UNIVERSITAT POLITÈCNICA DE VALÈNCIA

DEPARTAMENTO DE TECNOLOGÍA DE ALIMENTOS

Desarrollo y optimización de nuevos procesos para la obtención de productos de la pesca ahumados

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Valencia, Octubre de 2016

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CONSIDERAN que la memoria titulada **DESARROLLO Y OPTIMIZACIÓN DE NUEVOS PROCESOS PARA LA OBTENCIÓN DE PRODUCTOS DE LA PESCA AHUMADOS** que presenta Dña. ARANTXA RIZO PÁRRAGA, para aspirar al grado de Doctora por la Universidad Politécnica de Valencia, que ha sido realizada bajo nuestra dirección en el departamento de Tecnología de Alimentos, reúne las condiciones adecuadas para constituir su tesis doctoral, por lo que AUTORIZAN al interesado para su presentación.

Valencia, 25 de Octubre de 2016

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RESUMEN

La industria de los salazones y ahumados de pescado necesita innovar en sus procesos productivos, optimizando los mismos para rentabilizar sus actividades, mejorando la calidad nutricional de sus productos y garantizando la inocuidad de los mismos. En la actualidad, la mejora de los procesos de salado y ahumado se centra principalmente en agilizar los procesos, aplicando nuevas técnicas alternativas que permitan reducir los tiempos de procesado, minimizar la generación de residuos y conseguir una mayor estandarización de sus productos.

En el presente trabajo se ha desarrollado un nuevo método de salado-ahumado simultáneo, empleando un salado controlado, humo líquido y bolsas permeables al vapor de agua, para obtener productos de la pesca ahumados. La viabilidad de la técnica se estudió en tres especies de pescado (salmón, bacalao y trucha), con el fin de optimizar las condiciones de procesado, en función de las características de la materia prima y de productos comerciales de referencia. Posteriormente, se evaluó la calidad y vida útil durante el almacenamiento en refrigeración de los productos obtenidos. En la siguiente etapa, se llevó a cabo una mejora en la calidad nutricional de los productos desarrollados de salmón y trucha ahumada a través de la reducción de su contenido en sal, mediante el reemplazo de NaCl por otras sales y se estudió su efecto sobre los parámetros fisicoquímicos y sensoriales, así como en la calidad y vida útil durante su almacenamiento. Finalmente, se estudió la aplicación de la técnica de espectroscopía de impedancia como herramienta de monitorización on-line del proceso de salado-ahumado de salmón.

Los resultados obtenidos han demostrado que el salado-ahumado simultáneo en bolsas permeables al vapor de agua permite obtener productos de pescado ahumado con características similares a los productos

comerciales, sin afectar a su aceptación por parte del consumidor y estables microbiológicamente durante el almacenamiento. El nuevo método aporta ventajas respecto a los procesos tradicionales de ahumado, como son la reducción de etapas de procesado, la disminución de residuos de salmuera y la protección del producto frente a contaminaciones durante el procesado. En el presente estudio, se ha conseguido sustituir un 50% de NaCl por KCl en el producto de salmón y trucha ahumada sin afectar a la calidad físico-química y sensorial, ni a la estabilidad microbiológica durante su almacenamiento en refrigeración. La técnica de espectroscopía de impedancia ha demostrado ser una herramienta eficaz en la monitorización on-line de los parámetros fisicoquímicos que caracterizan el salado-ahumado de salmón.

ABSTRACT

Fish salting and smoking industry need to innovate in their production processes, optimizing them to increase the profitability of its activities, improving the nutritional quality of their products and ensuring food safety. Currently, salting and smoking improvements focus primarily on speed up processes, using alternative techniques, which allow reducing processing steps, minimize waste and achieve a greater standardization of their products.

In this study, a simultaneous salting-smoking method has been developed, employing a controlled salting, liquid smoke and water vapour permeable bags, to obtain smoked fish products. The feasibility of this technique was studied on three fish species (salmon, cod and trout), with a view to optimizing processing conditions, according to the characteristics of the raw material and the commercial products used as a reference. Afterwards, the quality and shelf-life during cold storage of the products obtained was evaluated. In the next step, the nutritional quality of salmon and trout products developed was improved, by means of the partial replacement of NaCl by other salts, and the effect on the physicochemical and sensory characteristics, as well as the quality and shelf-life during storage was evaluated. Finally, the application of impedance spectroscopy as an on-line monitoring technique on the salting-smoking was studied.

The results show that the simultaneous salting-smoking in water vapour permeable bags is suitable to obtain smoked fish products, with similar characteristics to the commercial products, without affecting consumer acceptance, and microbiologically stable during storage. The new method offers advantages compare with the traditional methods such as the reduction of processing steps, less brine waste, and the protection against contaminations during processing.

In the present study, replacement of 50% NaCl by KCl has been achieved in the smoked salmon and trout products, whithout affecting physicochemical and sensory quality or the microbial stability during cold storage. The spectroscopy impedance has shown to be an effective tool in the on-line monitoring of the physicochemical parameters in the salting-smoking.

RESUM

La indústria de les saladures i fumats de peix necessita innovar en els seus processos productius, optimitzant els mateixos per a rendibilitzar les seues activitats, millorant la qualitat nutricional dels seus productes i garantint la innocuïtat dels mateixos. En l'actualitat, la millora dels processos de salat i fumat se centra principalment a agilitzar els processos, aplicant noves tècniques alternatives que permeten reduir els temps de processat, minimitzar la generació de residus i aconseguir una major estandardització dels seus productes.

En el present treball s´ha desenvolupat un nou mètode de salat-fumat simultani, emprant un salat controlat, fum líquid i bosses permeables al vapor d'aigua, per a obtindre productes de la pesca fumats. La viabilitat de la tècnica es va estudiar en tres espècies de peix (salmó, abadejo i truita), a fi d'optimitzar les condicions de processat, en funció de les característiques de la matèria prima i de productes comercials de referència. Posterioment, es va avaluar la qualitat i vida útil durant l'emmagatzemament en refrigeració dels productes obtinguts. En la següent etapa, es va dur a terme una millora en la qualitat nutricional dels productes desenvolupats de salmó i truita ahumada a través de la reducció del seu contingut en sal, per mitjà del reemplaçament de NaCl per altres sals i es va estudiar el seu efecte sobre els paràmetres físico-químics i sensorials, així com en la qualitat i vida útil durant el seu emmagatzemament. Finalment, es va estudiar l'aplicació de la tècnica d'espectroscòpia d'impedància com a ferramenta de monitorització on-line del procés de salat-fumat de salmó.

Els resultats obtinguts han mostrat que el salat-fumat simultani en bosses permeables al vapor d'aigua permet obtindre productes de peix fumat, amb característiques semblants als productes comercials, sense afectar la seua acceptació per part del consumidor i estables microbiològicament durant

l'emmagatzemament. El nou mètode aporta avantatges respecte als processos tradicionals de fumat, com són la reducció d'etapes de processat, la disminució de residus de salmorra i la protecció del producte davant contaminacions durant el processat. En el present estudi, s'ha aconseguit substituir un 50% de NaCl per KCl en el producte de salmó i truita ahumada sense afectar la qualitat físico-química i sensorial, ni a l'estabilitat microbiològica durant el seu emmagatzemament en refrigeració. La tècnica d'espectroscòpia d'impedància ha demostrat ser una ferramenta eficaç en la monitorització on-line dels paràmetres fisicoquímics que caracteritzen el salat-fumat de salmó.

Agradecimientos

Quiero dar las gracias a mis directoras de tesis Ana e Isabel por estos años de esfuerzo y dedicación para que este trabajo pudiera salir adelante. A José Manuel Barat por ofrecerme la oportunidad de trabajar en el grupo y realizar esta tesis.

A los alumnos que han realizado su Trabajo fin de Carrera y Fín de Máster conmigo, por su colaboración y compañía en el laboratorio.

A todos los compañeros que han compartido este periodo conmigo en el laboratorio y en la sala de becarios, a los que continúan, a los que terminaron y a los que se quedaron por el camino.

A los compañeros del "Coffee break", Carlos, Amparo, Chus, Javi y Puri por su amistad y su compañía.

A mis nuevas compañeras del IATA por todo su apoyo y por darme ánimos en la recta final de este camino.

A Fernando por aguantar todo este tiempo de distancia, por ser optimista y por sostenerme siempre.

ÍNDICE

INTRODUCCIÓN

1.1. LA INDUSTRIA DE LOS AHUMADOS DE PESCADO

Las técnicas tradicionales de salado y ahumado de pescado fueron desarrolladas hace miles de años para preservar los excedentes en las capturas, permitiendo que el pescado pudiera ser almacenado largos periodos de tiempo. Evidencias encontradas dentro de cuevas situadas lejos de la costa (huesos de peces marinos, cenizas), demuestran la práctica de alguna forma de secado y/o ahumado de pescado por parte del hombre primitivo hace más de 20.000 años (Gallart-Jornet et al., 2005).

El ahumado era una manera frecuente de conservar el pescado en el Norte de Europa debido a las condiciones meteorológicas del lugar, con climas fríos y lluviosos, mientras que el salado lo era en los países mediterráneos al contar con reservas de sal y veranos cálidos que favorecen el secado del pescado (Gallart-Jornet, 2006). El proceso original de ahumado de pescado consistía en posicionar el pescado cerca del fuego, en contacto directo con el humo y con ello se conseguía proteger el producto del ataque de los insectos y otros animales.

Hasta el siglo XX, el pescado era habitualmente sometido a salados intensos, casi llegando al punto de saturación, además de ser fuertemente secado y ahumado para alargar su vida útil de forma sustancial, sin la necesidad de almacenarlo a bajas temperaturas. Posteriormente, gracias al desarrollo de los sistemas de refrigeración y distribución, así como de las nuevas tecnologías de envasado, se hizo posible la comercialización del pescado fresco o congelado a nivel mundial, terminando con la necesidad ineludible de recurrir a las técnicas tradicionales de salado y/o ahumado, como forma de conservar el producto para su almacenamiento y/o transporte a largas distancias hasta ser consumido. En consecuencia, los procesos de ahumado y salado de pescado empezaron a ser más valorados por las características sensoriales que aportan al producto que como método de conservación. En este sentido, el creciente interés por el consumidor actual

hacia alimentos con contenidos en sal moderados y los aspectos negativos para la salud asociados con el ahumado, también han contribuido en gran medida a la reducción del contenido de sal y la intensidad de humo en estos productos (Birkeland & Skåra, 2008; Rørå et al., 2005). Por este motivo, hoy en día los ahumados de pescado son una semiconserva con menos sal, más humedad y menos aroma de humo que en el pasado, lo que implica que su vida útil sea más limitada. Su distribución y almacenamiento se realiza bajo temperaturas de refrigeración, lo que contribuye por otra parte, a una mejor conservación del producto.

En la actualidad, entre los productos de la pesca ahumados, el salmón ahumado es el más vendido, seguido de la trucha y el arenque (Arvanitoyannis & Kotsanopoulos, 2011). No obstante, en el mercado internacional es también común el ahumado de otras especies de pescado como el bacalao, la caballa y el atún. Algunos ejemplos de productos típicos de la gastronomía alemana son los ahumados en caliente, como el arenque "buckling" y las ventrescas de cazón "Schillerlocken'. En Grecia la anguila y la dorada ahumada son también populares (Arvanitoyannis & Kotsanopoulos, 2011).

Hace unas décadas, los ahumados de pescado eran considerados artículos de lujo sujetos a una gran estacionalidad. Hoy en día, la gran oferta comercial de estos productos y el abaratamiento del precio de los mismos ha hecho que pasen a ser productos de consumo popular y estén presentes de forma habitual en cualquier supermercado. El crecimiento del mercado de los ahumados fue impulsado por el desarrollo generalizado de la acuicultura, que comenzó a partir de los años 80, siendo el caso del salmón ahumado el más representativo. El gran aumento de la producción de salmón Atlántico "*Salmo salar*" de crianza, desplazó drásticamente a las especies de salmón de captura procedentes del Pacífico como materia prima para el ahumado. La introducción del salmón procedente de acuicultura supuso para la industria de

ahumados la disponibilidad de materia prima fresca, de calidad más o menos uniforme y a precio asequible todo el año. Este hecho propició a partir de la década de los 90, una intensa promoción del salmón ahumado en frío y el incremento de su consumo a nivel global, manteniéndose hasta nuestros días. No obstante, la utilización de materia prima de piscifactoria también implicó la aparición de ciertos problemas de calidad, relacionados con excesivo contenido de grasa, inestabilidad en el color, así como fenómenos de "gaping" y textura blanda (EC, 2001, Løje, 2007). La producción de salmón Atlántico de acuicultura a nivel mundial ha aumentado un 428% desde 1994 (Marine Harvest, 2015). En la actualidad, los principales países productores de salmón Atlántico en el mundo son Noruega, Escocia, Irlanda, Islas Faroe, Canadá, la costa noreste de los EE.UU., Chile y Australia (Tasmania) (FAO, 2014). En Europa, se estima que la producción en 2014 fue de aprox. 175,000 toneladas, siendo Alemania y Francia los principales mercados (MarineHarvest, 2015). Noruega es el mayor productor y se estima que alrededor de un 15% del salmón que sale de sus industrias es destinado a la elaboración de salmón ahumado para el mercado internacional (Birkeland & Skåra, 2008). En Europa, las mayores industrias de salmón ahumado manejan más del 60% del mercado y la producción se lleva a cabo principalmente en Polonia, Francia, Reino Unido, países bálticos y Holanda (Marine Harvest, 2015).

En España, el salmón ahumado es, al igual que en el resto de Europa, el pescado ahumado más consumido, con un 90% de las ventas de de este tipo de productos, seguido por la trucha y el bacalao. El mercado español de salmón ahumado se encuentra dominado por la marca de distribuidor, que supone un 90% de la oferta total (Rodriguez, 2010). España ocupa el séptimo lugar en el ranking europeo de consumo de salmón ahumado, con un consumo total de 6.300 toneladas en 2014 (Anónimo, 2015).

1.2. EL AHUMADO

1.2.1. El proceso de ahumado

El proceso tradicional de ahumado consta principalmente de tres etapas que se realizan consecutivamente: salado, secado y/o ahumado. El efecto conservante del proceso se debe a la combinación de la incorporación de la sal y la deshidratación, que reducen la a_w, inhibiendo el crecimiento de los microorganismos responsables del deterioro y la actividad enzimática. Asimismo, la deposición de sustancias fenólicas procedentes del humo ejercen también un efecto antioxidante y antimicrobiano en el pescado.

Existen diferentes técnicas para obtener pescado ahumado, el cual ha ido variando con el paso del tiempo para adaptarse a los nuevos medios tecnológicos y al gusto del consumidor actual. Tal y como se menciona anteriormente, en el pasado eran habituales procesos de salado y ahumado intensos que podían prolongarse hasta 1 semana. Hoy en día estos procesos son más suaves y pueden ser completados en 1-2 días, siendo necesario almacenar el producto envasado a vacío o en atmosfera modificada y a temperaturas de refrigeración para facilitar la conservación.

El esquema del proceso de ahumado en frío de salmón se muestra en la figura 1. El proceso comienza con la selección y clasificación de la materia prima en función de su calibre, para adaptar las condiciones de procesado a las características de la materia prima. Posteriormente, el pescado es acondicionado mediante el eviscerado, fileteado y limpiado, eliminando las espinas y el tejido adiposo superficial. Las especies de pequeño tamaño como los arenques y la caballa son normalmente ahumadas enteras y sin eviscerar.

