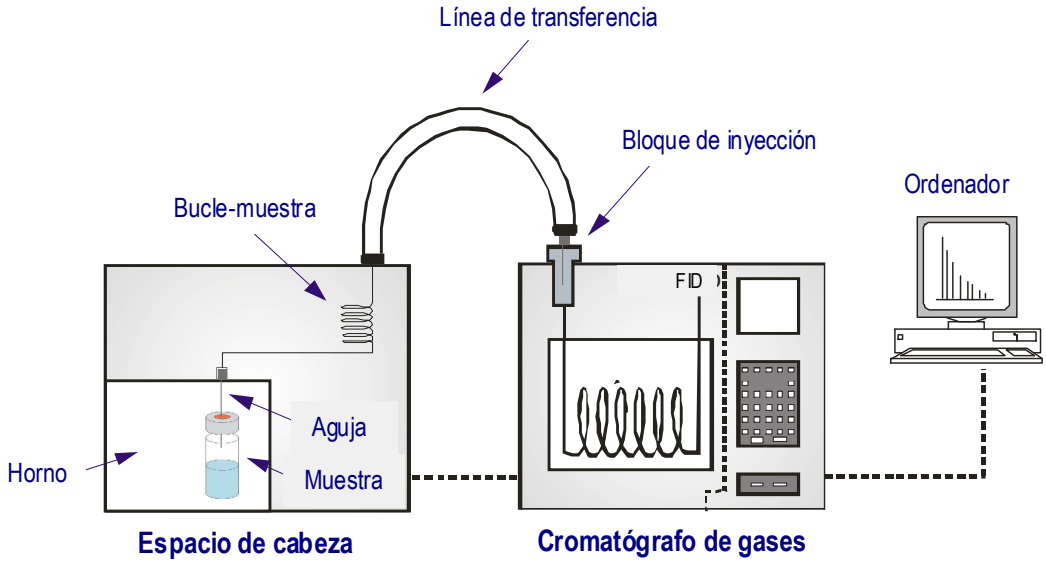


Figura 1.5. *Diagrama de flujo del equipo en espacio de cabeza.*

En muchas ocasiones, la selectividad de la membrana, debido a la baja concentración de los metabolitos a separar, limita la posibilidad del empleo de este tipo de tecnologías a escala industrial. Para mejorar la capacidad de este tipo de procesos se estudia la incorporación a la recuperación de los mismos de procesos de condensación en etapas múltiples. Uno de los trabajos más importantes llevados a cabo en este sentido ha sido realizado por Marin y col. (37, 38) con mezclas modelo agua – acetato de etilo, agua – etanol – acetato de etilo. Los condensadores parciales operan a distintas temperaturas en orden decreciente. En la Figura 1.6 aparece un esquema del sistema de recogida empleado utilizando dos condensadores en serie. En este tipo de disposiciones, el primer condensador opera a una mayor temperatura, en el que se recogerán la mayoría de los compuestos menos volátiles del permeado, mientras que en el segundo condensador se recogerá la fracción más volátil.

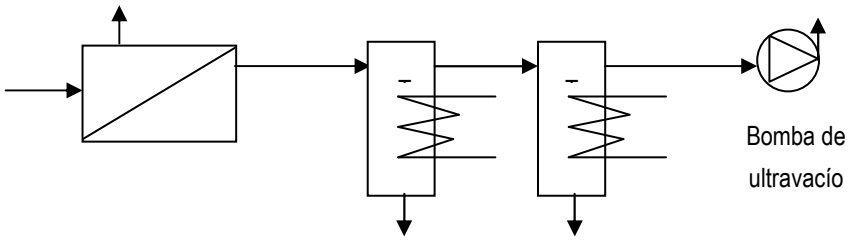


Figura 1.6. *Condensación en dos etapas*

1.4 Extracción con fluidos supercríticos.

La extracción con fluidos supercríticos (EFSC) es una operación cada vez más utilizada para la recuperación y estudio de los componentes volátiles responsables del aroma de diversas sustancias naturales.

La EFSC puede considerarse una operación intermedia entre la destilación y la extracción ya que tanto la presión de vapor de los compuestos como su solubilidad en el disolvente supercrítico son parámetros que influyen en la selectividad y el rendimiento de la operación. La mayoría de los compuestos responsables del aroma se extraen con dióxido de carbono supercrítico, pese a que este disolvente también es capaz de extraer ciertos compuestos indeseados presentes en la matriz.

El dióxido de carbono es el disolvente supercrítico más utilizado ya que no es tóxico y permite operar a presiones moderadas y cerca de temperatura ambiente. Se comporta como un disolvente lipofílico pero, en comparación con los disolventes líquidos, tiene la ventaja de que su poder disolvente se puede ajustar y puede establecerse en valores correspondientes a los gases o a los líquidos simplemente cambiando sus condiciones de presión y temperatura. Todos estos aspectos han hecho del dióxido de carbono el disolvente por excelencia en la mayor parte de las aplicaciones de la EFSC, fundamentalmente en la Industria Alimentaria, sin olvidar el creciente interés en reemplazar técnicas tradicionales como la destilación por arrastre de vapor o la extracción con disolventes, debido al deterioro que pueden sufrir los compuestos termolábiles, la posibilidad de reacciones de hidrólisis o la imposición de severas restricciones legales para eliminar residuos de disolventes en este tipo de productos cuando se emplean en la Industria Cosmética, Farmacéutica y Alimentaria (39).

1.4.1 Variables en el proceso de extracción con fluidos supercríticos.

Las variables de proceso más importantes en EFSC son la temperatura y la presión de extracción y el flujo de disolvente utilizado por unidad de masa de sustrato. Asimismo el pretratamiento del sustrato a extraer influye en el rendimiento de la extracción

- Temperatura. La temperatura debe estar fijada entres 35 y 60ºC, es decir, en la proximidad del punto crítico del dióxido de carbono (Tc = 32 ºC) y tan bajo como sea posible para evitar la degradación. El aumento de la temperatura reduce la densidad del SC-CO_2 (para una presión determinada), por tanto disminuye su poder disolvente, pero aumenta la presión de vapor de los componentes a extraer por lo que aumenta la

tendencia de estos compuestos a pasar a la fase fluida. Sin embargo el parámetro más importante es la presión de extracción.

- Presión. De manera general, a mayor presión mayor poder disolvente y menor selectividad en la extracción. La extracción de volátiles con dióxido de carbono en condiciones supercríticas se ha llevado a cabo a presiones elevadas (por encima de 400 bar) incluso cuando los compuestos a extraer son relativamente solubles en CO_2 supercrítico. Operando de esta forma, únicamente se aumentaba el poder disolvente del CO_2. Por tanto, se ha aplicado el concepto de optimización entre poder disolvente y selectividad y se han escogido las condiciones de operación de la EFSC para obtener la extracción selectiva de los compuestos de interés, reduciendo al mínimo la co-extracción de compuestos indeseados (40).

  Para realizar una extracción con éxito no sólo se debe tener en cuenta la solubilidad de los compuestos de interés e indeseados, ya que las resistencias a la transferencia de materia debidas a la estructura del material de partida y a la localización específica de los compuestos a extraer también juega un papel importante. Un análisis microscópico o experimentos específicos desarrollados variando el tamaño de partícula y el tiempo de residencia del disolvente supercrítico pueden facilitar el conocimiento de donde se encuentran dichas resistencias a la transferencia de materia.

- Relación de disolvente. Otro parámetro crucial es el flujo de disolvente utilizado por unidad de masa de sustrato (relación de disolvente). Este parámetro es muy relevante si el proceso está controlado por la resistencia a la transferencia de materia externa o por equilibrio; la cantidad de dióxido de carbono en condiciones supercríticas que se suministra al extractor determina la velocidad de extracción.

- Tamaño de partícula. El tamaño de partícula es un parámetro relevante si el proceso está controlado por la resistencia a la transferencia de materia interna, ya que tamaños de partícula pequeños reducen el camino de difusión del disolvente. Sin embargo, si las partículas son demasiado pequeñas, pueden dar problemas de canalizaciones en el lecho de extracción. Parte del disolvente puede fluir a través de canales formados en el interior del lecho y no entrar en contacto con el material a extraer, causando una pérdida de eficiencia y de rendimiento en el proceso. Se suelen emplear partículas entre 0.25-2mm.

  La dimensión óptima puede escogerse en cada caso teniendo en cuenta el contenido en agua en la matriz y la cantidad de componentes líquidos extraíbles que pueden producir fenómenos de coalescencia entre las partículas y favorecer una extracción irregular a lo largo del lecho. Asimismo, la producción de partículas de pequeño tamaño mediante molienda podría producir la pérdida de compuestos volátiles.

La duración del proceso está relacionada con el tamaño de partícula y la velocidad de flujo y deber ser seleccionado apropiadamente para maximizar el rendimiento del proceso de extracción.

1.4.2 Equipo de extracción con fluidos supercríticos.

En términos generales, el estudio de los procesos de extracción de volátiles se lleva a cabo a escala analítica. Las partes fundamentales de un **equipo analítico de EFSC** son el extractor, en el que se sitúa la materia prima previamente tratada para optimizar el proceso de extracción, y una trampa para volátiles situada a presión atmosférica donde tiene lugar la recogida de volátiles. La descompresión se realiza mediante una válvula o restrictor de flujo situados antes de la trampa.

Se emplean diferentes **métodos de separación** en función del tipo de compuestos volátiles a extraer, pudiéndose clasificar en función del fenómeno en el que se basan:

- Adsorción sobre la superficie de un sólido, se emplean materiales que presenten un tamaño de partícula pequeño, con elevada área superficial para asegurar un buen contacto entre el sólido y el fluido circulante, de manera que el proceso sea lo más eficiente posible. Se emplean materiales de distinto tipo, como bolas de vidrio (41, 42), ODS (43-47), Tenax TA (48) o Tenax GC (49). Dado que los procesos de adsorción son generalmente exotérmicos, al aumentar la temperatura disminuye la cantidad adsorbida, por ello, el proceso se desarrolla a temperaturas bajas, casi siempre por debajo de 0 ºC. Los flujos empleados en estos equipos son pequeños, del orden de g/h de CO2, asegurándonos que el tiempo de contacto entre el sólido y el fluido sea suficiente para que tenga lugar la retención de los compuestos de interés. La desorción de los compuestos de interés se efectúa térmicamente o mediante el empleo de un disolvente.
- Absorción en el seno de un líquido (50-52) donde el factor determinante es la solubilidad de los compuestos de interés en el disolvente adecuado, por tanto la elección del disolvente o mezcla de disolventes adecuados es crucial. Al igual que en el proceso de adsorción, la temperatura y el flujo de disolvente juegan un papel importante en el proceso. Un inconveniente de estos procesos es que el disolvente empleado normalmente es volátil en las condiciones en las que se efectúa la recogida, por lo que éste puede evaporarse y fluir hacia el interior del equipo, pudiendo causar problemas en el funcionamiento de la bomba (53).
- Condensación sobre un recipiente refrigerado (54-56). La eficiencia del proceso depende fundamentalmente de la temperatura empleada en el proceso, empleándose en algunos casos mezclas etanol/hielo seco, que aseguran temperaturas de -68ºC.

Por otro lado, los **procesos de extracción llevados a cabo a escala semi-piloto o piloto** se llevan a cabo de manera diferente. Las plantas de extracción constan de un extractor y varios separadores en los cuales tiene lugar el fraccionamiento del extracto. La separación por fraccionamiento de los extractos es un concepto que puede ser útil para mejorar la selectividad del proceso de EFSC. En muchos casos, es imposible evitar la co-extracción de algunas familias de compuestos con diferentes solubilidades o resistencias a la transferencia de materia en el material de partida. En estos casos, es posible desarrollar una extracción en pasos sucesivos a distintas presiones para obtener un extracto fraccionado de los compuestos solubles contenidos en la matriz orgánica. La separación fraccionada permite fraccionar el extracto, operando en la planta con varios separadores en serie a distintas presiones y temperaturas. La finalidad de esta operación es inducir una precipitación selectiva de las diferentes familias de compuestos en función de las distintas condiciones del FSC. Generalmente se emplean dos separadores, precipitando todas las sustancias indeseadas co-extraídas en el primer separador y recogiéndose un producto rico en componentes volátiles en el segundo. Se emplean flujos de disolvente mucho más elevados, del orden de kg/h. Las condiciones en los separadores se establecen de forma que la selectividad del proceso hacia los componentes volátiles de interés sea lo más elevada posible (39, 47, 57-61).

Para el desarrollo de estos objetivos se utilizó la planta piloto de EFSC diseñada y construida en el área de Ingeniería Química de la Universidad de Burgos y cuyo diagrama de flujo se presenta en la Figura 1.7. Las especificaciones máximas de dicha planta son 650 bar de presión y 200 C de temperatura, existiendo en la planta una zona de alta presión, desde la bomba hasta el extractor, y una zona de baja presión que engloba la zona donde tiene lugar la separación del extracto y la recirculación del fluido circulante. La planta consta de los elementos que se detallan a continuación:

a) Un extractor de acero inoxidable AISI-316 con capacidad de 2 litros, diseñado para operar hasta 65 MPa. Un sistema de difusión del fluido en la parte inferior del mismo, evita los canales preferenciales y volúmenes muertos. Asimismo dispone de un sistema de calefacción que consiste en una resistencia alrededor del cuerpo del extractor que permite el control y mantenimiento de la temperatura de extracción, junto con un baño termostático que calienta el fluido a la entrada del extractor.

b) Una bomba de diafragma, de membrana metálica y cabezal refrigerado que impulsa el fluido y consigue la presión de trabajo. La regulación del flujo se realiza de forma manual, ajustando la carrera del vástago que acciona las membranas, y con ello la cantidad de fluido que se pretende impulsar.

c) Dos separadores de acero inoxidable, de un litro y medio litro de capacidad. El fondo de los separadores tiene forma cónica y una válvula inferior para facilitar la descarga posterior de los extractos. Ambos separadores se encuentran encamisados para mantener la temperatura deseada en los mismos.

d) Las tuberías son de acero inoxidable, de diámetro externo ¼" y de espesor variable en función de la presión que lleve el fluido que circula.

e) Las válvulas instaladas en la planta son diferentes dependiendo de la función que desempeñen y la zona en la que se encuentren. Las hay de cierre-apertura, de regulación y de purga.

f) Medidor de flujo de tipo Coriolis que puede operar a presión máxima de 35 MPa y temperatura entre 220 y 450K. Permite la medida de flujo másico, densidad y masa total.

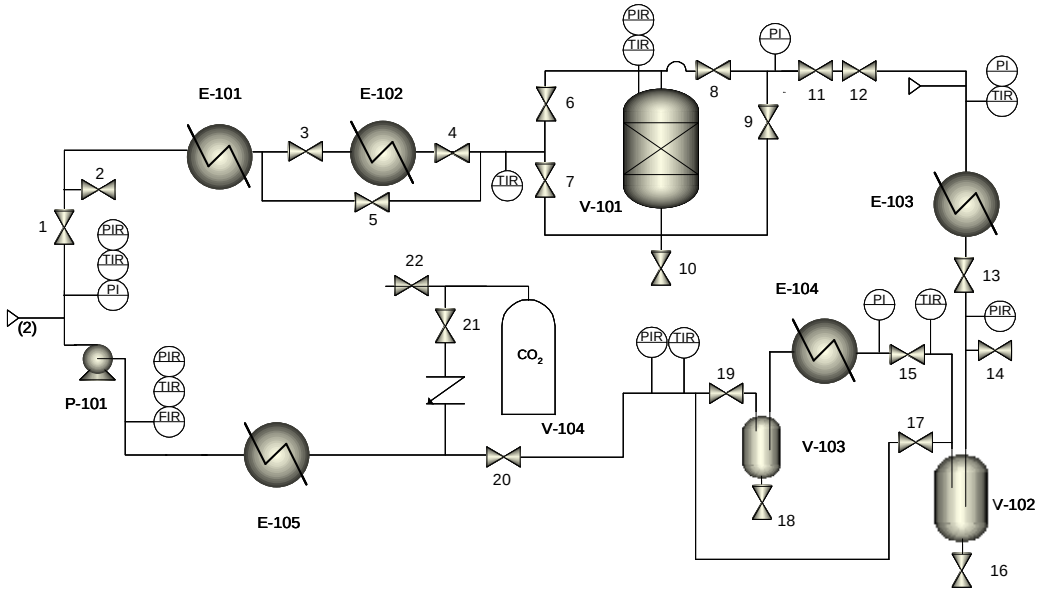
g) Medidores de temperatura, son termorresistencias del tipo Pt-100, instaladas dentro del tubo cerrado adecuado a la presión que debe soportar en cada caso, y colocadas en las distintas zonas de interés del equipo. Los datos son registrados en el ordenador a través de un sistema de adquisición de datos. En la cabeza del extractor se dispone de un termopar tipo J que indica la temperatura de operación.

h) Medidores de presión de dos tipos: tipo Bourdon que dan lectura directa de la presión y otros que incorporan transductores de presión que permiten monitorizar los valores recogidos gracias a un sistema de adquisición de datos.

i) Baños termostáticos, equipo de frío y resistencias que permiten mantener la temperatura adecuada en las distintas zonas de la planta, según las necesidades. Los baños y equipos de frío intercambian calor mediante serpentines o pueden estar conectados a encamisados, al cabezal de la bomba, etc. Las resistencias están reguladas gracias a potenciómetros y permiten calentar el extractor hasta la temperatura de operación.

j) Mecanismos de seguridad que garantizan un manejo seguro de la misma:

- Discos de ruptura en las distintas zonas de operación.
- Estructura de acero en la que todos los elementos se encuentran fijados.
- Pantalla de policarbonato de seguridad que separa la planta de la zona de manejo y control de la misma.



P-101	E-101	E-102	V-101	E-103	V-102	E-104	V-103	V-104	E-105
Bomba de CO_2	Cambiador de calor	Cambiador de calor	Extractor	Cambiador de calor	Separador 1	Cambiador de calor	Separador 2	Botella de CO_2	Cambiador de calor

Figura 1.7. *Diagrama de flujo de la planta de extracción con CO_2 supercrítico empleada durante el proceso.*

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2. OBJETIVOS GENERALES

Como ya se ha indicado anteriormente, el objetivo global de la presente tesis doctoral es el estudio de la obtención de concentrados aromáticos a partir de subproductos generados en la Industria Alimentaria, en concreto en el procesado de buey de mar, mediante el empleo de tecnologías limpias: extracción con CO2 supercrítico y tecnología de membranas, en concreto pervaporación.

Para la consecución del objetivo global de la tesis se han considerado distintos objetivos concretos:

1. Determinación de propiedades termodinámicas en el intervalo de dilución infinita.

Para optimizar los procesos de separación de aromas es necesario conocer el comportamiento termodinámico de estos compuestos en el intervalo de dilución infinita en el que se encuentran en la materia prima de partida. Por ello, otro de los objetivos de la tesis doctoral es la determinación experimental de los coeficientes de actividad a dilución infinita de algunos de los compuestos volátiles más representativos en este tipo de sustratos.

2. Estudio de la recuperación de mezclas sintéticas acuosas diluidas mediante pervaporación de compuestos aromáticos presentes en los caldos de cocción de buey de mar.

Previo a la recuperación de compuestos aromáticos a partir de los caldos de cocción, de gran complejidad, es de gran utilidad un estudio detallado con mezclas sintéticas para evaluar la influencia de distintas variables de operación. Para ellos se ha estudiado la pervaporación de mezclas sintéticas diluidas formadas por siete de los compuestos aromáticos más representativos encontrados en los caldos de cocción del buey de mar utilizando dos tipos de polímeros.

Se han considerado dos membranas cuya capa activa se encontraba compuesta por polioctimetilsilosano (POMS) y polidimetilsiloxano (PDMS) respectivamente. Para ambos tipos de polímeros se ha estudiado el efecto de algunas de las variables de operación más importantes como presión (afecta directamente a la fuerza impulsora del proceso y al perfil aromático), temperatura (afecta la velocidad de permeado y el perfil aromáticos) y concentración de la alimentación. Asimismo se ha estudiado el fenómeno de la polarización por concentración, ya que en este tipo de mezclas diluidas puede llegar a ser importante, evaluando así la importancia de la capa límite. Una vez obtenidos los resultados con ambos tipos de polímeros se ha comparado los distintos perfiles aromáticos obtenidos con ambas membranas

En el estudio de la pervaporación con membranas PDMS se ha considerando además un estudio de la recuperación de aromas en estado no estacionario y el efecto de la presencia de componentes no volátiles como sales. Asimismo, se ha estudiado la optimización del proceso mediante fraccionamiento en el lado del permeado.

3. Pervaporación de los caldos de cocción de buey de mar.

Finalmente se ha llevado a cabo la pervaporación de los caldos de cocción de buey de mar. Se ha analizado el efecto de la temperatura en los distintos perfiles aromáticos. Además se ha estudiado la optimización del proceso mediante procesos de fraccionamiento en el lado de permeado, para poder obtener una mayor concentración de los compuestos aromáticos en los permeado obtenidos.

4. Estudio de la obtención de concentrados aromáticos mediante extracción con CO_2 supercrítico a partir de los residuos sólidos generados en el procesado de buey de mar.

Este estudio engloba la optimización de los diferentes parámetros involucrados en el proceso, a partir de los datos de caracterización y cuantificación de los diferentes compuestos presentes en el extracto final. La presión y la temperatura de extracción determinan el poder disolvente del fluido, así como sus propiedades físicas (viscosidad y difusividad) que delimitan la capacidad de penetrar del fluido en la matriz sóilda. El estudio de la presión en el separador permite alcanzar un óptimo en el grado de separación del soluto del fluido comprimido y mejorar la eficacia del proceso.

3. OBTENCIÓN DE CONCENTRADOS AROMÁTICOS MEDIANTE PERVAPORACIÓN

3.1 Activity coefficients at infinite dilution in water: Effect of temperature and salt concentration.

ABSTRACT.

Activity coefficients at infinite dilution in water have been determined for some aroma compounds detected in brown crab liquid effluent produced during boiling (1-octen-3-ol, 1-penten-3-ol, 3-methylbutanal, hexanal, benzaldehyde, 2,3-pentadione and ethyl acetate) by using the headspace gas chromatography technique (HSGC). Experimental data have been obtained over the temperature range of 40ºC to 50ºC. In this work, activity coefficients at infinite dilution for different kinds of systems have been considered: one component solute + water and multicomponent solute + water. No significant differences were observed between activity coefficients obtained in these two kinds of systems. Additionally the effect of salt concentration at 40ºC has been studied by varying the salt concentration from 0 to 1.71 mol/kg. Experimental data were fitted as a function of salt concentration by using the Setschenow equation obtaining the salting out coefficient.

Keywords: aroma compounds, activity coefficients at infinite dilution, salt effect, temperature effect.

3.1.1 Introduction

The knowledge of the thermodynamic behavior of dilute aqueous solutions is necessary for a correct design of processes to separate dilute compounds such as the aroma recovery from food streams. In previous work (1), it has been reported that pervaporation technique is a promising alternative to recover the volatile fraction from brown crab processing effluent. Pervaporation is a membrane process that has been considered in the last years as an alternative to conventional methods to recover aroma compounds since the addition of chemical solvents is avoided (2). Based on the solution/diffusion model the flux of component i through the membrane is proportional to the difference in partial vapor pressure at both sides of the membrane $\left(x_i \gamma_i p_i^s - y_i p_p\right)$. In case of pervaporation of dilute aqueous solutions, activity coefficients at infinite dilution in water (γ_i^∞) are used as feed-side activity coefficients due to the very low concentrations of aroma compounds in the feed. Activity coefficients at infinite dilution provide an insight into the chemical and physical interactions

between water (solvent) and aroma compounds (solute molecules) in the absence of solute-solute interactions.

The affinity of a solvent towards a volatile compound can be expressed using Henry's law:

$$H = \lim_{x_i \to 0} \frac{p_i}{x_i} \qquad [3.1]$$

where p_i is the partial pressure of the volatile component and x_i its mole fraction at infinite dilution. The activity coefficient at infinite dilution of the volatile compound in the solvent is determined by taking into account the vapour pressure of the volatile compound at the same temperature, p_i^s (3):

$$\gamma_i^\infty = \frac{H}{p_i^s} \qquad [3.2]$$

Both, Henry constant and γ_i^∞, allow the evaluation of affinity of the aroma compound and the solvent (water) (4).

Different techniques have been developed to determine γ_i^∞ in water. Sherman et al. (5) emphasize that each method is most suitable in a certain range of relative volatility at infinite dilution, $\alpha_{i,w}^\infty$, defined as:

$$\alpha_{i,w}^\infty = \frac{p_i^s}{p_w^s}\gamma_i^\infty \qquad [3.3]$$

In this study the headspace gas chromatography has been used to determine γ_i^∞. The headspace gas chromatography is useful not only for analytical purposes (6), but also provides a valuable tool to obtain thermodynamically reliable data (7). Static headspace methods are based on measurements at thermodynamic equilibrium between liquid and gas phases. This technique has a range of applicability from low relative volatility systems (around 0.5) to systems of relative volatilities up to 1000 (5).

In this work, the activity coefficients at infinite dilution of seven volatile compounds found in the brown crab processing effluent (1-octen-3-ol, 1-penten-3-ol, 3-methylbutanal, hexanal, benzaldehyde, 2,3-pentadione and ethyl acetate) have been determined by using the headspace gas chromatography technique (HSGC). These coefficients were determined at three different temperatures over the range 40ºC to 50ºC. In food systems the volatility of aroma compounds is dependent on the presence of nonvolatile components such as sugars, salts, lipids and other macromolecular compounds (8). In the particular case of aroma recovery from brown crab effluent the effect of the presence of salts must be considered. Therefore the effect of sodium

chloride on activity coefficients at infinite dilution has been analyzed by varying the salt concentration in the range 0 to 1.7 mol/kg at 40ºC, since it is well known that the presence of salts and other electrolytes may increase or decrease the value of γ_i^{∞} (9).

3.1.2 Experimental section.

3.1.2.1. Materials.

The volatile compounds belong to different chemical classes: 1-octen-3-ol (Sigma Aldrich, 98 %), 1-penten-3-ol (Sigma Aldrich, 99 %), 3-methylbutanal (Sigma Aldrich, 97 %), hexanal (Sigma Aldrich, 98 %), benzaldehyde (Sigma Aldrich, >=99 %), 2,3-pentadione (Sigma Aldrich, 98 %) and ethyl acetate (Sigma Aldrich, HPLC grade). The chemicals were used without further purification. Sodium chloride was supplied by Sigma (≥ 99.5 % purity).

3.1.2.2. Systems.

Activity coefficients at infinite dilution, γ_i^{∞}, have been determined for different type of systems. First, γ_i^{∞} has been obtained for binary systems consisting of an aroma compound and water at three different temperatures, 40ºC, 45ºC and 50ºC. The headspace oven could not be regulated below 40ºC and this value was the lowest temperature studied in this work. Subsequently, γ_i^{∞} has been obtained for a multicomponent solute system formed for the seven aroma compounds and water at 40ºC. Finally the effect of salt on γ_i^{∞} has been determined for a multicomponent solute and water system at 40 ºC in the range 0 to 1.7 mol/kg of salt concentration.

3.1.2.3. Apparatus and procedure

Activity coefficients at infinite dilution were determined by headspace gas chromatography (HSGC). The HSGC consists of a gas chromatograph (Hewlett Packard GC 6890) and a headspace sampler (Hewlett Packard 7694E).

To determine γ_i^{∞}, glass vials (ca. 20 cm^3) were filled gravimetrically with different mixtures of the corresponding system varying the mole fraction of the volatile compound in the liquid phase. To obtain one activity coefficient at infinite dilution, seven measurements were performed to determine the vapor solute partial pressure as a function of the solute concentration. The interval of infinite dilution region cannot be evaluated a priori. For highly associated compounds, this region is smaller than 10^{-4} mole fraction and can be as small as 10^{-6} or 10^{-7} (10). In our case, mole fraction of the aroma compounds was kept below $2 \cdot 10^{-4}$ in all cases. In this concentration range results are obtained under Henry's law conditions (see

section 3.1). Solutions were prepared from a concentrated solution (approximately 1000 ppm) in water or in a salty water mixture. This mixture was stored at 4ºC to avoid losses of volatile compounds and diluted for the different solutions concentrations. The vials were filled about half way (ca. 10 cm^3) and immediately sealed properly with a pressure-tight proper rubber septum and a special aluminum lid to ensure that the headspace gases do not escape. Equilibrium between gas and liquid phases is reached in the headspace oven. After reaching equilibrium, an aliquot of the vapor phase is withdrawn and transported and analyzed in the GC. The GC column was a 007 FFAP 25 m × 0.25 mm bonded phase fused silica capillary column. The injector and flame ionization detector were at 200 ºC and 250ºC respectively. The oven was operated at programmed temperature, from 40ºC to 220ºC. At least three samples at the same concentration were used in the experiments.

Equilibrium time was determined for each of the investigated systems. For that, different glass vials were prepared with the same concentration and kept in the headspace oven for different increasing time intervals. When the peak areas obtained in the GC were constant, phase equilibrium was assumed to be reached.

The calibration was performed according to Whitehead and Sandler (11) by using pure components at different temperatures to determine the relationship between solute vapor pressure and peak area. This way for mixtures, the solute partial pressure in equilibrium with the dilute solution can be obtained from the saturation pressure calibration curves. For all the components and range of conditions considered in this work the pure component peak area was linearly proportional to vapor pressure with a linear correlation coefficient above 0.99.

Table 3.1. *Coefficients for the extended Antoine Equation. (Equation 3.4). (*) Predicted*

Compound	C_1	C_2	C_3	C_4	C_5	C_6	C_7	Ref.
Water	66.74	-7258.2	0	0	-7.30	$4.17 \cdot 10^{-6}$	2	Aspen Plus
Ethyl acetate	59.92	-6227.6	0	0	-6.41	$1.79 \cdot 10^{-17}$	6	Aspen Plus
3-methylbutanal	54.11	-6192.1	0	0	-5.52	$1.17 \cdot 10^{-17}$	6	Aspen Plus
2,3-pentadione	13.77	-2756.6	-82.1	0	0	0	0	Soni et al. (13)
1-penten-3-ol	87.19	-8981.7	0	0	-9.98	$1.72 \cdot 10^{-17}$	6	Aspen Plus*
Hexanal	75.60	-7776.8	0	0	-8.45	$1.51 \cdot 10^{-17}$	6	Aspen Plus
Benzaldehyde	109.3	-9331.2	0	0	-14.6	$1.19 \cdot 10^{-2}$	1	Aspen Plus
1-octen-3-ol	91.50	-10339.1	0	0	-10.5	$9.46 \cdot 10^{-18}$	6	Aspen Plus*

Vapor pressure correlations of the pure compounds were obtained or predicted considering experimental data found in the literature by using Aspen Plus (2008) (12) except for 2,3-pentadione which Antoine constants were obtained from the literature (13). The equation for the extended Antoine vapor pressure model is:

$$\ln\left(p_i^s / kPa\right) = C_{1,i} + \frac{C_{2,i}}{(T/K) + C_{3,i}} + C_{4,i}(T/K) + C_{5,i}\ln(T/K) + C_{6,i}(T/K)^{C_{7,i}} \qquad [3.4]$$

Coefficients for the extended Antoine equation are listed in Table 3.1. The uncertainty in the pure solute vapor pressure has not been considered in γ_i^∞ calculation since the way vapor pressure data have been obtained is unknown. Similar procedure has been followed in the literature (14, 15).

3.1.3 Results and discussion.

When determining γ_i^∞ by headspace it must be taken into account that the liquid phase composition in equilibrium with the vapour phase does not correspond with the liquid composition calculated from the amounts weighed since a certain amount has been vaporized during equilibration. This correction has been calculated as indicated by Brendel and Sandler (9). Due to the large difference in the molar volume of a liquid and a gas, the correction in the liquid phase was not very important. For the systems studied in this work the relative deviation between the initial and the real liquid phase composition was always lower than 2 %. This correction, though small, was included in all the results. Additionally, as it was pointed out by Whitehead and Sandler (11) the greatest source of experimental error in the activity coefficient at infinite dilution calculation comes from the solute peak area determination.

3.1.3.1. Binary systems: solute + solvent.

The values of γ_i^∞ for the aroma compounds selected in this work have been determined at three different temperatures, 40ºC, 45ºC and 50ºC. The partial pressures of the volatile compounds in the vapor phase in the vials were calculated from the calibration with pure components and the detector response. This solute partial pressure has been found to be a linear function of the aroma mole fraction in the liquid phase. As an example Figure 3.1 shows this behavior for benzaldehyde at the three different temperatures studied in this work. In this graph the uncertainties for the mole fraction and the partial pressure have been also represented. Henry's law constant can be calculated from the slope of the variation of partial pressure with mole fraction according to Equation 3.1 (3). This slope is independent of mole

fraction which indicates that results were obtained under Henry´s law conditions in the interval of infinite dilution. Activity coefficients were directly deduced from H values by using Equation 3.2. The H and γ_i^∞ values for each volatile compound at the three temperatures are listed in Table 3.2 together with the uncertainties for the activity coefficients calculated. The uncertainties for γ_i^∞ are expressed through the relative standard deviations calculated from the uncertainties of the experimental variables (24). Relative standard deviations range from 4% to a maximum of 17%. For most experimental points relative standard deviation is less than 10 %, with a mean value of 9%. The maximum value of 17% corresponds to 1-octen-3-ol. Brendel and Sandler (9) pointed out that the error of the HSGC technique can be as high as 25% especially for compounds with low solubility and high values of activity coefficient at infinite dilution, as is the case of 1-octen-3-ol.

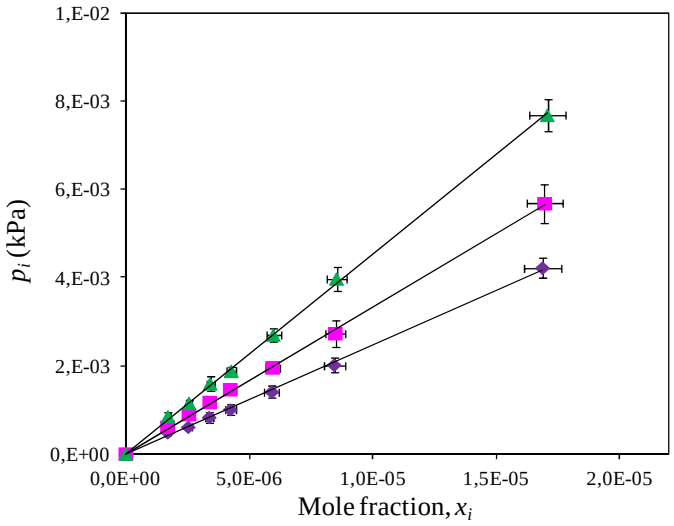

Figure 3.1. *Partial pressure of benzaldehyde as a function of mole fraction in the liquid phase at three different temperatures (♦ 40ºC, ■ 45ºC, ▲ 50ºC). Standard deviation for each data has been drawn.*

The temperature dependence of γ_i^∞ can be expressed by an Arrhenius type relationship (26):

$$\ln \gamma_i^\infty = a + \frac{b}{(T/K)} \qquad [3.5]$$

In general γ_i^∞ slightly increase with increasing temperature. Figure 3.2 shows the logarithms of γ_i^∞ as a function of the reciprocal temperature and the corresponding Arrhenius fit.

Table 3.2 also reports some activity coefficients found in the literature for the compounds studied in this work. For some of the compounds differences can be appreciated among the different values reported in the literature. In this regard Barrera Zapata et al. (25) emphasize that accurate data for γ_i^∞ are not abundant and even for common systems like ethanol in water at room temperature, the experimental values reported for γ_i^∞ can vary by a factor of two.

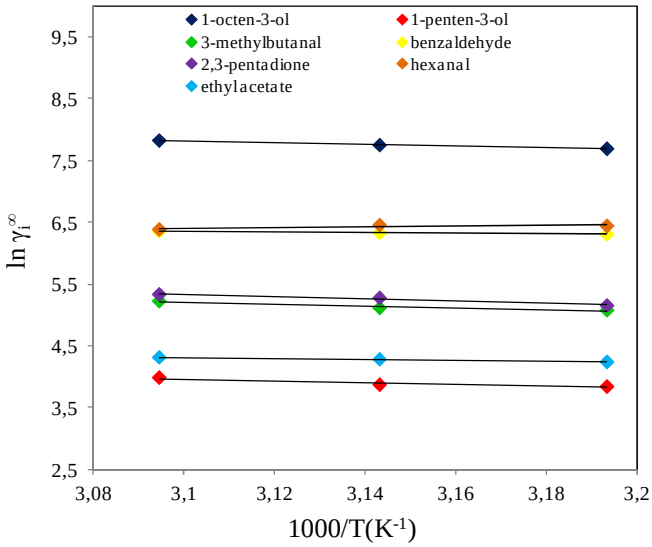

Figure 3.2. *Arrhenius plot of the activity coefficients at infinite dilution for the volatile compounds studied in this work. Standard deviation for each data has been drawn.*

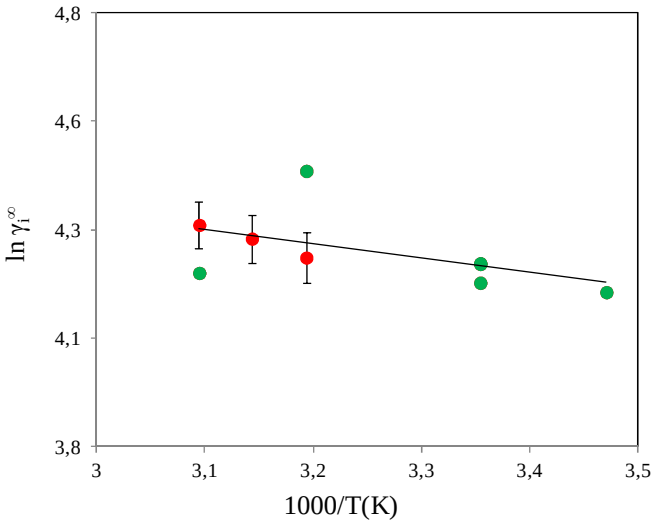

Figure 3.3. *Values of the activity coefficients at infinite dilution for ethyl acetate as a function of temperature (● data obtained in this work ● literature values from Table 3.2).*

Table 3.2. *Experimental values of Henry's law constant (H_i), γ_i^{∞} obtained with Equation 3.2 and literature values of γ_i^{∞}.*

Compound	T (K)	H_i, kPa	γ_i^{∞}	RSD (%)	γ_i^{∞} (literature)
Ethyl acetate	313.15	1761 ± 102	69	6	$68.3^{25°C,(16)}$ $68.2^{25°C,(17)}$
	318.15	2267 ± 125	72	4	$63.9^{15°C,(18)}$ $65.3^{25°C,(18)}$
	323.15	2860 ± 165	74	4	$84.5^{40°C,(18)}$ $66.8^{50°C,(18)}$
3-Methylbutanal	313.15	2190 ± 193	160	9	$161^{-,(19)}$
	318.15	2870 ± 240	166	9	$46.4^{98-99°C,(14)}$
	323.15	3982 ± 320	185	8	
2,3-Pentadione	313.15	1087 ± 94	173	9	
	318.15	1582 ± 138	195	9	
	323.15	2133 ± 146	207	7	
1-Penten-3-ol	313.15	153 ± 13	47	9	
	318.15	211 ± 23	48	10	
	323.15	315 ± 54	54	11	
Hexanal	313.15	2097 ± 283	625	14	$813^{25°C,(5)}$ $121.1^{98-99°C,(14)}$
	318.15	2754 ± 321	634	14	$1213^{25°C,(16)}$
	323.15	3291 ± 431	591	13	$1012-1000^{20-25°C,(20)}$
Benzaldehyde	313.15	241 ± 19	545	8	$559^{20-22°C,(21)}$ $1001^{25°C,(16)}$
	318.15	333 ± 26	559	8	$1485.5^{25°C,(22)}$ $251^{100°C,(15)}$
	323.15	454 ± 28	574	6	
1-Octen-3-ol	313.15	413 ± 70	2179	17	$3568^{25°C,(17)}$ $3386^{25°C,(23)}$
	318.15	623 ± 73	2310	12	
	323.15	943 ± 84	2487	9	

Figure 3.3 shows the logarithms of the activity coefficients at infinite dilution obtained in this work for ethyl acetate as a function of the reciprocal temperature together with the data found in the literature. From linear regression analysis of all the data an activity coefficient of 67.8 can be estimated for ethyl acetate at 298.15 K. For the rest of the compounds limited published data do not allow precise comparison with the results of the present work. For hexanal, benzaldehyde and 1-octen-3ol a value of γ_i^{∞} of 694, 501 and 1753 respectively at 298.15 K can be extrapolated from experimental data reported in this work.

3.1.3.2. Multicomponent solute + solvent system.

Activity coefficients at infinite dilution for each volatile compound, previously considered in this work, were also determined in a mixture formed by all the volatile compounds (multicomponent solute) and the solvent (water). Table 3.3 shows the γ_i^{∞} values obtained for each compound in this multicomponent mixture at 40ºC and the values obtained at the same temperature in a single component solute mixture.

***Table 3.3**. Activity coefficients at infinite dilution obtained in a single component solute aqueous solution and in a multicomponent solute aqueous solution at 40ºC.*

Compound	γ_i^{∞} Single component solute mixture	RSD (%)	γ_i^{∞} Multicomponent solute mixture	RSD (%)
Ethyl acetate	69	6	71	8
3-Methylbutanal	160	9	179	9
2,3-Pentadione	172	9	160	9
1-Penten-3-ol	47	9	49	8
Hexanal	625	14	601	14
Benzaldehyde	545	8	570	9
1-Octen-3-ol	2179	17	2164	10

This Table also presents the uncertainty of γ_i^{∞} through the percentage of the relative standard deviation. As an example, Figure 3.4 shows the solute partial pressure in the vapor phase as a function of mole fraction in the liquid phase in the single component solute mixture and in the

multicomponent solute mixture for ethyl acetate. The values obtained for multicomponent solute-water mixtures at 40ºC are similar to those obtained for single solute component-water mixtures concluding that no interactions take place among the volatile compounds in the range studied in this work. So, in this case, a mixture with (n-1) components at a composition close to zero and the solvent at a composition close to 1 is similar to the situation of having (n-1) binary mixtures formed by the solvent and the (n-1) components always infinitely diluted (10). Similar results were obtained for Bao and Han (27) in the study of the infinite dilution activity coefficients for various types of systems.

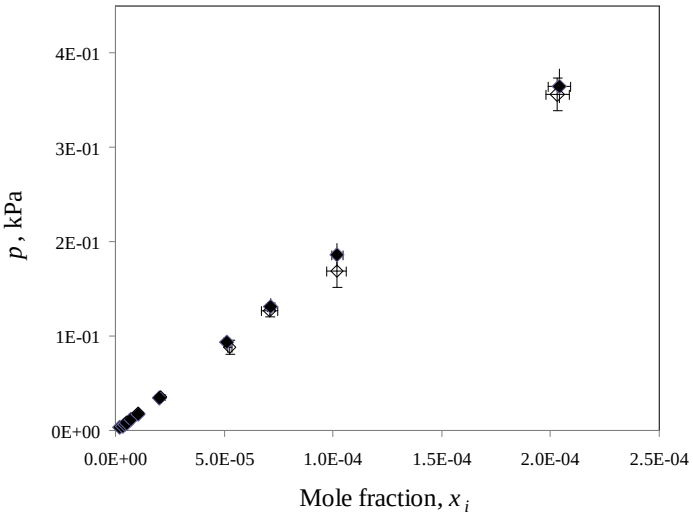

***Figure 3.4**. Partial pressure of ethyl acetate as a function of mole fraction in the liquid phase at 40ºC (♦ Multicomponent solute aqueous solution, ◊ single component aqueous solution). Standard deviation for each data has been drawn.*

3.1.3.3. Effect of salt on activity coefficients at infinite dilution.

The effect of salt concentration on γ_i^{∞} has been evaluated by varying the sodium chloride concentration from 0 to 1.71 mol/kg (0% to 10 w%) at 40ºC. Henry's constants have been calculated through the slope of solute partial pressure in the vapor phase as a function of mole fraction of the solute in the liquid phase. Activity coefficients were then deduced from Equation 3.2. The experimental values are listed in Table 3.4. As a general trend the values of γ_i^{∞} increase with increasing salt concentration, this effect is referred as "salting out" since an increase of the activity coefficient value involves lower solubility values.

***Table 3.4**. Activity coefficients at infinite dilution at 40ºC at different salt concentrations and salting out coefficient, k_i, at 40ºC.*

Compound	c_s (mol/kg)	γ_i^∞	RSD (%)	r^2	k_i
Ethyl acetate	0.00	71	8	0.995	0.58 ± 0.05
	0.34	86	8		
	0.86	121	6		
	1.71	186	6		
3-Methylbutanal	0.00	179	9	0.996	0.39 ± 0.03
	0.34	200	12		
	0.86	244	9		
	1.71	350	9		
2,3-Pentadione	0.00	160	6	0.984	0.43 ± 0.07
	0.34	196	18		
	0.86	236	17		
	1.71	324	18		
1-Penten-3-ol	0.00	49	8	0.990	0.37 ± 0.03
	0.34	56	14		
	0.86	66	14		
	1.71	--			
Hexanal	0.00	601	14	0.996	0.62 ± 0.07
	0.34	768	10		
	0.86	1029	10		
	1.71	1644	10		
Benzaldehyde	0.00	570	9	0.992	0.34 ± 0.04
	0.34	646	4		
	0.86	788	5		
	1.71	1003	6		
1-Octen-3-ol	0.00	2164	10	0.995	0.56 ± 0.05
	0.34	2493	11		
	0.86	3537	11		
	1.71	5679	9		

Brendel and Sandler (9) proposed the following equation to correlate γ_i^∞ in salty solutions based on the Setschenow empirical equation to correlate solubility of substances in salty solutions:

$$\ln\left(\frac{\gamma_i^\infty}{\gamma_{i,o}^\infty}\right) = k_i c_s \qquad [3.6]$$

where γ_i^∞ is the activity coefficient at infinite dilution in salty solutions, $\gamma_{i,o}^\infty$ the activity coefficient at infinite dilution in pure water, c_s the salt concentration and the proportionality factor, k_i, is the salting-out coefficient. Brendel and Sandler (9) report a dependence of k on temperature but this effect has not been studied in this work. The values of the salting out coefficients at 40ºC are provided in Table 3.4.

The correlation factor was higher than 0.98 for the all the compounds considered in this work. According to Equation 3.3, the relative volatility would also increase with salt concentration due to the salting out effect.

3.1.4 Conclusions.

The Henry's law constant and the activity coefficient at infinite dilution of seven volatile compounds found in brown crab boiling effluent have been determined by using the headspace gas chromatograpy technique. Experimental data have been obtained at three different temperatures 40ºC, 45ºC and 50ºC. The temperature dependence of activity coefficients at infinite dilution can be expressed by an Arrhenius type expression.

Comparing the γ_i^∞ obtained in a single component solute aqueous solution with those obtained in a multicomponent solute aqueous solution it can be concluded that no, or little, interactions take place among the volatile compounds in the concentration range studied in this work. However, one should keep in mind that the number of volatile compounds identified in the brown crab boiling effluent was more than 150 compounds, including aldehydes, ketones, alcohols, esters, aromatic compounds and sulphur and nitrogen-containing compounds (1).

Finally the effect of salt concentration has been studied by varying the sodium chloride concentration from 0 to 1.71 mol/kg. As a general rule, activity coefficients at infinite dilution for all the volatile compounds considered in this work increase as the salt concentration increases, showing a salting out effect.

3.1.5 References.

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NOMENCLATURE.

c_s = salt concentration

H = Henry constant

k = salting out coeffficient

p = pressure

R = gas constant

T = temperature

γ = activity coefficient

α = relative volatility

Upperscripts:

s: saturation

∞: Infinite dilution

Subscripts:

i = component

w = water

3.2 Concentration by pervaporation of brown crab most representative volatile compounds from dilute model solutions with POMS membrane.

ABSTRACT.

In this work, the pervaporation technique is investigated in the separation of dilute solutions of volatile compounds from brown crab effluent in order to obtain a valuable food flavouring fraction. A systematic study of the pervaporation process has been carried out on dilute model solutions of some of the compounds identified in the brown crab effluent as typical volatile compounds. The membrane used in this work was a hydrophobic membrane with a selective layer of POMS (polyoctylmethyl siloxane). The effect of some operating variables, such as feed flow rate, feed concentration, feed temperature and permeate pressure was analyzed on the pervaporation performance of the membrane.

Keywords: volatile compounds, concentration, pervaporation, POMS membrane

3.2.1 Introduction.

Brown crabs are found in the Eastern Atlantic and are heavily exploited commercially being available throughout the year. The brown crab liquid effluent produced during boiling is believed to contain important amounts of volatile flavour components (1). This work is part of a wider study to consider the conversion of this by-product into valuable volatile concentrate. Concentrates of the volatile species have considerable commercial utility, especially in the food industry due to longer shelf life, reduced packaging and lower distribution and storage costs (2). Additionally, organic removal from water at low concentrations involves an important environmental challenge.

Cha et al. (1) studied the concentration of the liquid effluent produced during snow crab boiling by steam distillation. However, this technique involves high energy consumption as well as physical aroma losses (3). In this work, the pervaporation process has been considered to recover the volatile fraction from brown crab effluent. Pervaporation is a membrane process which has been developed rapidly in the last 20 years for aroma concentration (2) since the addition of chemical solvents is avoided. Additionally moderate operating temperatures help to minimize degradation of aroma character.

Brown crab effluent was supplied by IDOKI SCF Technologies S.L. (Spain). The first step in this work was to determine the main volatile organic compounds present in the industrial

effluent. More than 150 compounds were identified in the brown crab effluent. These included mainly aldehydes, ketones, alcohols, esters, aromatic compounds and sulphur and nitrogen-containing compounds. To study the ability of pervaporation process to recover the volatile fraction from the brown crab effluent seven of the identified compounds have been selected for a model aqueous solution of brown crab effluent: 1-octen-3-ol, 1-penten-3-ol, 3-methylbutanal, hexanal, benzaldehyde, 2,3-pentadione and ethyl acetate.

A systematic study of the pervaporation process of the dilute model solution was performed in order to analyze the influence of some operating variables on the pervaporation performance. The permeation flux and enrichment factor of the selected volatile compounds were analyzed at different operating conditions: feed flow rate, feed temperature, feed concentration and permeate pressure.

3.2.2 Theory.

On the basis on the solution/diffusion model the flux of component i through the membrane is proportional to the difference in partial vapor pressure at both sides of the membrane (4):

$$J_i = Q_{OV,i}\left(x_i \gamma_i p_i^s - y_i p_p\right) \qquad [3.7]$$

where J_i is the partial permeation flux, Q_{OVi} the pressure-normalized permeation flux (permeance), x_i the mole fraction of component i in the feed, γ_i the activity coefficient and p_i^s the saturation vapor pressure at the temperature of the feed, y_i, the mole fraction in the permeate and p_p the permeate pressure. In case of pervaporation of dilute aqueous solutions activity coefficients at infinite dilution in water (γ_i^∞) are used as feed-side activity coefficients due to the very low concentrations of aroma compounds in the feed (5). In this work, the activity coefficients at infinite dilution in water were estimated with the help of the software Aspen plus (6) by using UNIQUAC equation when binary interaction parameters were available, otherwise the predictive method UNIFAC-Dortmund was used.

According to the resistance-in-series model, the two main mass transfer resistances that affect the pervaporation process are the liquid boundary layer resistance and the membrane resistance. At steady state the flux through the different mass transfer layers is equal:

$$J_i = Q_{ov,i}\left(x_i \gamma_i p_i^s - y_i p_p\right) = k_{bl,i}\rho(x_i^f - x_i^m) = Q_{m,i}\left(p_i^m - p_i^p\right) \qquad [3.8]$$

where k_{bl} is the liquid boundary layer mass transfer coefficient, ρ the total mass volume concentration of the feed, x_i^m mol fraction of i at the membrane-fluid interface, $Q_{m,i}$ the pressure normalized permeation flux across the membrane and p_i^m the partial vapor pressure of i at the membrane-fluid interface. The rest of the symbols are the same as in Equation 3.7.

The overall mass transfer coefficient in the steady state can be expressed as the sum of these two resistances:

$$\frac{1}{Q_{ov,i}} = \frac{\gamma_i \, p_i^s}{k_{b,l}\rho} + \frac{1}{Q_{m,i}} \qquad [3.9]$$

The term $\gamma_i p_i^s / \rho$ is the conversion factor from a concentration driving force to a partial vapour pressure driving force. The overall mass transfer coefficient $Q_{OV,i}$ of Equation 3.7 can be obtained from experimental measurements of the permeate flux and feed concentration of the permeating component i. The liquid boundary layer mass transfer coefficient, k_{bL}, is related to the feed hydrodynamic conditions and it can be estimated from the Sherwood correlation in terms of Reynolds (Re) and Schmidt (Sc) numbers for a plate-and-frame module (7):

$$Sh = \frac{k_{bl} d_h}{D_{i,water}} = 1.86 \, \mathrm{Re}^{1/3} \, Sc^{1/3} \left(\frac{d_h}{L} \right)^{1/3} \qquad [3.10]$$

where d_h is the hydraulic diameter, L a characteristic measure of the module defined by Dotremont et al. (1994) for a similar plate and frame module and $D_{i,water}$ the diffusion coefficient of i in water estimated using the Wilke-Chang correlation (8).

For pervaporation of dilute organic solutions, the boundary layer mass transfer resistance for water transport is assumed to be negligible (9):

$$J_w = Q_w^m \left(p_w^s \gamma_w x_w - y_w p_p \right) \qquad [3.11]$$

for dilute aqueous solutions activity coefficient and molar fraction of water are approximately equal to 1.

The separation performance of a pervaporation membrane can be described in terms of the permeation flux and the separation factor of the membrane (10). The enrichment factor of a given component is the relationship between the concentration in the permeate and the feed:

$$\beta_i = w_{i,p} / w_{i,f} \qquad [3.12]$$

In dilute systems, as aroma recovery systems, the solvent enrichment factor is close to one, so aroma enrichment factors can be considered equal to the corresponding separation factors.

3.2.3 Experimental section.

3.2.3.1. Materials.

Pervaporation membrane

The membrane used in this work was a hydrophobic membrane kindly supplied by GKKS Research Center (Germany). This membrane has a selective layer of POMS (polyoctylmethyl siloxane) on a PEI (poly ether imide) support (batch 03/011).

Volatile compounds

The identification of the main volatile components present in the brown crab effluent was performed by using a headspace-solid phase dynamic extraction-gas chromatography/mass spectrometry (HS-SPDE-GC/MS). More than 150 compounds were identified in the brown crab effluent. Among them, seven compounds have been selected for a model aqueous solution of brown crab effluent. The selected volatile compounds belong to different chemical classes: 1-octen-3-ol (Sigma Aldrich, 98 %), 1-penten-3-ol (Sigma Aldrich, 99 %), 3-methylbutanal (Sigma Aldrich, 97 %), hexanal (Sigma Aldrich, 98 %), benzaldehyde (Sigma Aldrich, >=99 %), 2,3-pentadione (Sigma Aldrich, 98 %) and ethyl acetate (Sigma Aldrich, HPLC grade).

These compounds are characteristic of seafood flavour: 1-octen-3-ol has been reported to be one of the volatile components widely distributed in fresh and saltwater fish, 1-penten-3-ol contributes to a butter-like odor (although its aroma treshold value is rather high), 3-methylbutanal is one of the most abundant volatile compound in boiled and pasteurized crabmeat, hexanal is one of the most abundant volatiles generated during lipid oxidation at moderate temperatures, benzaldehyde contributes to characteristic cooked crab flavour and ketones such as 2,3 pentadione contribute to the sweet floral, fruity flavour of many crustacean (1,12,13). Ethyl acetate was also found in the brown crab effluent and it was included in the model solution since could be considered as model molecule (Baudot et al., 1999).

Table 3.5 summarizes the organoleptic characteristics of the selected volatile compounds including the aroma threshold values (ATV), defined as the lowest concentration in a water solution at which an aroma compound is perceptible. Table 3.6 lists some thermodynamic properties of the selected compounds, including activity coefficients at infinite dilution and vapor pressure of the volatile compounds. Vapor pressure correlations were obtained or predicted by using Aspen Plus (2008) except for 2,3-pentadione which Antoine constants were obtained from the literature (Soni et al., 2008).

Table 3.5. *Aroma compounds used in the model solution (11).*

Aroma compound	Organoleptic characteristics	ATV, ppb
1-octen-3-ol	Very strong, sweet, earthy mushroom, odor taste	1.0
1-penten-3-ol	Pungent, grassy, alliaceous-like, green vegetable, fruity taste	400
3-methylbutanal	Powerful, penetrating, cheesy-sweaty-fruity in dilution	0.2-2
Hexanal	Strong, penetrating, fatty-green, grassy unripe fruit odor	4.5
Benzaldehyde	Odor of bitter almond oil, characteristic sweet cherry taste	350
2,3-pentadione	Oily-buttery, fatty odor, butter, cream, milk taste	30
Ethyl acetate	Ethereal, sharp, wine-brandy like odor	5.0

Table 3.6. *Physicochemical properties of the volatile compounds*

Compound	MW	BP (ºC)	p^s (26ºC), Pa	γ_i^∞ (26ºC)	VMBP (cc/mol)
1-octen-3-ol	128.2	174-5	65	4955.4	180
1-penten-3-ol	86.1	114.4	1348	17.0	117
3-methylbutanal	86.1	92.5	7035	164.6	118.9
Hexanal	100.2	128.3	1540	1047.2	140.0
Benzaldehyde	106.1	178.8	181	273.7	118.6
2,3-Pentadione	100.1	108	2918	282.4	121
Ethyl acetate	88.1	77.1	13045	75.3	106.3

Figure 3.5 shows the vapor pressure of the volatile compounds including water vapor pressure as a function of temperature. This Figure clearly shows that 1-octen-3-ol and benzaldehyde are the less volatile components while ethyl acetate and 3-methylbutanal are more volatile than water.

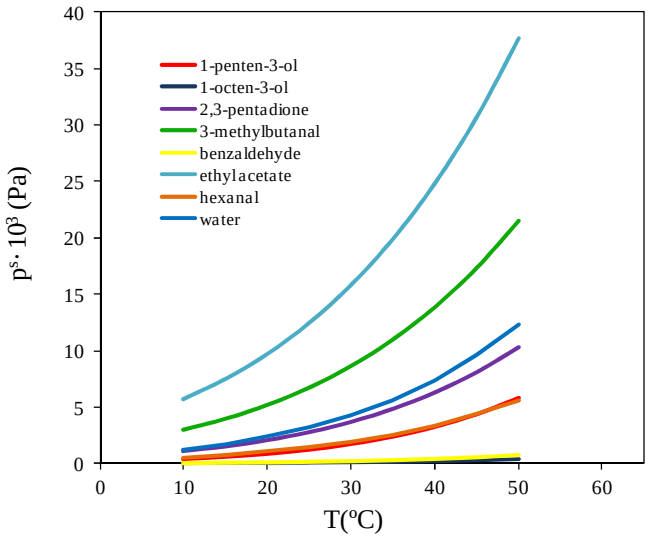

Figure 3.5. *Vapour pressure of water and volatile compounds as function of temperature.*

3.2.3.2. Feed solution.

Different feed solutions were used in this work. First, pervaporation experiments were performed using pure water as feed solution to check the performance of the POMS membrane. Further, separations of binary mixtures (water/1-octen-3-ol) and multicomponent mixtures were carried out in order to evaluate the influence of some operating variables such as: feed flow rate, feed concentration, feed temperature and permeate pressure on pervaporation performance.

3.2.3.3. Pervaporation experiments.

The pervaporation experiments were performed under steady state with a plate and frame laboratory stainless steel permeation cell (Sulzer Chemtech®) with an effective membrane area in contact with the feed mixture of 170 cm^2 (14). The temperature of the feed liquid mixture was kept constant (± 0.5 ºC) by using a thermostat to heat the stirred tank feed reactor of 5 L capacity. Permeate pressure was regulated with an air-inlet located between the condensers and the vacuum pump. The chemical stability of the membrane was checked between each experiment, measuring pure water flux at reference operating conditions.

3.2.3.4. Sample analysis.

Permeate and feed concentrations were measured off-line using a Hewlett Packard (6890) gas chromatograph (GC) equipped with series connected thermal conductivity (TCD) and flame ionization (FID) detectors. Helium, 99.999 % pure, was used as carrier gas. The GC column was a 007 FFAP 25 m × 0.25 mm bonded phase fused silica capillary column. The injector and detectors were at 200 ºC and 250ºC respectively. The oven was operated at programmed temperature, from 40ºC to 220ºC. 1-hexanol was used as internal standard for analysis of the samples.

3.2.4 Results and discussion.

3.2.4.1 Pure water as feed solution.

The effect of feed temperature and permeate pressure on membrane performance was studied using pure water as feed solution to check the behaviour of the POMS membrane. Feed temperature was varied in the range 26 ºC to 35ºC.

By increasing feed temperature, water permeation flux also increases mainly due to the increase of saturated water pressure on the feed side of the membrane (Equation 3.7). The temperature dependence of water permeation flux, J_{water}, can be expressed by an Arrhenius-type relation:

$$J_{water} = J_{water,o} \exp\left(-E_{a,water} / RT\right) \qquad [3.13]$$

where $E_{a,water}$ is the apparent activation energy of permeation, $J_{water,o}$ the preexponential factor and T the absolute temperature.

An apparent activation energy of 46.65 kJ/mol (Figure 3.6) was found by fitting water permeation flux obtained in this section (pure water as feed solution) as well as water permeation flux obtained in the pervaporation of volatile compounds dilute aqueous solutions (section 3.2.4.3).

Permeate pressure was varied in the range 100 Pa to 1200 Pa. Figure 3.7 shows the water permeation flux dependence on permeate pressure. This Figure shows experimental data obtained using pure water as feed solution and the results obtained in subsequent studies (section 3.2.4.3). According to Equation 3.11 water flux decreases with increasing permeate pressure (Figure 3.7). For the POMS membrane calculated water permeances were constant whatever the feed temperature and permeate pressure considered ($1.95 \cdot 10^{-7} \pm 1.23 \cdot 10^{-8}$ $mols^{-1}m^{-2}Pa^{-1}$ for all the experiments performed in this work).

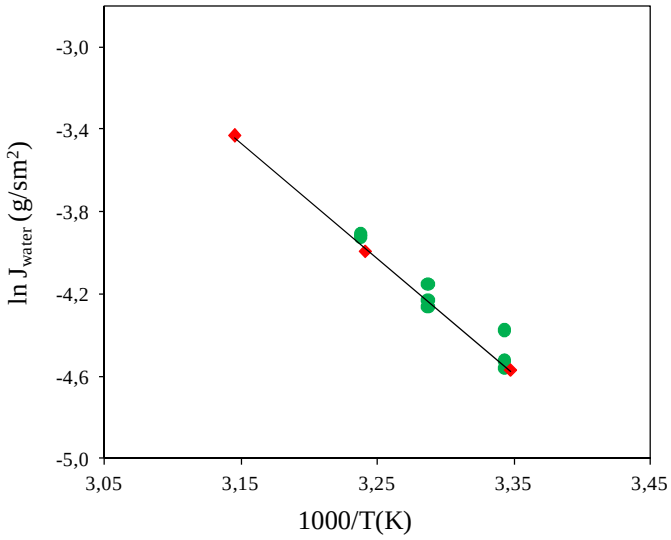

Figure 3.6. *Effect of temperature on water permeation flux at p_p = 300 Pa (♦ pure water ● multicomponent mixture).*

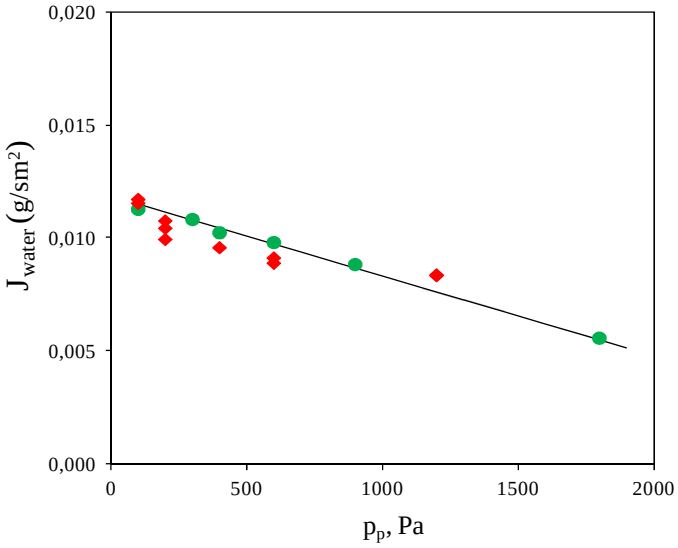

Figure 3.7. *Effect of permeate pressure on water permeation flux at p_p = 300 Pa. (♦ pure water ● multicomponent mixture).*

3.2.4.2. Binary feed solution.

Boundary layer effect

First, the boundary layer effect was studied in the pervaporation of the binary system water/1-octen-3-ol by varying the feed flow rate between 25 kg/h to 92 kg/h. According to resistance-in-series model when boundary layer is dominant resistance, mass transfer across the membrane increases with feed flow rate due to a decrease of the boundary layer thickness. Figure 3.8 shows the effect of increasing feed flow rate on partial (water and 1-octen-3-ol) permeation flux. Water and organic permeation fluxes were approximately constant inferring that little concentration polarization takes place. The mass transfer coefficient k_{bl} was calculated according to Sherwood correlation (Equation 3.10). The relative significance of the boundary layer mass transfer resistance was estimated less than 2% of the total resistance in the range of feed flow rates studied in this work. However this result must be carefully considered since Olsson and Tragardh (17) in their study of the influence of feed flow velocity on pervaporative aroma recovery pointed out that Sherwood correlation could overestimates the mass transfer coefficient of the liquid feed boundary layer.