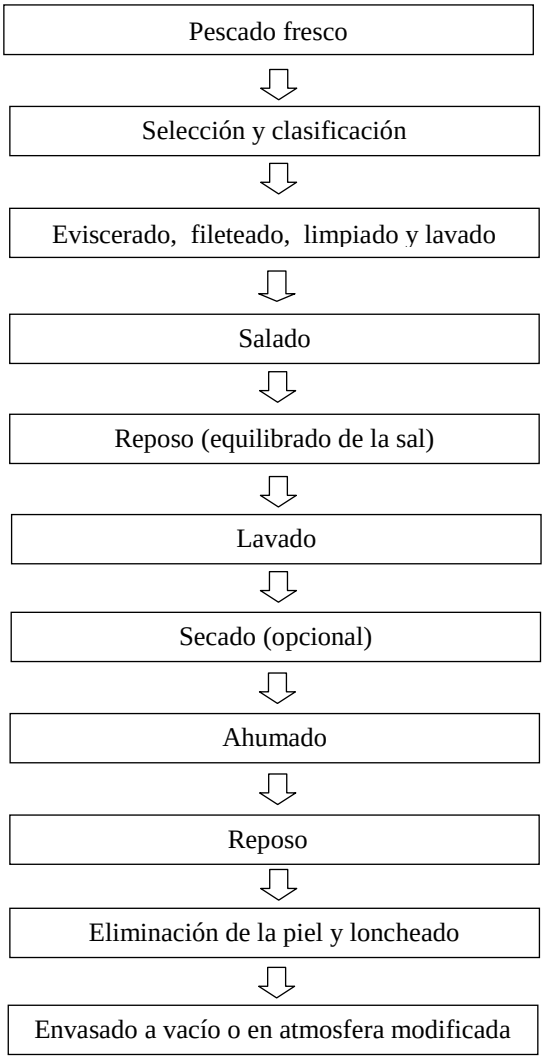

Fig. 1. Esquema general del proceso de ahumado en frío

A continuación, los filetes se someten a una etapa de salado. La incorporación de la sal al producto se realiza principalmente por medio de la salazón en seco, el salado por inmersión en salmuera o por técnicas más

modernas, como la inyección de salmuera (Røra et al., 2004). La utilización de una u otra técnica está condicionada por las características de la materia prima y, de manera significativa, por la duración del proceso de elaboración, el rendimiento esperado, así como las características físico-químicas y sensoriales requeridas del producto final (Løje, 2007). Actualmente, los productos de pescado ahumado comerciales suelen ser sometidos a salados ligeros, cuyo contenido en sal suele estar en el rango de 1,3-5 g /100 g de pescado (Birkeland & Skåra, 2008). El tiempo de salado puede variar entre 6-24 h y se recomienda que sea llevado a cabo a temperaturas de refrigeración por debajo de 10-15ºC (Birkeland et al., 2004; Codex 2003; FDA, 2001; Løje, 2007). Factores como el contenido lipídico del pescado, el tiempo, la temperatura y la humedad relativa afectan de forma importante a la penetración y difusión de la sal en el producto. En el caso del salado en seco, tras dosificarse la sal, el pescado se coloca en rejillas de metal y se deja un tiempo determinado en reposo para que la sal penetre dentro del músculo y asegurar su distribución de forma homogénea. Posteriormente se retira el exceso de sal de la superficie por medio del lavado de los filetes.

Una vez salado, el pescado es enjuagado y posteriormente sometido a un secado y/o ahumado por medio de la exposición al humo. En técnicas más actuales, el pescado puede ser también aromatizado con sabor a humo sin ser necesario el uso de humo natural, ni cámaras de ahumado.

El humo es generado por la combustión de la madera a niveles limitados de oxígeno, la cual a modo general se compone de un 50% de celulosa, un 25% de hemicelulosa y un 25% de lignina. Los materiales utilizados para la producción del humo son generalmente serrín, virutas y leños, y la combustión de los mismos se produce a temperaturas entre 180 y 500ºC. La composición del humo depende del tipo de madera utilizada, de la temperatura de generación del mismo, del contenido de oxígeno, pero

también de los procesos de limpieza del humo que pueden aplicarse inmediatamente después de ser generado (Simko, 2005).

El ahumado produce en el pescado una deshidratación superficial que origina una barrera protectora frente al ataque de los microorganismos. Paralelamente, varios componentes del humo como aldehídos, cetonas, alcoholes, ácidos, hidrocarburos, ésteres, fenoles y éteres, son depositados en la superficie del producto ahumado y van penetrando gradualmente en las capas interiores del músculo. Asimismo, las sustancias que se generan procedentes de la reacción de proteínas, de la oxidación lipídica y de las reacciones de Maillard imparten al pescado el color, sabor y aroma característico. El ahumado, al igual que el salado, tiene la capacidad de alargar la vida útil del pescado, protegiéndolo del crecimiento microbiano y bloqueando las reacciones enzimáticas (Arvanitoyannis & Kotsanopoulos, 2011; Sampels, 2015; Varlet et al., 2007). La humedad relativa y la temperatura afectan en gran medida a la difusión del agua, que se produce durante el ahumado/secado, al igual que sucede en el salado, por lo que estos parámetros deben ser cuidadosamente controlados. Una vez completado el ahumado, el pescado se deja reposar nuevamente. Posteriormente, se retira la piel del filete y éste es habitualmente cortado en lonchas. La congelación ligera de las piezas es a menudo practicada por las empresas para facilitar el loncheado (Løje, 2007). Finalmente, el producto es envasado a vacío o en atmosfera modificada para su comercialización (Truelstrup-Hansen, 1995).

1.2.2. Tipos de ahumado

La tecnología de ahumado ha evolucionado en las últimas décadas. Además del ahumado tradicional, que como se ha comentado, consiste en la exposición al humo procedente de la combustión lenta de madera, existen otros métodos más modernos.

En el pasado, los alimentos eran directamente expuestos al humo en la misma cámara en la que se generaba. En la actualidad, las cámaras de ahumado son cámaras climáticas que pueden ser usadas, no solo para el ahumado, sino también para el curado, secado y cocinado, etc. En los sistemas modernos, la cámara de producción de humo y la cámara donde se produce el ahumado del alimento se encuentran separadas. Esto provoca el enfriamiento del humo durante el camino, desde la cámara donde se produce hasta la zona de ahumado, lo que implica que parte de los compuestos nocivos para la salud como los hidrocarburos aromáticos policíclicos (HAPs) y el alquitrán, se queden en las paredes de la conducción, evitando en gran medida que éstos sean depositados sobre el alimento (Andrés et al., 2006).

En general, se reconocen cuatro procedimientos de ahumado según el sistema generador de humo utilizado: por combustión lenta sin llama, por placas termostáticas, por procedimientos de fricción y a través de condensados de humo (Codex, 2009).

En Europa, durante el año 2002, el 65% de los pescados ahumados fueron obtenidos por combustión lenta de madera, el 30% mediante el uso de placas termostáticas y el 5% mediante la vaporización de humo líquido (Varlet et al., 2007).

En función de la temperatura de procesado, el ahumado se divide en dos categorías:

El ahumado en frío, comentado anteriormente, se realiza a temperaturas inferiores a la temperatura de coagulación de las proteínas del músculo, normalmente alrededor de 30ºC, pudiendo variar entre 27 ºC y 38 ºC (Codex, 2003). En la industria, la humedad relativa utilizada suele estar comprendida entre 50-70% y el tiempo de procesado suele oscilar entre las 2 y las 12 h (Løje, 2007). El uso de temperaturas bajas durante el ahumado favorece un

ahumado ligero, texturas suaves y una menor oxidación (Espe et al., 2004; Rørå et al., 2005).

El ahumado en caliente se realiza habitualmente a temperaturas entre 70-80ºC. Estas temperaturas dan como resultado cierto grado de cocción en el pescado y por lo tanto, una textura diferente a la que resulta del ahumado en frío. La humedad relativa durante el proceso debe ser alta para evitar una deshidratación excesiva. Con este procedimiento el tiempo de ahumado suele ser generalmente más corto que en el ahumado en frio, reduciéndose a pocas horas o minutos (Andrés et al., 2006).

1.2.3. Los aromas de humo

El ahumado con aromas de humo se realiza empleando condensados de humo o mezclas artificiales de sabor, las cuales otorgan al pescado sabor a humo sin entrar en contacto con humo natural (Codex, 2003).

La utilización de aromas y condensados de humo se ha convertido en una alternativa muy interesante al ahumado tradicional para las industrias, ya que proporcionan el sabor a humo característico y presenta menos inconvenientes que el proceso tradicional.

Con el uso de los aromas de humo es posible controlar de forma efectiva la aparición de HAPs, perjudiciales para la salud humana. Por otro lado, se reduce considerablemente el tiempo necesario para conseguir las características organolépticas deseadas de los productos ahumados y requiere una menor inversión en equipos e instalaciones, siendo la manipulación más sencilla y segura, lo que supone para las empresas una reducción de costes y de tiempos de producción (Muratore et al., 2007; Simko, 2005).

La producción de los aromas de humo comienza con la condensación del humo. Los condensados de humo son productos obtenidos mediante la degradación térmica controlada de madera, en condiciones de suministro limitado de oxígeno (pirólisis) (Codex, 2009). Los vapores obtenidos son posteriormente condensados y separados por procesos físicos, dando como resultado un condensado primario de humo de base acuosa, una fase de alquitrán y otra fase oleosa insolubles en agua. La fase oleosa supone un subproducto que se elimina, mientras que el condensado de humo primario y la fase de alquitrán insoluble son purificadas para eliminar los componentes potencialmente nocivos para la salud. De esta forma resulta un condensado de humo cuyos componentes mayoritarios son los ácidos carboxílicos con grupos carbonilo y los compuestos fenólicos (Lin et al., 2008). Estos procesos de fraccionamiento y purificación hacen que la eliminación de los HAPs en la producción de los aromas de humo sea relativamente sencilla, en comparación con los métodos tradicionales de generación de humo (Montazeri et al., 2013; Simko et al., 2005).

En la industria alimentaria, el sabor a humo puede aplicarse de distintas formas, mediante diversas tecnologías y en diferentes fases del procesado del alimento. Es común el empleo de soportes líquidos o físicos para retener los componentes del humo y facilitar su incorporación a los alimentos. Los soportes líquidos más utilizados son el agua, el aceite y las soluciones hidroalcohólicas, mientras que los soportes sólidos más empleados son azúcares, almidones, dextrinas, goma arábiga, especias, sales, hidrolizados de proteínas y sistemas coloidales como gelatinas (Fuentes et al., 2007; Simko et al., 2005). Los aromas a humo puede aplicarse por atomización, inmersión, inyección o adición directa del condensado en la formulación del producto (Codex, 2003; Fuentes, 2007). Este tipo de ahumado también puede ser realizado en cámaras de secado bajo unas condiciones de tiempo y temperatura similares a las del ahumado tradicional (Codex, 2003; 2009).

1.2.4. Alteración del pescado ahumado. Evolución de la calidad durante el almacenamiento

La alteración del pescado es un proceso complejo, en el que están implicados mecanismos físicos, químicos y microbiológicos. Los cambios que se producen en el pescado, tanto fresco como procesado, con el paso del tiempo son principalmente causados por la actividad oxidativa, enzimática y/o microbiana.

-**Deterioro autolítico**: La actuación de las enzimas endógenas determinan los cambios autolíticos que se producen en el pescado. En el pescado ahumado, éstos afectan principalmente a la textura, ablandando los tejidos y provocando pérdida de la capacidad de retención de agua (Truelstrup-Hansen et al., 1996). Estos cambios texturales están relacionados con la degradación de las proteínas del músculo del pescado. Cuando la alteración está muy avanzada, las proteínas son también descompuestas por las proteinasas bacterianas, dando lugar a pequeños péptidos y aminoácidos libres (Fuentes, 2007).

-**Deterioro químico**: El oxígeno es uno de los principales responsables de estos cambios, que tienen lugar principalmente en la fracción lipídica insaturada del pescado. En los procesos oxidativos, como la autooxidación, inicialmente se produce la formación de hidroperóxidos, que no afectan al sabor, pero pueden provocar una coloración marrón y amarilla en el músculo. La degradación de los hidroperóxidos da lugar a la formación de aldehídos y cetonas, que confieren sabores y olores rancios (Fernández-Segovia, 2003).

-**Deterioro microbiológico**: Los microorganismos son los principales responsables del sabor y el aroma del pescado alterado que se desarrollan con el paso del tiempo, provocados por sus metabolitos. Respecto a los microorganismos presentes en este tipo de productos, diversas

investigaciones indican que las bacterias acido-lácticas constituyen la microflora dominante (González-Rodríguez et al., 2002; Gram & Huss, 1996; Truelstrup-Hansen et al., 1998). *Enterobacteriaceae* y vibrios marinos (*Photobacterium phosphoreum*) están habitualmente presentes (Gram & Dalgaard, 2002; Lyhs et al., 1998), y otro tipo de bacterias como *Brochotrix thermospacta*, *Aeromonas spp.*, *Shewanella putrefaciens* son también aisladas a menudo en estos productos (Leroi et al., 1998; Løvdal, 2015). Por otro lado, diversos estudios sugieren que *P. phosphoreum* y algunos tipos de bacterias acido-lácticas podrían ser los principales microrganismos responsables del deterioro en el pescado ahumado en frío (Truelstrup-Hansen et al., 1995; Løvdal, 2015). Factores relacionados con el procesado, como el tipo de salado y ahumado, así como las condiciones y prácticas de manipulación de las empresas de ahumado parecen condicionar el crecimiento de un tipo u otro de microorganismos. Truelstrup-Hansen et al. (1996) observaron grandes diferencias en la microflora predominante en el salmón ahumado en frío, en función del tipo de salado al que había sido sometido (en seco o con salmuera inyectada) y según la empresa de ahumado donde se realizó la producción.

El ahumado en frío de pescado es un sistema de conservación con ciertas limitaciones ya que el nivel de sal y de ahumado de estos productos no son lo suficientemente intensos como para conservar por sí solo un producto tan altamente perecedero como el pescado, sin depender de los sistemas de refrigeración. Además no existe un tratamiento térmico en el proceso que pueda eliminar eficazmente los microorganismos, lo que hace que estos productos sean vulnerables a la contaminación microbiológica. Estas particularidades hacen imprescindible que este tipo de alimentos deban ser sometidos a un control exigente en todos los aspectos del proceso para evitar posibles riesgos para el consumidor. El consumo de pescado ahumado en frio ha estado implicado en brotes de intoxicación alimentaria producidos por *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes* y *Clostridium*

botulinum (ICMSF, 2005). La presencia de aminas biógenas generadas por cierto tipo de bacterias también puede suponer un riesgo para los consumidores de este tipo de productos (FDA, 2001). No obstante, modificaciones en el proceso, como una mayor adición de sal o la introducción de algún tipo de tratamiento térmico podrían hacer el producto más estable microbiológicamente, pero probablemente dichas modificaciones, no serían bien acogidas por los productores ni consumidores, ya que modificarían significativamente los atributos sensoriales que caracterizan el producto (FDA, 2001). Por ello, el empleo de materia prima de óptima calidad higiénica, el diseño correcto y el control del proceso, así como unas buenas prácticas de manipulación son vitales para garantizar la seguridad del consumo de este tipo de productos durante toda su vida útil, la cual suele estar comprendida entre 3 y 6 semanas a temperaturas de refrigeración (Codex, 2013; FDA, 2001; Løvdal, 2015).

En este sentido, existen diversas metodologías y parámetros específicos que permiten evaluar la calidad del pescado, los cuales se dividen principalmente en cuatro categorías:

-Métodos microbiológicos: Su objetivo es proporcionar información acerca de la calidad higiénica del pescado y de la posible presencia de microorganismos potencialmente dañinos para la salud del consumidor (Huss, 1995).

-Métodos sensoriales: El análisis sensorial es uno de los métodos más eficaces para medir el grado de frescura en el pescado. Son empleados de manera rutinaria como método de inspección y control en los canales de distribución.

-Métodos químicos: En la evolución de la frescura del pescado se utilizan diferentes índices químicos, como la determinación del nitrógeno básico volátil total (N-BVT), nitrógeno de trimetilamina (N-TMA),

determinación del contenido aminas biógenas, la evaluación de la oxidación lipídica mediante el índice de peróxidos o el índice del TBA, el análisis de compuestos de degradación del ATP y valor K_1 y el análisis de compuestos volátiles.