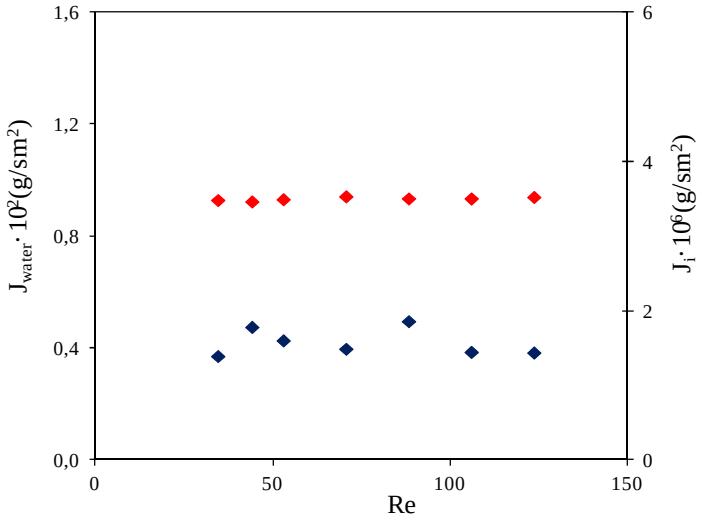

Figure 3.8. *Water (♦) and 1-octen-3-ol (i, ♦) permeation flux at different Reynolds (p_p = 400 Pa, T = 26ºC, $C_{i,feed} \approx$ 5 ppm).*

Effect of feed concentration

The effect of organic feed concentration was studied for water/1-octen-3-ol by varying the volatile feed concentration in the range of 0.1 to 10 ppm. By increasing the concentration of

the volatile component in the feed solution, organic partial permeation flux increases. A linear dependence of organic permeation flux can be assumed in the range of concentrations studied in this work ($r^2 = 0.97$). However, water permeation flux remained constant whatever the feed concentration (within the experimental error) and similar to the values obtained when using pure water as feed solution. The mean value found for 1-octen-3-ol enrichment factor was 37 ± 5.

Finally the effect of feed concentration, feed temperature and permeate pressure was study in the pervaporation of a feed model solution consisting of seven volatile compounds: 1-octen-3-ol, 1-penten-3-ol, 3-methylbutanal, hexanal, benzaldehyde, 2,3-pentadione and ethyl acetate.

3.2.4.3. Multicomponent feed solution.

Effect of feed concentration

The feed concentration of all the organic compounds studied in this work was varied in the range of 0.1 to 10 ppm at a fixed feed temperature (26ºC) and permeate pressure (300 Pa). Volatile organic concentration found in the brown crab effluent was rather low (less than 2 ppm). A wider range has been studied to minimize errors in the volatile organic compound determination. Figure 3.9 shows an acceptable linear relationship between organic permeation flux and feed concentration ($r^2 > 0.95$, except for benzaldehyde and 3-methylbutanal, $r^2 = 0.93$). This behaviour indicates that a constant normalized permeation flux (permeance) can be assumed in the studied concentration range.

Figure 3.10 shows the membrane permeance for the volatile compounds studied in this work as a function of organic concentration in the feed. The greatest deviations were shown for benzaldehyde. The lower values of the permeances correspond to 3-methylbutanal and ethylacetate, these are the organic compounds with the greater vapour pressure. The enrichment factor for the volatile compounds was independent of concentration in the range investigated in this work. The observed tendency of enrichment factor was the following: $\beta_{1\text{-octen-3-ol}}$ (≈121) > $\beta_{\text{benzaldehyde}}$ (≈93) > $\beta_{1\text{-penten-3-ol}}$ (≈25) > β_{hexanal} (≈22) > $\beta_{2,3\text{-pentanedione}}$ (≈7) ≈ $\beta_{\text{ethylacetate}}$ (≈7) > $\beta_{3\text{-methylbutanal}}$ (≈5).

Water permeation flux remains constant and equal to the flux of pure water (within the experimental error) whatever the feed concentration of the different aroma compounds.

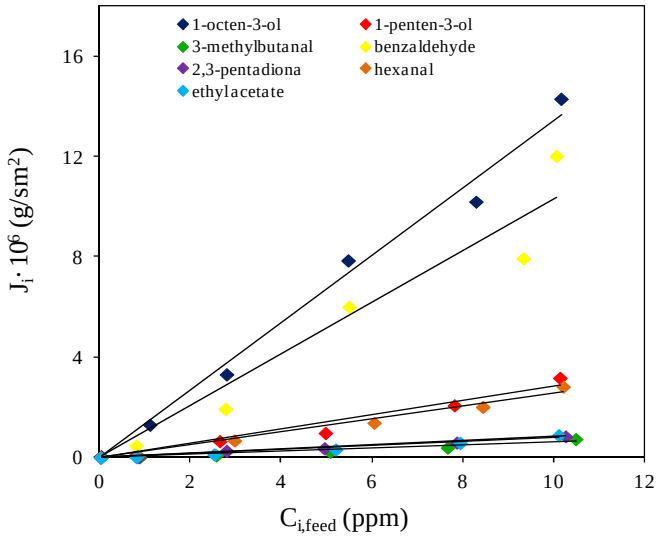

***Figure 3.9**. Effect of volatile feed concentration on volatile compound permeation flux (T = 26 ºC, pp = 300 Pa).*

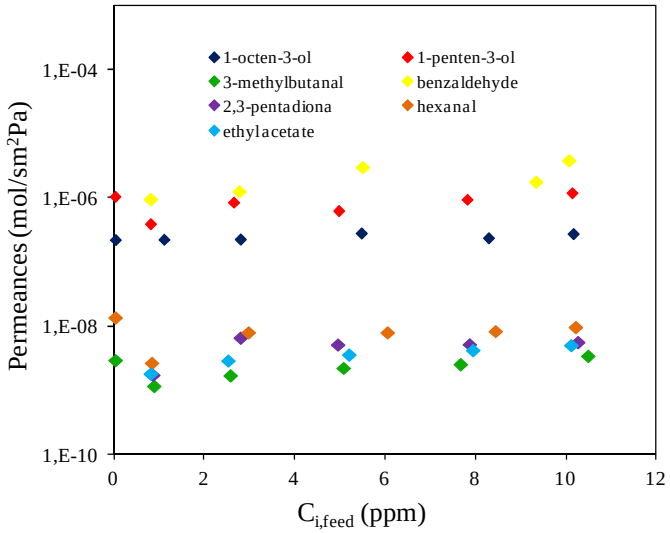

***Figure 3.10.** Volatile compound permeance as a function of volatile feed concentration (T = 26 ºC, p_p = 300 Pa).*

Compared to the binary system previously studied 1-octen-3-ol shows an increase in permeability in model multicomponent mixtures. This indicates that the presence of other organic compounds in the feed solution can affect the membrane selectivity due to interactions between the different aroma compounds. In this case a positive effect was observed in the 1-octen-3-ol permeation. Other studies of the pervaporation of organic compounds multicomponent mixtures (18-20) have also observed positive or negative interactions between the permeating aroma compounds. Isci et al. (2006) explained that higher fluxes than expected can be obtained when a permeant of low diffusivity is dragged through the membrane polymer by a permeant of higher diffusivity, the opposite can also happen.

Effect of feed temperature

Feed temperature is an important operating variable since it affects the feed/membrane characteristics and the driving force of the process.

The operating temperature was changed in the range 26 ºC to 35.7 ºC at a fixed permeate pressure (300 Pa) and different fixed feed concentration (0.1, 5 and 10 ppm). Moderate feed temperature is recommended in the study of pervaporation of flavour compounds to avoid any damage to heat-sensitive compounds. (2). Figure 3.11 shows the effect of temperature on volatile compounds permeation fluxes at an organic feed concentration of 10 ppm for all the volatile compounds. For all the organic compounds, when the temperature increases the organic permeation flux increases. The variation of the volatile compounds permeation flux with temperature was found to follow an Arrhenius type relationship (Equation 3.13). Apparent activation energy for permeation of aroma compounds follows the order: $E_{a,benzaldehyde}$ (49.47 kJ/mol) < $E_{a,1\text{-octen-3-ol}}$ (58.64 kJ/mol) < $E_{a,hexanal}$ (79.55 kJ/mol) < $E_{a,1\text{-penten-3-ol}}$ (84.77 kJ/mol) < $E_{a,ethylacetate}$ (86.81) < $E_{a,3\text{-methylbutanal}}$ (87.66 kJ/mol) < $E_{a,2,3pentadione}$ (155.01 kJ/mol). The apparent activation energy found for all volatile compounds is higher than that of water ($E_{a,water}$ = 46.65 kJ/mol). A higher value of the apparent activation energy indicates a more sensitive behaviour towards temperature changes, inferring that water permeation flux is less temperature dependence than that of volatile compounds. Therefore, the enrichment factor of all volatile compounds increases with an increase in the feed temperature. According to the values found for the apparent activation energy this trend was more noticeable for the most volatile component than for the less volatile components considered in this work.

Similar behaviour has been described in the literature in the recovery by pervaporation of different volatile aroma compounds through different pervaporation membranes (21-23). The results found in this section show a strong dependence on temperature for 2,3-pentadione. Further studies are necessary to confirm such behaviour.

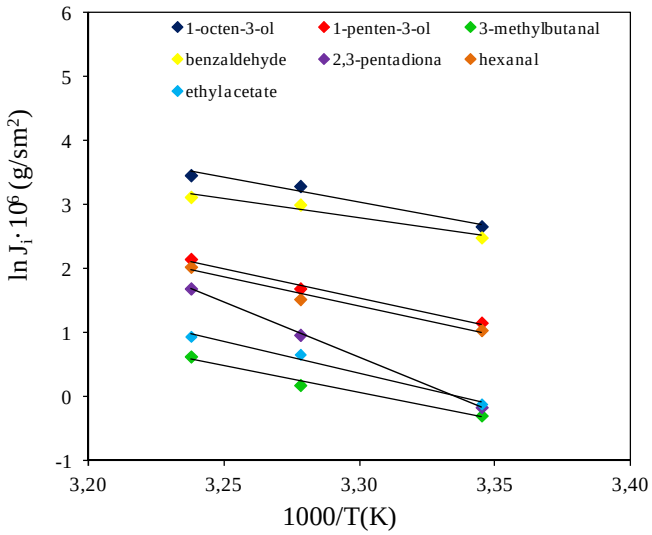

Figure 3.11. *Effect of feed temperature on volatile compound permeation flux (Ci, feed ≈ 10 ppm, pp = 300 Pa).*

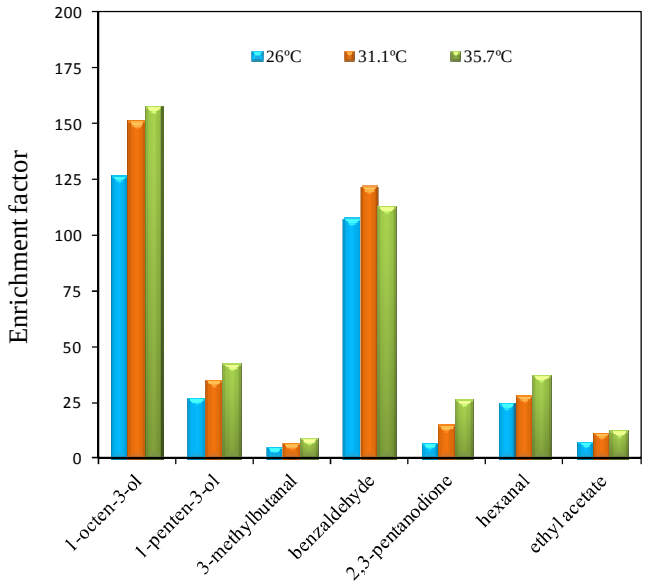

Figure 3.12. *Enrichment factor of volatile compound at different operating temperatures ($C_{i,feed}$ ≈ 10 ppm, p_p = 300 Pa).*

Figure 3.12 shows the enrichment factor at the three temperatures studied in this work at a fixed feed concentration of 10 ppm for the volatile compounds. With increasing temperature, the driving force increases because of the increasing vapour pressure, and therefore the permeate flux will also increase (see Equation 3.7). Additionally, an increase in the operating temperature causes an increase in the motion of the polymer chains improving the diffusion of the permeant molecules. Figure 3.13 shows the ratio of partial permeation fluxes obtained at 35.7 ºC and 26 ºC for all the volatile compounds and water. According to Olsson and Trägardh (1999b) the contribution from improved diffusion and increasing driving force to the increase of partial permeation flux has been also shown. This Figure shows that for water and the less volatile components (benzaldehyde, 1-octen-3-ol and 1-penten-3-ol) the increase in partial permeation flux is mainly due to an increase in the driving force. However the contribution due to an increasing diffusion becomes important for the more volatile compounds.

Enrichment factor values seem to decrease with the apparent activation energy values. The activation energy that characterizes the temperature dependence of the membrane can be estimated by subtracting the heat of vaporization (ranging from 35 $kJ \cdot mol^{-1}$ for ethyl acetate to 57 $kJ \cdot mol^{-1}$ for 1-penten-3-ol) from the calculated apparent activation energy (Feng and Huang, 1996).

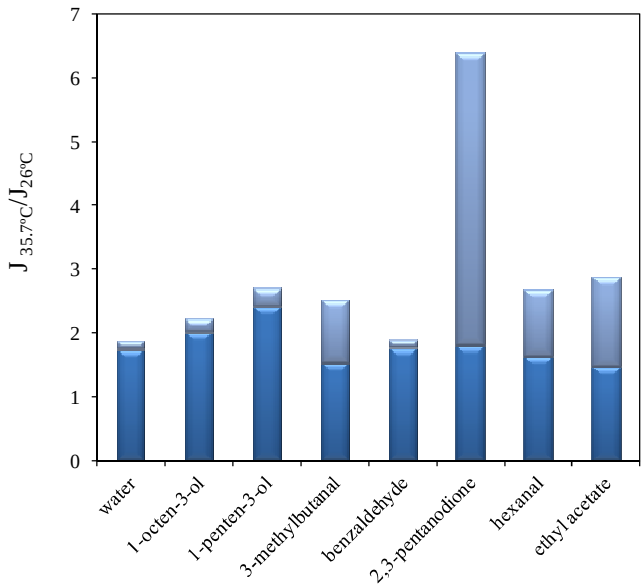

Figure 3.13. *Ratio of volatile compound permeation flux at 35.7 ºC and 26ºC.*

Activation energy of aroma compounds follows the order: $E_{a,benzaldehyde}$ (-0.17 kJ/mol) < $E_{a,1\text{-}octen\text{-}3\text{-}ol}$ (8.26 kJ/mol) < $E_{a,1\text{-}penten\text{-}3\text{-}ol}$ (27.72 kJ/mol) < $E_{a,hexanal}$ (36.73 kJ/mol) < $E_{a,3\text{-}methylbutanal}$ (50.23 kJ/mol) < $E_{a,ethylacetate}$ (51.57) < $E_{a,2,3pentadione}$ (116.82 kJ/mol).

From the values of activation energy, it could be concluded that sorption contributes more to permeation of 1-octen-3-ol and benzaldehyde molecules (the more hydrophobic compounds and less volatile). In contrast, the permeation for the rest of the volatile compounds studied in this work seems to be a diffusion dominating process.

Effect of permeate pressure

Permeate pressure is another operating parameter that affects the pervaporation performance as well as the operating cost of the process (Raisi et al., 2008). Different behavior was observed for organic permeation fluxes when varying permeate pressure in the range studied in this work (100 Pa – 1800 Pa). Trifunovic et al. (2006) state that in general components that are less volatile are more sensitive to changes in permeate pressure than compounds with higher volatility due to their smaller driving force. Figure 3.14 presents the effect of permeate pressure on the enrichment factor of the aroma compounds considered in this work.

For the low volatile components (1-octen-3-ol and benzaldehyde) the enrichment factor decreases as permeate pressure increases. However, other components such as 1-penten-3-ol, hexanal and 2,3-pentadione are less sensitive to changes in permeate pressure. Figure 3.14 shows that the enrichment factor of the components with higher equilibrium vapour pressure than water (3-methylbutanal and ethyl acetate) tends to increase as permeate pressure increases.

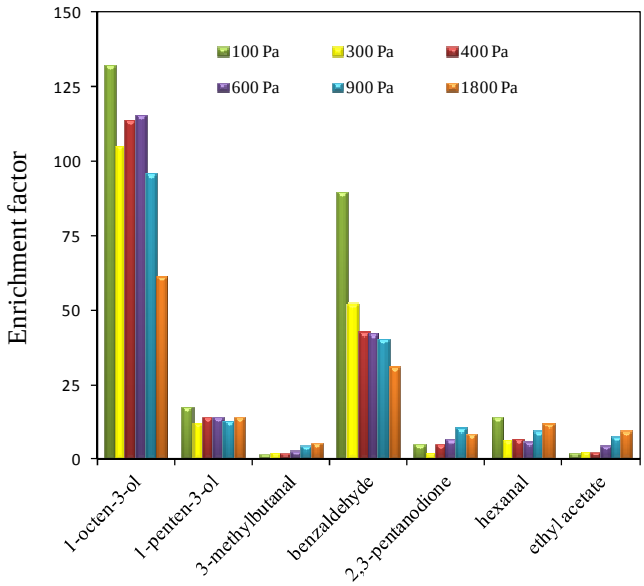

Figure 3.14. *Enrichment factor of volatile compound at different operating permeate pressure ($C_{i,feed} \approx 10$ ppm, T = 26 ºC).*

These results agree with other findings that appear on literature. According to Aroujalian and Raisi (2007) if the less volatile component is the more rapidly permeating species, selectivity decreases as permeate pressure increases. On the other hand, if the more rapidly permeating species are also the more volatile, selectivity increases as permeate pressure increases. As pointed out by Wijmans et al. (26), this indicates a unique characteristic of pervaporation process since separation can be improved by decreasing the driving force of the process.

3.2.5 Conclusions.

In this work, the recovery of volatile components from a model solution was performed by pervaporation with a POMS membrane. Pervaporation seems to be a promising technique for the recovery of aroma compounds from brown crab effluent. POMS membrane has been able to separate the organic compounds although organic partial permeation fluxes were not very high. A constant water permeance was observed for all the experiments carried out in this study. The membrane used in this work has shown higher selectivity towards the less volatile components. Organic permeation fluxes increase with feed concentration as a consequence of a higher driving force for the mass transport. In general partial permeation fluxes and enrichment factors increase as the feed temperature increases. However different behavior was observed for organic permeation fluxes with permeate pressure. As permeate pressure increases enrichment factor of the less volatile component was found to decrease, however for the most volatile components enrichment factors tend to increase by decreasing the driving force of the process. Operating conditions can be optimized to obtain permeates with a maximum organoleptic quality in its aroma profile. Further research is needed to account the influence of other substances present in brown crab effluent such as salt content.

3.2.6 References.

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NOMENCLATURE.

d_h = hydraulic diameter, m

D = diffusion coefficient, $m^2 \cdot s^{-1}$

E_a = apparent activation energy of permeation, $kJ \cdot mol^{-1}$

J = mass permeation flux, $g \cdot s^{-1} \cdot m^{-2}$

k_{bl} = liquid boundary layer mass transfer coefficient, $m \cdot s^{-1}$

L = characteristics of the module, m

p = pressure, Pa

Q_{OV} = pressure-normalized permeation flux, $g \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$

Q_m = pressure-normalized permeation flux across the membrane, $g \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$

R = gas constant, $kJ \cdot mol^{-1}K^{-1}$

T = absolute temperature, K

x, y = mol fraction

ρ = total mass volume concentration of the feed, $g \cdot m^{-3}$

γ = activity coefficient

β = enrichment factor

Upperscripts

i = component

m = membrane

p = permeate

s = saturation

3.3 Concentration by pervaporation of brown crab volatile compounds from dilute model solutions: Evaluation of PDMS membrane.

ABSTRACT.

Pervaporation experiments with PERVAPTM 4060 membrane have been performed to study its ability to concentrate some aroma compounds identified in the brown crab boiling effluent from a model dilute aqueous solution. The effect of feed concentration, permeate pressure and pervaporation temperature on the pervaporation performance of the membrane has been analyzed. The results obtained with PERVAPTM 4060 membrane were compared with experiments performed in a previous work with a POMS/PEI membrane. Membrane material seems to be a key factor in the permeate aroma profile since different aroma profiles were obtained with both membranes. The presence of non-volatile compounds, such as sodium chloride, in brown crab boiling effluent slightly improves the pervaporation performance in the concentration range studied in this work. Batch pervaporation experiments prove that the loss percentage during long operation time is high, especially for the most volatile compounds. Additionally, it has been shown that pervaporation performance can be significantly improved by modifying the permeant circuit by means of two stage condensation step.

Keywords: volatile compounds, pervaporation, salt effect, PDMS membrane

3.3.1 Introduction.

Shellfish flavour is a high value food product. The liquid effluent of the seafood industry, produced during boiling process, contains important amounts of flavour components (1). Brown crabs are found in the Eastern Atlantic and are heavily exploited commercially being available throughout the year. This work is part of a wider study to consider the conversion of the brown crab effluent produced during boiling into a valuable volatile concentrate. More than 150 compounds were identified in the brown crab boiling effluent. These included mainly aldehydes, ketones, alcohols, esters, aromatic compounds and sulphur and nitrogen-containing compounds

Membrane separation techniques represent a potential pathway for the production of a natural aroma concentrate and a disposable effluent (2,3). In a previous study (4), it has been shown that organophilic pervaporation, performed with a polyoctylmethylsiloxane membrane, is able to concentrate some components of the liquid effluent of brown crab from a model dilute solution. Pervaporation allows the use of moderate operating temperatures to minimize degradation of aroma compounds and avoids addition of chemical solvents.

In this work, pervaporation through a polydimethylsiloxane membrane has been performed in order to compare the ability of the two different polymers, POMS and PDMS, to recover aroma compounds found in the brown crab effluent from a dilute model solution.

The effect of some important variables that determine the final aroma profile, such as feed temperature, permeate pressure and feed concentration has been studied. Brown crab boiling liquid effluent contains organic compounds and non-volatile compounds. The influence of the presence of non-volatile components, such as salts (e.g.: sodium chloride) was also analysed by varying the salt content in the feed solution.

For industrial applications, a batch operation is preferred to a continuous operation if the aroma recovery is a short-term operation with relatively small amount of extracts (5). In this work, batch processes have been performed to study the recovery of aroma compounds from model solutions.

Finally, fractioning experiments have been also performed to improve the performance of pervaporation by using a two stage condensation in the permeate side.

3.3.2 Theory.

Solution/diffusion model is used to describe the transport of permeating components through the membrane being proportional to the difference in partial vapor pressure at both sides of the membrane (6):

$$J_i = Q_{OV,i}\left(x_i \gamma_i p_i^s - y_i p_p\right) \qquad [3.14]$$

where J_i is the partial permeation flux, Q_{OVi} the pressure-normalized permeation flux (permeance), x_i the mole fraction of component i in the feed, γ_i the activity coefficient and p_i^s the saturation vapor pressure at the temperature of the feed, y_i, the mole fraction in the permeate and p_p the permeate pressure. In case of pervaporation of dilute aqueous solutions, activity coefficients at infinite dilution (γ_i^∞) are used as feed-side activity coefficients. In this work, the activity coefficients at infinite dilution in water were obtained from a previous work in which activity coefficients at infinite dilution were obtained by using the headspace gas chromatography technique (7).

Separation performance of a pervaporation membrane, in case of pervaporation of dilute aqueous solutions, can be described in terms of the enrichment factor. The enrichment factor of a given component is the relationship between the concentration in the permeate and the feed:

$$\beta_i = w_{i,p} / w_{i,f} \qquad [3.15]$$

In dilute systems, as aroma recovery systems, the solvent enrichment factor is close to one, so aroma enrichment factors of organic compounds can be considered equal to the corresponding separation factors. There is usually an opposite trend between permeation flux and separation factor i.e.: when one factor increases the other decreases. This way, a pervaporation separation index (P.S.I.) has been defined as a measure of the separation ability of a membrane (8):

$$\text{P.S.I.} = J_{tot} \cdot \text{ Separation factor} \qquad [3.16]$$

To describe the batch operation systems, the following expression inferred by She and Hwang (5) was used:

$$\ln\left(\frac{x_i}{x_{i,o}}\right) = \left(-\frac{a + \left(\frac{K_L}{C_{tot}}\right)}{V_o}\right) t \qquad [3.17]$$

This expression was obtained by combining the material balance to the feed tank and membrane system with the expression of the organic permeation flux considering negligible the partial pressure of organics in downstream ($y_i p_p << x_i \gamma_i^{\infty} p_i^s$). She and Hwang (5) introduced a term, K_L, to consider the flavor loss rate in unit of mol/s, which represents how quickly the flavor organic compound is lost due to several reasons such as system leakage, partial condensation or incomplete collection in the pervaporation system. In Equation 3.17 x_i is the concentration of residue of component i in feed tank, $x_{i,o}$ is the initial mole fraction of component i at the beginning of the process, V_o is the initial volume of the feed solution, an t is the operation time. Parameter a is defined as:

$$a = \frac{Q_{OV,i} p_i^s \gamma_i^{\infty} A}{C_{tot}} - K_V \qquad [3.18]$$

where C_{tot} is the total mole concentration of the feed solution (for dilute pervaporation C_{tot} becomes approximately the pure water molar density), A is the membrane area and K_V is the total permeation volume flow rate ($dV = -K_V dt$). According to She and Hwang (5) the residue

percentage and recovery percentage are calculated by Equation 3.19 and Equation 3.20 respectively:

$$\text{residue}(\%) = \left(1 - \frac{K_V}{V_O} t\right)^{1+\left(a+K_{L,i}/C_{tot}\right)/K_V} \cdot 100 \qquad [3.19]$$

$$recovery(\%) = \frac{1+a/K_V}{1+\left(a+K_{L,i}/C_{tot}\right)/K_V}\left[1-\left(1-\frac{K_V}{V_O}t\right)^{1+\left(a+K_{L,i}/C_{tot}\right)/K_V}\right]\cdot 100 \qquad [3.20]$$

3.3.3 Experimental section.

3.3.3.1. Materials

A dense membrane was used in this study: PERVAP™ 4060 (Sulzer Chemtech®, Switzerland), a membrane whose active layer is based on polydimethylsiloxane (PDMS).

A multicomponent aqueous dilute solution was prepared with seven selected volatile compounds [4] belonging to different chemical classes: 1-octen-3-ol (Sigma Aldrich, 98 %), 1-penten-3-ol (Sigma Aldrich, 99 %), 3-methylbutanal (Sigma Aldrich, 97 %), hexanal (Sigma Aldrich, 98 %), benzaldehyde (Sigma Aldrich, >=99 %), 2,3-pentadione (Sigma Aldrich, 98 %) and ethyl acetate (Sigma Aldrich, HPLC grade). Some thermodynamic properties of the selected compounds are listed in Table 3.7.

3.3.3.2. Pervaporation experiments

The pervaporation experiments were performed with a plate and frame laboratory stainless steel permeation cell (Sulzer Chemtech®) with an effective membrane area in contact with the feed mixture of 170 cm^2 (9). The temperature of the feed liquid mixture was kept constant (± 0.5 ºC) by using a thermostat to heat the stirred tank feed reactor. The permeate was condensed on two parallel glass cold traps cooled by liquid nitrogen to ensure that all permeates were fully collected. Permeate pressure was regulated with an air-inlet located between the condensers and the vacuum pump. For steady state operation the feed reactor has 5 L capacity. This way, due to the small amount of permeate product, the concentration of the volatile compounds in the feed tank was kept approximately constant. For unsteady state operation the ratio membrane area to initial feed volume ratio (A/Vo) was higher than for steady state operation so the feed concentration of volatile compounds continuously decreases as pervaporation takes place. In the case of fractioning in the permeate side, two condensers

were placed in series in one of the two parallel permeate circuits. The first condenser was cooled in a refrigerant bath using a Julabo FP50 cryostat. The second condenser was cooled with liquid nitrogen and act as a total condenser. The chemical stability of the membrane was checked by measuring pure water flux at reference operating conditions.

Table 3.7*. Physicochemical properties of the volatile compounds.*

Compound	MW, g/mol	BP (ºC)	p_i^s, Pa	γ_i^∞	$H \cdot 10^4$, Pa
1-octen-3-ol	128.2	174-5	65	1779	11.6
1-penten-3-ol	86.1	114.4	1348	38	5.1
3-methylbutanal	86.1	92.5	7035	127	89.3
Benzaldehyde	106.1	178.8	181	504	9.1
2,3-pentadione	100.1	108	2918	133	38.8
Hexanal	100.2	128.3	1540	599	92.3
Ethyl acetate	88.1	77.1	13045	62	80.9

3.3.3.3. Sample analysis

Permeate and feed concentrations were measured off-line using a Hewlett Packard (6890) gas chromatograph (GC) equipped with series connected thermal conductivity (TCD) and flame ionization (FID) detectors. Helium, 99.999 % pure, was used as carrier gas. The GC column was a 007 FFAP 25 m × 0.25 mm bonded phase fused silica capillary column. The injector and detectors were at 200 ºC and 250ºC respectively. The oven was operated at programmed temperature, from 40ºC to 220ºC. 1-hexanol was used as internal standard for analysis of the sample.

3.3.4 Results and discussion.

First, the effect of different operating variables, such as feed temperature, permeate pressure and feed concentration in the pervaporation performance of PERVAPTM 4060 membrane is presented. Further, these results are compared with a previous work (4), where pervaporation was carried out with a POMS/PEI membrane. Afterwards, the results obtained in batch pervaporation performance are presented. Then, the effect of sodium chloride is analysed by

varying the salt concentration in the feed. Finally some preliminary results of optimization of pervaporation by two step condensation are discussed.

3.3.4.1. Evaluation of PERVAP™ 4060 performance.

Effect of feed concentration

The effect of feed concentration on membrane performance was investigated in the range of 0.1 to 50 ppm at a fixed feed temperature (26ºC) and permeate pressure (300 Pa). Since the experiments were carried out at very dilute concentration, the total permeate flux is close to the water permeate flux and remains almost constant (0.0054 mol/m^{2}s) whatever the volatile compounds feed concentration.

Figure 3.15 shows that volatile organic compounds fluxes present a linear relationship with the feed concentration for all the compounds studied in this work ($r^2 > 0.99$ except for 2,3-pentanedione that shows a $r^2 = 0.97$). Since the activity coefficients at infinite dilution are constant, and the polymeric membrane swelling can be considered negligible, the normalized permeation flux (permeance) of the organic compounds and water, and consequently the separation factor, remain constant. This fact was proved by the linear behavior of organic permeate fluxes with feed concentration in the concentration range studied in this work.

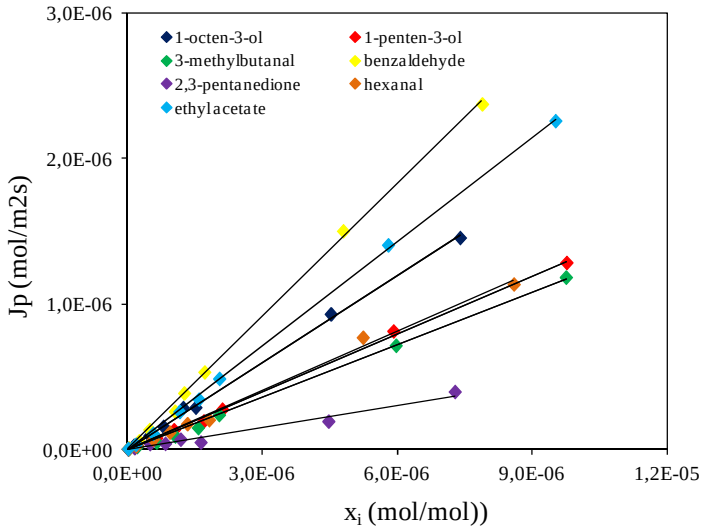


Figure 3.15. *Effect of volatile feed concentration on volatile compound permeation flux (T_{feed} = 26 ºC, p_p = 300 Pa) in the experiments performed with PERVAP™4060.*

The enrichment factors obtained through the PDMS membrane are reported in Table 3.8. Benzaldehyde was found to have the highest enrichment factor. This way, Baudot and Marin (10) assessed that the pervaporation membranes are very permselective for aldehydes containinig a benzene ring, which enhances their hydrophobicity and, consequently, their solubility in the pervaporation membrane. Ethyl acetate and 1-octen-3-ol also exhibit high separation factors. On the other hand, the selectivity for 2,3-pentanedione was found to be the lowest, exhibiting a separation factor value of 10.

Table 3.8. *Pervaporation parameters of PERVAP™ 4060. Comparison of the performance of PERVAP™ 4060 and POMS/PEI membranes at a fixed feed temperature (26ºC) and permeate pressure (300 Pa).*

	PERVAP™ 4060		**POMS/PEI**	
Compound	β	PSI (mol/m^{2}s)	β	PSI (mol/m^{2}s)
1-octen-3-ol	36	$1.98 \cdot 10^{-1}$	120	$8.77 \cdot 10^{-2}$
1-penten-3-ol	25	$1.36 \cdot 10^{-1}$	25	$1.92 \cdot 10^{-2}$
3-methylbutanal	18	$9.95 \cdot 10^{-2}$	5	$3.87 \cdot 10^{-3}$
Benzaldehyde	51	$2.78 \cdot 10^{-1}$	93	$7.51 \cdot 10^{-2}$
2,3-Pentadione	10	$6.77 \cdot 10^{-2}$	7	$5.20 \cdot 10^{-3}$
Hexanal	23	$1.26 \cdot 10^{-1}$	22	$1.74 \cdot 10^{-2}$
Ethyl acetate	39	$2.15 \cdot 10^{-1}$	7	$5.55 \cdot 10^{-3}$

Effect of feed temperature

The operating temperature studied was varied in the range 26 ºC to 40 ºC, to prevent thermal decomposition of the thermolabile aromatic compounds. The experiments were carried out at three different feed concentrations (1, 5 and 10 ppm) and at a fixed permeate pressure (300 Pa). In the temperature range studied, water permeation flux increased exponentially from 0.0054 mol /m^{2}s to 0.012 mol/m^{2}s.