-Métodos físicos: En esta categoría se engloban métodos relacionados con la evaluación del color, el pH, la textura o la capacidad de retención de agua.

1.3. INNOVACIÓNES EN EL SECTOR DE LOS PRODUCTOS AHUMADOS

1.3.1. Necesidades y retos del mercado

El consumo actual de pescado ahumado en nuestro país se encuentra en auge. En las últimas décadas el volumen de producción y ventas ha aumentado significativamente. La imagen "delicatesen" que tenían estos productos ha quedado atrás, al igual que en gran parte su estacionalidad navideña, pasando a ser alimentos de consumo popular, como se ha comentado anteriormente (Anónimo, 2012).

No obstante, a raíz de la crisis económica de los últimos años, los ahumados han sufrido una bajada de precios generalizada, al igual que muchos productos de alimentación. En contraste, el precio de las materias primas ha aumentado significativamente, debido al gran aumento en la demanda mundial de pescado procedente de acuicultura. Tal es el caso del salmón noruego, cuyo precio se ha incrementado un 57,2% desde 2009 (FAO, 2014; Rodriguez, 2010)

Para afrontar estas dificultades las empresas productoras deben responder adaptándose a las nuevas necesidades del mercado para ser más competitivas. En este sentido, la apuesta por el I+D para desarrollar nuevos productos de mayor valor añadido es fundamental.

La demanda actual en este tipo de productos se dirige hacia el "listo para consumir" y las raciones individuales, centradas en la rapidez y comodidad para el consumidor, en donde la tecnología del envasado juega un papel cada vez más importante. En este sentido, recetas elaboradas que tratan de innovar para atraer al consumidor, como el bacalao desalado listo para su consumo, o los platos preparados especiales de aperitivo como los mix de ahumados son cada vez más comunes en los comercios.

Por otro lado, el proceso de salado y ahumado ha variado muy poco a través de los años desde el punto de vista tecnológico, si se compara con otros procesos de transformación de alimentos. La producción de pescado ahumado no está exenta de problemas, si se tiene en cuenta la generación de efluentes salinos contaminantes que implica, o la alta vulnerabilidad que presenta el producto frente al crecimiento microbiano. Por ello, es muy recomendable para el sector, la búsqueda de nuevos procesos productivos y/o la optimización de los ya existentes, para rentabilizar sus actividades y mejorar el control y la calidad de sus productos. En este sentido, la reducción de tiempos de procesado, la minimización de la generación de residuos, la mejora de la calidad higiénica, la reducción del contenido en sodio, así como una mayor estandarización de sus productos son las principales mejoras que necesita el sector.

1.3.2. Impacto medioambiental

Las industrias de procesado de pescado se enfrentan a problemas de gestión de los residuos que generan sus procesos productivos, ya que las normas de vertido y tratamiento son cada vez más estrictas. Por este motivo, es imperativo para las empresas invertir recursos para minimizar su generación y ajustar sus actividades a los requisitos del desarrollo sostenible.

Las operaciones que más contribuyen a la generación de residuos de este tipo de industrias son el secado, fermentado, la elaboración de conservas y el ahumado. Desde el punto de vista medioambiental, se considera que las fases más críticas son el corte, el eviscerado y el pelado, por el alto volumen de residuos de pescado a que dan lugar (IHOBE, 2000). Estas operaciones implican además un consumo elevado de agua, el cual, al igual que en el caso de los residuos, debe ser gestionado a través de planes formales de control, implementando medidas específicas en los diferentes etapas de procesado.

En las industrias de ahumado de pescado, la etapa de salado es especialmente crítica respecto a la generación de residuos. Uno de los efluentes de mayor volumen derivado de este proceso, lo constituyen los residuos generados durante el lavado al que es sometido el pescado al finalizar la etapa de salado. Otro de los efluentes de alta carga salina es el generado por el propio pescado durante el salado con sal sólida. En este proceso, la sal se deposita sobre la superficie del pescado y la cantidad no absorbida por el músculo es drenada hacia el exterior, junto con parte del líquido de constitución que pierde el pescado, debido a la deshidratación que se produce. Este exudado resultante es básicamente una salmuera saturada, con un alto nivel de proteínas disueltas y otros compuestos orgánicos solubles procedentes del pescado, los cuales no pueden ser vertidos directamente al sistema de alcantarillado sin un tratamiento previo. Sin embargo, en ocasiones se eliminan diluidos con otros efluentes, para reducir los niveles de salinidad del vertido y cumplir así con los límites máximos de conductividad establecidos por la legislación (Ferraro et al., 2011; IHOBE, 2000). Estos residuos son considerados como ecotóxicos y suponen un alto impacto ambiental. Sus elevados valores de materia orgánica, combinados con la alta conductividad eléctrica de la salmuera, dificultan en gran medida su tratamiento físico-químico o biológico por la inhibición que produce el contenido salino en su tratamiento (Barat & Grau, 2009; Muñoz-Herrero, 2010).

La gestión de estos residuos supone un elevado coste para las empresas. En algunos casos, empresas especializadas externas son las que gestionan la eliminación de estos residuos, aunque es obligación del productor realizar una adecuada gestión de los mismos hasta su cesión a dichas empresas. Sin embargo, en otras ocasiones son las propias industrias las que utilizan diferentes estrategias de depuración, en función de las características y de los límites de vertido al medio receptor (IHOBE, 2000).

Existen varias técnicas de tratamiento para gestionar las salmueras residuales, que por su alta conductividad no pueden ser vertidas directamente al medioambiente y cuya descarga en la red de saneamiento puede ocasionar problemas en la planta de tratamiento de aguas residuales. Hasta el momento el sistema más extendido para gestionar los residuos es la evaporación solar de los efluentes en balsas y el vertido controlado del residuo seco resultante (Muñoz-Guerrero, 2010). Otras técnicas, como el tratamiento mediante tecnología de membranas y la descarga líquida cero (ZLD), son opciones prometedoras (Meca & Martínez, 2016; Muñoz-Guerrero et al., 2010). Las buenas prácticas operativas proporcionan también altos porcentajes de reducción de la problemática ambiental con inversiones mínimas. No obstante, la carga contaminante de los vertidos líquidos no puede eliminarse del todo, lo que la convierte en la principal problemática ambiental del sector de las conservas y salazones de pescado. Por todo lo expuesto, el desarrollo de nuevas tecnologías de salado que impliquen una menor generación de efluentes salinos, sería de gran interés tanto para el sector como para la sociedad.

1.3.3. Aspectos nutricionales

En general, los productos ahumados presentan un contenido de sal elevado, desde 1,3-5% (Birkeland & Skåra, 2008), perteneciendo a uno de los grupos de alimentos que más contribuye a aumentar el nivel de la ingesta de sodio en la dieta de los españoles (fig. 2).

Fig. 2. Origen de la ingesta de sodio en España según los diferentes grupos de alimentos en %. Fuente: Encuesta Nacional de Dietética. /El País (2013).

El consumo excesivo de sodio ha sido relacionado directamente con enfermedades como la hipertensión, la cual aumenta el riesgo de infarto y muerte por enfermedades cardiovasculares (Lydakis et al., 1997). Hoy en día, la hipertensión constituye uno de los problemas de salud pública más preocupantes de las últimas décadas y las enfermedades cardiovasculares una de las principales causas de mortalidad en los países desarrollados.

Ante este problema de salud pública, el Ministerio de Sanidad y Consumo, a través de la AECOSAN puso en marcha en 2005 la Estrategia NAOS (Estrategia para la Nutrición, Actividad física y Prevención de la Obesidad), cuyo objetivo es fomentar una alimentación saludable y reducir de 9,8 a 5 g el consumo de sal/día. Con este objetivo, se solicitó a las industrias disminuir el contenido en sal de los productos elaborados, ya que éstos constituyen la principal fuente de sodio en la dieta (70-75% de la ingesta total).

La reducción del contenido total de sodio procedente del NaCl en los alimentos procesados, básicamente se puede llevar a cabo reduciendo la cantidad de sal añadida a los productos formulados. Sin embargo, esta reducción en los alimentos resulta compleja, ya que puede originar defectos en los productos terminados como por ejemplo, problemas microbiológicos, texturas blandas o sabores desagradables.

Existen diferentes estrategias posibles para reducir el contenido de Na en los alimentos, además de la reducción directa del contenido de sal. Una de estas estrategias consiste en amplificar el sabor del NaCl a través de la optimización de su forma física. Se ha comprobado que a menor tamaño de los cristales de sal, mayor rapidez en la percepción del sabor salado, siendo posible el uso de una cantidad menor de sal. No obstante, esta estrategia de reducción de sal es adecuada cuando la sal es adicionada en el alimento con función saborizante y no con fines tecnológicos, relacionados con la textura o la conservación. Por ello, después de una reducción de sal significativa, es a menudo necesaria la revisión y adaptación de las técnicas de procesado, considerando las importantes funciones tecnológicas que ésta desempeña en el producto final (Aliño et al., 2006; Toldrá et al.2002).

Por otro lado, los potenciadores del sabor pueden ser una herramienta útil en el desarrollo de productos bajos en sodio, ya que ayudan a realzar el sabor salado de los alimentos, permitiendo el uso de una menor cantidad de sal. Uno de los más destacados es el glutamato monosódico (GMS) que permite, tal y como se ha demostrado en algunos estudios, una reducción entre un 30 y un 40% de NaCl sin afectar al sabor del producto. Otros potenciadores del sabor, cuya aplicación ha comenzado a ser estudiada en los últimos años, son los extractos de levaduras y nucleótidos (Campagnol et al., 2011a; 2011b).

Otra de las posibles opciones para reducir el contenido de sodio, es la sustitución del NaCl por otras sales como el KCl, $MgCl_2$, $CaCl_2$, lactato

potásico, etc. Los efectos del NaCl sobre la solubilidad de las proteínas y la retención de agua se deben principalmente a la fuerza iónica del ión cloruro, por lo que es posible su sustitución por otras sales sin pérdida de funcionalidad, evitando los problemas de salud relacionados con el consumo de sodio (Viadel, 2012). Sin embargo, existen inconvenientes en su aplicación práctica, como son la posible modificación en la aceptación del producto, debido a una menor intensidad en el sabor salado, la aparición de sabores anómalos aportados por otros cationes, el desconocimiento de las dosis necesarias para producir un producto seguro desde el punto de vista microbiológico, así como la posible aparición de coloraciones y texturas anómalas (Aliño et al., 2006).

1.3.4. Nuevas técnicas para el procesado y control de los productos de pescado ahumado

Tal y como se ha evidenciado en apartados anteriores, algunos aspectos del proceso de salado/ahumado relacionados con el control, la calidad y la generación de residuos son susceptibles de mejora. Por ello, es muy interesante la optimización de los métodos existentes y/o la aplicación de nuevas técnicas, así como el diseño de nuevos productos.

En la actualidad, uno de los principales intereses de la industria de productos salados cárnicos o de la pesca se centra principalmente en la reducción de los tiempos de procesado. Con este fin se han desarrollado diversas técnicas alternativas. Una de estas técnicas, la cual está enfocada hacia el control del salado fue desarrollada por Fuentes et al. (2008), a través del estudio de las variables que definen el proceso. El **salado con control termodinámico** consiste en dosificar sobre el pescado la cantidad exacta de sal necesaria para alcanzar la concentración de sal óptima en el producto, adaptada en cada caso en función de variables como las características

iniciales de la materia prima, las condiciones de procesado, la especie de pescado, etc. Esta técnica permite además controlar de forma efectiva el volumen de residuos de sal generados por un uso excesivo de sal en estos procesos.

La **inyección de salmuera** es una técnica de salado relativamente nueva desarrollada inicialmente en los años 90 por la industria cárnica. En la actualidad es frecuentemente usada por la industria de pescado ahumado (Løje, 2007). En el salado por inyección, la salmuera es inyectada directamente al interior del alimento a través de agujas. El proceso se realiza generalmente de forma automática, y la salmuera y otros posibles ingredientes son introducidos a través de diferentes puntos de entrada y a alta presión en el músculo (Albarracín, 2009). Este hecho conduce a un incremento de la velocidad de salado y a una distribución más homogénea de la sal, comparado con los métodos tradicionales, ya que depende menos de los fenómenos de difusión que el salado en seco o el salado por inmersión en salmuera (Birkeland et al., 2007; Røra et al., 2004). Este método permite obtener mayores rendimientos y un color más homogéneo, así como reducir tiempos de procesado, comparado con otras técnicas (Løje, 2007). Sin embargo, al ser un método más agresivo con el músculo que otras técnicas, puede conducir a determinados defectos de calidad en el pescado ahumado, como una mayor separación de los miotomos ("gaping") y una textura más blanda (Birkeland et al., 2007; Løje, 2007; Sigurgisladottir et al., 2000). Aspectos como la presión de inyección de la salmuera, la velocidad y la orientación de la agujas deben ser controlados, ya que influyen en el contenido final de sal del producto y en otros aspectos de calidad (Løje, 2007).

Otra técnica que se centra en la reducción de tiempos de procesado es el **salado-descongelado simultáneo,** el cual consiste en realizar conjuntamente la descongelación y el salado cuando se utiliza materia prima

congelada. El empleo de materia prima congelada es habitual por las industrias, ya que facilita la planificación de la producción en función de la demanda y la rotación de stocks. Asimismo permite la selección de los lotes de materia prima que va a ser salada, en función del peso o tamaño para obtener producciones más homogéneas. La desventaja del uso de materia prima congelada es el paso previo de descongelación que requiere, lo que aumenta el tiempo de procesado en horas e incluso días, respecto a la utilización de materia prima fresca, sobre todo en el caso de piezas de gran tamaño. Esta limitación puede ser resuelta mediante el uso de esta técnica, que propone la descongelación y el salado del producto al mismo tiempo en una cámara frigorífica. El procedimiento aplicado a piezas de carne o pescado, puede ser llevado a cabo mediante el uso de sal seca o por inmersión del alimento en salmuera (Grau & Barat, 2009). En el caso del salado-descongelado simultáneo con salmuera, se requiere de un sistema que permita controla la temperatura y agitación. La salmuera se prepara a la concentración requerida en un depósito, desde el cual se alimentan, tanto el tanque de tratamiento de las piezas como un equipo refrigerador, forzándose la circulación de la salmuera en circuito cerrado mediante una bomba. Además, esta técnica se puede combinar con la utilización de pulsos de vacío para acelerar la captación de sal (Barat et al., 2004). Su aplicación en la obtención de jamón curado ha sido estudiada. Albarracín (2009) observó que el tiempo total de salado/descongelado de los jamones es de 5 días cuando se utiliza salmuera saturada a 3ºC, frente a los 13 días que supone el proceso tradicional (descongelado en cámara frigorífica de 4 días, más salado posterior con sal sólida de 9 días) (Barat et al., 2004; 2005). Este proceso también ha sido estudiado para la obtención de atún seco-salado "mojama", tanto en su modalidad con salmuera, como con sal seca. Con esta técnica, el tiempo de procesado necesario para conseguir la misma concentración de sal en base seca en el producto final que el producto obtenido del modo tradicional, se redujo un 89,5% y un 42,8% cuando el salado-descongelado

simultáneo se realizó con salmuera saturada y con sal seca respectivamente (Barat & Grau, 2009).

La **impregnación a vacío** es uno de las tecnologías aplicadas para mejorar los procesos de salado. Concretamente, este método ha sido estudiado en el salado de jamón curado, bacalao (Chiralt et al., 2001) y salmón ahumado (Bugueño et al., 2003). No obstante, la impregnación a vacío tiene aplicaciones en el procesado de un gran número de alimentos de naturaleza porosa con fines diferentes, como es el caso de la deshidratación osmótica de muchos tipos de frutas. Estas técnicas consisten en sumergir el alimento en una solución osmótica y aplicar presión subatmosférica durante un intervalo corto de tiempo seguido de un periodo a presión atmosférica, por lo que el gas ocluido en los poros se expande para equilibrarse con la presión impuesta al sistema (Chiralt et al., 2001).

La impregnación a vacío aplicada en el salado de pescado se realiza introduciendo el pescado en un tanque con salmuera, aplicando pulsos de vacío. En una primera fase, se aplica una presión de vacío al sistema durante un periodo corto de tiempo en un tanque cerrado. Así se promueve la expansión y la salida del gas interno del producto, que arrastra consigo líquido procedente de los poros. En una segunda fase, la presión atmosférica es restablecida en el tanque durante un tiempo. La compresión conduce a una gran reducción del gas restante en los poros y al subsiguiente flujo de líquido externo hacia el interior de la estructura porosa. Esta técnica consigue acelerar la captación de sal y disminuir las pérdidas de peso, aumentando el rendimiento en comparación con los procesos tradicionales (Chiralt *et al*., 2001).

Otras tecnologías novedosas orientadas a la optimización del salado y la aceleración en la captación de sal son los pulsos eléctricos (Taiwo, et al., 2003) y los ultrasonidos (Cárcel et al., 2007; Siró et al., 2009).

Por otra parte, de los numerosos avances tecnológicos acontecidos en las últimas décadas en el sector de la alimentación, la innovación en materiales y sistemas de envasado es uno de los más relevantes. El envasado permite prolongar la vida útil de los alimentos que contiene, y juega un papel fundamental para garantizar la calidad y seguridad de los mismos hasta que son consumidos. Aunque el envase es aplicado fundamentalmente para proteger el alimento durante su almacenamiento y distribución hasta que llega al consumidor, éste puede tener interesantes aplicaciones en el procesado de los alimentos.

Un ejemplo de la incorporación del envase como una variable más del proceso es la **cocción sous-vide**, que consiste en cocinar los alimentos bajo condiciones contraladas de temperatura y tiempo dentro de bolsas termoestables selladas a vacío. El empleo de la cocción sous-vide comenzó en los años 70 y su empleo ha crecido enormemente en las últimas décadas, sobre todo en el sector de la alta cocina (Baldwin, 2012). Uno de los aspectos interesantes de esta técnica radica en su capacidad para extender la vida útil de alimentos mínimamente procesados, al eliminar el riesgo de re-contaminación durante el almacenamiento y reducir el crecimiento de bacterias aerobias. El envasado a vacío en el cocinado aporta otros beneficios, ya que permite que el calor sea eficientemente transferido del agua de cocinado al alimento, ayuda a controlar la oxidación y previene pérdidas por evaporación de compuestos aromáticos volátiles y de humedad durante el cocinado (Baldwin, 2012).

Otro ejemplo de la aplicación de envases en el proceso con fines tecnológicos se encuentra en la maduración de carne de vacuno que se lleva a cabo dentro de bolsas selladas a vacío o "**wet ageing**". Esta técnica se utiliza para retener en contacto con la pieza todos sus jugos naturales durante el reposo/maduración de la carne, obteniéndose así una textura más tierna y características sensoriales deseables.

Basado en la técnica de salado con salmuera mediante impregnación a vacío, comentado previamente (Chiralt et al., 2001), se ha propuesto una técnica llamada **"salado a vacío" o "salado en bolsa"**, que consiste en la fijación de la sal mediante el empleo de vacío en el salado con sal sólida. Tanto el salado como el reposo de las piezas son realizados dentro de bolsas selladas a vacío, con el fin de favorecer la penetración de la sal. Esta técnica ha sido aplicada en el proceso de obtención de jamón curado (Armenteros, 2010). El uso del envasado a vacío permite una difusión más intensa de la sal en el interior del producto, ayuda a hacer el proceso más higiénico, al evitar el goteo de los exudados dentro de las cámaras de salado y protege al producto de posibles contaminaciones a la vez que facilita su manipulación y transporte.

Por otro lado, nuevos polímeros altamente permeables al vapor de agua, de reciente aparición en el mercado, podrían ser aplicados para optimizar el proceso de salado en bolsa. El uso de estos envases especiales podría permitir el secado óptimo de las piezas como si no hubiera envase, pero con las ventajas que supone el tener el producto envasado durante todo el proceso, como la protección frente a posibles contaminaciones. Las bolsas permeables al vapor de agua han sido aplicadas con éxito en productos del sector cárnico, como en la maduración de carne en seco ("dry ageing") (Ahnström et al., 2006; DeGeer et al., 2009; Dikeman et al., 2013; Li et al., 2013) y en el secado de jamones reestructurados (Serra et al., 2009).

Respecto al ahumado, la optimización de estos procesos se centra actualmente en el control de la generación de HAPs y también en la aceleración de la absorción del humo, para reducir tiempos de procesado.

Una técnica orientada a agilizar estos procesos es el **ahumado electrostático.** Esta técnica que consiste en aplicar un campo eléctrico entre las partículas del humo y el alimento (Andrés et al., 2006). Las partículas ionizadas precipitan de forma rápida en el producto, lo que acelera

significativamente la deposición de las sustancias de humo, reduciendo el tiempo de ahumado.

En otros estudios se propone el **ahumado a bajas temperaturas** (0-5ºC) (Yamaoka et al., 1996). La ventaja fundamental que aporta realizar el ahumado a temperaturas reducidas, radica en que la cadena de frío se mantiene en todo momento, a diferencia de lo que sucede en el ahumado tradicional, donde se utilizan temperaturas alrededor de 30ºC. Este hecho ayuda a mantener una óptima calidad higiénica en estos productos y además previene la oxidación lipídica, que puede darse cuando se emplean altas temperaturas de ahumado.

La implementación de nuevas tecnologías en el salado-ahumado de pescado, no solo es interesante para la optimización del proceso, sino también para el desarrollo de sistemas de control, que permitan asegurar la calidad de las materias primas, del producto terminado y la monitorización durante el procesado. Existe una gran diversidad de técnicas utilizadas para ahumar el pescado, así como grandes diferencias en las características del pescado utilizado como materia prima, incluso dentro de una misma especie. Esta variabilidad conduce a que los pescados ahumados comerciales presenten grandes diferencias en los contenidos de sal y humedad, lo que provoca características de calidad heterogéneas, que afectan directamente a la calidad sensorial y a la vida útil del producto, pudiendo tener implicaciones sobre la seguridad de su consumo (Fuentes et al., 2010). En este sentido, muchos estudios han encontrado grandes diferencias en la vida útil de pescados ahumados en frío, la cual puede llegar a oscilar entre 1 y 8 semanas (Cardinal et al., 2004; Jørgensen & Dalgaard 2000).

En respuesta a estos problemas de control, la industria alimentaria emplea de forma rutinaria todo tipo de técnicas analíticas, basadas en métodos físicos, químicos, sensoriales y microbiológicos, para vigilar la calidad y seguridad de sus productos. Muchas de estas técnicas son de gran

fiabilidad, pero alta complejidad tecnológica e implican largos periodos de tiempo para la toma de muestras y el ensayo, además de requerir personal especializado para el manejo de los equipos. Para agilizar la vigilancia y control en el proceso industrial, una de las tendencias actuales es el desarrollo de métodos de análisis rápidos on-line y no destructivos. Algunos ejemplos de este tipo de métodos, cuya aplicación en la industria alimentaria está siendo estudiada, son la espectroscopía de infrarrojo cercano, la tomografía, la resonancia magnética o los sensores electrónicos como la espectroscopia de impedancia.

La técnica de **espectroscopía de impedancia** consiste en el estudio de la oposición que presenta un determinado material al paso de una corriente a través de él, siendo consecuencia directa de su composición y de su naturaleza interna. La conductividad y la impedancia de las células de los tejidos, sometidos a un campo eléctrico, están relacionadas principalmente con la presencia de sales en disolución, como Na^+, K^+, Cl^- (conductores electrolíticos) presentes en el líquido extra e intracelular. La respuesta depende, no solo de las propiedades dieléctricas del producto, sino también de las características y geometría de los electrodos utilizados (Masot, 2010).

El procedimiento experimental utilizado en esta técnica consiste en aplicar una pequeña señal eléctrica, a través de unos electrodos a diferentes frecuencias, obteniendo como resultado un valor de impedancia para cada frecuencia, expresado a través de números complejos constituidos por módulos y fases (espectro de impedancia) (Masot et al., 2010). La gran cantidad de datos obtenidos a través de estos espectros, se procesa posteriormente a través de técnicas estadísticas de tipo multivariante, como el análisis de componentes principales (PCA), que permiten explicar la variabilidad de los datos reduciendo la dimensionalidad del conjunto de los mismos a una matriz de dos dimensiones. A partir de estos datos, también es posible relacionar determinadas características del alimento susceptibles de

ser detectadas y/o cuantificadas por el equipo, mediante la construcción de modelos estadísticos de predicción.

Estos sensores son de aplicación en la determinación de la composición y las propiedades fisicoquímicas de los alimentos y pueden ser utilizados como herramienta de control de calidad y monitorización en línea. Esta técnica ha sido aplicada en productos de la pesca, para estudiar los cambios dieléctricos que se producen en el músculo del pescado durante su almacenamiento y poder establecer su grado de frescura, así como para detectar si éste ha sido sometido a congelación y posterior descongelación (Fernández-Segovia et al., 2012; Vidacek et al., 2008). La utilización de sensores electrónicos basados en la espectroscopía de impedancia también ha demostrado ser una herramienta útil en la determinación del contenido de humedad y sal en alimentos (Chevalier et al, 2006; Greiff et al., 2014; Masot et al., 2010), por lo que su aplicación puede ser de gran interés para el control de los procesos de salado y ahumado de pescado.

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OBJETIVOS

2.1 Objetivo general

El objetivo general de la presente tesis doctoral es desarrollar un proceso de salado-ahumado simultáneo de pescado, empleando un salado controlado, humo líquido y bolsas permeables al vapor de agua (WP), para obtener productos de la pesca ahumados.

2.2. Objetivos específicos

Para alcanzar este objetivo general se plantearon los siguientes objetivos específicos:

-Desarrollar un proceso de salado-ahumado simultáneo en bolsas WP para la obtención de un producto de salmón ahumado.

-Optimizar el salado-ahumado simultáneo en bolsas WP para la obtención de un producto de bacalao ahumado.

-Optimizar el salado-ahumado simultáneo en bolsas WP para la obtención de un producto de trucha ahumada.

-Evaluar la calidad físico-química y microbiológica durante el almacenamiento de los productos de salmón y trucha ahumados.

- Estudiar el efecto de la temperatura de procesado sobre la calidad físico-química y microbiológica durante el almacenamiento del producto de bacalao ahumado

-Desarrollar productos de salmón y trucha ahumados con un contenido en sodio reducido.

- Evaluar la calidad físico-química y microbiológica durante el almacenamiento de los productos de pescado ahumado con contenido reducido de sodio.

- Evaluar la aplicación de la técnica de espectroscopía de impedancia en la monitorización on-line del proceso de salado-ahumado de salmón.

PLANTEAMIENTO EXPERIMENTAL

3. PLANTEAMIENTO EXPERIMENTAL

La presente tesis doctoral se estructura en cuatro capítulos, los cuales engloban artículos publicados o en proceso de publicación en revistas científicas internacionales. Para dar una visión general del trabajo experimental realizado, en este apartado se detalla la metodología utilizada en cada capítulo.

El **capítulo 1** consiste en el desarrollo de un proceso de salado-ahumado simultáneo en bolsas permeables al vapor de agua para la obtención de productos de la pesca ahumados. Estos objetivos se abordan en los artículos 1, 2 y 6. Cada artículo se centra en el estudio del proceso en una especie de pescado diferente; salmón, bacalao y trucha, siguiendo el plan experimental que se detalla a continuación:

-Caracterización de la materia prima y del producto comercial:

Para establecer las condiciones óptimas de procesado, fue necesario determinar las características fisicoquímicas iniciales de la materia prima, así como los parámetros fisicoquímicos de diferentes pescados ahumados comerciales (salmón, bacalao y trucha ahumados), para ser utilizados como valores de referencia.

El pescado utilizado como materia prima, así como los pescados ahumados comerciales fueron caracterizados a través de análisis de contenido de humedad, grasa, NaCl, pH y a_w.

-Estudio del proceso de salado-ahumado: Para estudiar la viabilidad de la técnica de salado-ahumado simultáneo, en la obtención de productos de pescado ahumado de características similares a los productos comerciales analizados, se ensayaron distintas condiciones de procesado. En el desarrollo del producto de salmón ahumado (artículo 1) se estudiaron varias condiciones de procesado como cantidad de sal dosificada, humedad relativa establecida en la cámara de secado y tipo de envase utilizado (bolsas

de alta barrera y bolsas WP). En el caso del producto de bacalao ahumado (artículo 2) las variables de procesado fueron la cantidad de sal dosificada, la humedad relativa y el tiempo de proceso, mientras que en el caso del producto de trucha ahumada (artículo 6) se estudió la cantidad de sal dosificada y el tiempo de procesado. Todas las muestras obtenidas a partir de cada uno de estos procesos, se analizaron para establecer los valores de humedad, grasa y NaCl, pH, a_w, así como pérdida de peso (ΔMt) y porcentaje de líquido exudado durante el salado-ahumado. En base a estos resultados se establecieron las condiciones óptimas de procesado para cada especie de pescado. Asimismo, la aceptación sensorial de los productos ahumados de salmón y trucha obtenidos fue evaluada a través de los atributos sensoriales que caracterizan a estos productos.

El **capítulo 2** engloba la evaluación de la calidad físico-química y microbiológica de los productos de pescado ahumado desarrollados durante su almacenamiento en refrigeración. Consta de dos publicaciones (artículos 3 y 4), donde se estudió la calidad de los productos de salmón y bacalao ahumado durante un periodo de almacenamiento de 40 días. En el caso del bacalao se estudió también el efecto de la temperatura de procesado (5 y 10ºC). Se llevaron a cabo periódicamente análisis microbiológicos: aerobios mesófilos, enterobacterias, bacterias ácido-lácticas y fisicoquímicos: nitrógeno básico volátil total, nitrógeno de trimetilamina, compuestos de degradación del ATP y valor K_1, índice de TBA pH y parámetros de color.

El **capítulo 3,** se centra en la mejora del perfil nutricional de los productos de pescado ahumado obtenidos, a través del desarrollo de productos de pescado ahumado con contenido reducido en sodio, para hacer más saludable su consumo. Este bloque consta de dos publicaciones (artículo 5 y 6). El artículo 5 aborda la reducción del contenido en sodio del producto de salmón ahumado desarrollado en el artículo 1, mediante la sustitución parcial del 50% de NaCl por dos mezclas de sales alternativas: sal sin sodio

comercial y KCl. En el artículo 6, a partir del producto de trucha desarrollado se evaluó el reemplazo de un 50% de NaCl por KCl. Los productos de pescado ahumado (salmón y trucha) con contenido reducido de sodio fueron caracterizados por medio de análisis de contenido de humedad, grasa, cloruros, sodio, potasio, pH y a_w. Paralelamente se evaluó el efecto de la sustitución parcial de NaCl en los productos de salmón y trucha ahumada en los atributos sensoriales a través de pruebas triangulares.

En ambos trabajos, la calidad fisicoquímica y microbiológica de los productos de pescado ahumado con contenido en sodio reducido desarrollados fue evaluada durante su almacenamiento en refrigeración. De forma similar a la planteada en el capítulo 2, se realizaron análisis microbiológicos: aerobios mesófilos, enterobacterias y fisicoquímicos: nitrógeno básico volátil total, nitrógeno de trimetilamina, compuestos de degradación del ATP y valor K_1, índice de TBA, pH, parámetros de color, y ensayos texturales de corte y doble compresión (TPA).