Figure 3.16 shows the effect of temperature on volatile compounds permeation fluxes at an organic feed concentration of 10 ppm for all the volatile compounds. Partial permeate fluxes increase exponentially with increasing feed temperature.

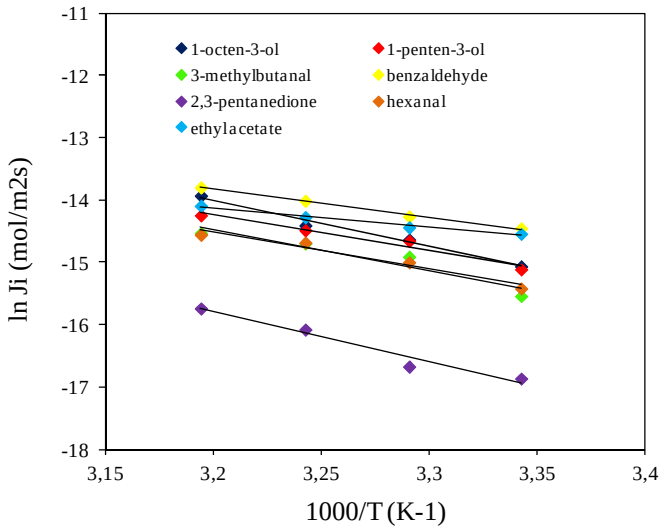


Figure 3.16. *Effect of feed temperature on volatile compound permeation flux ($C_{i.\ feed} \approx$ 10 ppm. p_p = 300 Pa) in the experiments performed with PERVAP™4060.*

The effect of feed temperature can be described by an Arrhenius type equation:

$$J_i = J_{i,o} \cdot \exp\left(-\frac{E_{a,i}}{RT}\right) \qquad [3.21]$$

where $E_{a,i}$ is the apparent activation energy of permeation, $J_{i,o}$ the preexponential factor and T the absolute temperature. Apparent activation energies for permeation of aroma compounds and water are reported in Table 3.9 for PDMS membrane.

This parameter characterizes the overall effect of temperature on the permeability and the driving force for permeation (11). A higher value of the apparent activation energy indicates a more sensitive behaviour towards temperature changes. Table 3.9 shows that $E_{a,i}$ for most of the organic compounds is higher than that of water, except for benzaldehyde and ethyl acetate. However, the difference in the apparent activation energy of organic compounds and water is not a big value. Therefore, it cannot be stated as a general trend that the enrichment factor of volatile compounds increases with temperature, although for some of the volatile compounds, such as 1-octen-3ol, the increase of the enrichment factor with temperature is remarkable. This behaviour can be observed in Figure 3.17 that shows the enrichment factor at the three

temperatures studied in this work at a fixed feed concentration of 10 ppm for the volatile compounds.

Table 3.9. *Apparent activation energies of the volatile organic compounds in the experiments performed with PERVAP™ 4060 and POMS/PEI membranes.*

	E_a (kJ/mol)	
Compound	PERVAP™ 4060	POMS/PEI
1-octen-3-ol	60.87	58.64
1-Penten-3-ol	48.21	84.77
3-Methylbutanal	54.41	87.66
Benzaldehyde	37.48	49.47
2,3-Pentanedione	68.99	155.01
Hexanal	47.86	79.55
Ethyl Acetate	26.67	86.81
Water	43.13	46.65

The effect of temperature on selectivity depends on changes of sorption of organic compounds on the membrane and its diffusion through the membrane with temperature. According to Feng and Huang (11) the activation energy, E_p, which characterizes the temperature dependence of the membrane can be estimated by subtracting the heat of vaporization from the calculated apparent activation energy. This calculated activation energy follows the order: $E_{p,1\text{-penten-3-ol}}$ (-8.8 kJ/mol) < $E_{p,ethylacetate}$ (-8.6 kJ/mol) < $E_{p,benzaldehyde}$ (-5.3 kJ/mol) < $E_{p,hexanal}$ (-1.8 kJ/mol) < $E_{p,\ water}$ (-0.5 kJ/mol) < $E_{p,1\text{-octen-3-ol}}$ (10.5 kJ/mol) < < < $E_{p,3\text{-methylbutanal}}$ (17.0 kJ/mol) < $E_{p,2,3pentadione}$ (28.1 kJ/mol). Negative values of E_p indicate that the membrane's permeability decreases with increasing temperature. Since the permeant flow rate depends on partition and diffusion through the membrane $P = D \cdot S$ (where D and S are the diffusivity and solubility coefficients respectively), E_p can also be expressed as the activation energy of permeating compounds to diffuse through the membrane, E_D, plus the enthalpy of dissolution, ΔH_s (11). Enthalpy of dissolution is usually negative due to exothermic sorption processes. However the diffusion coefficient increases with increasing operating temperature. According to the level of contribution, E_p will be positive or negative (12). Considering that for some of the volatile compounds the value of the activation energy of permeation is negative, it can be

concluded that for PDMS membrane temperature has greater effects on sorption than on diffusion. Similar results were obtained by She and Hwang (13) for PDMS membranes. This fact can be also appreciated in Figure 3.18.

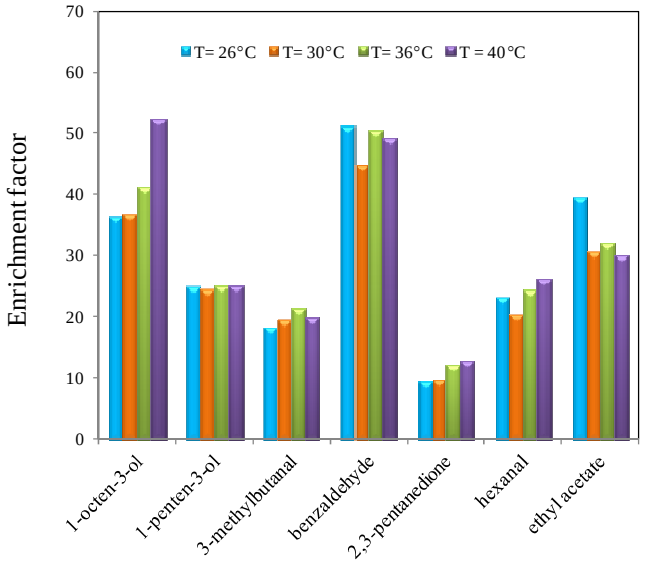


Figure 3.17. *Effect of the feed temperature on the separation factor of the volatile compounds operating ($C_{i.\,feed} \approx$ 5 ppm. P_p = 300 Pa) in the experiments performed with PERVAP^{TM}4060.*

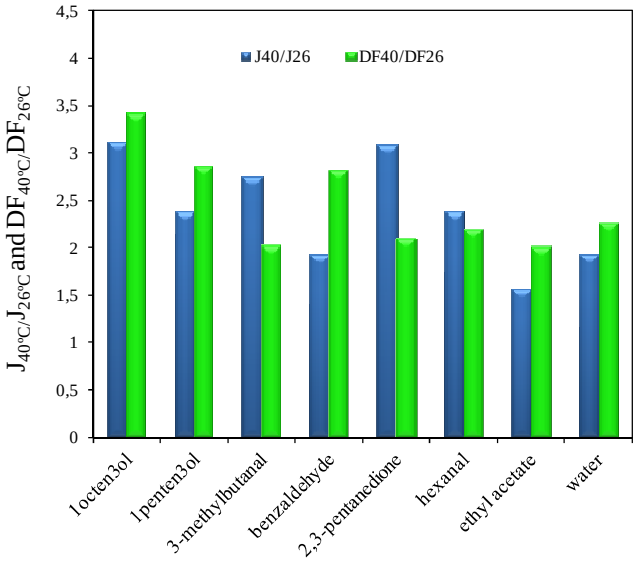


Figure 3.18. *Effect of the feed temperature on the flux and driving force ratio for PERVAP^{TM}4060 ($C_{feed} \approx$ 10ppm, P_p = 300 Pa). DF =driving force of the process.*

According to Olsson and Trägardh (14), this Figure shows the ratio of partial permeation fluxes obtained at 40 ºC and 26 ºC showing also the contribution from increasing driving force. This Figure shows that in general, the increase in partial permeation flux is mainly due to an increase in the driving force. For some of the volatile compounds a greater effect of temperature on sorption can be appreciated since the ratio of partial permeation fluxes is even lower than the corresponding increase in driving force. This result agrees with the trend of enrichment factor with temperature (Figure 3.17).

Effect of permeate pressure.

Permeate pressure was varied in the range 300-1800 Pa at a fixed feed concentration (1ppm) and a fixed temperature (26ºC). Water permeation flux decreased from 0.0054 mol/m^{2}s to 0.0027 mol/m^{2}s. According to Equation 3.14 increasing the permeate pressure will decrease the permeate flux. This fact can be observed in Figure 3.19 where organic permeation flux has been plotted as a function of permeate pressure.

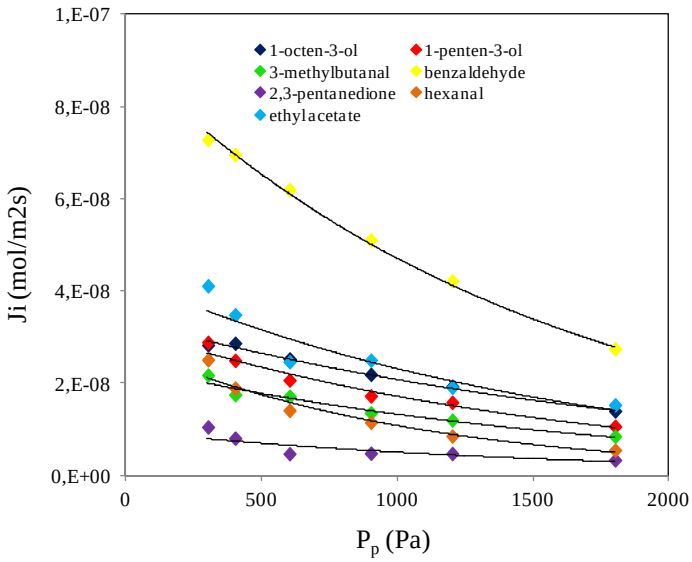


Figure 3.19. *Effect of the permeate pressure on the partial permeation flux of the volatile compounds in the experiments carried out with PERVAP^{TM}4060 ($C_{i.\ feed} \approx 1$ ppm. T_{feed} = 26ºC). The continuous lines are to guide the eye.*

Figure 3.20 illustrates the effect of the permeate pressure on enrichment factors of the volatile compounds. The effect of the permeate pressure depends on the thermodynamic properties of

the compounds. As a general trend, for organic compounds with low values of Henry's law constant (see Table 3.7) the pressure in the permeate side is not negligible respect to pressure in the feed side and the driving force of the process decreases with increasing permeate pressure. This way, volatile compounds such as 1-octen-3-ol, benzaldehyde and 1-penten-3ol are more sensitive to changes in permeate pressure. However, for other organic compounds, such as 3-methylbutanal, 2,3-pentadione and ethyl acetate enrichment factor remains more or less constant with the permeate pressure. For these compounds the driving force of the process remains almost constant in the pressure range studied in this work, due to the high values of the Henry's law constant (see Table 3.7). It must be pointed out that in our case, hexanal exhibits high values of the Henry's law constant, but enrichment factor tends to decrease with increasing permeate pressure.

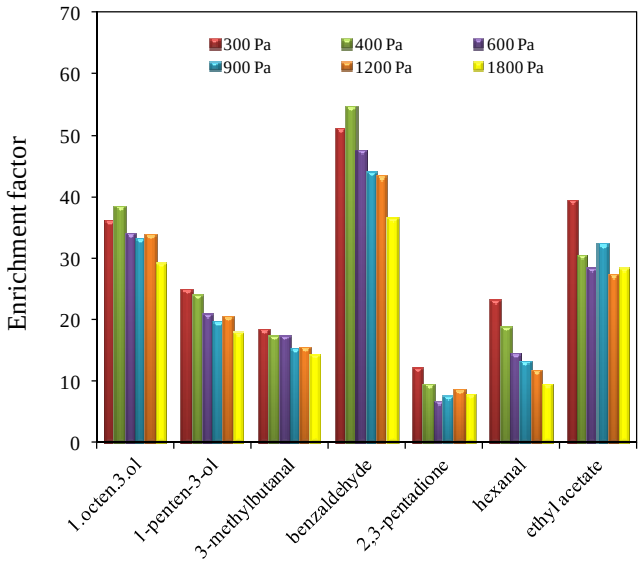


Figure 3.20. *Effect of permeate pressure on the enrichment factor of the volatile compounds in the experiments carried out with PERVAP™4060 ($C_{i.\,feed} \approx$ 1 ppm. T=26°C).*

3.3.4.2 Comparison of PERVAP™ 4060 and POMS/PEI performance.

The main objective of this work is the study of the ability of pervaporation processes to concentrate the volatile compounds found in the brown crab liquid boiling effluent. In this study pervaporation has been performed through a PDMS membrane, while in a previous work, results were obtained by using a POMS membrane.

Table 3.8 compares the performance of PERVAP™ 4060 and POMS/PEI membranes at a feed temperature of 26ºC and at a permeate pressure of 300 Pa. Volatile compounds from dilute

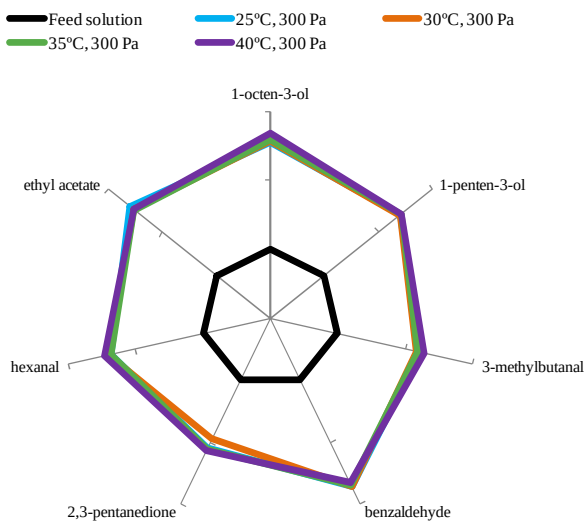
aqueous solutions have been successfully concentrated by these two hydrophobic membranes. However, POMS membrane produced a lower total flux. According to She and Hwang (5) this can be due to the bulky octyl group in the POMS polymer that could rejects more water molecules from passing through the membrane.

Regarding the enrichment factors (Table 3.8) it can be concluded that membrane material has a great influence in the aroma profile of the permeate. The volatile compounds with high vapor pressure, ethyl acetate and 3-methylbutanal, showed the lowest separation factor with POMS/PEI membrane. However PERVAP^{TM} 4060 membrane is more selective to these compounds, achieving acceptable separation factors for both compounds. On the other side, the less volatile compounds, 1-octen-3-ol and benzaldehyde, show a decrease in the enrichment factor when comparing POMS/PEI membrane with PERVAP^{TM} 4060

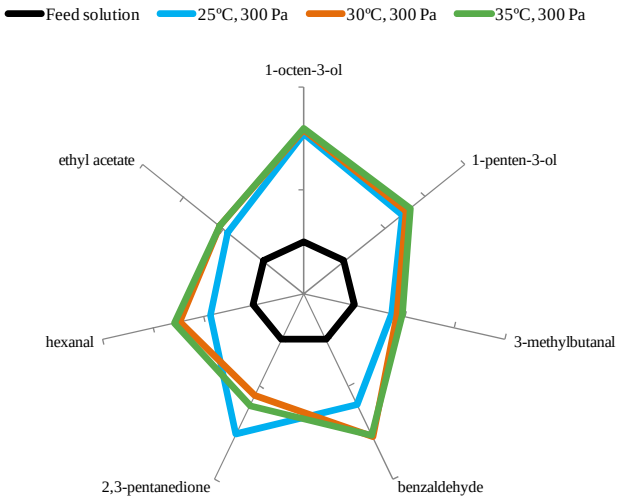
Table 3.8 also presents the pervaporation separation index (Equation 3.16) for the organic compounds obtained with the two different membranes polymers. PERVAP^{TM} 4060 membrane exhibits higher pervaporation separation index than those corresponding to POMS/PEI membrane for all the aroma compounds studied due to the higher permeation flux through PERVAP^{TM} 4060.

Table 3.9 lists the apparent activation energies obtained in this work for water and the organic compounds and those obtained in a previous work with a POMS membrane. $E_{a,water}$ has similar value for both polymers. However $E_{a,organic\ compounds}$ presents higher values for POMS/PEI membrane showing that it is more sensitive towards changes in temperature. In fact, in a previous work (4), it was found that enrichment factor increased with temperature for all the organic compounds, while this behavior was not observed with PDMS membrane (Figure 3.17). Additionally, it was reported (4) that diffusion contribution was found to be important in POMS membrane. However, as it has been explained in section 3.3.4.1, for PDMS membrane, temperature has greater effects on sorption than on diffusion. These results show that the effect of temperature on pervaporation performance is strongly influenced by membrane polymer. Similar conclusions were raised by She and Hwang (13). Based on these results, POMS membrane would be a superior choice when working at high temperature, but pervaporation working temperature is usually not high to avoid damage of the aroma compounds. The different aroma profile obtained in the permeate for a 10 ppm feed concentration for all the aroma compounds at different temperatures for both polymers, are compared in Figure 3.21.a and 3.21.b. These diagrams are based on the Weber-Fechner law that establishes that the odor intensity is dependent on the logarithm of concentration of odorant. In this work, it was assumed that the contribution of all the organic compounds to the overall aroma is qualitatively the same. Therefore, the aroma profile of the feed solution consists in a regular heptagon whose distortion in the permeate estimates the difference between the initial aroma in the feed solution and the final aroma collected after the pervaporation process. This distortion depends on the selectivity that the membrane shows for

the different aroma compounds. From Figures 3.21.a and 3.21.b it can be easily observed that the aroma profile of permeate is more similar to the feed solution profile for PERVAP^{TM} 4060 than for POMS/PEI membrane.

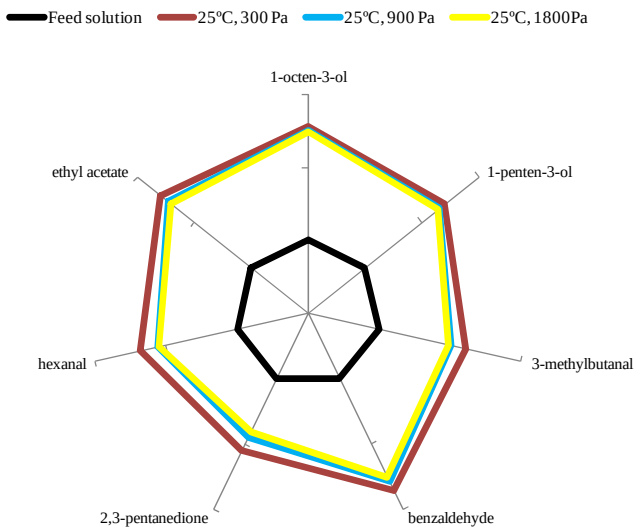


(a)

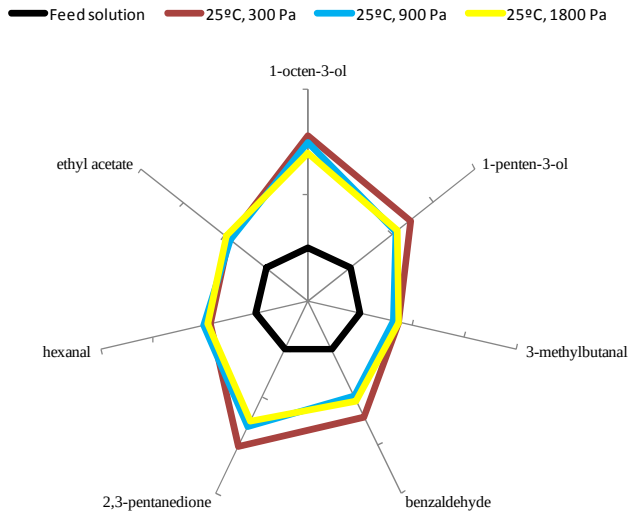


(b)

Figure 3.21. *Effect of the feed temperature on the aroma profile of the multicomponent solution (Ci = 10 ppm) at 300 Pa. a) PERVAP^{TM} 4060. b) POMS/PEI.*



(a)



(b)

Figure 3.22. *Effect of the permeate pressure on the aroma profile of the multicomponent solution (Ci = 10 ppm) at 300 Pa. a) PERVAP™ 4060. b) POMS/PEI.*

The effect of the permeate pressure on the process is similar for both type of membranes. As it was concluded in section 3.3.4.1, organic compounds with low values of Henry's law constant (low saturation vapour pressure and/or low activity coefficient at infinite dilution) are more sensitive to an increase in permeate pressure due to a decrease in the driving force of the process. Figures 3.22.a and 3.22.b compares the aroma profile for both membranes at different

permeate pressure. Similar to the effect of temperature in the aroma profile for both polymers, less distortion was observed in the permeate aroma profile with PERVAP™ 4060 when comparing with the feed.

3.3.4.3. Unsteady state batch pervaporation of dilute multicomponent solution.

The unsteady state pervaporation experiments were carried out at a feed temperature of 26ºC and a permeate pressure of 300 Pa. Aroma feed concentration was in the range 1 – 3 ppm for all the organic compounds and the ratio membrane area to initial feed volume was 0.23 cm^{-1}. Figure 3.23 shows how the residue concentration decreases as a function of time for batch operation of dilute aqueous solution. Experimental results were fitted to the model proposed by She and Hwang (5) (see section 2). These authors consider that recovery and loss percentage depends on the flavor permeation rate constant (a) and flavor loss rate (K_L). According to She and Hwang (13) flavor loss rate, K_L, is evaluated considering the difference between ideal behavior, with no flavor loss (K_L = 0), and experimental data. From the difference in slopes of the straight lines (lnxi/xi,o vs t) between ideal and experimental data, organic compounds loss rate was determined. Permeation rate constant, a, was evaluated by Equation 3.18 by using the permeability coefficients obtained in the previous steady state experiments (section 3.3.4.1). In Figure 3.23 solid lines represent the results obtained with Equation 3.17.

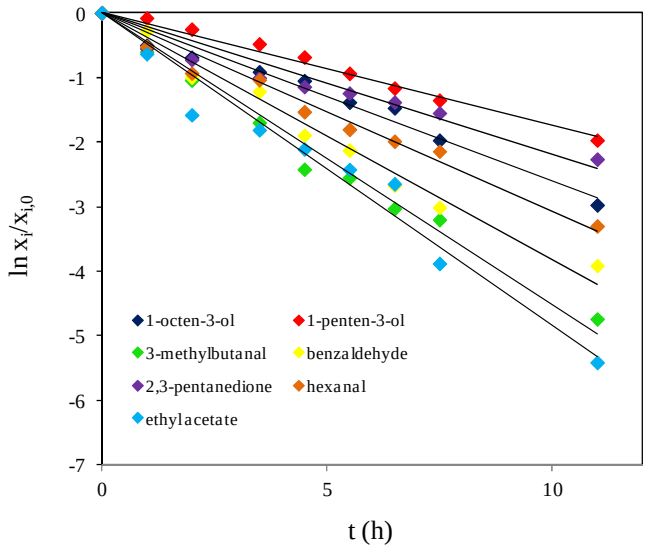


Figure 3.23. *Evolution of feed concentration over time in the pervaporative recovery of aroma compounds (T_{feed} = 26ºC. P_p = 300 Pa, t = 11 h). The continuous lines represent the model proposed by She and Hwang (5).*

Table 3.10 reports the values of the flavor permeation rate constant, a, flavor loss rate, K_L, and the residue (Equation 3.19), recovery (Equation 3.20) and loss percentage of the aroma compounds. All the organic compounds were recovered successfully. The recovery percentage depends on flavor permeation rate and flavor loss rate (5). Aroma compounds with high values of permeation rates (higher enrichment factors: 1-octen-3-ol, benzaldehyde) present the highest recovery percentage after 11 h batch operation time. Ethyl acetate shows high values of permeation rate constant, however its flavor loss rate is high which means lower recovery than 1-octen-ol and benzaldehyde. 1-penten-3-ol presents high recovery yield due to its low flavor loss rate, similar to that of 1-octen-3-ol and benzaldehyde.

Table 3.10. *Experimental results in batch pervaporation of most representative compounds of brown crab aroma carried out with PERVAP™ 4060 (feed temperature 26ºC, permeate pressure 300 Pa, t = 11 h).*

Compound	a (m^3/s)	K_L (mol/s)	Recovery (%)	Residue (%)	Loss (%)
1-octen-3-ol	$6.51 \cdot 10^{-8}$	$3.83 \cdot 10^{-4}$	86	5	9
1-penten-3-ol	$4.50 \cdot 10^{-8}$	$1.81 \cdot 10^{-4}$	81	13	6
3-methylbutanal	$2.83 \cdot 10^{-8}$	$5.37 \cdot 10^{-3}$	24	1	76
Benzaldehyde	$1.00 \cdot 10^{-7}$	$3.02 \cdot 10^{-4}$	94	1	5
2.3-pentanedione	$1.24 \cdot 10^{-8}$	$2.70 \cdot 10^{-3}$	21	8	72
Hexanal	$3.76 \cdot 10^{-8}$	$2.64 \cdot 10^{-3}$	44	3	53
Ethyl acetate	$7.19 \cdot 10^{-8}$	$3.46 \cdot 10^{-3}$	54	0	46

She and Hwang (5) reported loss percentage in the range of 4% to 54% for 2-methyl-1-butanol and ethyl acetate respectively by batch pervaporation through a POMS-PVDD-PP membrane after 10 h batch operation time. These authors consider that due to the volatile nature of these organic compounds. flavor loss is unavoidable. Flavor loss is mainly due to evaporation. loss of aroma compounds through the vacuum (incomplete condensation) or loss during sample collection (5). So. it is expected that flavor loss is strongly dependent on the thermodynamic properties of aroma compounds. In this work. the highest loss percentage was found for aroma compounds with high values of flavor loss rate. In this sense. aroma compounds with low Henry's constant values. 1-penten-3-ol. 1-octen-3-ol and benzaldehyde.

show the lowest flavor loss rate. On the other hand. compounds with high Henry's constant values. manifested a high flavor loss. from 46 to 76%. depending on the permeation rate. since the higher the permeation rate. the lower the loss percentage.

In order to improve the low recovery yield that high volatile compounds present in this study. changes in the process design must be made. She and Hwang (5) propose a better sealing. condensation and collection method in pervaporation process to reduce the flavor loss. Additionally. since the production capacity of pervaporation processes (15) depends on the residue mass. time and membrane area. higher membrane area would lead to higher flavor permeation rate. reducing the flavor loss percentage.

3.3.4.4. Effect of the salt content in the feed solution.

Boiling brown crab liquid effluent contains different nonvolatile components such as salts. e.g. sodium chloride (NaCl). The salt concentration in brown crab liquid effluent was about 0.43 mol/kg. To study the effect of salt concentration. pervaporation experiments were carried out by varying the salt feed concentration in the range 0-0.85 mol/kg. at a fixed feed temperature of 40ºC and a permeate pressure of 300 Pa.

There is a tradeoff in the salt feed concentration on the pervaporation performance. The positive effect of the presence of salts in the feed solution is related to the "salting out" effect. This effect is based on the reduction of the solubility of the organic compounds in aqueous solutions. increasing the activity coefficients of the organic compounds present in the feed solution. The negative effects are related to the increase in the density and viscosity of the feed solution and the fouling effect of the salts which could penetrate into the structure of the membrane (16). There are different studies in the literature considering the effect of salts on pervaporation performance. Most of the studies observed a positive effect in the membrane selectivity. However other studies reflected no changes or even fouling of the membrane (12).

Water permeation flux remained more or less constant in the salt concentration range studied in this work. On the other hand. partial permeation flux of the organic compounds was found to slightly increase as the salt concentration in the feed increased. Based on this result. it can be concluded that the salting out effect could dominate over the retarding effect on mass transport in the salt concentration range studied in this work. Activity coefficients at infinite dilution at different sodium chloride concentrations were obtained in a previous work (7) showing that activity coefficients increase with NaCl concentration. This higher activity coefficient can generate higher driving force and higher permeation flux (Equation 3.14). Based on these results. the enrichment factor of aroma compounds slightly increases in the salt concentration range considered in this work (Figure 3.24).

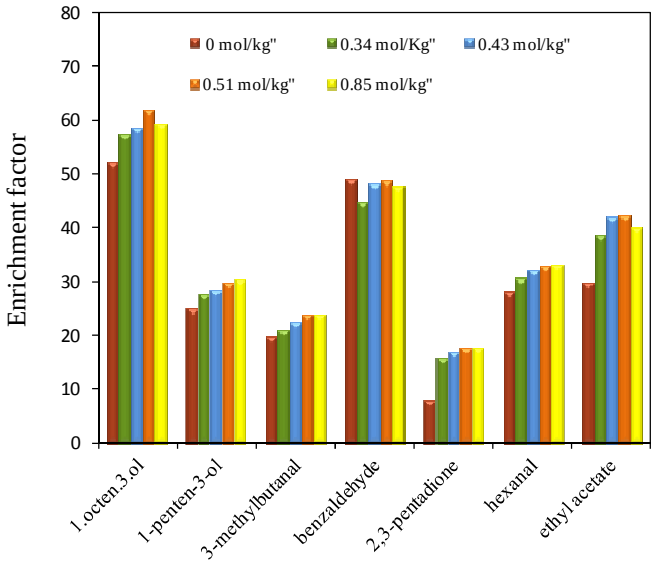


Figure 3.24. *Influence of salt concentration on the enrichment factor of the organic compounds (C_{feed} = 1ppm. T_{feed} = 40ºC. P_p = 300 Pa).*

Kujawski and Krajewski (17) proposed the applicability of the empirical Setschenov equation:

$$\ln\left(\frac{\gamma_i^{\infty}}{\gamma_{i,o}^{\infty}}\right) = k_i c_s \qquad [3.22]$$

to describe the increase of organic permeation flux with salt concentration. In Equation 3.22 γ_i^{∞} is the activity coefficient at infinite dilution in salty solutions. $\gamma_{i,o}^{\infty}$ the activity coefficient at infinite dilution in pure water. c_s the salt concentration and the proportionality factor. k_i. is the salting-out coefficient. This way the ln ($J_{i.\ salty\ solutions}/J_{i.non\text{-}saltysolution}$) can be plotted as a function of NaCl concentration and compared with data of the activity coefficients obtained in previous work (7).

Table 3.11 lists the slopes of the linear relationship for the relative permeation flux of organic compounds and salt concentration together with the values previously reported in the study of the activity coefficients at infinite dilution. It can be observed that lower values of the slope were obtained for the ln ($J_{i.\ salty\ solutions}/J_{i.non\text{-}saltysolution}$) than the slopes for the activity coefficients at infinite dilution previously reported (7). Based on these results it can be concluded that blocking effect cannot be considered negligible in the salt concentration range considered in this work since the slightly increase in partial permeation flux is much lower than the corresponding increase in driving force due to an increase in the activity coefficients at infinite dilution with salt concentration.

Table 3.11. *Salting out coefficients obtained from the curves that represent ln ($J_{i.salty\ solutions}$ /$J_{i.non\text{-}salty\ solutions}$) and ln ($\gamma_i^{\infty}$ /$\gamma_{i,o}^{\infty}$) versus salt concentration in the feed solution.*

Compound	log ($J_i/J_{i.0}$)	log (γ_i^{∞}/ $\gamma_{i.0}^{\infty}$)
1-octen-3-ol	0.15	0.56
1-penten-3-ol	0.26	0.37
3-methylbutanal	0.31	0.39
Benzaldehyde	0.14	0.34
2.3-pentadione	0.40	0.43
Hexanal	0.19	0.62
Ethyl acetate	0.25	0.58

As an example Figure 3.25 represents the applicability of the Setschenov equation for one of the organic compounds (ethyl acetate). In this Figure. the values of the activity coefficients at infinite dilution obtained in a previous work have been also plotted.

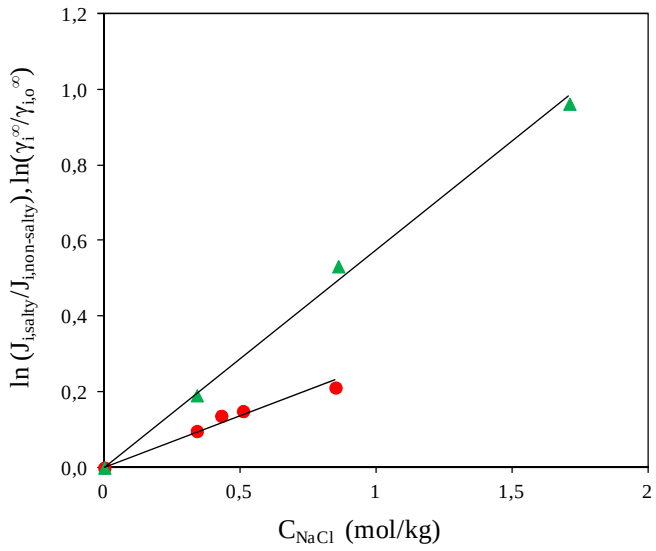


Figure 3.25. *Comparison between the effect of salt concentration on ethyl acetate partial permeation flux (●) and activity coefficients at infinite dilution (▲).*

3.3.4.5. Multistage condensation of the aroma compounds.

In section 4.1 enrichment factors were reported in the range of 10 for 2.3-pentadione to 39 for ethyl acetate. To improve the performance of pervaporation Marin et al. (18) proposed to modify the downstream section with a multi-stage condensation step. In this work. some previous pervaporation experiments have been performed with a two stage condensation step by using two condensers placed in series. This way. a partition of the permeate can lead to higher efficiency in the recovery of volatile compounds in one of the condensers. The components of the vapor permeate have different condensation potentials. thus yielding an additional separation factor (19). The distribution of water and organic compound in each condenser depends on the temperature of the first condenser. the volatility of the components. the flow of the condensable and inert gases in the permeate side and the stripping effect between condensers (20. 21).