El **capítulo 4** estudia la aplicación de la técnica de espectroscopía de impedancia (EI) como herramienta de monitorización on-line del proceso de salado-ahumado de salmón. En este caso se empleó un proceso de salado-ahumado con un sazonador con sabor a humo y se seleccionaron como condiciones de procesado: la cantidad de sal dosificada, el tiempo de salado y el envasado (a vacío y en aire). Se llevaron a cabo análisis fisicoquímicos de contenido de humedad, NaCl, pH y a_w y paralelamente se evaluaron las mediciones efectuadas con tres electrodos diferentes: electrodo doble, punta de flecha y aguja. Posteriormente a partir de las condiciones de procesado y el electrodo seleccionados se realizó un seguimiento del proceso de salado-ahumado durante 25 h de procesado, realizando mediciones con el electrodo de impedancia y los análisis fisicoquímicos descritos a intervalos periódicos de 5 horas.

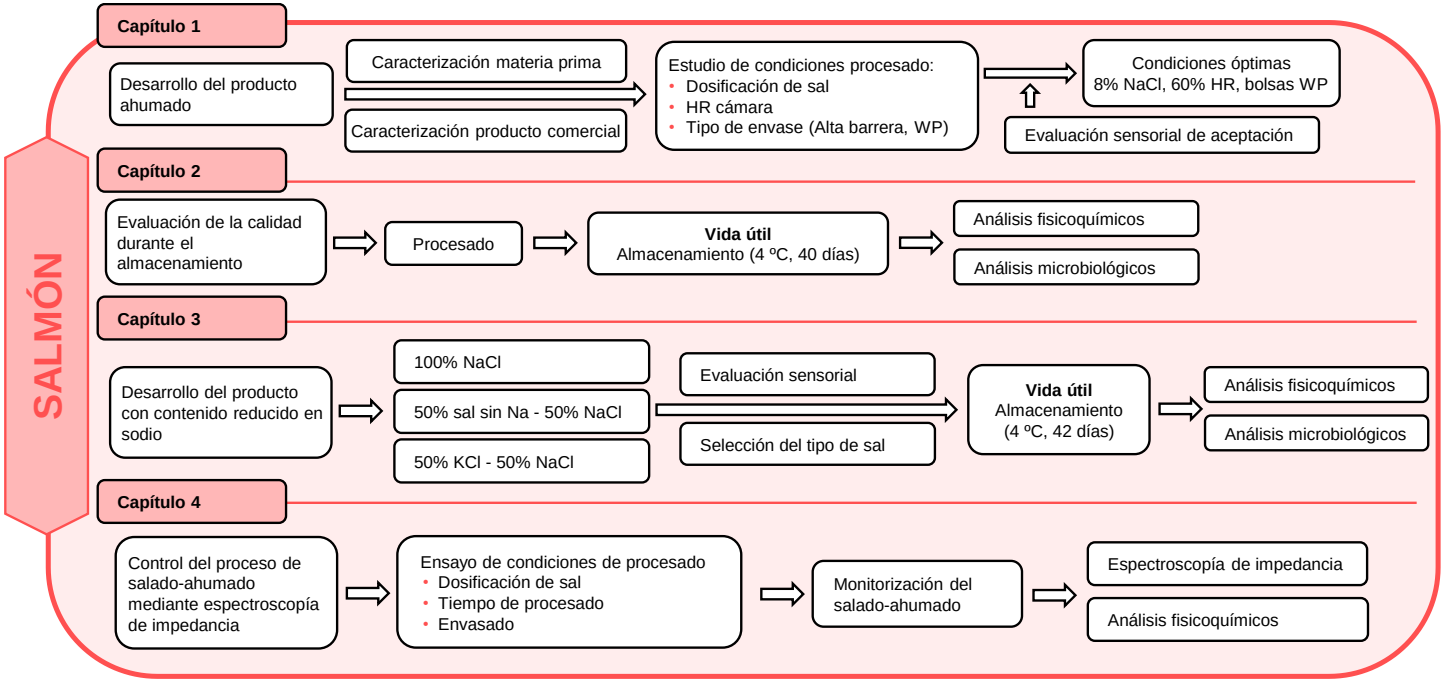

Fig.1. Esquema general del trabajo experimental realizado con salmón.

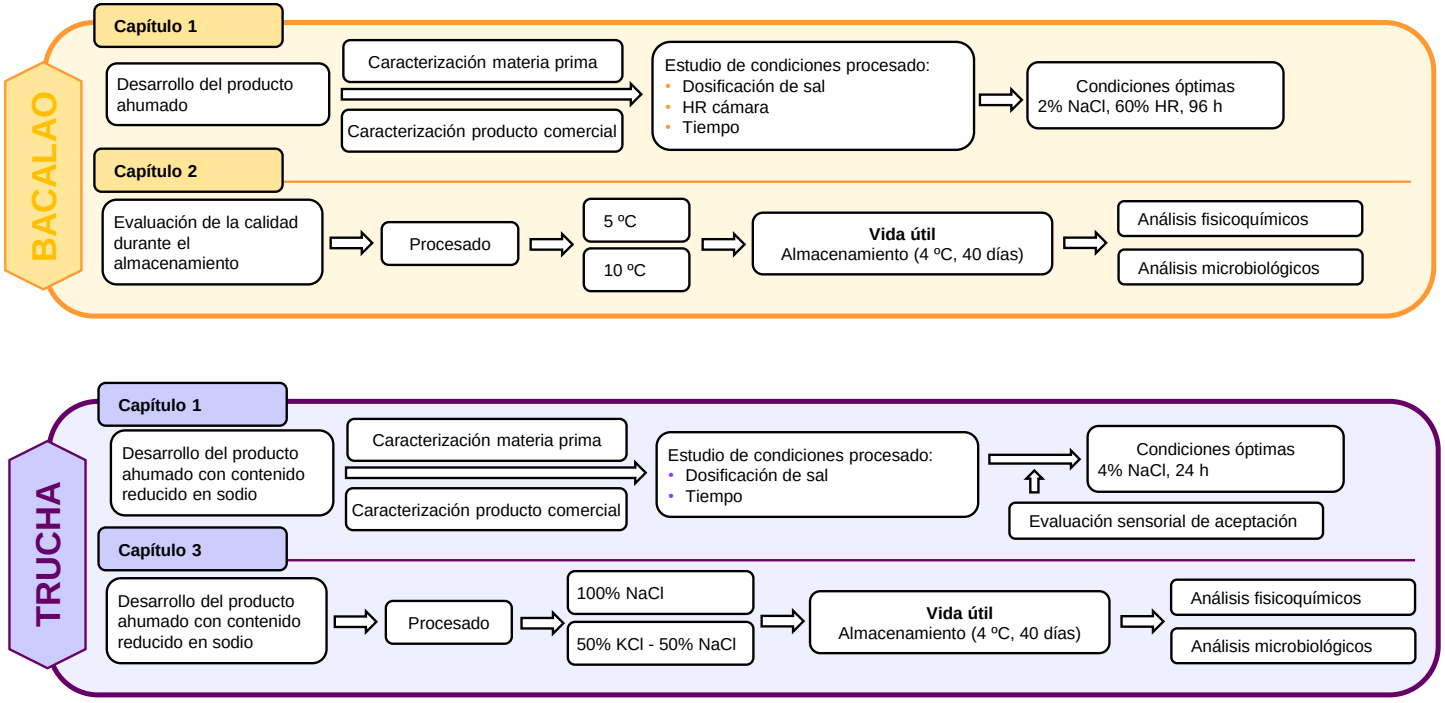

Fig.2. Esquema general del trabajo experimental realizado con bacalao y trucha

CAPITULOS

CAPITULO 1

DESARROLLO DE PRODUCTOS DE PESCADO AHUMADO

ARTÍCULO 1

A NOVEL PROCESS FOR OBTAINING SMOKE-FLAVOURED SALMON USING WATER VAPOUR PERMEABLE BAGS

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Journal of Food Engineering 149 (2015) 44–50

Versión adaptada para la tesis doctoral

Abstract

We aimed to optimise a new smoking-salting method using water-vapour permeable bags to obtain smoke-flavoured salmon. This involved a smoking-salting process with three salt doses (4, 6, 8 g salt/100 g fresh salmon) and two packaging types: high barrier (HB) bags and water vapour permeable bags (WP) with different humidity levels (50%, 60%, 70% RH). The salting and vacuum packaging combination increased the salt concentration in the final product versus WP bag packaging. The lower the RH for WP-packed samples in the drying chamber, the lower the moisture and a_w values. The 8% salt dose/60% RH samples came closer to the a_w, salt content and moisture levels determined in commercial samples. In WP samples, 50% and 60% RH completely evaporated the water released by muscle. Sensory attributes of the new product obtained similar scores to the commercial product. The new methodology is suitable to obtain smoke-flavoured salmon with similar physico-chemical characteristics and consumer acceptance to a commercial smoked salmon reducing processing steps and brine wastes.

Keywords: salting, smoking, smoke-flavoured salmon, water vapour permeable bags, liquid smoke

1. Introduction

Smoking is a preservation method that has been employed since ancient times. The preservative effect of smoking is due to a combination of different factors, including addition of salt, partial dehydration of tissues which occurs throughout the different stages of the process, and the preservative action of the smoke components. The smoking process slows down the biological processes and oxidative damage, and confers the final product sensory characteristics that consumers greatly appreciate.

The traditional smoking process involves different stages such as salting, drying and/or smoking. There is increasing interest in modifying the traditional smoking and salting processes according to industrial demands. Producers seek new methods to reduce processing times, minimise salt waste, reduce overall weight loss and/or improve the hygienic quality of the final product. Hence the salting step is especially critical. Currently, salting process improvements focus primarily on reducing processing times and using alternative techniques, such as direct brine injections (Thorarinsdottir et al., 2010), simultaneous salting-thawing (Barat et al., 2005) or vacuum impregnation (Chiralt et al., 2001).

A new salting process in which the exact amount of salt to be absorbed by the fish is directly dosed has been proposed by Fuentes et al. (2008). This procedure has been demonstrated capable of reducing waste and obtaining homogeneous products. The combination of this controlled salting process, by using smoke-flavoured salt, and vacuum packaging has also been studied with a view to obtaining smoked salmon with similar characteristics to currently marketed products (Rizo et al., 2013). This methodology was able to accelerate NaCl absorption and dehydration, and can therefore cut the total processing time without affecting physicochemical parameters and sensory traits as compared with traditional smoked salmon. Other studies have been focus on the use of smoke condensates and liquid smoke as an

alternative to the traditional smoking process (Muratore & Licciardello, 2005; Muratore et al., 2007; Suñen et al., 2003). The use of smoke flavourings apart from providing the typical smoke flavour to this type of products, can also avoid the occurrence of harmful components for human health and the environment generated by traditional smoking techniques (Martinez et al., 2007)

Recently, materials with high water vapour transmission rates have been investigated. Currently there are very few applications for these new materials in food technology, being these focused mainly on dry ageing of beef (Ahnström et al., 2006; DeGeer et al., 2009; Dikeman et al., 2013; Li et al., 2013). The use of highly water vapour-permeable bags (WP) facilitates the control of product dehydration by managing the temperature and humidity conditions, as with the traditional methods (unpacked). Yet at the same time, it minimises the risk of microbial contamination. DeGeer et al., (2009) stated that this type of bags helps obtain higher meat yields and lower microbial counts as compared with the traditional dry ageing processes, and does not affect the sensory traits of the meat product. For this reason, we considered that a combination of a one-step smoking-salting process with WP bags would help obtain smoke-flavoured fish with similar sensory traits to smoke products obtained by traditional methods and would optimise yields, reduce waste, speed up processes, maintain the hygienic quality during processes, and facilitate transportation and distribution at the same time. This could improve the methodology previously described, in which a controlled salting process was combined with vacuum packaging.

The aim of this study was to establish the optimal processing conditions (salt dose, type of packaging and RH of drying) to develop a new smoking-salting procedure using water vapour permeable bags in order to obtain smoke-flavoured salmon.

2. Material and methods

2.1. Materials

Aquacultured salmon (*Salmo salar*) fillets (n=9) from a land-based farm in Norway (Marine Harvest, Bergen, Norway), of the commercial size 1.4-1.8 Kg were purchased from a local market in the city of Valencia (Spain) and were transported to the laboratory under refrigeration. Upon arrival at the laboratory, the fillets were trimmed to remove bones, fins and any visible adipose tissue. Then salmon fillets were cut into 4 cm portions to obtain six portions per fillet (54 samples in all were obtained). Average weight of fish portions was 151 ± 35 g and thickness between 2 and 3 cm.

The sodium chloride used for the smoking-salting process was supplied by Panreac Química, S.A (Barcelona, Spain), and the natural liquid smoke HARDWOOD AFS 10 was provided by Amcan ingredient Ltd. (Le Chesnay, France). Two types of plastic bags packaging were used for this study: water vapour permeable bags (WP) were supplied by TUB-EX ApS (Taars, Denmark) (size: 200×300×0.04 mm; water vapour transmission rate: 5,000g/50μ/m²/24h (38°C/50% RH); high barrier bags (HB) (size 200×300×0.12 mm, water vapour transmission rate 1.8g/120μ/m²/24h (23°C/85% RH), supplied by Productos Pilarica, S.A. (Valencia, Spain). Two batches of smoked salmon from two different brands were used to determine the target physico-chemical parameters for the new product. Raw material of these products was Norwegian aquacultured salmon and they were processed using traditional cold-smoking techniques (dry salting, followed by smoking in a smoking chamber).

2.2. Experimental design

In order to establish the optimal salting conditions of the new method, the effect of the amount of salt dosed, RH in the drying chamber, and packaging permeability on the physico-chemical properties of the final product were all studied. These conditions were set in order to obtain smoke-flavoured salmon with similar characteristics to currently marketed products. Values considered as reference were obtained from the commercial products analysed (moisture, salt content and a_w).

Salmon portions were submitted to a simultaneous smoking-salting process (Figure 1). The smoking conditions and salting parameters to be tested were selected based on previous studies with minor modifications (Fuentes et al., 2008). Fish samples were smoked by spraying the fillet surface with liquid smoke that was diluted with distilled water (60 ml/100 ml solution) for 30 s, followed by a salting procedure based on thermodynamic control. Salting was carried out by dosing a previously established amount of NaCl over the fillet surface. The amount of salt added to each sample was calculated from the initial weight of the fish portion and the initial water weight fraction (x^w), according to procedure established by Fuentes et al. (2008). In this study, three salt dose concentrations were considered: 4, 6, and 8 g salt/100 g fresh salmon. Then the salmon portions were randomly divided into two groups, one group was packed into WP bags and the other group was packed into HB bags. All the samples were vacuum-packaged with a vacuum packaging machine (Tecnotrip EV-25-CD, Barcelona, Spain). It should be noted that the vacuum conditions for the WP samples were not maintained throughout the process, vacuum packaging was used to ensure the initial contact between fish and the packaging material since air can pass through the bag.