Pervaporation experiments have been carried out at a fixed feed temperature of 26ºC and a fixed permeate pressure of 300 Pa. Feed concentration was about 1 ppm for all the organic compounds. Two different temperatures were tried in the first condenser -4 ºC and -10ºC.

Table 3.12 shows the permeation flux percentage collected in both condensers as a function of temperature in the first condenser. As it can be observed. the percentage collected in the first condenser increases as the condensation temperature in the first condenser decreases. Due to the efficiency in water removal in the first condenser. the enrichment factors of the organic compounds collected in the second condenser improve considerably (Table 3.13) compared to one condenser. Additionally the concentration of organic compound in the second condenser increases as the temperature in the first condenser increases due to a higher efficiency in water removal.

Table 3.12. *Percentage of permeate collected in both condensers at different temperatures in the first condenser.*

T in the first condenser	% condenser one	% condenser two
T_1 = -4 ºC	79	21
T_1 = -10 ºC	90	10

Table 3.13 reports also the separation factor. relative volatility. which would be obtained on the basis on vapour-liquid equilibrium (VLE) at 40ºC based on the activity coefficients at infinite dilution reported in a previous work (7). This relative volatility at infinite dilution. $\alpha_{i,w}^{\infty}$. is defined as:

$$\alpha_{i,w}^{\infty} = \left(\frac{p_i^s}{p_w^s} \right) \gamma_i^{\infty} \qquad [3.23]$$

where p_i^s and p_w^s are the saturation vapour pressure of organic compounds and water respectively. The separation factors obtained with one condenser were lower than the separation that would be achieved on the based on VLE. However. with two stage condensation step the new separation factors obtained are even higher than the values of the relative volatility.

Table 3.13. *Comparison of enrichment factors obtained during the fractionation experiments in two condensers and one condenser (T_{feed} = 26ºC. p_p = 300 Pa). Relative volatility at infinite dilution at 40ºC*

T_1	-4ºC		-10ºC			
Compound	β_1	β_2	β_1	β_2	$\beta_{\text{one condenser}}$	$\alpha_{i,w}^{\infty}$
1-octen-3-ol	16	78	20	179	36	56
1-penten--3-ol	14	79	16	149	25	22
3-methylbutanal	<1	68	<1	139	18	293
benzaldehyde	18	132	17	347	51	33
2.3-pentadione	<1	40	<1	54	10	147
hexanal	<1	97	<1	164	23	284
ethyl acetate	<1	145	<1	261	39	232

For the most volatile compounds. especially the most volatile. the concentration in the permeate collected in the first condenser was very diluted. That indicates that most of the permeant molecules of these organic compounds were mainly collected in the second condenser probably due to a stripping effect. However. the concentration of the aromatic compounds in the second condenser is less than expected based on mass global balance with the results obtained with one condenser. This fact can be due to the very high loss rates found

for these compounds in the studies performed in non-steady state (section 3.3.4.3). The non-steady state experiments revealed that the concentration of the high volatile compounds is largely conditioned by the time employed in each run due to the high value of the flavor loss rate. The time necessary in the fractionation experiments is longer than those carried out with one total condenser. due to the fact that insufficient quantities for the analysis were collected in the second condenser. especially in the experiments carried out with at the lowest temperature in the first condenser.

3.3.5 Conclusions.

PERVAP™ 4060 membrane has been found to be effective to recover some key aroma compounds found in the brown crab liquid boiling effluent from a model dilute aqueous solution. The effect of feed concentration. permeate pressure and pervaporation temperature on pervaporation performance has been analyzed. An increase of the aroma feed concentration increases organic permeation flux due to an increase in the permeation driving force. Partial and total permeation flux increased with temperature. However permeation flux decreased as permeate pressure increased.

The results obtained under steady state operation with PERVAP™ 4060 were compared with experiments performed in a previous work with a POMS/PEI membrane. Higher total permeation flux was obtained with the PERVAP™ 4060 membrane compared to POMS/PEI. Membrane material seems to be a key factor in the permeate aroma profile since different aroma profiles are obtained with both kinds of polymers essayed. Based on the different effect of temperature on pervaporation performance of both types of membranes it was concluded that sorption contribution is more important in PERVAP™ 4060 membrane than in POMS/PEI membrane.

From batch pervaporation experiments it was found that the loss of volatile compounds increases with operation time. Loss percentage was high for volatile compounds with low permeation rate and high flavour loss rate. Higher membrane area would help to reduce the loss percentage. The efficiency of aroma recovery from dilute aqueous solutions has been slightly improved for the presence of NaCl. This fact can be attributed to the salting out effect that leads to an increase in the activity coefficients at infinite dilution with salt concentration. However this increase is much lower than the corresponding salting out effect concluding that blocking effect cannot be neglected.

Additionally. modifying the permeate circuit with a two stage step condensation can be an efficient way of improving the pervaporation performance since it has been observed a considerable increase of the enrichment factors obtained compared to one total condenser.

However. the design must be optimized since important loss of some of the volatile compounds. especially the most volatile. has been observed.

Further studies will be carried out with the real brown crab boiling liquid effluent to study the ability of pervaporation technique.

3.3.6 References.

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NOMENCLATURE.

a = permeation rate constant. $m^3 \cdot s^{-1}$ (Equation 3.17)

A = membrane area. m^2

C_{tot} = total molar concentration in liquid phase. $mol \cdot m^{-3}$ (Equation 3.17)

E_a = apparent activation energy of permeation. $kJ \cdot mol^{-1}$

H = Henry's law constant. Pa

J = permeation flux. $mol \cdot s^{-1} \cdot m^{-2}$

K_L = flavor loss rate. $mol \cdot s^{-1}$ (Equation 3.17)

K_V = total permeation volume flow rate. $m^3 \cdot s^{-1}$ (Equation 3.18)

Q_{OV} = pressure-normalized permeation flux. $mol \cdot s^{-1} \cdot m^{-2} \cdot Pa^{-1}$

p = pressure. Pa

P.S.I. = pervaporation separation index

R = gas constant. $kJ \cdot mol^{-1}K^{-1}$

T = absolute temperature. K

V = volume of feed solution. m^3

w = mass fraction

x. y = mol fraction

t = time. s

γ = activity coefficient

β = enrichment factor

Subscripts

i = component

p = permeate

f = feed

Upperscripts

m = membrane

s = saturation

∞ = infinite dilution

3.4 Study of the recovery by pervaporation of volatile compounds in brown crab boiling effluent.

ABSTRACT.

Pervaporation has been used to obtain aroma concentrates from brown crab boiling effluent. The boiling effluent and the obtained permeate have been analyzed by Headspace Solid Phase Dynamic Extraction Gas Chromatography/Mass Spectrometry. The effect of feed temperature on the pervaporation performance of the membrane has been analyzed. Enrichment factor for some of the volatile compounds were much lower than those obtained in model aqueous dilute solutions. Pervaporation performance can be significantly improved by modifying the permeant circuit by means of two stage condensation step.

Keywords: volatile compounds, pervaporation, brown crab effluent.

3.4.1 Introduction.

The liquid effluent of the seafood industry, produced during boiling process, contains important amounts of flavour components (1). The recovery of this valuable flavour fraction present in the seafood wastewaters would allow to reduce the waste water treatment for the industries (1) as well as to obtain a valuable product. The volatile components of crabs are regarded as the most determinant components of the flavour quality. In this work, the conversion of the brown crab effluent produced during boiling into a valuable volatile concentrate has been studied.

Cha et al. (2) studied the concentration of the liquid effluent produced during snow crab boiling by steam distillation. However, this technique involves high energy consumption as well as physical aroma losses. Membrane separation techniques represent a potential pathway for the production of a natural aroma concentrate and a disposable effluent (3). Pervaporation represents an alternative to the techniques based on distillation evaporation or partial condensation to concentrate aroma compounds since it allows the use of moderate operating temperatures to minimize degradation of aroma compounds and avoids addition of chemical solvents. Several studies have been focused on the study of the aroma recovery by pervaporation. However, the number of publications using real feeds is still limited (4-8).

The main objective of this work is to apply the pervaporation process to recover the valuable volatile fraction present on an industrial brown crab boiling effluent. In previous work (9, 10) a study of pervaporation was done on a model aqueous solution containing seven typical aroma compounds (1-octen-3-ol, 1-penten-3-ol, hexanal, 3-methylbutanal, benzaldehyde, 2,3-pentadione and ethyl acetate) found in the brown crab boiling effluent to choose the polymer and to study the effect of the some operating variable such as pressure and temperature on the aroma profile in the permeate. Two different membrane polymers were considered polioctimetilsiloxane (POMS) and polidimethylsiloxane (PDMS). The efficacy of this process was evaluated in terms of total and partial permeation flux and enrichment factor of the volatile compounds through the investigated membranes. Both membranes were able to concentrate successfully the volatile compounds from the dilute aqueous solutions, but PDMS membranes presented higher separation pervaporation index than POMS/PEI membranes. It was observed that the effect of temperature on pervaporation performance is strongly influenced by membrane polymer being POMS/PEI membrane more sensitive towards changes in temperature.

The first step of this work was to determine the volatile organic compounds present on the industrial brown crab boiling effluent. The effect of feed temperature on the different aroma profile of the permeate has been studied. Finally, fractionation experiments have been also performed to improve the performance of pervaporation by using a two stage condensation in the permeate side.

3.4.2 Experimental section.

3.4.2.1. Boiling effluent characterization.

Boiling brown crab effluent was provided by IDOKI SCF Technologies S.L. (Bizkaia, Spain). Chemical oxygen demand (COD), total solids (TS), volatile solids (VS), suspended solids (SS), suspended volatile solids (SVS), total nitrogen content (NKT) and ammoniacal nitrogen content ($N\text{-}NH_4^+$) were determined according to Standard Methods for the examination of water and wastewater (11). The sodium ion concentration was determined using a Perkin-Elmer 3300 atomic absorption spectrophotometer, using high purity sodium chloride as external standard. Chloride concentration was determined using a Hach Lange DR 2800 spectrophotometer, employing the chloride cuvette test 1-70 mg/l. The total ion concentration (TIC) was determined using a conductivity meter (Basic 30, Crison) employing high purity potassium chloride as external standard. pH was measured using a micropH *2002 Crison pH-meter*. Effluent was stored at -20 ºC and was filtered before pervaporation experiments.

3.4.2.2. Pervaporation experiments.

The pervaporation membrane used in this work was the commercial hydrophobic membrane PERVAP™ 4060 supplied by Sulzer Chemtech® whose active layer is based on polydimethylsiloxane (PDMS). The pervaporation experiments were performed with a plate and frame laboratory stainless steel permeation cell (Sulzer Chemtech®) with an effective membrane area in contact with the feed mixture of 170 cm^2 (12). The temperature of the feed liquid mixture was kept constant (± 0.5 ºC) by using a thermostat to heat the stirred tank feed reactor. The permeate was condensed on two parallel glass cold traps cooled by liquid nitrogen to ensure that all permeates were fully collected. In the case of fractioning in the permeate side, two condensers were placed in series in one of the two parallel permeate circuits. The first condenser was cooled in a refrigerant bath using a Julabo FP50 cryostat. The second condenser was cooled with liquid nitrogen and act as a total condenser.

3.4.2.3. Sample analysis

The volatile fraction of boiling brown crab effluent as well as the different collected permeate was evaluated by Head Space-Solid Phase Dynamic Extraction-Gas Chromatography/Mass Spectrometry (HS-SPDE-GC/MS). Qualitative analysis were carried out with an Agilent 6890N GC equipped with a 5973i MS (Agilent Technologies, Palo Alto, CA, USA) and a CTC-Combi-Pal auto sampler (GTC Analytics AG, Zwingen, Switzerland).

The SPDE analysis uses a 2.5 ml gas tight syringe, with a PDMS (10% AC) internally coated needle. The needle was conditioned at 260ºC and flushed with Helium for 5 min. Samples were heated at 70ºC for 1 min and agitated at 500 rpm in order to reach equilibrium conditions. An aliquot (1ml) of the headspace was pulled through the needle at 40 µl/s for 50 cycles. The syringe was automatically transferred to the injection port and volatile compounds were desorbed with 0.5 ml of Helium at 15 µl/s.

GC conditions were as follows: capillary column 007-CW-60W-0.25F capillary column (007-WAX Quadrex Corporation, New Haven, USA) 60 m × 0.32 mm x1.0 µm, port injection temperature: 250ºC, splitless mode injection, oven temperature was programmed from an initial temperature of 30ºC (11 min holding), rising to 35ºC at 3ºC/min (5 min holding) and rising to 240ºC at 3ºC/min., carrier gas was Helium with an initial flow rate of 0.8 ml/min (18 min holding), rising to 1.5 ml at 1.4 ml/min^2 (13 min holding) and descending to 0.8 ml/min at 0.1 ml/min^2. MS conditions were as follows: interface temperature at 280ºC, ionization voltage at 70 eV, mass range at 30-300 amu and electron multiplier voltage at 1835 V. Analyses were performed three times and the values reported in this work are the mean values calculated from the three chromatography determinations. 1-hexanol was used as internal standard.

3.4.3 Results and discussion.

3.4.3.1. Characterization of boiling brown crab effluent.

Some physic-chemical properties of brown crab boiling effluent are presented in Table 3.14. The flavour of most fresh shellfish has been described as sweet, distinctly plant-like, often accompanied by metallic and very slight to pronounced fishy attributes, primarily generated by unsaturated alcohols and aldehydes of less than ten carbon atoms, alkylpyrazines and sulphur containing compounds (13). Nearly 100 compounds were identified in the boiling effluent. These included mainly aldehydes, ketones, alcohols, esters, aromatic compounds and sulphur and nitrogen-containing compounds. Appendix A reports the chromatographic areas obtained for the different aroma compounds detected in the boiling brawn crab effluent. Alcohols, ketones and aldehydes were found to be the majority of the family of volatile compounds.

***Table 3.14**. Physicochemical properties of brown crab boiling effluent.*

Parameter	Value
Total ion concentration. g/L	36.9
Sodium. g/L	24.5
Chloride. g/L	23.7
pH	8.3
Total solids. g/kg	46.4
Volatile solids. g/kg	8.3
Suspended solids. g/kg	21.3
Volatile suspended solids. g/kg	2.7
Chemical oxygen demand. g/L	1.4
Total nitrogen. g/L	0.56
Ammonical nitrogen. g/L	0.06

Saturated alcohols contribute hardly to the overall aroma because of their high odor threshodls values (14). The two alcohols choosen in previous pervaporation experiment with model dilute aqueous solutions were found in the boiling effluent. 1-octen-3-ol has been been

reported to be one the volatile components widely distributed in fresh and saltwater fish (14) and possess a mushroom like odor. 1-penten-3-ol is obtained via-enzymatic-mediated conversion of polyunsaturated fatty acids and contributes to a butter-like odor (14).

Ketones contribute to the sweet floral, fruity flavour of many crustaceas (2). 2-alkanones, such as 2-hexanone, 2-heptanone, 2-octanone, 2-nonanone and 2-undecanone have a distinct green and fruity aroma and give a more floral note as the chain length increase. Diketones provides a desirable balance of meaty and buttery notes (15). Among them only 2,3-pentadione has been identified in the boiling brown crab effluent.

Aldehydes play an important role in food product (2). Aldehydes with 6-10 carbon were widely reported in crab, squid, prawn, crayfish and sardine. In this work, 3-methylbutanal, hexanal, heptanal, octanal and nonanal were successfully detected. 3-methylbutanal is one of the most abundant aldehydes in boiled and pasteurized crabmeat (16). Benzaldehyde contributes to characteristic cooked crab flavour having a pleasant almond, nutty and fruity aroma crustacean.

Three alkylpirazines were identified in the boiling brown crab effluent. These compounds are important due to their low odor thresholds values, having a roasted, nutty, potato boiled aroma (13).

Three organosulphur compounds have been detected. Among sulphur compounds dimethyl trisulfide and dimethyl disulfide have been found in thermally processed sea foods and meat products (13). These compounds greatly affect the overall food aroma because of their low threshold values and generally, they are considered undesirable, exhibiting a cooked cabbage and spoilage odor. Thiazols have been reported to be important in generating meaty flavors in marine crustacean (2), 4-methylthiazole, was identified in small amounts in the bowling brown crab effluent. Trimethylamine was also found in the boiling effluent and it has been reported to contribute to boiling-crab odor (14). Five saturated hydrocarbons were detected in the boiling brown crab effluent, but they contribute very little to the overall flavour of foods due to their high aroma thresholds.

3.4.3.2. Effect of the pervaporation temperature

Pervaporation experiments were carried out at two different temperatures, 25 ºC and 40 ºC. At 25ºC total permeation flux was found to be of the same order than this obtained in a previous study for model dilute aqueous solution. However, at 40ºC total permeation flux was about 85 % of the model dilute aqueous solutions. These could be due to the presence of non-volatile compounds that could affect the total permeation flux of the system. For instance in previous work it has shown that partial permeation flux of the organic compounds was found to increase as sodium chloride concentration in the feed increased (in the range 0-0.85 mol/kg). However blocking effect could not be considered negligible since the increase in partial

permeation flux was lower than the corresponding increase due to an increase in the activity coefficient values at infinite dilution with salt concentration (10). Figure 3.26 shows the Arrhenius plot for the model dilute aqueous solution. In this Figure the experimental data obtained in this work at 25ºC and 40ºC for the real boiling crab effluent has been also included.

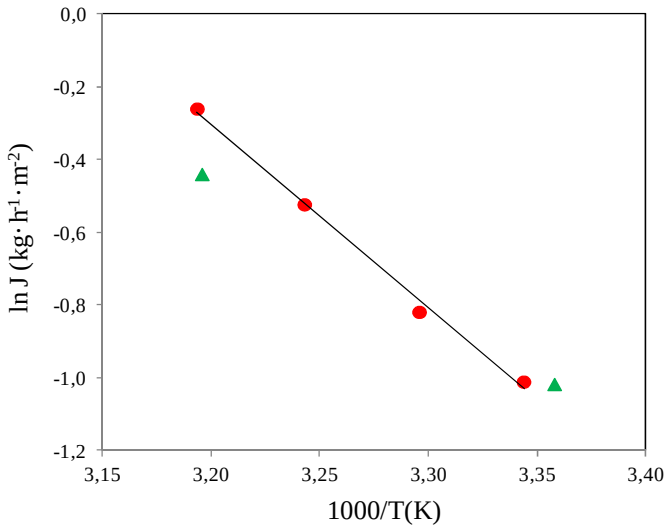


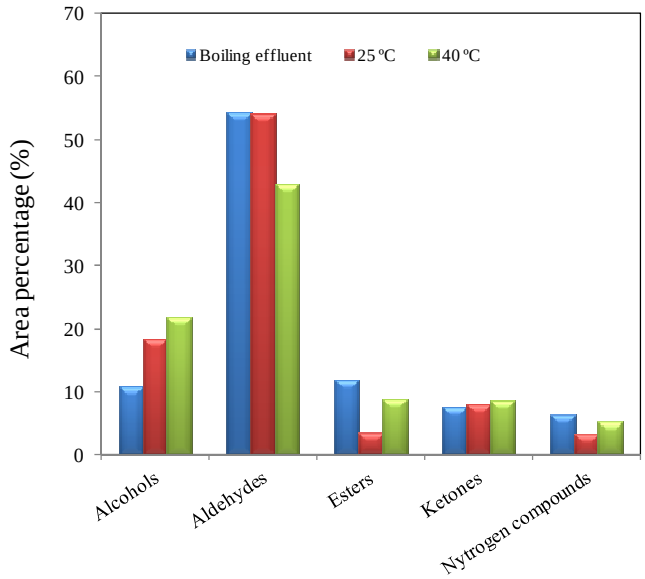
Figure 3.26. *Effect of feed temperature on total permeation flux:* ● *model dilute aqueous solution.* ▲ *brown crab boiling effluent.*

The change in the aroma profile in the permeate obtained at the two different temperatures was evaluated considering the percentage of chromatographic area for each family of compounds. Figures 3.27a and 3.27b show this percentage for the brown crab boiling effluent along with permeate obtained at 25ºC and 40ºC. At the two temperatures studied in this work, the permeate aroma profile is different from that of the boiling effluent, due to different selectivity of the membrane for the aroma compounds. As it has been describe in the literature, one of the difficult in the pervaporation concentration is to maintain the original flavor profile (6, 17). In both cases, boiling effluent and permeate, aldehydes compounds were found to be the most abundant volatile compounds, mainly due to the presence of benzaldehyde, acetaldehyde, hexanal and (Z,Z-2-butyl-2-octenal). Alcohols, ketones, esters and nitrogen compounds are also present in considerable amount in the permeate at the two different temperatures.

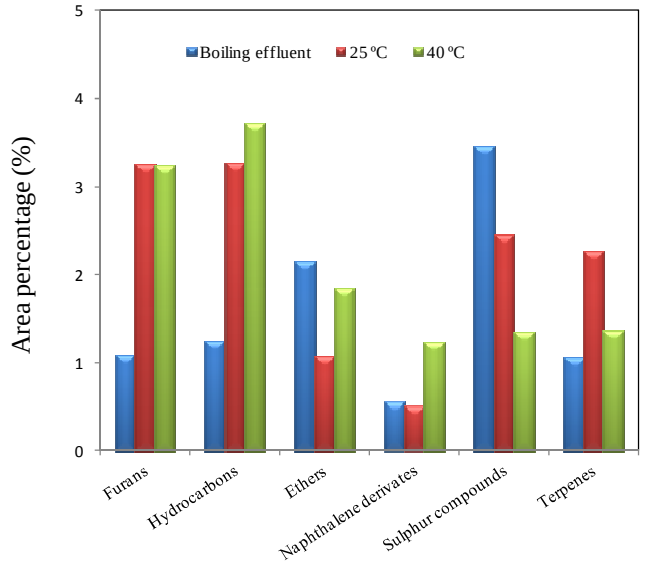
Enrichment factor for all the volatile compounds have been evaluated by determining the ratio of the chromatographic areas of the volatile compound and for the internal standard in the

permeate obtained at the different temperatures and in the brown crab boiling liquid effluent:

$$\beta = \frac{\left(A_i / A_{IS}\right)_{p,T}}{\left(A_i / A_{IS}\right)_{effluent}} \qquad [3.24]$$



(a)



(b)

Figure 3.27. *Effect of temperature on the aroma profile evaluated with the percentage chromatographic area for the different family of compounds.*

Enrichment factors determined by using equation (24) are listed in Appendix A. The highest enrichment factor was found for the 2-ethyl-1-hexanol. Some ketones, such as 2-octanone, exhibit also relative high enrichment factors. In any case, enrichment factors were very low when comparing these values with those obtained in a previous work in a model dilute aqueous solutions formed by seven representative compounds.

Table 3.15 reports the ratio of the enrichment factor for the seven selected compounds in a model dilute aqueous solution and in the boiling effluent. For some of the volatile compounds, such as ethyl acetate, 2,3-pentadione and 1-octen-3ol enrichment factors obtained in the pervaporation of the brown crab boiling effluent is around five times lower than the one obtained on model solution. But for other compounds, such as hexanal enrichment factor has found to be up to fifteen times lower on the boiling effluent. Similar results have been found in the literature in the pervaporation of aroma compounds from cauliflower blanching water when comparing the membrane selectivity for three sulfur compounds on a model dilute aqueous solutions and the values obtained on the real industrial effluent (5). This fact was explained in terms of the presence of an important boundary layer due to the extremely low concentration of the aroma compounds. In this case, concentration polarization may have a large influence on the removal of organic traces and it could be the limiting step of the process.

Table 3.15. *Ratio of the enrichment factor for some volatile compounds in a model dilute aqueous solutions and in the brown crab boiling effluent at 25ºC and 40 ºC.*

Compound	$\beta_{\text{model solution}}/\beta_{\text{effluent.}}$ 40ºC	$\beta_{\text{model solution}}/\beta_{\text{effluent.}}$ 25ºC
1-octen-3-ol	4	6
1-penten-3-ol	13	8
3-Methylbutanal	11	4
Hexanal	13	15
Benzaldehyde	9	9
2.3-pentadione	5	3
Ethyl acetate	7	5

Regarding the effect of pervaporation temperature on the enrichment factors obtained at the two studied temperatures, slightly higher values were obtained at 40ºC than at 25ºC. However

this increase is not very marked. This result agrees with previous study on a model dilute aqueous solution where it has shown that for PDMS membrane temperature has greater effects on sorption than on diffusion.

In general terms, pervaporation is able to successfully concentrate the volatile fraction found in the brown crab boiling effluent. For instance, as it has been previously explained in section 4.1 diketones provides a desirable balance of meaty and buttery notes. Only 2,3-pentadione was detected in the boiling effluent. However other diketones such as 2,3-butanedione and 2,3 octanedione were successfully concentrated in the permeate at 40 ºC. As it has been describe in the literature 2,3-butadione is a characteristic product in cooked food having a low threshold value of 2.6 ppb in water (18).

3.4.4.3. Two step condensation of the aroma compounds.

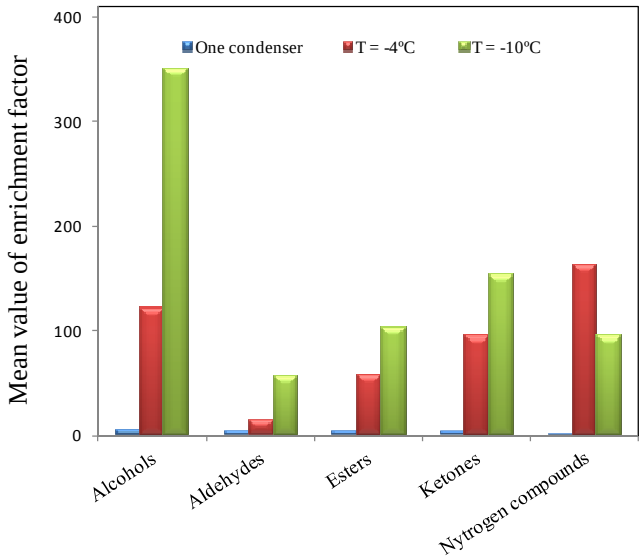
In section 3.4.4.2 enrichment factors were found to be not very high, probably due to low concentration of the aroma compounds in the brown crab boiling effluent and the consequently influence of the concentration polarization. In a previous work a two stage condensation step was proposed to improve the pervaporation performance. This way, an additional separation factor is included based on the different condensation potential of the aroma compounds (19). In that work, the enrichment factors of the organic compounds collected in the second condenser improved considerably compared to one condenser and they were found to be even higher than the values of the relative volatility.

In this work, pervaporation has been carried out at a fixed feed temperature of 26ºC and two different temperatures were tried in the first condenser, - 4ºC and -10ºC. Table 3.16 shows the permeation flux percentage collected in both condensers as a function of temperature in the first condenser.

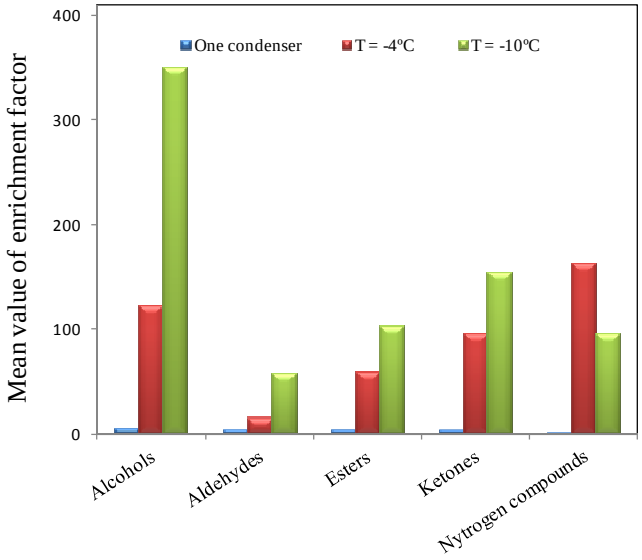
Table3.16. *Percentage of permeate collected in both condensers at different temperatures in the first condenser.*

T in the first condenser	% condenser one	% condenser two
T_1 = -4 ºC	83	17
T_1 = -10 ºC	89	11

As it can be observed, the percentage collected in the first condenser increases as the condensation temperature in the first condenser decreases. Appendix B shows the aroma profile in the permeate expressed as the chromatographic area of the volatile compound divided by the area of the internal standard.



(a)



(b)

Figure 3.28. *Effect of temperature of the first condenser on the mean value of the enrichment factor for the different family of compounds.*

From this Appendix it is clear that concentration of organic compounds in the first condenser is much lower than organic concentration reached in the second condenser. In general, more than 90% of the volatile compound is recovered in the second condenser, probably due to a stripping effect. Additionally, the recovery of aroma compounds in the second condenser seems to increase as the temperature in the first condenser decreases due to a higher efficiency in water removal. In any case, considerable loss of aroma compounds are expected based on

the results obtained in previous work on a model dilute aqueous solutions (10).Enrichment factors were evaluated through Equation 3.24. This parameter has been listed in Appendix B. As it can be expected from the values of the chromatographic areas, much bigger values of enrichment factors are obtained than when using one condenser.

For a better comparison enrichment factors obtained with one condenser and in the second condenser at the two different temperatures studied in the first condenser have been plotted in Figure 3.28a and 3.28b. In these Figures naphthalene derivates have not been included due to the extremely high value of enrichment factor obtained for 1,6-dimethylnaphtalene. As a general trend, higher enrichment factors are obtained in the second condenser with a decrease of temperature in the first condenser. It seems that higher enrichment factors are obtained for alcohols than for aldehydes and ketones. Moreover new aroma compounds have detected in the permeated obtained with fractionation: 2,6-dimethyl-4-heptanol, dihydromircenol, 2,5-dimethylbenzaldehyde, tetradecane, 2-pentanone, trans-beta-ionone, naphthalene, 5-methylthiazole, benzothiazole. This also proves the efficiency of fractionation process.

3.4.1 Conclusions.

Pervaporation has been used to recover the volatile fraction of the brown crab boiling effluent. Experimental results confirm that pervaporation can successfully concentrate the aroma compounds from the real boiling effluent. Pervaporation experiments were carried out at two different temperatures. Total permeation flux increases with temperature. However for the highest temperature studied in this work, 40ºC, total permeation flux is slightly lower than permeation flux obtained in a model dilute aqueous solution. Enrichment factor for the aroma compounds obtained at the two temperatures are much lower than the one obtained on a model solution, probably due to the boundary layer effect. Modifying the permeate circuit with a two stage step condensation seems to be an efficient way of improving the pervaporation performance since it has been observed a considerable increase of the enrichment factors obtained compared to one total condenser

In any case, the suitability of pervaporation technique to obtained aroma concentrates would depend on the degree of concentration that it would provide an acceptable sensory profile.

3.4.2 References.

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4. EXTRACCIÓN DE COMPUESTOS AROMÁTICOS CON CO_2 SUPERCRÍTICO

4.1 Volatile compounds in supercritical carbon dioxide extracts of brown crab (Cancer Pagurus) processing by-product.

ABSTRACT.

Supercritical CO_2 extraction has been used to obtain aroma concentrates from brown crab (*Cancer Pagurus*) processing by-product. The SF extracts has been analyzed by Headspace-Solid Phase Dynamic Extraction Gas Chromatography/Mass Spectrometry. Volatile profile of the brown-crab processing by products is quiet different respect to the SF extracts one. Extracts presents higher quantities of aldehydes, phenols, pyrazines, pyridines, furans and organosulphur compounds formed during extraction process. Some operating parameters such as extraction pressure (100-500 bar), extraction temperature (40-80 ºC) and pressure in the separator have been analyzed. Increasing the CO_2 density (above 600 kg/m^3) a higher amount of volatile compounds was obtained, as many volatile compounds were thermally formed in at higher temperatures and co-extraction of fatty acids takes place. Decompostion of fatty acids causes the formation of hydrocarbons, which impart our extracts undesirable organoleptic characteristics. The pressure in the separator plays also an important role in the volatile recovery.

Keywords: CO_2, supercricrical extraction, brown crab, by-products.

4.1.1 Introduction.

The brown crab (*Cancer pagurus*) is found in the Eastern Atlantic, from the northern Morocco extending along the Atlantic coast of Europe, to the British Isles and northern Norway and in the north coast of the Mediterranean (Marseille. Napoli. Greece). Brown crabs are heavily exploited commercially throughout their range and are available throughout the year. The total catch reported for this species in 2006 was 46016 ton (FAOSTAT). Remaining by-product comprises as much as 70% of total crab weight and only a small amount of this by-product is processed into value-added products.

Flavor is considered a high value product and good quality shellfish flavors are in high demand, employing them in sauces, soups, instant noodles, snacks, etc. (1). Fresh shellfish flavour has been defined as sweet, distinctly plant-like, often accompanied by metallic and very slight to pronounced fishy attributes (2). Volatile compounds in seafoods are formed in

the living species, at the time of death, and during food processing (3). The volatile components of crabs are regarded as the most determinant compon ents of the flavour quality.

Several methods have been employed to isolate crab flavour from processing by-product. such as simultaneous distillation extraction (4-8) and enzymatic hydrolysis (9). Depending on the extraction method used, the volatile oil composition may change leading to deviations from the natural odour. By using a thermal process to obtain volatiles does not actually allow to distinguish volatiles generated as the result of heat-induced mechanism.