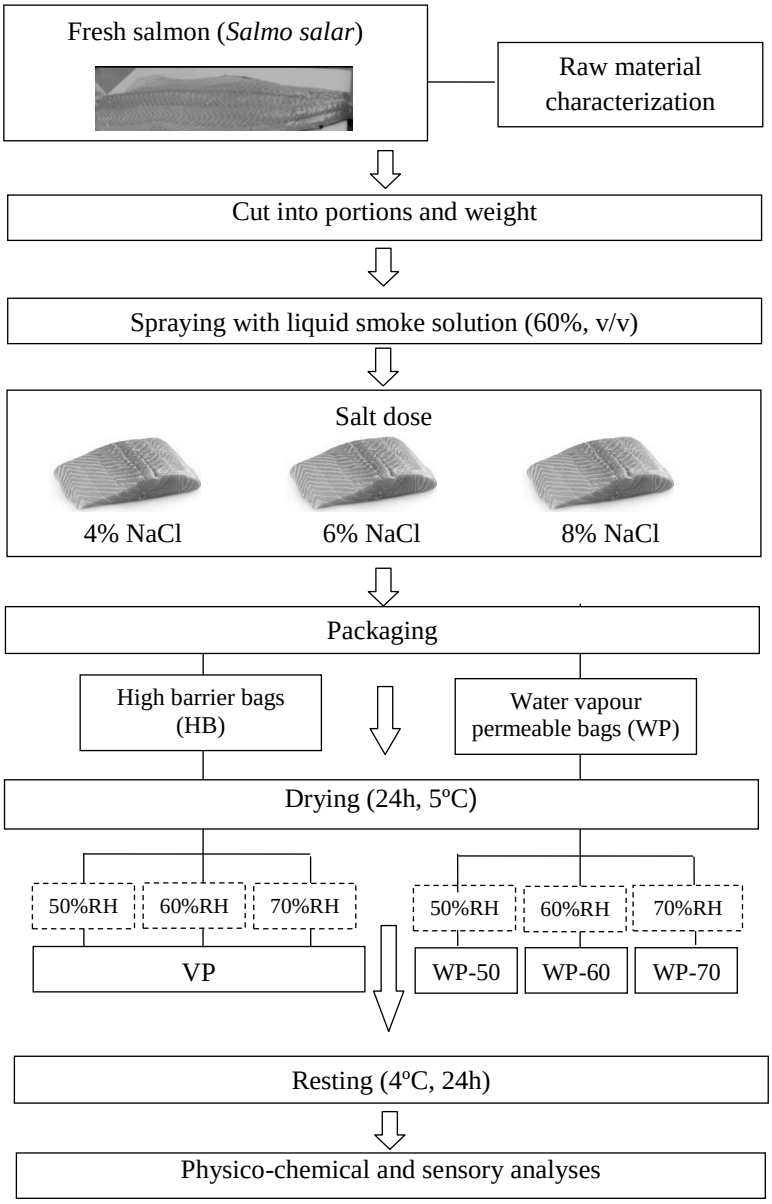

Fig. 1. Smoking-salting process of salmon.

Samples from the two groups were randomly divided into three new batches (6 batches in all); one batch per group of samples (WP and HB) was introduced into a drying chamber at 5 °C for 24 h (Binder mod. KBF Tuttlingen, Germany) with an established RH (50%, 60% or 70%). Afterwards, the salmon portions were removed from the bags and the exudate formed during the process was weighed. Fish samples were introduced into saturated brine under constant stirring for 30 s to remove any traces of salt attached to the surface. Finally, they were dried with absorbent paper, weighed and left at 4ºC for 24 h to ensure a homogeneous salt distribution onto the pieces.

Analyses of moisture, lipid content, NaCl content, pH and a_w were carried out on the fresh salmon and the smoked samples. Three samples were used for each condition (n=3), and the analyses were done on each sample in triplicate, except for pH, which was measured in quintuplicate.

2.3. Analytical determinations

Moisture content was determined according to the AOAC method 950.46 (1997). The lipid content of the samples was determined by Soxhlet extraction using petroleum ether according to the AOAC method 991.36 (AOAC, 1997). Sodium chloride content was determined following the procedure described by Fuentes et al., (2010b) with an automatic Sherwood Chloride Analyzer Model 926 (Sherwood Scientific Ltd., Cambridge, UK). pH measurements were taken using a digital pH-meter micropH 2001 (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) in five different sample locations. Water activity (a_w) was measured in minced samples with a fast water activity-meter (GBX scientific FA-st/1, Cédex, France).

Changes in the total mass of the salmon portions (ΔM_t) throughout the salting process were estimated from the weight (M) of the samples (at sampling time t and 0), respectively by Eq. (1). Likewise, the total sodium chloride concentration on a dry basis (X^{NaCl}) and in the liquid phase (z^{NaCl}) was estimated from the weight fractions of water (x^w) and sodium chloride (x^{NaCl}), in accordance with Eqs. (1) and (2) .

$$\Delta M_t = \left(\frac{M_t - M_0}{M_0}\right) \quad (1)$$

$$z^{NaCl} = \left(\frac{x^{NaCl}}{x^w + x^{NaCl}}\right) \quad (2)$$

2.4. Sensory analysis

After having established the appropriate smoking-salting conditions for the new process, a sensory assessment was made in order to evaluate the sensory acceptance of the obtained products. The sensory analysis was conducted with the smoke-flavoured salmon developed by the new process. In this case the smoking process was carried out by using the selected conditions (WP-50, WP-60, and WP-70 with a salt dose of 8 g NaCl/100 g fish) and commercial smoked salmon. The smoking process was similar to that previously described, but in this case, only the selected salt dose level was employed. Then the smoked salmon samples were filleted, vacuum-packed and kept at 4º C until the sensory evaluation was made (24 h approximately after finishing the whole process).

The sensory analysis of the smoke-flavoured salmon samples was undertaken by 32 non-expert and untrained assessors who are regular

consumers of smoked products. Tests were done with the semi-structured scales (AENOR, 2006) by which attributes such as appearance, colour, odour, intensity of smoke odour, taste, intensity of saltiness and global acceptance were evaluated. These attributes were selected as being the most important and representative for both industry and consumers (Rodrigues et al., 2005 cited by Fuentes et al., 2010b). Each assessor answered a questionnaire with 8 cm lines and three anchor points (0=unpleasant, 4 =acceptable, and 8=pleasant) for all the attributes, except for smoke odour and saltiness intensity, where the anchors corresponded to insufficient, optimum, and excessive (0, 4, and 8, respectively). Each assessor evaluated four samples served at room temperature and coded with a 3-digit random number.

2.5. Statistical analysis

A multifactor ANOVA was conducted in the first part of the study for each physico-chemical parameter to evaluate the effect of salt dose, packaging conditions, RH during salting-smoking, and their interactions. For the sensory analysis, a one-way ANOVA was performed for each sensory attribute evaluated in order to test if there were significant differences between the smoke-flavoured samples. The least significance procedure (LSD) was used to test for the differences between averages at the 5% level of significance. The data were statistically processed using Statgraphics Centurión XVI (Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Characterization of raw material and commercial product

Moisture, lipid content, sodium chloride, pH and a_w values of the raw material and the commercial smoked salmon samples are shown in Table 1.

Table 1. Physico-chemical parameters of raw material and commercial smoked salmon. Mean values ± SD (n = 3).

	Raw material	**Commercial smoked salmon**	
		Brand 1	**Brand 2**
Moisture (g/100g)	65.39 ± 1.93	60.10 ± 0.06	60.01 ± 0.22
Lipid (g/100g)	12.48 ± 3.47	11.43 ± 0.63	12.36 ± 0.94
NaCl (g/100g)	0.20 ± 0.01	4.31 ± 0.08	4.00 ± 0.22
pH	6.19 ± 0.08	6.30 ± 0.06	6.28 ± 0.02
a_w	0.993 ± 0.003	0.950 ± 0.008	0.949 ± 0.001

The results obtained in the physico-chemical characterisation of the fresh salmon used as raw material were similar to those reported in other studies (Duun & Rustad, 2008; Fernández-Segovia et al., 2012; Rørå et al., 2004). In general, the uptake and distribution of salt into the fillets in the salting processes depends on several factors, such as the salting method, salt dose, muscle thickness and other intrinsic fish factors like composition and post-mortem state (Rørå et al., 1998; Mørkøre & Rørvik, 2001). Accordingly, the correct characterisation of the raw material is especially important for selecting the most appropriate processing conditions. Some physico-chemical parameters, such as the moisture and lipid content of the raw material,

strongly influence muscle behaviour during processing because these parameters modify salt uptake and moisture loss, and can therefore determine the final product characteristics. For this reason, it is important to adjust the smoking-salting conditions depending on the moisture and lipid content of the raw material in order to ensure typical product characteristics (Barat et al., 2006).

For the commercial smoked salmon, the observed values agree with those for cold smoked salmon of diverse origins reported by other authors (Cardinal et al., 2004; Fuentes et al., 2010a). Smoked salmon showed lower values of moisture, but higher salt values, which implied a lower a_w value if compared to fresh fish. The analysed smoked product fulfilled the French standard for smoked salmon (NF V45-065 (1997) cited by Rørå et al., (2004), which establishes a maximum lipid content of 18% and 74% water content in the fat-free product. No significant differences were found among batches in any of the analysed parameters. However, some studies have found high variability for such products for their moisture content, salt, $a_{w,}$ and their sensory attributes (Truelstrup Hansen et al., 1998; Cardinal et al., 2004). The selection of homogeneous raw material would reduce this variability which is, in most cases, due to differences in fish muscle composition (Espe et al., 2002; Mørkøre & Røvik, 2001); however, this variability cannot be completely eliminated since the process highly affects the final product characteristics.

As Table 1 shows, the pH values were higher for the commercial smoked salmon if compared with fresh salmon. However, salting processes are associated with lower pH values of fish muscles in relation to the greater ionic strength of the internal solution in cells due to the effect of salt (Leroi & Joffraud, 2000). A higher pH value might be explained by the production of basic compounds such as ammonia, trimethylamine and other biogenic amines during storage which cause a progressive increase in pH (Goulas &

Kontominas, 2007). According to the characteristics of our raw material and the values of the physico-chemical parameters of the reference product, the processing conditions of the new method should be selected to allow moisture reduction over 5% and the salt dose should be established to achieve a final salt content close to 4% by considering salt loss with exudates.

3.2. Study of the smoking-salting process

The smoking-salting process, regardless of the employed conditions, significantly lowered the water content and a_w values, and also increased the NaCl concentration, if compared with fresh salmon (Figure 2). Dehydration and NaCl absorption into fish flesh lead to lower a_w values. As expected, the salt concentration increased with higher salt doses regardless of type of packaging and RH employed, and the a_w values consequently lowered. For the same salt dose level, the salting procedure combined with HB led to a higher salt content in the final product as compared with WP. The effect of vacuum packaging on salt absorption has been observed in other studies. Rizo et al., (2013) established that vacuum packaging speeds up NaCl absorption and could, therefore, accelerate the whole process, reducing the total processing time.

The moisture and a_w values of the WP samples were affected by RH since the samples processed at the lowest RH (WP-50) exhibited the lowest moisture and a_w values. It is interesting to note that the salt content of the WP samples did not differ among samples, which indicates that salt absorption is not affected by RH at the levels studied. The WP-70 samples showed the highest a_w value and moisture content irrespectively of salt dose, and the established reference value was not reached in any case.

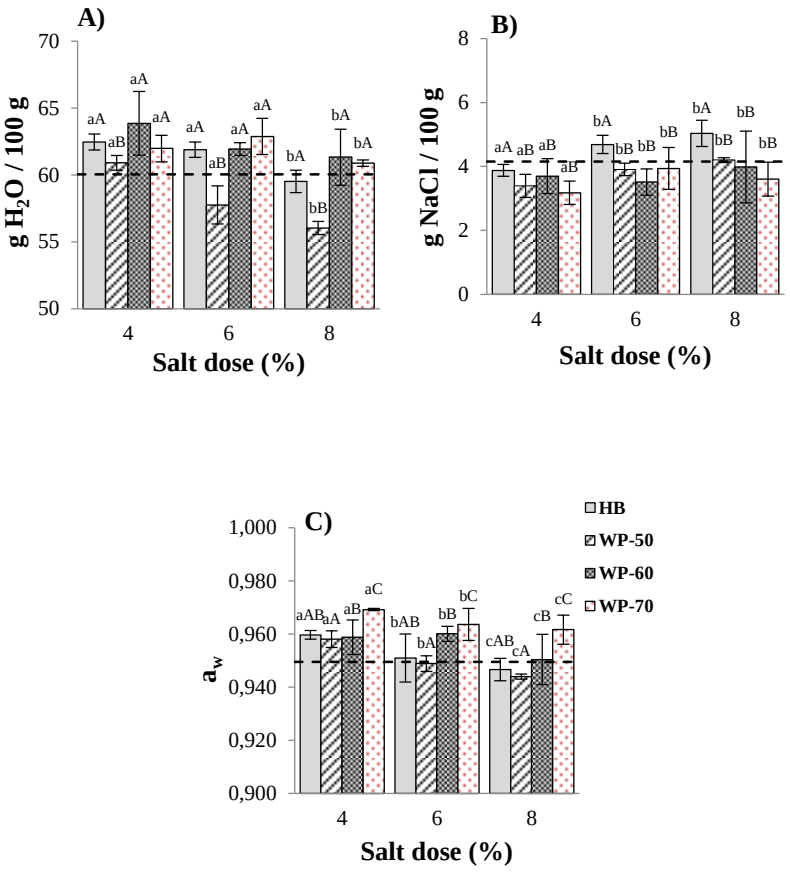

Fig.2. Moisture (a), salt content (b), and water activity (c) of the smoke-flavoured salmon samples obtained by using different salt dosage (4, 6, and 8% NaCl) and by different packaging conditions (HB, WP-50, WP-60, and WP-70). Mean values ± SD (n = 3). Bars indicate the standard deviation. The dashed line represents the reference value. Different lower-case and capital letters indicate significant differences for salt dose (S) and packaging conditions (P) factors, respectively ($p < 0.05$).

The WP-60 salmon samples with an 8% salt dose gave the closest moisture and salt content and a_w values to the reference value. Water activity (a_w) is directly related with the microbiological load of salted and smoked products. Therefore, it can be considered a decisive parameter to ensure smoked fish safety. In this sense, although the use of 50% RH implied a lower moisture and a_w in the fish samples, a higher risk of hardening and crust formation in the surface could appear. Crust formation leads to heterogeneous dried product with a highly dried surface and a poorly dried core (Andrés et al., 2007). These conditions should be corroborated with the sensory analysis.

The physico-chemical parameters of the smoke-flavoured salmon obtained by the new smoking-salting process using the different conditions are provided in Table 2. Weight loss increased in samples when the salt dose increased, and these data were higher as RH lowered. Regardless of the salt dose, weight loss was found to be lower in the HB samples as compared with the WP ones; this fact can be attributed to the higher dehydration observed in the WP samples, which led the water from fish muscle to flow out. Regarding lipid content, differences between samples were observed, and are possibly due to the existence of the initial variability among the fresh fish portions employed since lipid content is typically heterogeneously distributed in fish fillets, just as other studies have described (Katikou et al., 2001). The smoking-salting process led to lower pH values in the smoked salmon samples, except for the WP-70 samples (Table 2) given the effect of salt, which increases the ionic strength of the intracellular solution, as earlier mentioned.

According to Codex standard for smoked fish, smoke-flavoured fish and smoked dried fish (Codex, 2013), the variability in amount of salt in the aqueous phase could have important implications on the likehood of *Clostridium botulinum* toxin formation. In particular, smoked fish and

smoke-flavoured fish where the smoke flavour is provided by artificial flavour blends, 5% aqueous phase salt (z^{NaCl} = 0.05) would be required in order to provide complete protection at temperatures between 3°C and 10°C. Based on this standard, the products obtained by any of the treatments tested fulfil this requirement.

Table 2. Weight loss (ΔM_t), lipid content, pH, and salt content in water phase (z^{NaCl}) of the smoke-flavoured samples obtained using different salt dose (S) and packaging conditions (P). Mean values ± SD (n = 3).

P	S (g salt/100g)	ΔM_t	% Lipid	pH	z^{NaCl}
HB	4	-0.032 ± 0.003^{aA}	10.84 ± 0.56^{aB}	6.09 ± 0.09^{aAB}	0.058 ± 0.003^{aC}
	6	-0.043 ± 0.010^{bA}	10.68 ± 0.81^{aB}	6.11 ± 0.14^{aAB}	0.070 ± 0.005^{bC}
	8	-0.053 ± 0.006^{cA}	12.92 ± 1.22^{bB}	6.13 ± 0.09^{aAB}	0.078 ± 0.007^{cC}
WP-50	4	-0.094 ± 0.003^{aB}	12.73 ± 0.43^{aC}	6.13 ± 0.03^{aA}	0.053 ± 0.006^{aB}
	6	-0.095 ± 0.004^{bB}	13.22 ± 0.05^{aC}	6.17 ± 0.03^{aA}	0.064 ± 0.005^{bB}
	8	-0.107 ± 0.006^{cB}	16.56 ± 1.59^{bC}	6.17 ± 0.04^{aA}	0.070 ± 0.002^{cB}
WP-60	4	$-0{,}069 \pm 0.013^{aC}$	8.96 ± 3.68^{aA}	6.09 ± 0.00^{aB}	0.055 ± 0.006^{aAB}
	6	-0.085 ± 0.008^{bC}	10.41 ± 0.50^{aA}	6.04 ± 0.04^{aB}	0.054 ± 0.006^{bAB}
	8	-0.085 ± 0.012^{cC}	9.39 ± 1.88^{bA}	6.08 ± 0.03^{aB}	0.062 ± 0.007^{bAB}
WP-70	4	-0.056 ± 0.011^{aD}	12.20 ± 1.90^{aB}	6.26 ± 0.01^{aC}	0.049 ± 0.005^{aA}
	6	-0.067 ± 0.012^{bD}	10.75 ± 1.90^{aB}	6.31 ± 0.04^{aC}	0.058 ± 0.008^{bA}
	8	-0.069 ± 0.004^{cD}	12.80 ± 0.56^{bB}	6.26 ± 0.04^{aC}	0.056 ± 0.008^{cA}

Different lower-case letters indicate significant differences for salt dose factor (S). Different capital letters indicate significant differences for packaging conditions factor (P). ($p < 0.05$).
AMt: weight loss; % Lipid: g lipid/100 g fish; z^{NaCl}: g salt/g liquid phase

The results obtained in the multifactor ANOVA for each physico-chemical parameter are shown in Table 3. In general, the statistical analysis revealed that the amount of salt dosed and the packaging conditions strongly influence all the parameters evaluated, where the effect of packaging was greater as compared to the salt employed for all the variables considered. The interactions between salt and packaging did not affect the variables studied except for "exudate"; however, the effect of this interaction was less important than factors considered individually.

Table 3. F-ratio values and significance levels obtained in multifactor ANOVA for the physico-chemical parameters according to the factors: salt dose (S) and packaging conditions (P) and their interaction (S x P).

	S	P	S x P
Moisture	12.44^{***}	13.62^{***}	1.59^{ns}
Lipid content	3.23^{*}	11.78^{***}	1.93^{ns}
NaCl content	8.83^{***}	9.81^{***}	1.33^{ns}
pH	0.14^{ns}	12.71^{***}	0.33^{ns}
$\mathbf{a_w}$	10.72^{***}	10.84^{***}	0.67^{ns}
$\mathbf{\Delta M_t}$	13.71^{***}	125.83^{***}	0.77^{ns}
$\mathbf{X^{NaCl}}$	2.69^{ns}	7.68^{***}	1.32^{ns}
$\mathbf{z^{NaCl}}$	15.68^{***}	11.77^{***}	1.39^{ns}
Exudate	8.61^{***}	135.43^{***}	3.51^{**}

ns: no significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

After finishing the smoking-salting process, the liquid released by fish was collected and weighed. This exudated fluid is composed mainly of water, unabsorbed salt and soluble proteins (Barat et al., 2003). The exudate weight values are provided in Figure 3. No exudate was observed in the WP-50 and 60 samples. This fact would confirm that WP bags allowed the complete evaporation of the water released by the muscle during processing. Nevertheless for the highest RH, a certain amount of exudate was collected after processing, which was lower than the exudate collected in HB. For the HB samples, the amount of exudate increased as the amount of dosed salt rose because of the dehydrating effect of salt, which led to a greater flow of water from fish muscle to the fish surface during the salting process.

These results confirm that this new method reduce the number of processing steps, since the salting, drying and smoking stages can be carried out in one-single step. Otherwise, brine waste is considered one of the most worrying pollution sources from the food industry given its major

environmental impact and the investments required for treatment and waste disposal. Evaporation of residual brine using WP bags, and reducing the amount of salt employed with controlled salting, can help diminish the final volume of brine waste generated by the process. By using WP bags, RH conditions can be adjusted according to the specific raw material characteristics of each batch, which helps achieve a product with optimal physico-chemical characteristics. By using this methodology, modifications in other process parameters can be studied, such as temperature, air renewal and drying cycles, as these parameters correlate directly with water flow. Furthermore, the smoking-salting process inside a bag can offer other advantages over traditional methods (unpacked), such as protecting the product against possible contaminations and maximising yields (DeGeer et al., 2009).

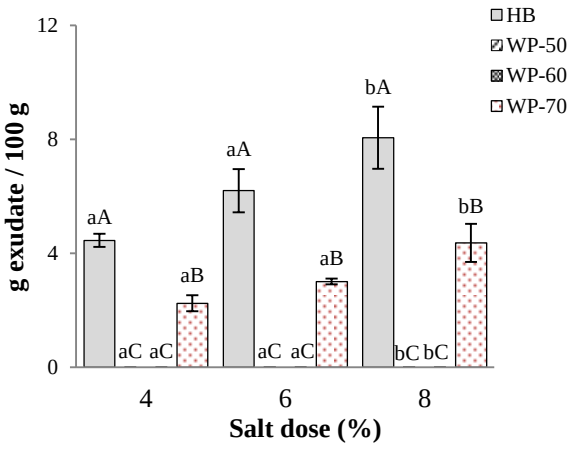

Fig. 3. Percentage of exudate generated during smoking-salting process. Mean values ± SD (n = 3). Bars indicate the standard deviation. Different lower-case and capital letters indicate significant differences for salt dose (S) and packaging conditions (P) factors, respectively ($p < 0.05$).

Among all the process conditions tested, the use of WP bags with 60% RH (WP-60), and an 8% salt dose was considered the most appropriate because it enables the achievement of smoke-flavoured fish with similar a_w, moisture and salt contents to the reference product.

3.3. Sensory analysis

After selecting the optimal conditions for the smoking-salting process, a sensory analysis was carried out to check the acceptability of the new smoke-flavoured salmon. The tested samples were WP-processed at different RHs (WP -50, WP-60, and WP-70) with the 8% sale dose. After processing, the moisture, lipid content, sodium chloride, pH, and a_w of smoke-flavoured product were determined. These analyses were also performed with the commercial product. The characterisation of the salmon products (those obtained by the new technology and the commercial ones) did not show significant differences in any of the parameters analysed (ANOVA data not shown). These values fell within the range of values for this type of products (Fuentes et al., 2010a). The overall scores marked by the assessors for the sensory attributes of the different evaluated samples are depicted in Figure 4. No significant differences were recorded between samples for any evaluated attribute ($p>0.05$; ns), although the scores for taste, odour and global acceptance were slightly higher for the commercial smoked salmon. Nevertheless, the commercial samples obtained lower scores for the colour attribute than those samples processed by the new smoking-salting process.

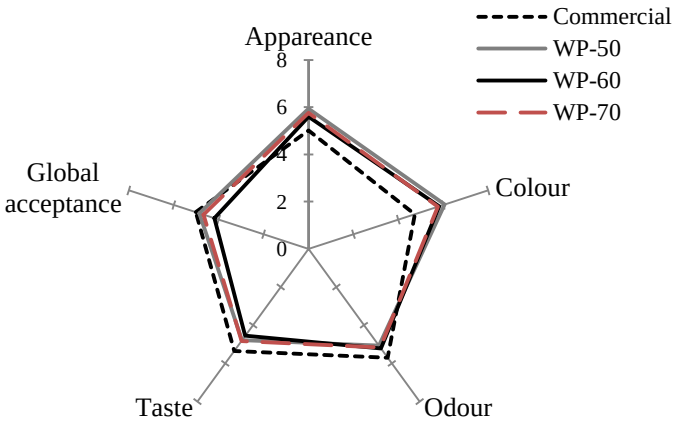

Fig. 4. Score average for the different attributes evaluated in samples of commercial smoked salmon and the new smoke-flavoured salmon products (WP-50, WP-60, and WP-70). 0: very unpleasant, 4: acceptable, 8: very pleasant.

The mean values scored by the assessors for smoke odour intensity and saltiness are shown in Figure 5.

For smoke odour intensity, all the samples obtained scores that came very close to the optimal value. No significant differences ($p>0.05$) were observed among samples. The WP-60 samples came closer to the score considered optimum, while the commercial smoked salmon and the samples WP-70 scores were the further away from the optimum value. In this case, the smoke odour intensity of commercial samples was considered by the panelists as excessive.

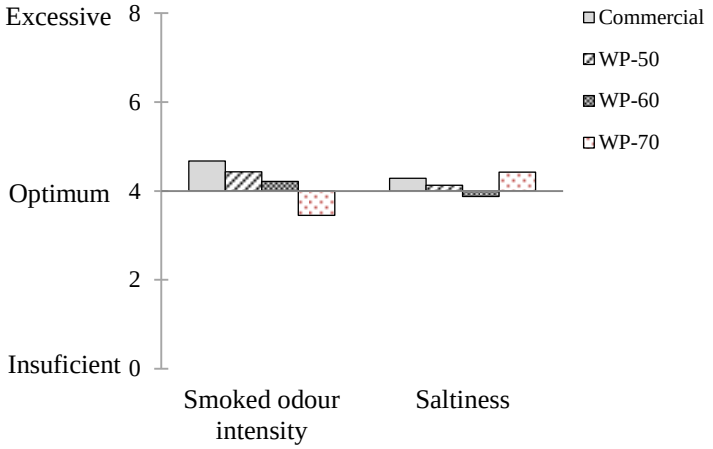

Fig. 5. Score average for the attributes "smoke odour intensity" and "intensity of saltiness", evaluated in samples of commercial smoked salmon and the new smoke-flavoured salmon products (WP-50, WP-60, and WP-70).

Likewise, no differences were found among samples for saltiness, and the scores for this attribute of the WP-70 and commercial smoked salmon were further from the "optimum" score. According to the sensory evaluation results, it may be stated that the sensory attributes of the smoked salmon obtained by the new methodology are perceived with the same degree of acceptance as the commercial smoked salmon.

This work provides producers a new alternative to the traditional salting and smoking processes. Further studies on the shelf-life of the product obtained will be necessary to establish if this product could be commercialized.

4. Conclusions

The salting procedure using HB led to a higher salt concentration in the final product as compared with the WP bags. In the WP samples, the decrease in RH in the drying chamber brought about a more marked reduction in the moisture and a_w values. However, salt uptake was not affected by the RH set. Among the salt levels employed, the WP-60 samples with an 8% salt dose exhibited the closest levels of a_w, salt content and moisture to the reference values. Use of 50% and 60% of RH led to the evaporation of the water released by the muscle. The sensory attributes of the smoke-flavoured salmon obtained similar scores to the commercial product. These results indicate that this new methodology is suitable to obtain smoke-flavoured salmon with similar physico-chemical characteristics and consumer acceptance to a commercial smoked salmon.

The new smoking-salting process minimises brine waste, reduces processing steps and facilitates fish handling during chain production, which could prove to be a major advantage as regards maintaining hygienic quality.

Acknowledgements

The authors gratefully acknowledge Tub-Ex Aps (Taars, Denmark) for supplying the water vapour permeable bags and for providing all the necessary information about their use. Author A. Rizo is grateful to the Universitat Politècnica de Valencia for the FPI grant.

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ARTÍCULO 2

SMOKE-FLAVOURED COD OBTAINED BY A NEW METHOD USING WATER VAPOUR PERMEABLE BAGS

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Journal of Food Engineering 179 (2016) 19-27

Versión adaptada para la tesis doctoral

Abstract

The objective of this study was to adapt and optimise a new smoking-salting process developed for salmon to obtain smoke-flavoured cod. Fish was processed at 60% relative humidity (RH)/5ºC for 24 h using different salt doses. During process optimisation, new conditions were studied (salt dose, RH, processing time). Smoke-flavoured cod showed higher salt and moisture content than the salmon samples, which required a higher salt concentration to reach similar a_w values. Process optimisation allowed the exudate to evaporate when the process lasted 72 and 96 h. The samples obtained with the 2% salt dose, 60% RH and 96 h gave the closest levels of moisture, salt and a_w to commercial products. This new smoking-salting could substitute traditional procedures as it minimises product handling and brine wastes, reduces processing steps and can be applied to different fish types by adapting processing parameters.

Keywords: salting, smoking, smoke-flavoured cod, water vapor permeable bags, liquid smoke

1. Introduction

The smoking process has been employed since ancient times to preserve fish. The traditional smoking process involves different stages, such as salting, drying and smoking. Currently, there is growing interest in improving traditional smoking and salting processes to minimise salt waste, reduce overall weight loss, improve hygienic quality and ensure final product safety. Regarding this last aspect, smoked foods generally involve health concerns, especially with respect to the possible presence of polycyclic aromatic hydrocarbons. However, using smoke flavourings is generally considered a less worrying health problem than the traditional smoking process since smoke flavourings are produced from smoke subjected to fractionation and purification processes (European Commission, 2003). For this reason, smoke-flavoured fish production could be a good alternative to traditional smoked products.

In order to meet food industry and consumer requirements related to increase yields, reduce wastes and improve the product safety, Rizo et al. (2013) proposed a new process to obtain smoke-flavoured salmon based on the combination of a controlled salting process with smoked-flavoured salt and vacuum packaging. This methodology was able to accelerate NaCl absorption and dehydration, reducing the total processing time without affecting physico-chemical parameters compared with traditional smoked salmon. In another study, Rizo et al. (2015a) proposed applying water vapour permeable (WP) bags to improve the previously described smoking-salting method. This methodology consists in simultaneous smoking-salting in WP bags under established temperature and humidity conditions to control product dehydration. This procedure allows smoke-flavoured salmon to be obtained with not only similar physico-chemical traits and sensory acceptance to the smoked products obtained by traditional methods, but also with good hygienic quality under cold storage (Rizo et al., 2015a, b). The use

of this new methodology could substitute traditional cold smoking of fish because the physico-chemical properties, consumer acceptance and final product safety are not affected, it minimises product handling and brine wastes, and reduces the number of processing steps.

For the fish industry, applying the new smoking-salting method using WP bags to other fish species could be of much interest. However, food processors need to optimise their processes according to the characteristics of the raw material, and it is not always possible to adapt previous procedures to new raw material characteristics or to final product requirements. There are many factors that affect salting and drying processes, such as species, muscle type, fish size, fillet thickness, weight, composition (lipid content and distribution), physiological state, salting method, brine concentration, salting step duration, fish-to-salt ratio, ambient temperature, freezing and thawing (Ismail and Wootton 1992; Wang et al., 2000). Lean fish species like cod display a different behaviour during salting and drying to fatty species like salmon (Cardinal et al., 2001; Gallart et al., 2007; Mørkøre et al., 2001).

The objectives of this study were to: (a) evaluate the feasibility of using a new smoking-salting process developed for salmon to obtain smoke-flavoured cod; (b) optimise the smoking-salting procedure to obtain smoke-flavoured cod.

2. Material and Methods

2.1. Materials

The fish employed as raw material were frozen cod (*Gadus morhua*) fillets of the 1.2-1.4 kg commercial size (Alimentos Friorizados, S.A., Barcelona, Spain), which is the commonest fish state used by the fish

industry. Before processing, frozen cod fillets (n=15) were thawed at 4°C for 24 h, trimmed to remove any bones and fins, and cut into 4-cm portions to obtain 5-6 portions per fillet (72 samples were obtained). The average weight and thickness of fish portions were 136±23 g and 3 cm, respectively.

Fine-grained salt used for the smoking-salting process was supplied by Panreac Química, S.A (Barcelona, Spain), and the natural liquid smoke HARDWOOD AFS 10 by Amcan ingredient Ltd. (Le Chesnay, France). Water vapour permeable bags were supplied by TUB-EX ApS (Taars, Denmark) (size: 200×300×0.04 mm; water vapour transmission rate: 5000g/50μ/m²/24h (38°C/50%RH)). To determine the target physico-chemical parameters for the new product, two different commercial smoked cod brands were used. From each brand, two different batches were analysed. These products were purchased from a local market in the city of Valencia (east Spain) and had been processed by traditional cold-smoking techniques: dry salting, followed by a smoking step in a smoking chamber.