Supercritical fluid extraction has received considerable attention from the food industry in the last years. Its ability to selectively extract the analytes of interest, without the presence of any contaminating solvent or other undesirable compounds, has made SFE a useful reasearch tool (10). Carbon disoxide is often used for SFE work due to the fact that it is non-flammable, odourless, chemically inert, easily disposed and available in good purity at a relatively low cost.

In this work, supercritical fluid extraction has been proposed as an alternative technique to obtain high quality crab flavors. Extraction with supercritical CO_2 has been previosusly employed to obtain aroma extracts of different food products (11-12). This technique takes advantage of using non toxic and volatile solvent, avoiding solvent contamination and protecting volatile compounds from thermal degradation employing gentle conditions. The effect of some important operating variables, such as pressure, temperature and operating pressure in the separator has been analyzed to obtain high quality flavors.

4.1.2 Experimental section.

4.1.2.1. Materials

Brown crab processing by-product, supplied by IDOKI SCF Technologies S.L. (Bizkaia, Spain), was stored at -20ºC. Brown crab by-product was mostly claws, viscera and shells. The moisture was determined by drying crab processing by-products in an oven at 100ºC until constant weight was obtained (AOAC, 1990). A medium value of 41.6 ± 5 has been obtained.Carbon dioxide was 99.8% (v/v) pure, supplied by Carburos Metalicos (Barcelona, Spain).

4.1.2.1. SC-CO_2 extraction.

For the SC-CO2 extraction a semi-pilot plan has been used. This equipment has been previously used to extract fat from pigskin (13) and the omega-3 rich oil contained in hake (*Merluccius capensis-Merluccius paradoxus*) by-products (14).

For each extraction, 600 g of brown crab processing by-product were weighed and filled into the 2 L extractor without any pretreatmentt. Carbon dioxide from the pressurized bottle (6MPa) was compressed and recirculated with a membrane pump (LEWA). Mass flow was 10 kg/h in all experiments. Extracts were collected in a 0.5 L separator, cooled at - 5ºC, and stored at - 18ºC.

Different SFE extraction conditions were used to study the influence of pressure, temperature and pressure in the separator on composition of volatile components. Extraction conditions are shown in Table 4.1. In this Table the CO_2 density and the total mass of CO_2 used in each extraction have been also listed.

Table 4.1. *Experimental conditions employed to obtain brown crab processing by-product aroma concentrates.*

Run	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14
$P_{ext,}$ bar	180	420	180	500	180	180	420	420	100	300	300	300	300	300
T_{ext}, ºC	70	70	70	60	50	50	50	50	60	40	80	60	60	60
P_{sep}, bar	48	48	42	44	48	42	42	48	44	42	42	50	44	38
w_{CO2}, kg	10.5	8.6	12.3	8.6	13	12.9	10.8	9.8	17.5	10.4	10.0	12.4	12.0	12.6
$\rho_{CO2,}$ kg/m^3	601	856	601	935	751	751	933	933	295	936	747	831	831	831

4.1.2.3. Sample analysis.

The identification of the volatile components was performed by using a Headspace-Solid Phase Dynamic Extraction-Gas Chromatography/Mass Spectrometry (HS-SPDE-GC/MS). Semiquantitative analysis were carried out with an Agilent 6890N GC equipped with a 5973i MS (Agilent Technologies, Palo Alto, CA, USA) and a CTC-Combi-Pal auto sampler (GTC Analytics AG, Zwingen, Switzerland).

HS-SPDE is a non-equilibrium sampling method close to the dynamic headspace approach (15). Solid Phase Dynamic Extraction was employed instead of concentration techniques, as

atmospheric evaporation or vacuum evaporation, which has been reported as non effective techniques because some of volatile compounds were lost in the process (16).

The SPDE analysis uses a 2.5 ml gas tight syringe, with a PDMS (10% AC) internally coated needle. The needle was conditioned at 260ºC and flushed with Helium for 5 min. Samples were heated at 70ºC for 1 min and agitated at 500 rpm. An aliquot (1ml) of the headspace was pulled through the needle at 40 µl/s for 50 cycles. The syringe was automatically transferred to the injection port and volatile compounds were desorbed with 0.5 ml of Helium at 15 µl/s.

GC conditions were as follows: capillary column Quadrex 07114D (silphenylene polysiloxane, 60 m x 0.320 mm I.D. x 1µm film thickness, Quadrex Corporation, New Haven, USA), port injection temperature: 250ºC, splitless mode injection, oven temperature was programmed from an initial temperature of 30ºC (11 min holding), rising to 35ºC at 3ºC/min (5 min holding) and rising to 240ºC at 3ºC/min, carrier gas was Helium with an initial flow rate of 0.8 ml/min (18 min holding), rising to 1.5 ml at 1.4 ml/min2 (13 min holding) and descending to 0.8 ml/min at 0.1 ml/min2. MS conditions were as follows: interface temperature at 280ºC, ionization voltage at 70 eV, mass range at 30-300 amu and electron multiplier voltage at 1835 V.

Analyses were performed three times for each extract and the values reported in this work are the mean values calculated from the three chromatography determinations. The reproducibility of this analysis technique has been checked by determining the composition of some volatile components found in the extracts in a standard (or synthetic) aqueous solution. The volatile components were: hexanal (11.2 ppm), E-2-hexenal (9.2 ppm) and nonanal (8.6 ppm). The relative standard deviation (RSD) obtained for each compound is presented in Table 4.2. A relative good repetibility for the SPDE analysis can be concluded taking into account the values of the RSD, around 9 %. Similar values were obtained by Bicchi et al. (15) in the study of this technique to analyse the volatile fraction of food matrices.

Table 4.2. *Relative standard deviation (RSD) for some characteristic volatile components found in the SC extracts using the SPDE technique analysis.*

Compound	RSD (%)
Hexanal	6.97
E-2-hexenal	10.87
Nonanal	8.35

Compounds identification was achieved by matching their mass spectra with those in Nist08 and Wiley libraries. Additionally, linear retention indices (17) were calculated using retention data from a serie of alkane satandars (C5-C18) run under the same chromatography conditions. These indices were compared with the values of the literature and used to identify the different volatile components.

4.1.2.4. Fatty acid composition and oil extracted.

The fatty acids profile was determined by the AOAC method. The fatty acids methyl esters were firstly prepared and then analyzed by gas chromatography (GC) in a Hewlett Packard gas chromatograph (6890 N Network GC System) equipped with an auto-sampler (7683B series) and a flame ionization (FID) detector. The separation was carried out with helium (1.8 mL/min) as carrier gas. A fused silica capillary column (OmegawaxTM-320, 30 m × 0.32 mm i.d.) was used. The column temperature was programmed starting at a constant temperature of 180 ºC during 20 min, heated to 200 ºC at 1 ºC/min, held at 200 ºC during 1 minute, heated again to 220ºC at 5 C/min and finally held at 220ºC for 20 min. A split injector (50:1) at 250 ºC was used. The FID was also heated at 250ºC. Most of the fatty acid methyl esters were identified by comparison of their retention times with those of chromatographic standards (Sigma Chemical Co.). Their quantification was made by relating the peaks area to the area of an internal standard (methyl tricosanoate) as indicated by the AOAC method (11). Calibration curves were made for each pair internal standard + chromatographic standards in order to find the corresponding response factors.

4.1.3 Results and discussion.

4.1.3.1 Qualitative analysis of brown crab processing by-products and SC-extracts.

The flavour of most fresh shellfish has been described as sweet, distinctly plant-like, often accompanied by metallic and very slight to pronounced fishy attributes, primarily generated by unsaturated alcohols and aldehydes of less than ten carbon atoms, alkylpyrazines and sulphur containing compounds (2). Eight-carbon volatile alcohols and ketones have been found to occur in most seafoods, where they contribute distinct to these fresh plant-like, metallic aromas, even though these compounds individually exhibit mushrrom and geranium aroma qualities (3).

Previous to the SC-CO_2 extractions, the volatile composition of the brown crab processing by-product was also determined by HS-SPDE. Volatile composition of the sample was

determined by following three different pre-treatments to the by-product: a) without performing any pretreatment and grinding the samples b) by using an automatic grinder and c) by using a manual grinder. In all cases 1.5 grams of the sample was introduced in a 20 ml vial of the HS-SPDE. The different volatile composition found using these three methods can be appreciated in Appendix C. Additionally the area percentage of the different family of compounds in brown crab by-product was shown in Table 4.3. Although the number of total volatile compnents found without performing any pretreatment was higher than for the two other methods, by grinding the sample a higher abundance of volatile components was obtained.

Table 4.3. *Area percentage of the different family of compounds in brown crab by-product for the different pretreatments.*

PT	% ald	% alc	% ket	% pyr	% py	% sul	% est	% alk	% alke	% arom	% other
WP	10.9	43.4	16.8	0.6	0.2	0.0	2.0	11.2	4.2	9.2	0.7
AG	0.0	47.5	25.2	0.2	0.0	1.3	4.8	9.5	2.4	3.5	5.1
MG	1.0	57.6	18.1	0.3	0.0	0.0	2.7	11.9	3.3	2.8	1.9

PT = pretreatment, WP = without pretreatment, MG = automatic grinder, MG = manual grinder, ald = aldehydes, alc = alcohols, ket = ketones, py = pyrazines, py = pyridines, sulf = organosulphur compounds, alk = alkanes, alke = alkenes, arom = aromatic compounds.

In Table 4.4 the area percentage of the different family of compounds found in the extracts has been listed. Despite having used different extraction conditions, aldehydes are the major components in all the samples, followed by alcohols and ketones. Among the aldehydes components, around 50-70 % of total aldehydes area correspond to unsaturated aldehydes. Other important components found in the extracts were pyrazines, pyridynes, sulfures and furans. Particularly, pyrazines and furans play a key role in the overall aroma.

As an example of the exact extract composition, volatile compounds found in the extract six, E6, have been presented in Appendix D, where the organolepthic characteristics for each compound and its corresponding threshold has been also listed according to its description in the Flavor Data Base (18). Comparing Tables 4.3 and 4.4, it can be easily appreciated that the volatile composition is quite different in the extracts than in the brown crab processing by-product. For instance, the percentage of aldehydes is 4 times higher in the extracts that in the by-product. Alcohols present in the extract were quiet different respect those present in the raw material. 3-methylbutanol is the most abundant compound in the raw material. Unsaturated alcohols might have been partially oxidized to their aldehyde counterparts, which

have been successfully identified in the extracts. Phenols were thermally formed during extraction process. In addition, most of the heterocyclic compounds, such as pyrazines, pyridines, furans, 2-acetylthiazole, were not found in raw material. These compounds, which contribute to the characteristic boiled aroma of the crustacean, were formed during extraction process via Maillard reactions, principally at temperatures between 50 and 60ºC.

Table 4.4. *Area percentage of the different family of compounds for the different SFE conditions*

Run	% ald	% alc	% ket	% pyr	% py	% sul	% fur	% alk	% alke	% arom	% other
E1	68.4	4.3	5.8	0.0	0.2	5.1	1.5	7.2	6.1	0.8	0.7
E2	62.1	8.9	17.6	0.8	0.5	3.0	2.7	1.1	1.3	0.6	1.4
E3	84.1	2.2	2.7	0.0	0.1	1.9	0.5	5.7	1.2	0.7	0.8
E4	46.8	14.4	15.9	3.9	0.0	0.4	5.6	1.7	4.9	1.3	5.0
E5	45.2	16.7	13.4	3.7	0.1	1.0	7.5	2.2	7.3	1.4	1.6
E6	42.8	15.2	13.5	3.6	0.0	0.8	9.9	1.6	10.4	1.0	1.2
E7	37.8	17.7	15.9	3.8	0.3	4.1	5.9	1.9	11.1	0.5	1.0
E8	36.3	22.3	10.2	2.1	1.0	4.7	3.3	1.8	9.8	1.0	7.6
E9	48.2	24.3	9.9	3.3	0.7	2.0	3.5	2.9	2.3	0.6	2.1
E10	26.0	26.8	6.0	9.6	1.3	2.8	1.7	6.4	11.0	3.2	5.3
E11	49.4	19.3	16.4	3.2	0.1	0.6	4.9	2.4	1.6	1.4	0.9
E12	34.5	23.1	9.8	4.4	0.1	0.8	20.7	1.7	3.5	0.8	0.4
E13	36.2	18.9	8.9	3.3	0.1	1.5	22.6	1.8	5.6	0.9	0.3
E14	53.1	17.6	13.4	2.0	0.3	1.6	3.9	1.2	5.7	0.9	0.5

ald = aldehydes. alc = alcohols. ket = ketones. py = pyrazines. py = pyridines. sulf = organosulphur compounds. alk = alkanes. alke = alkenes. fur = furans. arom = aromatic compounds.

4.1.3.2 Family of volatile compounds

In this section a qualitative description of each family of volatile compounds found in the different extracts is presented.

Aldehydes

Forty seven aldehydes were found in brown crab processing by-products in the different extracts, however only around thirty were present in most extracts. Many of these compounds arise through autoxidative degradation of polyunsaturated lipids at moderate temperatures (2). Hexanal, heptanal, benzaldehyde, (E)-2-hexenal, (E)-2-pentenal, 2-methyl-3-butenal, (E,Z)-2,6-nonadienal and (E,E)-2,4-heptadienal were the main volatile compounds in the headspace of the extracts. Hexanal was the most abundant aldehyde, present in all extract with a medium area percentage of 15%.

3-methylbutanal was the most abundant of its class, followed by pentanal, in boiled and pasteurized crabmeat (20). These two aldehydes have similar aroma characteristics and have been reported to have green, fruity, nutty, cheesy, or sweaty odors, depending on dilution. However, in our extracts, only 3-methylbutanal was found in some of the extracts, but its area percentage was much less than 1% in all cases.

Unsaturated aldehydes impart important organoleptic properties to the extracts (5). Unsaturated aldehydes represent 50-70 % of total aldehydes area encountered in the extracts. Extracts obtained at 80ºC yield in the maximum number of aldehydic compounds. This might be probably due to a highest level in thermal degradation of PUFAs.

Regarding to aromatic aldehydes, phenylacetaldehyde and benzaldehyde were the most abundant compounds. Benzaldehyde was thermally generated and partially contributes to characteristic cooked crab flavour. It has a pleasant almond, nutty and fruity aroma in crustacean. In addition β-cyclocitral, an aromatic aldehyde formed via carotenoids decomposition, was found in the aroma concentrates.

Alcohols

Thirty alcohols were found in supercritical extracts, but only half of them were found in most extracts. Alcohols are formed by the decomposition of secondary hydroperoxides of fatty acids, action of lipoxygenase on fatty acids, oxidative decomposition of fat or reduction carbonyl to an alcohol.

Saturated alcohols, such as 1-hexanol, 2-ethyl-1-hexanol, 1-heptanol, 3-octanol and 1-dodecanol, contribute hardly to the overall aroma because of their high odor thresholds. Odor threshold concentrations were generally higher for alcohols than the aldehyde counterparts (3). Unsaturated alcohols comprise 80-90 % of the alcohol total area registered

Unsaturated alcohols which are present in all extracts are 1-octen-3-ol and 1-penten-3-ol. 1-octen-3-ol, and enzymatic reaction product derived from lipids, has been reported to be one of the volatile components widely distributed in fresh and saltwater fish (3), possesses a mushroom-like odour (2). This alcohol is one of the most abundant with a medium value of 15 % of all the alcohols area found in the extracts.

1-penten-3-ol is obtained via enzymatic-mediated conversion of polyunsaturated fatty acids (3) and contributes to a butter-like odor. It was found in high quantities (around 30% of all the alcohols area), however its relatively high recognition threshold value (400 ppb) indicates that this compound would not be a major contributor to fresh seafood flavors (3).

Phenols, formed via decarboxylation of phenolic carboxylic acids and thermal degradation of lignin, posses a woody, smoky and burnt aroma (2). 9 phenolic compounds were identified in the extracts. Quantities of phenolic compounds are low and only represent 3-12 % of the total alcohol area of the extract.

α-terpineol is a degradation product by oxidation and hydrolysis of d-limonene. This off-flavor is important to determine quality or effects of storage time and temperature in orange juice.

Ketones

Ketones contribute to the sweet floral, fruity flavor of many crustaceans [19]. Ketones may be produced by thermal oxidation/degradation of PUFAs, aminoacid degradation or microbial oxidation. Twenty four ketones were identified in the extracts but only 10 of them appear in most extracts.

2-alkanones, as 2-heptanone, 6-methyl-2-heptanone, 2-nonanone and 2-undecanone, have a distinct green and fruity aroma and give a more floral note as the chain length increase.

The unsaturated ketones, 1-penten-3-one, 1-octen-3-one, (E,E)-3,5-octadien-3-one, (E)-3-octen-2-one and 3-undecen-2-one, were present in high quantities in most of the extracts, and are important compounds in the overall aroma of the extracts. Comparing to 1-penten-3-ol, 1-penten-3-one appears also in most of the extracts but in less quantities (medium value of 10% total ketones area). However, 1-penten-3-one has a lower threshold value (1.3 ppb) compared to the corresponding alcohol and might be expected to contribute to seafood flavors in certain species (3).

Diketones provides a desirable balance of meaty and buttery notes. 2,3-butanedione, which has been reported as an important contributor in crab meat [8], was not identified in our extracts. However, 2,3-pentanedione, which imparts a desirable buttery and desirable aroma to foods [2], and 2,3-octanedione has been identified as major compounds in most of the extracts (32 and 11 % area of the total ketones area registered, respectively).

2,6-bis(1,1-dimethylethyl)-2.5-cyclohexadiene-1,4-dione has been reported as a seawater contaminant.

Pyrazines

Pyrazines are generally considered as key flavour components in many heat processed foods, because they contribute nutty, roasted o toasted aromas. Pyrazines are formed via Maillard and pyrolysis reaction in heat processed products, they contribute greatly to the flavour of cooked crab (2). Formation of alkylpyrazines has been found to increase when food samples were heated at higher temperatures (3). These compounds are important in the overall aroma of the extract because of their low odor thresholds.

10 pyrazines were identified in the extracts. Most of them were alkylpyrazines which have been reported as having a roasted, nutty, potato boiled aroma. 2,5-dimethylpyrazine, 3-methyl-2-vinylpyrazine and 2-acetylpyrazine were the most abundant pyrazines in the extracts. In particular 2,5-dimethylpyrazine represents around the 50 % of the total area of pyrazines.

Furans

Eleven furans were identified in the extracts, however only 2-n-pentylfuran and trans-2-(1-pentenyl) furan were found in most extracts. 2-ethylfuran and 2-pentylfuran are important contributors to the overall aroma of the extracts and have been reported to contribute burnt, sweet, bitter and cooked meat flavour to foods (19). Nevertheless, 2-pentylfuran contributes negatively to the flavour quality of the extract. 2-(1-pentenyl)furan is found in oxidized fish oil as a degradation product of fatty acids

Organosulphur compounds

Volatile sufur compounds have been traditionally associated with seafood spoilage, but in some species its formation at the time of harvest supporting the fact that can be also produced by live seafoods (3). Eight organosulphur compounds were identified, among them 2-acetylthiazole, which has a nutty, cereal, popcorn like aroma, was one of the main sulphur components of the headspace of the extracts. N-butylsulfonamide was also relative abundant and it has been identified as an off-flavor in the extracts (plasticizer).

Among sulphur compounds dimehtyl trisulfide and dimethyl disulfide have been found in thermally processed seafoods and meat products (3) they have been reported to affect overall food aroma because of their low threshold values. These compounds generally are considered undesirable, exhibiting a cooked cabbage and spoilage odor. In the supercritical extracts only dimehtyl trisulfide was found in 20% of the extracts.

Other compounds

Trimethylamine and related amines have been classified as other important source of volatile seafood flavour compounds. Tri- and dimethylamine modify the flavors of staling seafoods and it has been reported to contribute to boiling-crab odor (3). Trimethylamine was found in the supercritical extracts, but it has been no included in the total are chromatographic since its integration with the GC was not satisfactory.

Hidrocarbons may be formed via lipid autoxidation processes through alkyl radical or from decomposition of carotenoids. Saturated hydrocarbons contribute very little to the overall flavour of foods due to their high aroma thresholds. Unsaturated hydrocarbons are formed via alcohol dehydration and their contribution to seafood aroma is expected to be significant. (3). (E,E,Z)-1,3,5-octatriene and (E,Z,Z)-undecatriene are majority components in most of the extracts.. With reference to aromatic hydrocarbons, 1,2,4-trimethylbenzene and naphthalene are reported to contribute overall aroma in crab (4).

Limonene and camphene were found in only two extracts. The presence of these terpenes might be due to the pollution of rivers and bays which exposed crab to untreated effluents from wood processing resulting in terpene tainting (21).

4.1.3.3 Effect of extraction parameters

Different SF extractions were performed to evaluate the effect of some important operating variables such as pressure, temperature and operating pressure in the separator. Pressure and temperature ranges were from 100 to 500 bar and from 40 to 80 ºC respectively. The pressure in the separator was varied from 38 to 42 bar. Extraction of volatile components depends on a complex balance between supercritical fluid density and solute vapour pressure. The results obtained are difficult to understand especially due to the heterogeneity of the raw material.

One of the main parameters that determine the solubility of a compound in CO_2 is the density of the CO_2 phase. The density determines the number of interactions between molecules of the compound and the CO_2 phase. If sufficient interactions occur, the cohesive forces between molecules of compound can be broken and solubilization can take place.

Figure 4.1 shows the total amount of volatile components extracted expressed as chromatograph area per CO_2 mass in each extraction as a function of CO_2 density. It can be observed that when CO_2 density is not high enough the total amount of volatile components extracted is the lowest for all the experiments (excluding the experiment E11, probably due to experimental errors such the heterogeneity of the raw material).

From this Figure seems that an increase in solvent density leads to an increase in the total amount of volatile components extracted. However from solvent density values high enough (around 600kg/m^3), the increase in the total amount of volatile components is not so clear.

Moderate conditions using solvent densities around 600 kg·m^{-3}, seem to be sufficient for efficient extraction of volatile components.

The CO_2 density is a function of both, pressure and temperature of the fluid. The CO_2 pressure is probably one of the main parameters that affect the extraction process. At a given temperature an increase in the extraction pressure means an increase in the CO_2 density and consequently a better solvent capacity. The temperature effect in the process yield is complex due to the combined effect of solvent density and solute vapour pressure. A temperature increase, although causing a decrease of the fluid density, could be responsible for an increase in the solvating power because of the increase in the solute vapour pressure (22).

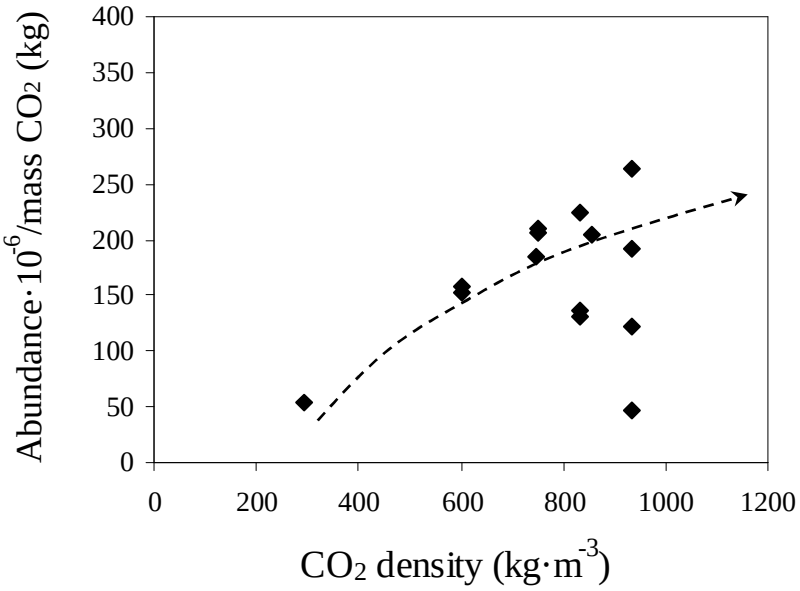

Figure 4.1. *Total amount of volatile compounds extracted as function of carbon dioxide density.*

Figure 4.2 shows the different effect of extraction temperature on the number of aldehydes, alcohols and ketones extracted. From this Figure could be concluded that the number of aldehydes and ketones increase with the extraction temperature, however in the case of alcohols this effect seems to be the opposite. This behaviour could be due to the fact that normally aldehydes and ketones have higher volatilities compared to alcohols, since the formation of hydrogen bonds is more difficult in a carbonyl group than in a hydroxyl group. In Figure 4.3 the total number of volatile components extracted has been represented as a function of CO2 density. It can be observed that the number of volatile components is more or less independent of CO2 density. However, it must be pointed out that the quality of the extracts was not the same in all the experiences.

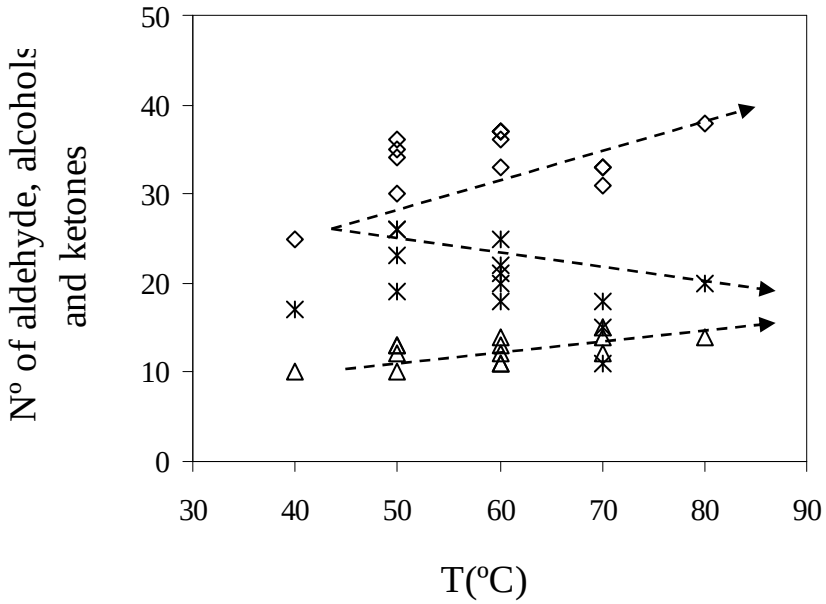

Figure 4.2. *Number of aldehydes (◊). alcohols (∗) and ketones (Δ) extracted as a function of extraction temperature. The lines are to guide the eye.*

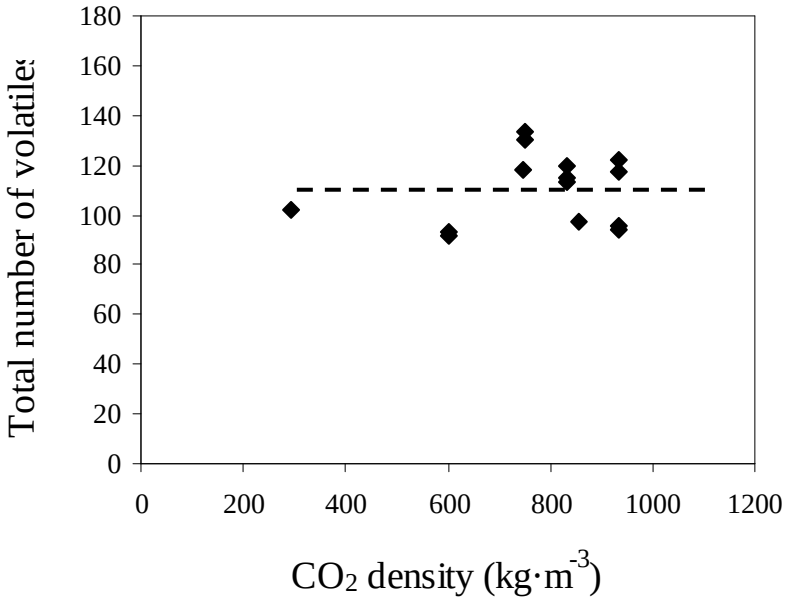

Figure 4.3. *Total number of volatile compounds extracted as a function of CO_2 density. The line is to guide the eye.*

In the literature, it can be found that in the extraction of plant volatile components high pressure is not always recommended for complex matrix due to the higher solubility of solutes when the pressure is high enough (23). This was also observed in this study of the extraction of volatile compounds of brown crab. At extraction pressures higher than 180 bar the co-extraction of some fatty acids was observed leading to an organic phase in the extracts. A further increase of the extraction pressure means a higher amount of the organic phase obtained in the extracts.

The fatty acids found in the organic phase corresponding to the extract number 5 (E5) has been listed in Table 4.5. The high content of unsaturated fatty acids (70%) makes organic phase very susceptible to lipid oxidation. These fatty acids are the "precursors" of the type of volatile compounds listed in Appendix E and found in the analysis of volatile of the organic phase. Storing organic phase at -18ºC, saturated hydrocarbons, (specially branched alkanes and cyclohexane derivates), unsaturated hydrocarbons and acids were formed. These compounds contribute a rancid, fishy, metallic odor to the overall aroma.

Table 4.5. *Fatty acid composition of brown crab oil in E10 extract.*

Fatty acid	Conc. (mg/g oil)
Total	391
Saturated	117.2
Monounsaturated	161.5
Polyunsaturated	112.3
EPA+DHA	84.2
ω6	16.7
ω3	95.6
ω 3/ ω 6	5.72
EPA/DHA	1.16

The pressure in the separator could also influence the amount and quality of the CO_2 extracts. In our study, there was a dependence of the total volatile components recovered in the separator on the pressure in the separator. In Figure 4.4, the total amount of volatile components expressed as chromatograph area per CO_2 mass in each extraction has been

plotted as a function of the pressure in the separator (extracts E9 and E10 have been excluded). The lower the pressure in the separator, the higher the amount of total volatile components extracted. By decreasing the pressure, the density of the CO_2 decreases, consequently its solvent power which leads to a better recovery.

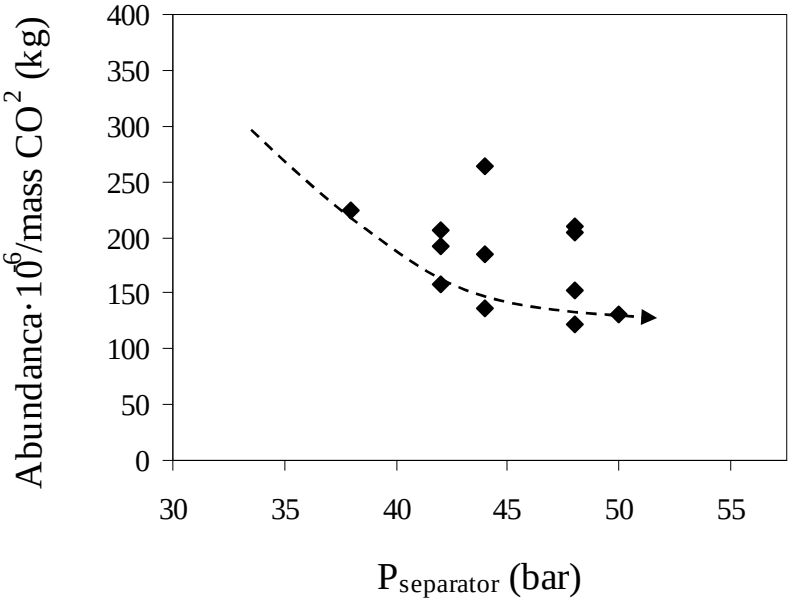

Figure 4.4. *Total amount of volatile compounds extracted as a function of the pressure in the separator. The line is to guide the eye.*

4.1.4 Conclusions.

Supercritical fluid extraction has been successfully employed for the extraction of brown crab aroma. Over 130 volatile components were identified in edible crab processing by-product. Extracts composition showed very large quantities of unsaturated aldehydes, unsaturated alcohols, ketones, pyrazines and furans, which provide good organoleptic properties to the extracts. Further research is needed to determine the sensory characteristics of the volatile compound in the extract by SFE of crab by-products.

4.1.5 References.

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5. CONCLUSIONES GENERALES

El trabajo desarrollado a lo largo de esta Tesis engloba el estudio de dos procesos de separación enmarcados como tecnologías verdes y que en los últimos años han cobrado bastante importancia sustituyendo a procesos habituales en la Industria Alimentaria. Los ensayos realizados con ambos procesos, enfocados a la obtención de sustancias aromatizantes útiles en la formulación de productos de alimentación, ha conducido a las siguientes conclusiones:

- Los subproductos de la industria del marisco, en concreto aquellos generados tras el procesamiento de buey de mar (*Cáncer Pagurus*) suponen una fuente de compuestos aromáticos importante, pudiendo ser reutilizados y reaprovechados para la obtención de productos aromáticos de interés en la Industria Alimentaria.

- La heterogeneidad de las muestras, así como la baja concentración, la diversidad en la naturaleza química y elevada volatilidad de los componentes aromáticos presentes en este tipo de subproductos son las principales dificultades que deben afrontar tanto los procesos de separación como los procedimientos analíticos.

- La temperatura en los procesos de separación que involucran este tipo de compuestos es muy importante ya que afecta enormemente en la calidad organoléptica del producto final, debido a la formación de nuevos compuestos y a la degradación de aquellos que presentan mayor sensibilidad térmica. Por tanto, los procesos que han sido empleados capaces de combinar temperaturas moderadas de trabajo con buenos rendimientos de proceso son definitivamente adecuados para desarrollar los objetivos descritos anteriormente.

- El proceso de pervaporación se ha llevado a cabo de forma exitosa para la recuperación de los componentes volátiles presentes en el caldo de cocción de buey de mar, habiéndose identificado más de un centenar de compuestos responsables del aroma presentes en los mismos. La elección del tipo de membrana, en concreto el tipo de material que constituye la capa activa de la membrana, es un factor determinante a la hora de conseguir una separación adecuada de los componentes, obteniéndose un permeado final rico en los componentes de interés y de características organolépticas adecuadas. Este enriquecimiento puede aumentar mediante un sistema de recogida de permeado consistente en dos condensadores en serie. No obstante, el proceso debe afrontar una serie de dificultades. La elevada volatilidad que presentan estos compuestos en medio acuoso provoca grandes pérdidas cuando los tiempos de operación son prolongados. Por otra parte, estos compuestos presentan una concentración extremadamente baja en el caldo de partida por lo que el efecto de polarización por concentración puede ser importante, afectando enormemente a la eficiencia en la separación.

- En los procesos de extracción con dióxido de carbono supercrítico de los compuestos orgánicos responsables del aroma de buey de mar a partir del subproducto sólido del

procesado del mismo, se han encontrado grandes cantidades de aldehídos y alcoholes insaturados, cetonas, pirazinas y compuestos organoazufrados característicos del aroma de las especies de marisco. En cuanto a las condiciones del proceso, la elevada presión de vapor que presentan estos compuestos hace que la solubilidad de los mismos sea alta cuando trabajamos con dióxido de carbono que presenta una densidad intermedia (600 kg/m^2). La temperatura de extracción es un parámetro determinante en el número de compuestos presentes en el extracto final. El empleo de presiones de extracción bajas (inferiores a 100 bar) evita la coextracción de los ácidos grasos presentes en el buey de mar, cuya degradación oxidativa provoca la formación de compuestos con características organolépticas indeseables.

Las perspectivas futuras se encuentran encaminadas a solucionar problemas técnicos encontrados durante la realización de ambos procesos, así como a la obtención de un producto final estable.

- Optimizar el diseño de la planta de pervaporación para minimizar. en la medida de lo posible las pérdidas de los compuestos aromáticos que se producen durante el proceso y paliar el efecto de polarización en la medida de lo posible.
- Sintetizar nuevos materiales para la fabricación de membranas que mejoren el rendimiento en el proceso de pervaporación.
- Estabilizar los permeados y extractos mediante técnicas de consistentes en la encapsulación de las biomoléculas de interés que prevengan su evaporación. oxidación. y reacción con otros componentes y ofrezcan protección contra los efectos perjudiciales provocados por la humedad. el pH y la exposición a la luz. Estas técnicas permiten. al mismo tiempo. la liberación controlada de los componentes aromáticos en una etapa determinada del procesado o la ingestión de un alimento.
- Analizar sensorialmente el producto final obtenido mediante ambos procesos.

APÉNDICE

Appendix A. *Aroma components of boiling brown crab effluent and permeate at 25ºC and 40ºC. β obtained at 25ºC and 40ºC (p = permeate, A_i = area of component I, A_{IS} = internal standard area, nd = not detected).*

			Chromatographic area			A_i/A_{IS}			Enrichment factor	
Family	Compound	L.R.I.	Effluent	p, 25ºC	p, 40ºC	Effluent	p, 25ºC	p, 40ºC	β, 25ºC	β, 40ºC
Acids										
	acetic acid	1463	348321	1520589	476082	1.515	8.453	3.229	5.6	2.1
	octanoic acid	>1800	221258	447213	642397	0.962	2.486	4.357	2.6	4.5
Alcohols										
	ethanol	<1100	298081	1131763	2752255	1.297	6.292	18.668	4.9	14.4
	(Z)-2-penten-1-ol	1196	88266	150615	160786	0.384	0.837	1.091	2.2	2.8
	1-penten-3-ol	1202	178409	273582	338585	0.776	1.521	2.297	2.0	3.0
	3-hexanol	1224	285647	330938	857042	1.243	1.840	5.813	1.5	4.7
	3-methylbutanol	1237	356722	1463345	983607	1.552	8.135	6.672	5.2	4.3
	2-hexanol	1246	29583	126422	133459	0.129	0.703	0.905	5.5	7.0
	1-pentanol	1262	115875	425681	257221	0.504	2.366	1.745	4.7	3.5
	2-ethyl-1-butanol	1323	209800	342904	280010	0.913	1.906	1.899	2.1	2.1
	2-heptanol	1330	74550	338644	316670	0.324	1.883	2.148	5.8	6.6
	3-octanol	1400	95896	587766	523746	0.417	3.267	3.552	7.8	8.5
	tetrahydrolinalool	1431	174314	290075	230022	0.758	1.613	1.560	2.1	2.1
	1-octen-3-ol	1455	85386	678437	454505	0.371	3.771	3.083	10.2	8.3
	1-heptanol	1460	77714	706840	566769	0.338	3.929	3.844	11.6	11.4
	2-ethyl-1-hexanol	1492	274145	5983113	7043691	1.193	33.261	47.776	27.9	40.1
	1-octanol	1558	15732	120938	115598	0.068	0.672	0.784	9.8	11.5
	(E)-3-nonen-1-ol	1563	142234	393964	1098632	0.619	2.190	7.452	3.5	12.0
	5-nonanol	1603	99415	867521	907564	0.432	4.823	6.156	11.2	14.2
	3-nonanol	1617	46495	123463	148885	0.202	0.686	1.010	3.4	5.0
	2-methyl-2-cyclohexen-1-ol	1642	211729	258875	255862	0.921	1.439	1.735	1.6	1.9

			Chromatographic area			A_i/A_{IS}			Enrichment factor	
Family	Compound	L.R.I.	Effluent	p, 25ºC	p, 40ºC	Effluent	p, 25ºC	p, 40ºC	β, 25ºC	β, 40ºC
Alcohols										
	1-nonanol	1656	46945	81192	233651	0.204	0.451	1.585	2.2	7.8
	benzyl alcohol	1701	198747	1296038	1576338	0.865	7.205	10.692	8.3	12.4
	1-decanol	1760	10852	53182	49276	0.047	0.296	0.334	6.3	7.1
	1-undecanol	>1800	15890	84665	132144	0.069	0.471	0.896	6.8	13.0
	1-dodecanol	>1800	11581	36534	52403	0.050	0.203	0.355	4.0	7.1
	phenol	>1800	114826	119441	80413	0.499	0.664	0.545	1.3	1.1
	BHT	>1800	505285	415910	659427	2.198	2.312	4.473	1.1	2.0
	trans-geraniol	>1800	423433	290095	832476	1.842	1.613	5.647	0.9	3.1
	2,5-di-tert-butylphenol	>1800	180032	222273	390242	0.783	1.236	2.647	1.6	3.4
Aldehydes										
	acetaldehyde	<1100	87689	128440	81894	0.381	0.714	0.555	1.9	1.5
	3-methylbutanal	<1100	256572	339955	786613	1.116	1.890	5.335	1.7	4.8
	hexanal	<1100	6338351	8825721	7438519	27.572	49.063	50.454	1.8	1.8
	heptanal	1197	145313	193297	234577	0.632	1.075	1.591	1.7	2.5
	octanal	1292	109369	1077128	826990	0.476	5.988	5.609	12.6	11.8
	trans-5-isopropyl-2-methyl-2-hexenal	1357	159485	502709	800694	0.694	2.795	5.431	4.0	7.8
	nonanal	1391	570411	2857624	1815634	2.481	15.886	12.315	6.4	5.0
	benzaldehyde	1521	3355139	14702346	1210667	14.595	81.732	82.117	5.6	5.6
	undecanal	1601	168954	590748	750493	0.735	3.284	5.090	4.5	6.9
	(E)-2-dodecenal	1638	211729	397540	599495	0.921	2.210	4.066	2.4	4.4
	(Z,Z)-2-butyl-2-octenal	1674	1804586	7948950	7657952	7.850	44.189	51.943	5.6	6.6
	4-ethylbenzaldehyde	>1800	282837	442799	1002598	1.230	2.462	6.800	2.0	5.5
Esters										
	ethyl acetate	<1100	5235	24546	18564	0.023	0.136	0.126	6.0	5.5
	ethyl caprylate	1276	3513	18527	26240	0.015	0.103	0.178	6.7	11.6

Family	Compound	L.R.I.	Chromatographic area			A_i/A_{IS}			Enrichment factor	
			Effluent	p, 25ºC	p, 40ºC	Effluent	p, 25ºC	p, 40ºC	β, 25ºC	β, 40ºC
Esters										
	isobornyl acetate	1580	898072	1634812	6106322	3.907	9.088	41.418	2.3	10.6
	diisobutyl phthalate	>1800	3907218	1564786	2602070	16.996	8.699	17.649	0.5	1.0
Ethers										
	phenyl ether	>1800	874026	1003337	1811738	3.802	5.578	12.289	1.5	3.2
Furans										
	2-butyltetrahydrofuran	1180	91891	378025.5	256143	0.400	2.101	1.737	5.3	4.3
	2-(2-propenylfuran)	1685	237068	264281	486953	1.031	1.469	3.303	1.4	3.2
	1-(3H)-isobenzofuranone	1718	57705	1761722	1042164	0.251	9.794	7.069	39.0	28.2
	2,5-dibutylfuran	>1800	52769	498060	1296561	0.230	2.769	8.794	12.1	38.3
	dibenzofuran	>1800	nd	153481	111550		0.853	0.757		
Hydrocarbons										
	tridecane	1300	98222	402782	563602.5	0.427	2.239	3.823	5.2	8.9
	pentadecane	1500	32184	421524	519630	0.140	2.343	3.525	16.7	25.2
	hexadecane	1600	71980	590748	750493	0.313	3.284	5.090	10.5	16.3
	heptadecane	1700	198747	1436587	1476995	0.865	7.986	10.018	9.2	11.6
	octadecane	1800	104266	228402.5	347569	0.454	1.270	2.357	2.8	5.2
Ketones										
	2,3-butanedione	>1100	nd	nd	56561			0.384		
	4-methyl-2-pentanone	1107	10623	35645	69676	0.046	0.198	0.473	4.3	10.2
	2,3-pentanedione	1123	12641	19002	36005	0.055	0.106	0.244	1.9	4.4
	2-hexanone	1132	105326	162171	91574	0.458	0.902	0.621	2.0	1.4
	5-methyl-2-hexanone	1192	87546	130620	114766	0.381	0.726	0.778	1.9	2.0
	2-heptanone	1198	146828	160215	327144	0.639	0.891	2.219	1.4	3.5
	cyclopentanone	1205	231487	266074	483174	1.007	1.479	3.277	1.5	3.3
	4-methyl-2-heptanone	1216	45268	192198	358967	0.197	1.068	2.435	5.4	12.4
	3-octanone	1259	154564	932174	1563322	0.672	5.182	10.604	7.7	15.8

Family	Compound	L.R.I.	Chromatographic area			A_i/A_{IS}			Enrichment factor	
			Effluent	p, 25ºC	p, 40ºC	Effluent	p, 25ºC	p, 40ºC	β, 25ºC	β, 40ºC
Ketones										
	2-octanone	1294	54779	688656	649778	0.238	3.828	4.407	16.1	18.5
	1-octen-3-one	1207	68349	860430	643208	0.297	4.783	4.363	16.1	14.7
	2,3-octanedione	1331	nd	25487	39997		0.142	0.271		
	(Z)-6-octen-2-one	1336	nd	nd	45238			0.307		
	6-methyl-5-hepten-2-one	1343	18797	154969	211098	0.082	0.861	1.432	10.5	17.5
	2-nonanone	1381	50460	224923	385381	0.220	1.250	2.614	5.7	11.9
	camphor	1505	213448	577282	402090	0.928	3.209	2.727	3.5	2.9
	3,5-octadien-2-one	1571	416189	195317	180263	1.810	1.086	1.223	0.6	0.7
	2-undecanone	1597	107463	287382	453571	0.467	1.598	3.076	3.4	6.6
	acetophenone	1623	127302	80174	110174	0.554	0.446	0.747	0.8	1.3
	corylone	1684	826093	1690429	1576422	3.593	9.397	10.693	2.6	3.0
	propiophenone	1719	325548	779408	520812	1.416	4.333	3.533	3.1	2.5
	cis-geranyl acetone	>1800	90765	103301	208680	0.395	0.574	1.415	1.5	3.6
Naphthalene derivatives										
	2-methylnaphthalene	>1800	141829	297017	509320	0.617	1.651	3.455	2.7	5.6
	2,7-dimethylnaphthalene	>1800	43343	105049	372234	0.189	0.584	2.525	3.1	13.4
	1,6-dimethylnaphthalene	>1800	38260	72954	331406	0.166	0.406	2.248	2.4	13.5
Nytrogenated compounds										
	butylamine	<1100	523007	705021	1488796	2.275	3.919	10.098	1.7	4.4
	trimethylamine	<1100	334470	365896	387965	1.455	2.034	2.631	1.4	1.8
	pyridine	1216	192198.5	256987	421415	0.836	1.429	2.858	1.7	3.4
	tributylamine	1233	nd	84433	41440		0.469	0.281		
	2,5-dimethylpyrazine	1333	422453	507966	588102	1.838	2.824	3.989	1.5	2.2
	2,4,6-trimethylpyridine	1376	83134	179427	254635	0.362	0.997	1.727	2.8	4.8
	2-ethyl-5-methylpyrzine	1406	149380	232534	268391	0.650	1.293	1.820	2.0	2.8
	2-butyl-3-methylpyrazine	1580	826093	856673	1592563	3.593	4.762	10.802	1.3	3.0

			Chromatographic area			A_i/A_{IS}			Enrichment factor	
Family	Compound	L.R.I.	Effluent	p, 25ºC	p, 40ºC	Effluent	p, 25ºC	p, 40ºC	β, 25ºC	β, 40ºC
Organosulphur compounds										
	dimethyl disulfide	1136	543933	997513	547550	2.366	5.545	3.714	2.3	1.6
	4-methylthiazole	1278	10568	65421	132598	0.046	0.364	0.899	7.9	19.6
	dimethyl trisulfide	1382	858362	1241567	654879	3.734	6.902	4.442	1.8	1.2
Terpenes										
	isocineole	1186	39173	718620	341847	0.170	3.995	2.319	23.4	13.6
	d-l-limonene	1208	243432	1145500	654306	1.059	6.368	4.438	6.0	4.2
	β-patchoulene	1553	144998	257458	349287	0.631	1.431	2.369	2.3	3.8

Appendix B. *Aroma profile of permeate with fractionation at two different temperatures in the first condenser. -4ºC and -10ºC (A_i=chromatographic area of component i, A_{IS} = chromatographic area, of internal standard, C1. C2 = condenser1. condenser2; nd = not detected;L.R.I. = linear retention index)*

Family	Compound	L.R.I.	A_i/A_{IS} effluent	T= -10ºC				T=-4ºC			
				A_i/A_{IS} C1	A_i/A_{IS} C2	β, C1	β, C2	A_i/A_{IS} C1	A_i/A_{IS} C2	β, C1	β, C2
Acids											
	acetic acid	1463	1.52	0.60	14.76	0.4	9.7	8.07	74.49	5.3	49.2
	octanoic acid	>1800	0.96	1.95	39.05	2.0	40.6	5.01	30.00	5.2	31.2
Alcohols											
	ethanol	<1100	1.30	2.90	1383.95	2.2	1067.3	10.48	641.47	8.1	494.7
	(Z)-2-penten-1-ol	1196	0.38	0.92	34.05	2.4	88.7	1.61	4.01	4.2	10.4
	1-penten-3-ol	1202	0.78	2.42	100.65	3.1	129.7	2.30	21.72	3.0	28.0
	3-hexanol	1224	1.24	4.43	86.14	3.6	69.3	1.91	35.72	1.5	28.7
	3-methylbutanol	1237	1.55	4.36	519.53	2.8	334.8	0.20	8.99	0.1	5.8
	2-hexanol	1246	0.13	1.85	33.13	14.4	257.4	1.18	3.25	9.2	25.3
	1-pentanol	1262	0.50	0.74	8.87	1.5	17.6	1.24	2.16	2.5	4.3
	2-ethyl-1-butanol	1323	0.91	3.83	83.07	4.2	91.0	5.80	20.01	6.4	21.9
	2-heptanol	1330	0.32	3.61	108.17	11.1	333.6		7.77	0.0	24.0
	2,6-dmethyl-4-heptanol	1350	nd	nd	nd			0.73	4.11		
	3-octanol	1400	0.42	0.91	417.76	2.2	1001.5	10.38	133.90	24.9	321.0
	tetrahydrolinalool	1431	0.76	0.91	417.76	1.2	550.9	1.18	1.58	1.6	2.1
	1-octen-3-ol	1455	0.37	0.32	37.31	0.9	100.5	2.82	40.21	7.6	108.3
	1-heptanol	1460	0.34	1.19	109.88	3.5	325.0	0.86	14.02	2.5	41.5
	dihydromircenol	1474	nd	nd	nd			2.52	3.63		
	2-ethyl-1-hexanol	1492	1.19	1.58	34.30	1.3	28.8	13.97	125.23	11.7	105.0
	1-octanol	1558	0.07	2.50	194.88	36.6	2847.6	10.96	13.42	160.	196.2

Family	Compound	L.R.I.	A_i/A_{IS} effluent	T= -10ºC				T=-4ºC			
				A_i/A_{IS} C1	A_i/A_{IS} C2	β, C1	β, C2	A_i/A_{IS} C1	A_i/A_{IS} C2	β, C1	β, C2
Alcohols											
	(E)-3-nonen-1-ol	1563	0.62	4.46	126.48	7.2	204.4	10.09	29.75	16.3	48.1
	5-nonanol	1603	0.43	0.99	24.59	2.3	56.9	2.79	1.15	6.4	2.7
	(Z)-2-nonen-1-ol	1614	0.20	2.93	165.56	14.5	818.6	7.44	51.94	36.8	256.8
	1-nonanol	1617	0.92	3.16	31.20	3.4	33.9	0.81	8.18	0.9	8.9
	2-methyl-2-cyclohexen-1-ol	1642	0.20	0.87	11.93	4.3	58.4	0.74	6.56	3.6	32.1
	benzyl alcohol	1701	0.86	1.88	27.94	2.2	32.3	11.13	37.25	12.9	43.1
	1-decanol	1760	0.05	0.58	43.05	12.3	912.0	10.60	28.68	224. 192.	607.5
	1-undecanol	>1800	0.07	1.08	17.93	15.6	259.3	13.33	50.91	76.5	736.6
	1-dodecanol	>1800	0.05	4.11	2.18	81.5	43.4	3.85	7.54		149.8
	phenol	>1800	0.50	1.75	15.87	3.5	31.8	1.14	4.10	2.3	8.2
	BHT	>1800	2.20	15.06	116.52	6.8	53.0	9.63	47.06	4.4	21.4
	trans-geraniol	>1800	1.84	10.02	82.18	5.4	44.6	1.18	5.99	0.6	3.2
	2,5-di-tert-butylphenol	>1800	0.78	2.50	28.90	3.2	36.9	5.59	66.41	7.1	84.8
Aldehydes											
	acetaldehyde	<1100	0.38	2.00	41.64	5.2	109.2	0.91	22.02	2.4	57.7
	3-methylbutanal	<1100	1.12	0.77	61.79	0.7	55.4	2.43	36.93	2.2	33.1
	hexanal	<1100	27.57	9.85	388.88	0.4	14.1	11.79	35.09	0.4	1.3
	heptanal	1197	0.63	1.61	56.75	2.5	89.8	0.72	3.21	1.1	5.1
	octanal	1292	0.48	1.96	19.97	4.1	42.0	1.87	3.19	3.9	6.7
	trans-5-isopropyl-2-methyl-2-hexenal	1357	0.69	1.43	14.90	2.1	21.5	0.81	5.01	1.2	7.2
	nonanal	1391	2.48	2.03	273.10	0.8	110.1	19.33	2.64	7.8	1.1
	benzaldehyde	1521	14.595	1.65	3440	0.1	235.7	1.65	1958	0.08	99.92
	undecanal	1601	0.73	1.45	16.28	2.0	22.2	1.53		2.1	0.0
	(E)-2-dodecenal	1638	0.92	8.43	159.74	9.1	173.4	4.67	19.37	5.1	21.0
	(Z,Z)-2-butyl-2-octenal	1674	7.85	12.41	69.87	1.6	8.9	25.88	69.17	3.3	8.8

Family	Compound	L.R.I.	A_i/A_{IS} effluent	T= -10ºC				T=-4ºC			
				A_i/A_{IS} C1	A_i/A_{IS} C2	β, C1	β, C2	A_i/A_{IS} C1	A_i/A_{IS} C2	β, C1	β, C2
Aldehydes											
	4-ethylbenzaldehyde	>1800	1.23	1.01	53.83	0.8	43.7	13.66	45.31	11.1	36.8
	2,5-dimethylbenzaldehyde	>1800	nd	nd	nd			1.74	42.98		
Ethers											
	Phenyl ether	>1800	3.80	4.51	586.99	1.2	154.4	2.70	100.23	0.7	26.4
Esters											
	ethyl acetate	<1100	0.02	nd	3.99		175.3	0.20	1.71	8.7	75.1
	ethyl caprylate	1276	0.02	0.13	1.33	8.6	87.2	nd	2.23		146.2
	isobornyl acetate	1580	3.91	42.65	579.56	10.9	148.4	0.96	9.80	0.2	2.5
	diisobutyl phthalate	57.9	17.00	28.83	10.64	1.7	0.6	22.89	160.61	1.3	9.4
Furans											
	2-butyltetrahydrofuran	1180	0.40	3.01	121.47	7.5	303.9	5.50	17.81	13.7	44.6
	2-(2-propenylfuran)	1685	1.03	5.04	46.65	4.9	45.2	4.06	26.65	3.9	25.8
	1-(3H)-isobenzofuranone	1718	0.25	4.70	82.81	18.7	329.9	9.94	1.82	39.6	7.2
	2,5-dibutylfuran	>1800	0.23	1.73	18.67	7.5	81.3	6.87	20.41	29.9	88.9
	dibenzofuran	>1800	nd	6.06	195.58			1.47	8.81		
Hydrocarbons											
	tridecane	1300	0.43	1.11	66.91	2.6	156.6	1.88	6.00	4.4	14.1
	tetradecane	1400	nd	nd	nd			1.03	1.22		
	pentadecane	1500	0.14	3.74	8.77	26.7	62.7	1.75	2.17	12.5	15.5
	hexadecane	1600	0.31	1.14	28.80	3.7	92.0	3.16	75.34	10.1	240.6
	heptadecane	1700	0.86	1.10	16.44	1.3	19.0	5.43	10.74	6.3	12.4
	octadecane	1800	0.45	1.82	34.68	4.0	76.5	2.37	27.79	5.2	61.3

Family	Compound	L.R.I.	A_i/A_{IS} effluent	T= -10ºC				T=-4ºC			
				A_i/A_{IS} C1	A_i/A_{IS} C2	β. C1	β, C2	A_i/A_{IS} C1	A_i/A_{IS} C2	β, C1	β, C2
Ketones											
	2,3-butanedione	<1100	nd	nd	27.43			2.91	11.70		
	2-pentanone	1102	nd	nd	nd			2.91	11.70		
	4-methyl-2-pentanone	1107	0.05	nd	7.79		168.6	0.41	15.63	8.8	338.3
	2,3-pentanedione	1123	0.05	nd	4.32		78.5	2.91	11.70	52.8	212.7
	2-hexanone	1132	0.46	0.21	14.60	0.5	31.9		3.53	0.0	7.7
	5-mehtyl-2-hexanone	1192	0.38	0.33	100.88	0.9	264.9		3.53	0.0	9.3
	2-heptanone	1198	0.64	0.34	143.15	0.5	224.1	2.26	4.34	3.5	6.8
	cyclopentanone	1205	1.01	0.13	177.97	0.1	176.7	0.69	3.52	0.7	3.5
	4-methyl-2-heptanone	1216	0.20	0.22	37.62	1.1	191.1	0.23	20.25	1.2	102.8
	3-octanone	1259	0.67	0.41	11.31	0.6	16.8	0.11	12.95	0.2	19.3
	2-octanone	1294	0.24	0.90	9.48	3.8	39.8	1.07	2.01	4.5	8.5
	1-octen-3-one	1207	0.30	0.29	7.57	1.0	25.5	1.90	12.27	6.4	41.3
	2,3-octanedione	1331	nd	1.22	7.36			7.42	23.21		
	(Z)-6-octen-2-one	1336	nd	0.44	22.98			0.81	5.01		
	6-methyl-5-hepten-2-one	1343	0.08	0.51	8.22	6.2	100.5	0.81	5.01	9.9	61.3
	2-nonanone	1381	0.22	5.83	36.67	26.6	167.1	1.15	9.72	5.2	44.3
	camphor	1505	0.93	0.00	24.04	0.0	25.9	1.01		1.1	0.0
	3,5-octadien-2-one	1571	1.81	3.58	338.80	2.0	187.1	16.02	258.05	8.8	142.5
	2-undecanone	1597	0.47	3.44	27.60	7.4	59.0	0.29	1.20	0.6	2.6
	acetophenone	1623	0.55	3.35	58.15	6.0	105.0	2.52	13.49	4.6	24.4
	corylone	1684	3.59	7.59	139.95	2.1	38.9			0.0	0.0
	propiophenone	1719	1.42	2.09	72.56	1.5	51.2	6.97	25.72	4.9	18.2
	cis-geranyl acetone	>1800	0.39	5.87	385.60	14.9	976.6	14.62	310.10	37.0	785.4
	trans-beta-ionone	>1800	nd	nd	nd			1.07	0.00		

Family	Compound	L.R.I.	A_i/A_{IS} effluent	T= -10ºC				T=-4ºC			
				A_i/A_{IS} C1	A_i/A_{IS} C2	β. C1	β. C2	A_i/A_{IS} C1	A_i/A_{IS} C2	β. C1	β. C2
Naphthalene derivatives											
	naphthalene	1732	nd	nd	nd			9.25	35.79		
	2-methylnaphthalene	>1800	0.62	0.42	10.79	0.7	17.5	1.89	8.22	3.1	13.3
	2,7-dimethylnaphthalene	>1800	0.19	1.56	86.15	8.3	457.0	2.88	11.77	15.3	62.4
	1,6-dimethylnaphthalene	>1800	0.17	4.51	586.99	27.1	3526.9	3.34	5.62	20.1	33.7
Nytrogenated compounds											
	butylamine	<1100	2.28	3.40	308.89	1.5	135.8	4.94	2096.91	2.2	921.7
	trimethylamine	<1100	1.45	2.94	38.68	2.0	26.6	19.51	229.02	13.4	157.4
	pyridine	1216	0.84	4.89	99.21	5.8	118.7		2.40	0.0	2.9
	tributylamine	1233			126.43			11.88	12.42		
	2,5-dimethylpyrazine	1333	1.84	1.38	171.77	0.8	93.5	3.39	1.55	1.8	0.8
	2,4,6-trimethylpyridine	1376	0.36	0.00	15.81	0.0	43.7	3.39	1.55	9.4	4.3
	2-ethyl-5-methylpyrazine	1406	0.65	5.70	142.98	8.8	220.0	1.06	29.15	1.6	44.9
	2-butyl-3-methylpyrazine	1580	3.59	3.92	107.22	1.1	29.8	15.92	49.07	4.4	13.7
Organosulphur compounds											
	dimethyl disulfide	1136	2.37	nd	549.67		232.3	1.02	4.01	0.4	1.7
	5-methylthiazole	1262	nd	nd	nd			1.44	1.72		
	4-methylthiazole	1278	0.05	nd	7.99		173.8	1.50	4.40	32.7	95.7
	dimethyl trisulfide	1382	3.73	nd	8.28		2.2	0.00	1.93	0.0	0.5
	benzothiazole	>1800	nd	nd	nd			1.69	19.52		
Terpenes											
	isocineole	1186	0.17	0.56	17.77	3.3	104.3	nd	4.07		23.9
	dl-limonene	1208	1.06	nd	19.90		18.8	nd	50.19		47.4
	β-patchoulene	1553	0.63	2.44	103.35	3.9	163.9	4.10	262.57		416.3

Appendix C. *Compounds identified in brown crab by-product (L.R.I. = linear retention index; A = abundance; n.d. = not detected; WP = without pretreatment; AG = automatic grinder; MG = manual grinder).*

			WP		AG		MG			
Family	Compound	L.R.I	A (10^{-6})	% area	A (10^{-6})	% area	A (10^{-6})	% area	Organoleptic characteristics	T (ppb)
Acids										
	nonanoic acid	1258	2.244	0.140	1.152	0.053	2.508	0.118		
Alcohols										
	2-methyl-1-propanol,	636	16.36	1.020	44.210	2.031	31.39	1.472	Breathtaking, sweet, sweaty-chemical, whiskey like in dilution.	7000
	1-butanol	672	1.957	0.122	1.470	0.068	1.619	0.076	Breathtaking, winey, fusel oil-like.	500
	1-penten-3-ol	688	155.84	9.717	131.41	6.036	201.8	9.461	Pungent, grassy, alliaceous-like, green vegetable, fruity taste.	400
	3-methyl-3-buten-1-ol	734	0.461	0.029	n.d.	n.d.	n.d.	n.d.	Sweet fresh green fruity.	
	1-butanol, 3-methyl-	739	269.09	16.77	628.56	28.87	638.3	29.92	Breathtaking, alcoholic odor, in dilution a winey-brandy.	250.0
	1-pentanol	770	96.044	5.988	79.939	3.672	129.2	6.059	Alcoholic-breathtaking, fusel-like odor with a burning taste.	4000
	(Z)-2-penten-1-ol	772	10.419	0.650	n.d.	n.d.	n.d.	n.d.	Ethereal, green, fruity odor reminiscent of cherry upon dilution with citrus connotation.	
	(Z)-3-hexen-1-ol	858	3.307	0.206	3.468	0.159	5.291	0.248	Strong, fresh, green, grassy odor.	70.0
	1-hexanol	870	22.156	1.381	26.148	1.201	46.03	2.158	Chemical, winey, slight fatty-fruity odor	2500
	3,5-octadien-2-ol	880	1.833	0.114	2.869	0.132	3.138	0.147		
	(E,E)-2,4-octadien-1-ol	882	2.710	0.169	2.262	0.104	2.923	0.137	Mild, pleasant fatty odor. Chicken fat with a creamy waxy nuance.	
	(Z)-4-hepten-1-ol	965	n.d.	n.d.	4.170	0.192	6.069	0.285	Creamy green, fruity aroma.	
	1-heptanol	970	10.13	0.632	8.018	0.368	12.02	0.564	Weak, fresh, green, fatty odor, winey, nut-like taste.	3.0
	(5Z)-octa-1,5-dien-3-ol	975	36.91	2.301	36.83	1.692	57.35	2.689	Earthy, mushroom-like	

Family	Compound	L.R.I	WP		AG		MG		Organoleptic characteristics	T (ppb)
			A (10^{-6})	% area	A (10^{-6})	% area	A (10^{-6})	% area		
Alcohols										
	1-octen-3-ol	979	25.373	1.582	37.66	1.730	45.91	2.153	Strong, sweet, earthy mushroom odor and taste.	1.0
	2-ethyl-1-hexanol	1029	17.70	1.104	6.480	0.298	10.97	0.514	Sweet, oily, weak rose odor, fatty-fruity musty, tea-floral taste.	830
	(Z)-5-octen-1-ol	1071	16.65	1.038	17.16	0.788	29.16	1.367	Green, melon, fruity and mushroom odor & taste.	
	2,6-dimethyl-1-heptanol	1081	5.622	0.351	2.990	0.137	4.829	0.226		
	phenethyl alcohol	1121	7.049	0.440	3.156	0.145	2.938	0.138	Floral, rose-like odor, floral taste.	750
Aldehydes										
	3-methylbutanal	661	8.041	0.501	0.712	0.033	0.543	0.025	Powerful, penetrating, cheesy-sweaty-fruity in dilution.	0.2-2
	2-methylbutanal	669	2.262	0.141	n.d.	n.d.	n.d.	n.d.	Strong, breathtaking odor, cocoa-like, weak fruity on dilution.	1.0
	pentanal	698	49.79	3.105	n.d.	n.d.	n.d.	n.d.	Strong, acrid, pungent odor, chocolate and nut-like below 10ppm.	12.0
	(E)-2-pentenal	756	8.248	0.514	n.d.	n.d.	9.099	0.427	Powerful, fruity, apple-like in dilution.	1500
	hexanal	799	59.79	3.728	n.d.	n.d.	n.d.	n.d.	Strong, penetrating, fatty-green, grassy unripe fruit odor.	4.5
	(E)-2-hexenal	854	2.469	0.154	n.d.	n.d.	n.d.	n.d.	Green, fruity, fresh, apple and woody with leafy and grassy.	
	(Z)-4-heptenal	899	5.67	0.354	n.d.	n.d.	10.00	0.469	Powerful creamy, green, vegetable odor.	
	heptanal	901	12.54	0.782	n.d.	n.d.	n.d.	n.d.	Fatty, in dilution sweet, fruity, nutty, fatty-cognac like.	3.0
	benzaldehyde	966	9.26	0.577	n.d.	n.d.	n.d.	n.d.	Odor of bitter almond oil, characteristic sweet cherry taste.	350
	octanal	1003	4.63	0.289	n.d.	n.d.	n.d.	n.d.	Fatty-fruity odor, sweet, citrus-orange-fatty taste.	0.7
	(E,E)-2,4-heptadienal	1012	5.207	0.325	n.d.	n.d.	n.d.	n.d.	Fatty, green, with an oily, greasy note.	

Family	Compound	L.R.I	WP		AG		MG		Organoleptic characteristics	T (ppb)
			A (10^{-6})	% area	A (10^{-6})	% area	A (10^{-6})	% area		
Aldehydes										
	2-phenylacetaldehyde	1062	n.d.	n.d.	n.d.	n.d.	1.495	0.070	Strong floral green odor (rose-hyacinth), floral almond taste.	4.0
	nonanal	1105	8.220	0.512	n.d.	n.d.	n.d.	n.d.	Fatty-floral-rose, waxy odor, citrus taste in dilution.	1.0
Esters										
	ethyl acetate	624	0.579	0.036	59.945	2.754	23.370	1.096	Ethereal, sharp, wine-brandy like odor.	5.0
	ethyl propionate	715	3.878	0.242	21.101	0.969	6.521	0.306	Strong, ethereal, fruity, rum-like odor and taste.	10.0
	ethyl isobutyrate	763	3.528	0.220	3.115	0.143	2.006	0.094	Sweet, ethereal, fruity odor and taste, apple note.	0.1
	ethyl isobutyrate	763	3.528	0.220	3.115	0.143	2.006	0.094	Sweet, ethereal, fruity odor and taste, apple note.	0.1
	ethyl 2-methylbutyrate	852	1.151	0.072	5.727	0.263	1.204	0.056		
	ethyl isovalerate	855	n.d.	n.d.	n.d.	n.d.	3.140	0.147	Strong, fruity apple odor and taste.	
	isoamyl acetate	878	2.618	0.163	6.589	0.303	7.738	0.363	Sweet, fruity, banana, pear odor & taste.	2.0
	2-methoxycarbonyl-2-(cis-2'pentenyl)-3-methoxycarbonylcethylcyclopentane	949	3.647	0.227	2.322	0.107	2.461	0.115		
	ethyl caproate	998	2.453	0.153	2.373	0.109	2.402	0.113	Strong, fruity, winey odor, apple, banana, pineapple notes.	1.0
	hexyl acetate	1011	n.d.	n.d.	1.368	0.063	4.006	0.188	Sweet, fruity, pear-apple like odor. apple like taste.	
	2-ethylhexyl acetate	1150	3.305	0.206	1.292	0.059	1.798	0.084	Sweet, raspberry, fresh fruity notes.	
	2-phenylethyl acetate	1264	10.427	0.650	0.867	0.040	3.116	0.146	Sweet, rose, fruity, honeylike odor, floral-honey taste.	
Ketones										
	2,3-butanedione	600	2.081	0.130	n.d.	n.d.	n.d.	n.d.	Strong, buttery odor and taste on dilution.	2.3

Family	Compound	L.R.I	WP		AG		MG		Organoleptic characteristics	T (ppb)
			A (10^{-6})	% area	A (10^{-6})	% area	A (10^{-6})	% area		
Ketones										
	2-butanone	607	10.30	0.642	415.2	19.07	190.5	8.934	Sweet, solvent, acetone-like ketonic odor.	50000
	3-pentanone	698	111.5	6.957	36.85	1.693	48.56	2.277	Ethereal, mild acetone-like, slight fruity cheese note.	
	3-hexanone	785	0.895	0.056	n.d.	n.d.	2.248	0.105	Alcoholic, grape, wine-like, ethereal, (medicinal) odor.	
	2-hexanone	790	n.d.	n.d.	n.d.	n.d.	5.949	0.279	Fruity, ketonic, banana, yeasty, cheese, dairy notes.	
	3-heptanone	887	1.012	0.063	0.758	0.035	0.910	0.043	Strong, fatty, green, fruity odor, fruity taste.	
	2-heptanone	891	16.61	1.036	12.21	0.564	16.39	0.768	Creamy, bitter, fruity, cheeese, cinnamon odor, in dilution-blue cheese-fruity.	140-3000
	6-methyl-2-heptanone	956	1.912	0.119	1.264	0.058	1.818	0.085		
	2,5-octanedione	983	4.478	0.279	n.d.	n.d.	2.530	0.119	Sweet, buttery-oily odor with herbaceous-cheesy undertone, yet not rancid or unpleasant on dilution.	
	3-octanone	987	31.92	1.990	52.58	2.415	67.32	3.156	Earthy, herbaceous-fruity, cheese odor, earthy-cheese taste.	28.0
	acetophenone	1074	1.861	0.116	2.373	0.109	1.588	0.074	Sweet, pungent, harsh, cherry-like odor and taste.	65
	2-nonanone	1092	15.40	0.961	13.82	0.635	16.9	0.793	Fruity, fatty-cheese odor, fruity cheese taste.	5-200
	3,5-octadien-2-one	1095	13.63	0.850	n.d.	n.d.	n.d.	n.d.	Woody, it provides freshness to green tea, mushroom, fatty notes.	100-150
	2-decanone	1194	6.218	0.388	2.046	0.094	3.234	0.152	Orange-like, floral odor, enhances oakmoss notes.	
	3-undecanone	1290	n.d.	n.d.	n.d.	n.d.	2.172	0.102		
	2-undecanone	1295	33.33	2.078	11.05	0.508	15.59	0.731	Fatty fruity-rosy-orange-like odor, oily-fruity, coconut.	
	2-dodecanone	1396	2.325	0.145	1.824	0.084	2.440	0.114	Fruity, citrus, floral odor.	

Family	Compound	L.R.I	WP		AG		MG		Organoleptic characteristics	T (ppb)
			A (10^{-6})	% area	A (10^{-6})	% area	A (10^{-6})	% area		
Ketones										
	benzophenone	>1600	16.80	1.048	n.d.	n.d.	7.882	0.370	Rose-like, geranium-like odor.	
Pyrazines										
	2,5-dimethylpyrazine	914	9.331	0.582	4.174	0.192	7.149	0.335	Chocolate, roasted nuts, earthy, chocolate taste.	1700
Pyridines										
	pyridine	753	2.774	0.173	n.d.	n.d.	0.744	0.035	Salty taste. Strong, amine-like, fishy, burnt odor.	820
Other nytrogenated derivative s										
	indole	1307	9.076	0.566	110.32	5.068	38.72	1.815	Floral odor and taste in dilution.	140
Saturated hydrocarbons										
	heptane	700	3.140	0.196	89.131	4.094	120.6	5.653		
	octane	800	39.16	2.442	35.33	1.623	48.62	2.279	Hydrocarbon odor (gasoline-like).	
	nonane	900	14.80	0.923	12.979	0.596	17.12	0.803	Hydrocarbon odor (gasoline-like).	
	2,2,4,6,6-pentamethylheptane,	995	4.748	0.296	n.d.	n.d.	n.d.	n.d.		
	decane	1000	n.d.	n.d.	n.d.	n.d.	14.72	0.690	Hydrocarbon odor (gasoline-like).	
	undecane	1100	14.91	0.930	12.88	0.592	11.37	0.533	Gassy, hydrocarbon odor.	
	dodecane	1200	9.839	0.613	2.954	0.136	3.362	0.158	Sweet hydrocarbon like, weak.	
	tridecane	1300	21.25	1.325	3.158	0.145	4.396	0.206	Hydrocarbon odor.	
	tetradecane	1400	9.405	0.586	4.076	0.187	4.830	0.226	Mild hydrocarbon odor.	
	pentadecane	1500	16.80	1.048	26.588	1.221	10.65	0.500	Mild hydrocarbon odor.	
	hexadecane	1600	19.97	1.245	4.732	0.217	7.870	0.369	Weak waxy hydrocabon odor.	
	2,6,10,14-tetramethylpentadecane	>1600	26.42	1.647	16.472	0.757	10.72	0.503	Waxy, oily, slight fish connotation.	
Unsaturated hydrocarbons										
	2-ethyl-1-hexene	791	14.60	0.911	6.380	0.293	5.456	0.256		
	2-propenylcyclopentane	793	17.26	1.076	13.468	0.619	14.47	0.679		

Family	Compound	L.R.I	WP		AG		MG		Organoleptic characteristics	T (ppb)
			A (10^{-6})	% area	A (10^{-6})	% area	A (10^{-6})	% area		
Unsaturated hydrocarbons										
	(E)-4-Octene	797	1.307	0.082	n.d.	n.d.	11.54	0.541	Hydrocarbon, gassy (gasoline) notes.	
	(E)-2-Octene	807	7.927	0.494	5.304	0.244	6.043	0.283	Hydrocarbon, gassy (gasoline) notes.	
	(Z,Z)-3,5-Octadiene	815	22.10	1.378	26.602	1.222	31.74	1.488		
	1-nonyne	835	2.141	0.133	0.551	0.025	0.772	0.036		
	2-methyl 1-octene	888	1.973	0.123	1.076	0.049	1.073	0.050		
Aromatic hydrocarbons										
	p-xylene	866	4.802	0.299	3.112	0.143	3.091	0.145	Resinous-balsamic pungent gassy-	730
	o-xylene	873	17.24	1.075	11.81	0.543	12.20	0.572		
	styrene	897	7.410	0.462	9.998	0.459	5.456	0.256		
	1-propylbenzene	960	2.134	0.133	0.890	0.041	0.937	0.044		
	1-ethyl-3-methylbenzene	968	8.715	0.543	3.919	0.180	3.208	0.150		
	1,2,4-trimethylbenzene,	1000	21.35	1.331	12.46	0.572	n.d.	n.d.		
	m-diethyl benzene	1032	7.075	0.441	4.108	0.189	4.366	0.205		
	o-diethyl benzene	1057	3.126	0.195	1.203	0.055	0.940	0.044		
	1-methyl-4-propylbenzene,	1059	5.033	0.314	1.511	0.069	1.492	0.070		
	1-methyl-2-propylbenzene	1065	5.768	0.360	2.500	0.115	2.745	0.129		
	2-ethyl-1,4-dimethylbenzene	1085	9.471	0.591	4.588	0.211	5.635	0.264		
	1-ethyl-2,3-dimethylbenzene	1089	7.588	0.473	8.299	0.381	9.808	0.460		
	cis-2-methyldecalin,	1113	3.532	0.220	1.792	0.082	2.068	0.097		
	trans-2-methyldecalin	1128	5.333	0.333	1.485	0.068	1.537	0.072		
	1-methyl-3,5-diethylbenzene	1132	3.647	0.227	1.543	0.071	1.652	0.077		
	(1-isopropenyll)benzene	1154	3.499	0.218	0.857	0.039		n.d.		
	2-ethenyl-1,4-dimethylbenzene	1166	8.083	0.504	1.876	0.086	1.922	0.090		
	Tetralin	1180	7.539	0.470	1.429	0.066	1.168	0.055		
	1,5-dimethyl-decalin	1184	5.344	0.333	0.940	0.043	0.844	0.040		

Family	Compound	L.R.I	WP		AG		MG		Organoleptic characteristics	T (ppb)
			A (10^{-6})	% area	A (10^{-6})	% area	A (10^{-6})	% area		
Aromatic hydrocarbons										
	naphthalene	1204	6.583	0.410	1.376	0.063	1.115	0.052	Characteristic dry tarry hydrocarbon odor, musty taste.	
	2-methyl-tetralin	1235	1.044	0.065	n.d.	n.d.	n.d.	n.d.		
	1-methylnaphthalene	1338	1.650	0.103	n.d.	n.d.	n.d.	n.d.	Green, musty taste, earthy, phenolic.	
	2,6-dimethylnaphthalene	1432	1.641	0.102	n.d.	n.d.	n.d.	n.d.		
Organosulphur compounds										
	dimethyl disulfide	748	n.d.	n.d.	28.436	1.306	n.d.	n.d.	Strong onion, cabbage-like odor.	
Terpenes										
	α-pinene	942	3.249	0.203	2.313	0.106	2.429	0.114	Resinous, pine odor, turpentine taste.	6.0
	Δ3-carene	1019	2.386	0.149	0.875	0.040	0.866	0.041	Fresh, harsh, terpentine notes.	
	dl-limonene	1037	1.653	0.103	n.d.	n.d.	1.075	0.050		
Total area			1603		2177		2133			
Number of compounds			103		81		91			

Appendix D. *Volatile compounds detected in the extract obtained with supercritical carbon dioxide (Pext = 180 bar, Text = 50ºC, Psep = 42 bar), L.R.I. = linear retention index; T = odor threshold; A = abundance (chromatographic area),*

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)	Organoleptic characteristics	T (ppb)
Alcohols						
	1-penten-3-ol	700	134.375	4.387	Pungent, grassy, alliaceous-like, green vegetable, fruity taste.	400
	3-methyl-1-butanol	754	4.059	0.133	Breathtaking, alcoholic odor, in dilution a winey-brandy.	250
	2-methyl-1-butanol	758	0.846	0.028		
	1-cyclopenten-3-ol	772	3.190	0.104		
	1-hexanol	885	7.274	0.238	Chemical, winey, slight fatty-fruity odor.	2500
	3,5-octadien-2-ol	892	4.073	0.133	Mild, pleasant fatty odor, Chicken fat with a creamy waxy nuance.	
	(E,E)-2,4-octadien-1-ol	895	9.510	0.311		
	5-methyl- 5-Hexen-2-ol	899	21.702	0.709		
	6-methyl-2-heptanol	980	4.231	0.138		
	p-methylanisole	982	1.255	0.041		
	(5Z)-octa-1,5-dien-3-ol	993	143.713	4.692	Earthy, mushroom-like.	
	1-octen-3-ol	996	77.415	2.528	Very strong, sweet, earthy mushroom odor and taste.	1
	3-octanol	1012	3.537	0.115	Herbaceous, oily-nutty, mushroom odor.	
	2-ethyl-1-hexanol	1044	15.896	0.519	Sweet, oily, weak rose odor, fatty-fruity musty, tea-floral taste.	830
	benzyl alcohol	1076	6.457	0.211	Faint, sweet, fruity aroma, sweet taste.	10000
	benzyl alcohol	1076	6.457	0.211	Faint, sweet, fruity aroma, sweet taste.	10000
	2,6-dimethyl-1-heptanol	1109	5.922	0.193		
	4-ethylphenol	1219	6.013	0.196		
	α-terpineol	1235	3.212	0.105	Sweet, floral (lilac), lime odor, very sweet taste in dilution.	330-350
	2-ethyl-4,5-dimethylphenol	1271	1.027	0.034		
	(Z)-4-decen-1-ol	1280	2.820	0.092		
	4-ethylbenzenemethanol	1329	16.846	0.550		
	2,5-diisopropylphenol	1336	6.541	0.214		

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)	Organoleptic characteristics	T (ppb)
Alcohols						
	2,6-diisopropylphenol	1370	4.090	0.134		
	BHT	1475	2.326	0.076		
	1-dodecanol	1493	11.987	0.391	Oily-fatty, slight waxy odor, fatty waxy taste.	
Aldehydes						
	3-methylbutanal	681	1.073	0.035	Powerful, penetrating, cheesy-sweaty-fruity in dilution.	0.2-2
	(E)-2-butenal	683	1.076	0.035	Sharp, warm, spciy odor.	
	2-methyl-2-butenal	767	5.803	0.189	Green, herbaceous, fruity-green-nutty character in dilution.	
	(E)-2-pentenal	782	111.646	3.645	Powerful, fruity, apple-like in dilution.	1500
	3-methyl-2-butenal	814	40.831	1.333	Spicy, cinnamon, fruiity, nutty odor with cheese undertone.	
	hexanal	821	266.333	8.696	Strong, penetrating, fatty-green, grassy unripe fruit odor.	4.5
	2-methyl-2-pentenal	853	13.983	0.457	Pungent fruity odor, sharp sweet, fruity, jammy brown taste.	
	(E)-2-hexenal	880	103.924	3.393	Green, fruity, fresh, apple and woody with leafy and grassy.	
	heptanal	925	54.901	1.793	Fatty, in dilution sweet, fruity, nutty, fatty-cognac like.	3
	(E)-2-heptenal	986	25.672	0.838	Intense green, sweet, fresh fruity apple skin nuances at 4 ppm.	13
	benzaldehyde	1008	44.289	1.446	Odor of bitter almond oil, characteristic sweet cherry taste.	350
	octanal	1026	13.572	0.443	Fatty-fruity odor, sweet, citrus-orange-fatty taste.	0.7
	(E,E)-2,4-heptadienal	1047	222.953	7.280	Fatty, green, with an oily, greasy note.	
	(E)-2-octenal	1087	106.271	3.470	Peculiar fatty, green-grassy-leafy odor, fatty-green taste.	3
	2-phenylacetaldehyde	1092	68.836	2.248	Strong floral green odor (rose-hyacinth), floral almond taste.	4
	4-hydroxybenzaldehyde	1095	8.120	0.265	Weak phenolic-woody, hay, bitter almond notes.	
	2-propyl-(E)-2-hexenal	1105	7.542	0.246		
	nonanal	1128	66.888	2.184	Fatty-floral-rose, waxy odor, citrus taste in dilution.	1
	(E,E)-2,4-octadienal	1148	7.481	0.244	Strong fatty odor, green fruity, melon, citrus, fatty and tallow.	1
	(E,Z)-2,6-nonadienal	1185	45.454	1.484	Odor like cucumber, green melon or violet leaf in dilution.	0.01
	(E)-2-nonenal	1189	14.452	0.472	Strong, fatty-orris odor, in dilution, waxy-cucumber-melon.	0.08
	4-ethylbenzaldehyde	1195	6.373	0.208	Bitter-almond, cherry-like.	
	2-phenylpropenal	1208	6.013	0.196		

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)	Organoleptic characteristics	T (ppb)
Aldehydes						
	2,6-dimethylbenzaldehyde	1213	4.140	0.135		
	decanal	1230	8.576	0.280	Strong, penetrating, sweet, orange peel odor, citrus taste.	0.1-2
	(E,E)-2,4-nonadienal	1254	1.974	0.064	Strong fatty type odor and taste, chicken fat on dilution.	0.09
	β-cyclocitral	1264	1.802	0.059	Sweet, mild green, grassy floral hay-like odor.	5
	(E)-2-decenal	1292	35.647	1.164	Waxy, fatty, earthy, coriander, mushroom, green, pork fat.	0.4
	(2-methyl-cyclohex-2-enylidene)-acetaldehyde	1318	19.076	0.623		
	2-phenyl-2-butenal	1322	1.734	0.057	Phenylacetaldehyde-like, floral, honey cocoa notes.	
	undecanal	1332	6.279	0.205	Sweet, fatty, waxy-floral-citrus odor, fatty citrus taste.	5
	(E,E)-2,4-decadienal	1358	7.022	0.229	Strong, deep fat flavor, fatty-citrus notes.	0.07
	α-methylcinnamaldehyde	1384	5.937	0.194	Sweet, cinnamon-spicy odor and taste.	
	(E)-2-undecenal	1393	25.038	0.818	Fresh, sweet-citrus, orange-lemon peel odor in dilution.	
	dodecanal	1433	3.948	0.129	Sweet, waxy-fatty-citrus herbaceous odor, in dilution violet.	2
Esters						
	2-methoxycarbonyl-2-(cis-2'pentenyl)-3-methoxycarbonylcethylcyclopentane	933	12.227	0.399		
	methyl caprate	1338	2.604	0.085	Fatty, oily, fruity-winey-cognac.	
	methyl laurate	1538	27.626	0.902	Waxy, creamy fatty taste.	
	ethyl phthalate	1639	5.147	0.168		
	methyl myristate	1736	10.240	0.334	Weak, fatty-cognac, oily-orris odor, weak fatty-waxy taste.	
Ketones						
	1-penten-3-one	706	48.593	1.587	Strong, penetrating pungent (alliaceous-like) odor.	1
	2,3-pentanedione	721	40.466	1.321	Oily-buttery, fatty odor, butter, cream, milk taste.	
	2,3-hexanedione	806	5.643	0.184	Strong, creamy, sweet buttery odor & butter-cheese taste.	
	2-heptanone	909	5.989	0.196	Creamy, bitter. Fruity, cheeese, cinnamon odor, in dilution-blue cheese-fruity.	140-3000
	1-octen-3-one	999	27.038	0.883	Powerful, harsh metallic mushroom like odor.	0.005

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)	Organoleptic characteristics	T (ppb)
Ketones						
	3,5-octadien-2-one	1099	81.465	2.660	Woody, it provides freshness to green tea, mushroom and fatty odor.	100-150
	2,5-octanedione	1003	54.884	1.792	Sweet, buttery-oily odor with herbaceous-cheesy undertone, yet not rancid or unpleasant on dilution.	
	(E)-3-octen-2-one,	1031	98.568	3.218	Earthy-mushroom, fruity, blueberry note.	
	2-nonanone	1111	8.589	0.280	Fruity, fatty-cheese odor, fruity cheese taste.	5-200
	p-isopropenylacetophenone	1360	7.573	0.247	Herbaceous, woody, orris odor, spicy-fruity taste.	
	3-Undecen-2-one	1367	14.006	0.457		
	2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione	1498	16.858	0.550		
Pyrazines						
	methylpyrazine	856	2.222	0.073	Green, nutty, cocoa, musty, potato, fishy-ammoniacal notes.	60
	2,5-dimethylpyrazine	945	45.889	1.498	Chocolate, roasted nuts, earthy, chocolate taste.	1700
	2,3,5-trimethylpyrazine	1032	14.820	0.484	Nutty, baked potato, roasted peanut, cocoa, burnt notes.	400
	2-ethyl-5-methylpyrazine	1036	5.708	0.186	Coffee bean, Nutty, roasted, somewhat "grassy".	100
	3-Methyl-2-vinylpyrazine	1054	10.589	0.346		
	3-ethyl-2,5-dimethylpyrazine	1103	10.350	0.338	Cocoa, chocolate, nutty (burnt almond) notes.	
	2,3,5,6-tetramethylpyrazine	1113	5.922	0.193	Weak, nutty, musty, chocolate odor, chocolate taste.	1000
	2-acetyl-3-methylpyrazine	1117	16.456	0.537	Nutty, popcorn, cereal, roasted grain, cocoa, coffee, shellfish nuances.	20
	2,3,5-trimethyl-6-ethylpyrazine	1181	1.234	0.040		
Pyridines						
	pyridine	780	1.099	0.036	Salty taste. Strong, amine-like, fishy, burnt odor.	820
	ethyl 3-pyridincarboxylate ester	963	0.906	0.030	Sweet, herbaceous tobacco-like odor.	
Other nytrogenated derivatives						
	indole	1361	18.979	0.620	Floral odor and taste in dilution.	
	3,3-dimethyl-1-indanone	1454	2.787	0.092		
Furans						
	2-ethylfuran	710	75.267	2.458	Strong, sweet-ethereal, burnt brown musty odor.	8000

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)	Organoleptic characteristics	T (ppb)
Furans						
	2-(2-propenylfuran	874	4.963	0.162		
	2-n-pentylfuran	1005	37.311	1.218	Ethereal rum, grassy, earthy, beany with vegetable and fruity notes.	600
	trans-2-(1-pentenyl)furan	1017	104.273	3.405		
	2-(1-cyclopentenyl)furan	1155	6.268	0.205		
Saturated hydrocarbons						
	dodecane	1200	3.533	0.115	Sweet hydrocarbon like, weak.	
	7-methyl-bicyclo[2,2,1]heptane	1279	3.059	0.100		
	tridecane	1300	0.617	0.020	Hydrocarbon odor.	
	tetradecane	1400	11.660	0.381	Mild hydrocarbon odor.	
	hexadecane	1600	6.138	0.200	Weak waxy hydrocabon odor.	
	2,6,10,14-tetramethylpentadecane	1694	17.785	0.581	Waxy, oily, slight fish connotation.	
	heptadecane	1700	5.452	0.178	Weak waxy hydrocabon odor.	
Unsaturated hydrocarbons						
	1,3,5-cycloheptatriene	784	35.645	1.164		
	1,3-octadiene	834	0.708	0.023		
	cyclooctene	837	0.286	0.009		
	(E,E,Z)-1,3,5-octatriene	893	4.916	0.161		
	(Z,Z,Z)-1,3,5-octatriene	895	2.387	0.078		
	3,3-dimethyl-6-methylenecyclohexene	978	0.706	0.023		
	(E,Z,Z)-1,3,5-undecatriene	1091	68.836	2.248		
	4-methyl-1-(1-methylethenyl)cyclohexene	1285	14.038	0.458		
	1-pentadecene	1493	14.137	0.462		
	1-hexadecene	1595	4.543	0.148		
	3',5'-diphenylcyclohexa-2,5-dienes	1709	0.697	0.023		
Aromatic hydrocarbons						
	p-xylene	891	0.651	0.021	Characteristic resinous-balsamic pungent gassy-plastic odor.	730

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)	Organoleptic characteristics	T (ppb)
Aromatic hydrocarbons						
	o-xylene	917	1.918	0.063		
	styrene	920	1.395	0.046		
	1,2,4-trimethylbenzene	1020	3.761	0.123		
	1-methyl-3-isopropylbenzene	1080	2.779	0.091		
	2-ethyl-1,3-dimethylbenzene	1158	2.157	0.070		
	m-diisopropylbenzene	1168	1.927	0.063		
	p-diisopropylbenzene	1174	1.839	0.060		
	1-isopropenyl-2-isopropylbenzene	1241	1.985	0.065		
	naphthalene	1249	2.525	0.082	Characteristic dry tarry hydrocarbon odor, musty taste.	
	1-isopropenyl-4-isopropylbenzene	1275	0.519	0.017		
	1,3-diisopropenylbenzene	1306	5.213	0.170		
	1,4-diisopropenylbenzene	1347	2.223	0.073		
	1,2-diisopropenylbenzene	1410	6.072	0.198		
	2-butenylbenzene	1525	3.609	0.118		
	1,1'-propylidenebisbenzene	1618	3.342	0.109		
Organosulphur compounds						
	2-acetylthiazole	1065	12.665	0.414	Nutty, cereal, cracker, popcorn, grill hazelnut, bread, toasted cereal notes.	
Terpenes						
	d-limonene	1050	59.497	1.943		
Total area			**3044**			
Total number of compounds			**132**			

Appendix E. *Volatile compounds in brown crab oil stored at -18ºC during two months. L.R.I= linear retention index; A = abundance.*

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)
Acids				
	2-methylpropanoic acid	764	0.334	0.005
	3-methylbutanoic acid	849	82.892	1.326
	2-methylbutanoic acid	857	52.368	0.838
	hexanoic acid	980	34.415	0.551
Alcohols				
	3-methyl-1-butanol	753	4.680	0.075
	benzyl alcohol	1079	8.016	0.128
	BHT	1532	2.297	0.037
Aldehydes				
	pentanal	722	49.801	0.797
	hexanal	823	88.791	1.420
	heptanal	925	100.197	1.603
	benzaldehyde	1008	48.713	0.779
	octanal	1026	89.256	1.428
	(E,E)-2,4-heptadienal	1047	78.617	1.258
	(E)-2-octenal,	1087	83.396	1.334
	benzeneacetaldehyde	1091	73.350	1.173
	4-hydroxybenzaldehyde	1094	24.767	0.396
	nonanal	1128	84.119	1.346
	(E,E)-2,4-octadienal	1148	13.292	0.213
	(E,Z)-2,6-nonadienal	1185	55.044	0.881
	(Z)-2-nonenal	1189	24.862	0.398
	2,6-dimethylbenzaldehyde	1213	5.048	0.081
	decanal	1230	14.439	0.231
	(E,E)-2,4-nonadienal	1255	2.065	0.033
	2-isopropylbenzaldehyde	1259	2.324	0.037
	(E,E)-2,4-decadienal,	1358	7.166	0.115
	undecanal	1434	5.234	0.084
Ketones				
	1-penten-3-one	708	23.019	0.368
	(E)-3-octen-2-one	1029	75.180	1.203
	2-nonanone	1111	29.007	0.464
	actophenone	1113	14.014	0.224
	2-undecanone	1313	5.372	0.086
	3-undecen-2-one	1367	14.857	0.238
	4,4a,5,6,7,8-2(3H)-naphthalenone	1371	6.278	0.100
	β-ionone	1513	2.310	0.037
	1,1'-(1,4-phenylene)bis-ethanone	1525	4.094	0.065
Furans				
	2-ethylfuran	713	36.733	0.588
	2-(2-propenyl)furan	875	6.365	0.102
	2-pentylfuran	1005	32.720	0.523
	trans-2-(2-pentenyl)furan	1017	116.288	1.860

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)
Nytrogenated derivatives				
	3,6-Dimethyl-1-indanone	1416	11.085	0.177
	2-methylindole	1459	2.008	0.032
	2,4-dimethylquimoline	1516	7.724	0.124
	3-ethenylcyclopentanecarboxamide	1727	21.138	0.338
Saturated hydrocarbons				
	2-methylpentane	576	25.791	0.413
	3-methylpentane	588	41.084	0.657
	methylcyclopentane	636	30.923	0.495
	isooctane	689	149.089	2.385
	heptane	700	93.770	1.500
	4-methyloctane	864	7.156	0.114
	2,3-dimethylheptane	870	1.278	0.020
	3-methyloctane	872	12.108	0.194
	1,2,4-trimethylcyclohexane	895	16.553	0.265
	nonane	900	191.354	3.061
	cis-1-ethyl-4-methylcyclohexane	905	25.370	0.406
	2,4,6-trimethylheptane,	914	13.676	0.219
	3,5-dimethyloctane	920	18.060	0.289
	2,7-dimethyloctane	929	12.611	0.202
	3,6-dimethyloctane	934	74.858	1.198
	3-ethyl-2-methylheptane	940	90.887	1.454
	propylcyclohexane	946	62.540	1.001
	4-ethyloctane	954	27.774	0.444
	5-methylnonane	959	38.217	0.611
	4-methylnonane	961	102.134	1.634
	2-methylnonane	966	100.234	1.604
	3-ethyloctane	968	21.775	0.348
	3-methylnonane	972	127.571	2.041
	1-ethyl-2,3-dimethylcyclohexane	989	12.878	0.206
	1,2,4,5-tetramethylcyclohexane	978	40.010	0.640
	1-methyl-3-isopropylcyclohexane	987	59.070	0.945
	1-methyl-4-propylcyclohexane	996	61.927	0.991
	decane	1000	755.301	12.083
	1-methyl-2-propylcyclohexane	1012	38.573	0.617
	4-ethylnonane	1020	138.378	2.214
	1,2-dibutylcyclopropane	1037	13.706	0.219
	2-ethyl-1,3-dimethylcyclohexane	1041	14.084	0.225
	isobutylcyclohexane	1044	45.315	0.725
	5-methyldecane	1055	58.255	0.932
	4-methyldecane	1059	35.951	0.575
	2-methyldecane	1064	67.638	1.082
	3-methyldecane	1071	52.731	0.844
	undecane	1100	175.674	2.810
	2-methylundecane	1164	8.617	0.138
	3-methylundecane	1168	12.502	0.200
	3,8-dimethyldecane	1171	6.585	0.105
	dodecane	1200	30.664	0.491

Family	Compound	L.R.I.	A. (10^{-6})	Area (%)
Saturated hydrocarbons				
	cyclododecane	1257	2.860	0.046
	tridecane	1300	12.391	0.198
	Decane, 3-cyclohexyl-	1311	1.790	0.029
	tetradecane	1400	17.549	0.281
	1-pentyl-2-propylcyclopentane	1465	1.158	0.019
	pentadecane	1500	30.854	0.494
	hexadecane	1600	7.086	0.113
	2,6,10,14-tetramethylpentadecane	1696	6.416	0.103
	heptadecane	1700	3.691	0.059
Unsaturated hydrocarbons				
	1,3-octadiene	836	7.040	0.113
	1-hexene	885	1.300	0.021
	1-dodecene	1195	88.396	1.414
	(Z)-2-dodecene	1218	3.951	0.063
	(Z)-6-tridecene	1288	5.465	0.087
	(E)-7-tetradecene	1354	0.924	0.015
	(E)-2-tetradecene	1396	59.332	0.949
	1-hexadecene	1597	18.959	0.303
Aromatic hydrocarbons				
	styrene	883	35.626	0.570
	p-xylene	891	137.935	2.207
	o-xylene	917	22.794	0.365
	1-ethyl-4-methylbenzene	985	17.907	0.286
	1-ethyl-2-methylbenzene	989	6.819	0.109
	1-ethyl-3,5-dimethylbenzene	1080	17.314	0.277
	1-ethyl-2,4-dimethylbenzene	1105	30.021	0.480
	1-ethyl-2,3-dimethylbenzene	1112	13.658	0.219
	1-methyl-2-(2-propenyl)benzene	1121	8.624	0.138
	1-methyl-3-(1-isopropyl)benzene	1135	9.253	0.148
	1,2,3,4-tetramethylbenzene	1151	15.591	0.249
	1-isopropenyl-3-isopropylbenzene	1241	6.229	0.100
	naphthalene	1249	8.438	0.135
	1-isopropenyl-2-isopropylbenzene	1275	1.263	0.020
	1,3-diisopropenylbenzene	1306	10.057	0.161
	1,4-diisopropenylbenzene	1347	3.833	0.061
	trimethyl(1-methylethyl)benzene	1360	9.742	0.156
Organosulphur compounds				
	2-propylthiophene	923	83.610	1.338
Terpenes				
	dl-Limonene	1050	150.925	2.414
Esters				
	methyl laurate	1539	3.437	0.055
	diethyl phthalate	1640	4.478	0.072
Total number of compounds		124		
Total volatile area		5268		