2.2. Experimental design

2.2.1. Phase I. Studying the feasibility of using a new smoking-salting process: comparison between salmon and cod

Cod portions were subjected to a simultaneous smoking-salting process (Fig. 1) following the method developed by Rizo et al. (2015a) to obtain smoke-flavoured fish. Fish samples were smoked by spraying the fillet surface with diluted liquid smoke (60 mL/100 mL solution) for 30 s. After applying liquid smoke, salting was carried out by dosing a previously established amount of NaCl on the fillet surface. Three salt dose concentrations were considered: 4, 6, and 8 g salt/100 g fresh fish. The amount of salt added to each sample was calculated from the initial fish

portion weight (after thawing) and the initial water weight fraction (x^w) according to the procedure by Fuentes et al. (2008). Samples were introduced into WP bags and were vacuum-packaged with a vacuum packaging machine (Tecnotrip EV-25-CD, Barcelona, Spain). It should be noted that vacuum packaging was used merely to ensure good initial contact between fish and the WP bag. Then portions were processed in a drying chamber (Binder mod. KBF Tuttlingen, Germany) at 60% RH for 24 h at 5ºC. At the end of the processing time, samples were removed from the bags and the exudate formed during the process was weighed. Fish samples were introduced into saturated brine under constant stirring for 30 s to remove any traces of salt attached to the surface. Finally, they were dried with absorbent paper, weighed and left at 4ºC for 24 h to ensure homogeneous salt distribution on the pieces.

Analyses of moisture, lipid content, NaCl content and a_w were carried out on the fresh fish and the final product. Values considered as references were obtained from the commercial smoked cod analysed (moisture, salt content and a_w).

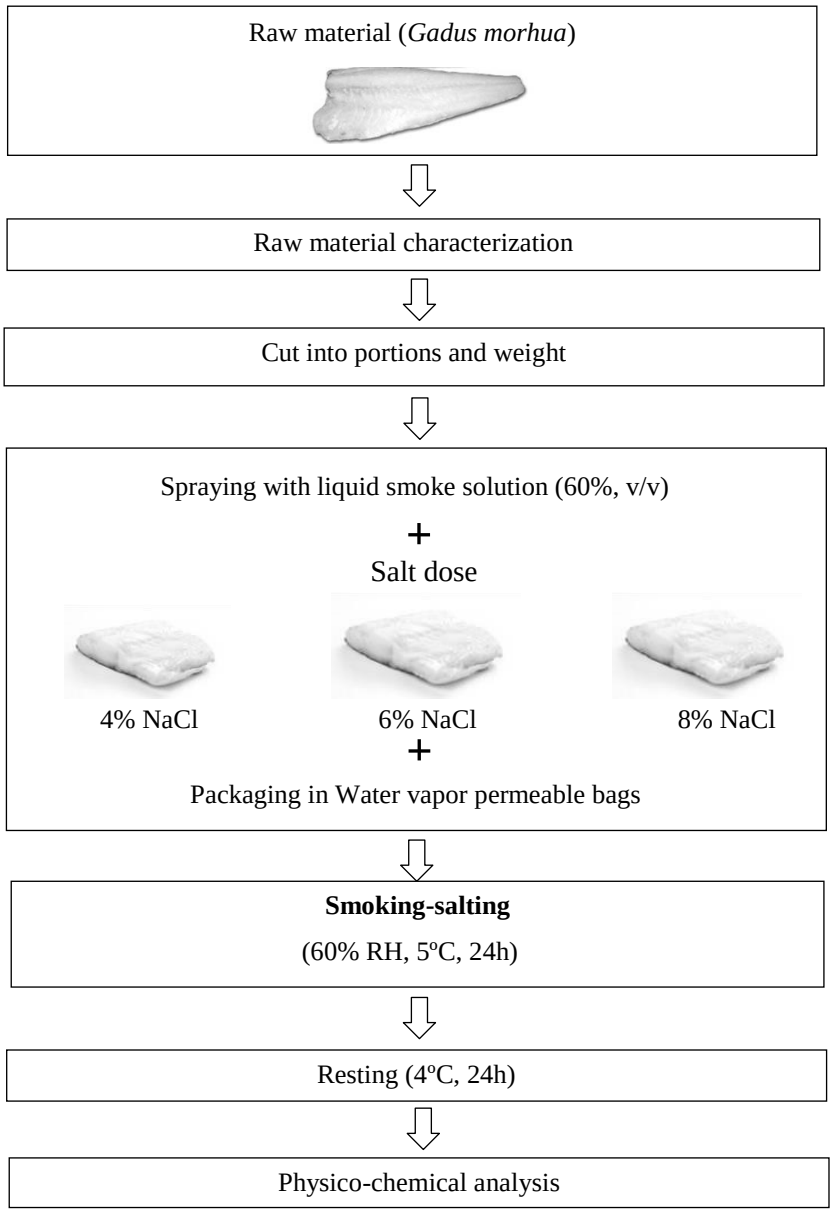

Fig. 1. Smoking-salting process in phase I.

2.2.2. Phase II. Optimisation of the smoking-salting process for cod

Cod portions were submitted to the smoking-salting process described in Phase I with some modifications (Fig. 2). In this study phase, the effects of different salt doses, RH in the drying chamber and processing time on the physico-chemical properties of the final product were studied. Processing variables were established after considering the results obtained in Phase I and the physico-chemical characteristics of the target product. Three salt dose concentrations (2, 3, and 4 g salt/100 g fresh cod), two levels of RH in the drying chamber (60% and 70% RH), and three processing times (48, 72, and 96 h) were studied. The smoking-salting process was carried out at 10 ºC.

In both phases, three samples were used per condition (n=3) and the physico-chemical analyses (moisture content, NaCl content and a_w) were run on each sample in triplicate.

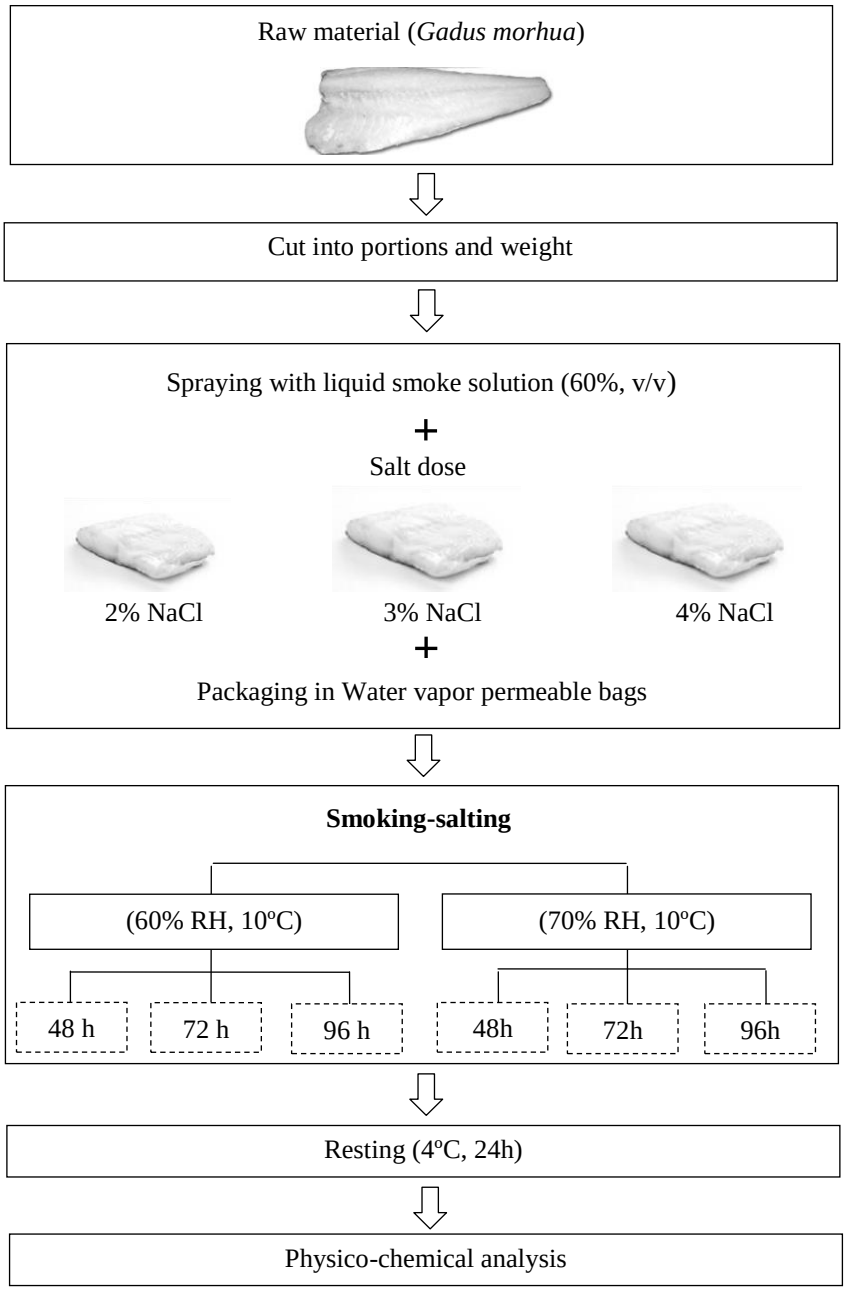

Fig. 2. Smoking-salting process in phase II.

2.3. Analytical determinations

Moisture content (x^w) was determined by oven drying until a constant weight was reached at 105°C (AOAC, 1997). The lipid content (x^f) of the samples was determined by the AOAC method (AOAC, 1997). Sodium chloride content (x^{NaCl}) was established by the procedure described by Fuentes et al. (2010b), but using an automatic Sherwood Chloride Analyzer Model 926 (Sherwood Scientific Ltd., Cambridge, UK). Water activity (a_w) was measured in minced samples with an Aqualab dew point hygrometer, model 4TE (Decagon Devices, Inc., Washington, USA).

The total sodium chloride concentrations on a dry basis (X^{NaCl}), on a dry fat-free basis (X_{ff}^{NaCl}) and in the liquid phase (z^{NaCl}) were estimated by Eqs. (1)-(3).

$$X^{NaCl} = \left(\frac{x^{NaCl}}{1-x^w}\right) \quad (1)$$

$$X_{ff}^{NaCl} = \left(\frac{x^{NaCl}}{1-x^w-x^f}\right) \quad (2)$$

$$z^{NaCl} = \left(\frac{x^{NaCl}}{x^w+x^{NaCl}}\right) \quad (3)$$

The total, water and sodium chloride weight changes (ΔM_t^o, ΔM_t^w and ΔM_t^{NaCl} respectively) of the fish samples were calculated by Eqs. (4)-(6).

$$\Delta M_t^o = \left(\frac{M_t^o - M_0^o}{M_0^o}\right) \quad (4)$$

$$\Delta M_t^w = \left(\frac{M_t^o \cdot x_t^w - M_0^o \cdot x_0^w}{M_0^o}\right) \quad (5)$$

$$\Delta M_t^{NaCl} = \left(\frac{M_t^o \cdot x_t^{NaCl} - M_0^o \cdot x_0^{NaCl}}{M_0^o} \right) \quad (6)$$

2.4. Statistical analysis

A multifactor ANOVA was conducted in Phase I for each physico-chemical parameter to determine whether there were significant differences between salt dose, fish species and their interactions. Likewise in Phase II, a multifactor ANOVA was performed for each physico-chemical parameter to evaluate the effect of salt dose, RH during smoking-salting, the processing time, and their interactions. The least significance procedure (LSD) was used to test for the differences between averages at the 5% level of significance. Data were statistically processed with Statgraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA).

3. Results and Discussion

3.1 Characterisation of raw material and commercial smoked fish

The physico-chemical parameters of the raw material and the commercial smoked cod (brands A and B) are shown in Table 1. The moisture, salt content and a_w values in the commercial samples were used to establish reference values.

The values obtained in the physico-chemical characterisation of the raw material were similar to those reported by other authors for fresh cod (Andrés et al., 2005; Gallart-Jornet et al., 2007). In our study, the moisture and the lipid content of raw material was 83.55% and 0.16%, respectively. Compared with the other fish species used traditionally for smoking, such as

salmon, herring or sardine, cod has a very low fat content, and therefore high moisture content. In salting processes, salt uptake depends on many factors: the salting method, salt dose, freezing and thawing, and on intrinsic fish factors like fish species, muscle thickness, postmortem state and composition (Fuentes et al., 2008; Gallart et al., 2007; Jittinandana et al., 2002; Rørå et al., 1998; Wang et al., 1998). For this reason, raw material characterisation prior to adjusting smoking-salting conditions is essential (Barat et al., 2006).

Table 1. Moisture, lipid, salt content, salt content in the liquid phase and a_w of raw material and commercial smoked cod (brand A and B). Mean values ± SD (n=3).

	x^w	x^f	x^{NaCl}	z^{NaCl}	a_w
Raw material	0.835±0.004	0.0016±0.0009	-	-	0.994±0.003
Smoked cod A	0.732±0.003	0.0013±0.0005	0.053±0.0003	0.068±0.003	0.959±0.002
Smoked cod B	0.751±0.013	0.0012±0.0001	0.023±0.0002	0.031±0.002	0.973±0.005

The physico-chemical parameters of the commercial smoked cod fell within the range of those reported by other authors (Karásková et al., 2011; Fuentes et al., 2010a). Significant differences were found between the two brands analysed (ANOVA data not shown). The largest difference was observed in salt content since the sodium chloride content in brand A was twice as high as in brand B. Brand A also gave lower moisture and a_w values compared to brand B, which could be attributed to greater intensity in salting and/or smoking. Some studies have found wide variability for such products in terms of their moisture content, salt and a_w, which could have implications for food safety (Cornu et al., 2006; Espe et al., 2004; Fuentes et al., 2010a). These differences directly influence sensory attributes and could determine shelf life, which can range between 1 and 8 weeks as reported in other studies

of smoked salmon (Cardinal et al., 2004; Jørgensen et al., 2000; Leroi et al., 2001).

Given the wide variability found between brands, establishing single reference values for moisture, salt and a_w is difficult. According to the Codex standard for smoked fish, smoke-flavoured fish and smoked-dried fish (Codex, 2013), 5% aqueous phase salt (z^{NaCl} = 0.05) would be required for smoke-flavoured fish in which smoke flavour is provided by artificial flavour blends to provide complete protection against *Clostridium botulinum* at temperatures between 3°C and 10°C. To fulfil this standard, the target value selected for sodium chloride content in this study was (x^{NaCl}=0.04), which corresponds to z^{NaCl} = 0.05 according to a moisture value of 74.2% (the average moisture of both brands).

3.2. Phase I. Studying the feasibility of using a new smoking-salting process: comparison between salmon and cod

The results obtained in the physico-chemical determinations carried out in smoke-flavoured cod under different salt dose conditions are shown in Table 2. These parameters were compared with those obtained for smoke-flavoured salmon by Rizo et al. (2015a), who followed the same smoking-salting procedure.

Table 2. Physico-chemical parameters and relative mass changes in smoke-flavoured cod and salmon obtained using different salt doses (4, 6, and 8% NaCl). Mean values ± SD (n = 3).

	Smoke-flavoured cod			Smoke-flavoured salmon*			α	
Salt dose (g/100g)	4	6	8	4	6	8	**S**	**F**
x^{w}	0.792±0.009^{aA}	0.771±0.009^{abA}	0.757±0.009^{bA}	0.636±0.02^{aB}	0.619±0.005^{abB}	0.613±0.029^{bB}	*	***
x^{NaCl}	0.045±0.004^{aA}	0.057±0.003^{aA}	0.067±0.008^{bA}	0.037±0.005^{aB}	0.035±0.004^{aB}	0.040±0.012^{bB}	*	***
X^{NaCl}	0.207±0.031^{aA}	0.249±0.019^{aA}	0.278±0.046^{aA}	0.103±0.021^{aB}	0.092±0.009^{aB}	0.103±0.035^{aB}	ns	***
X_{ff}^{NaCl}	0.209±0.032^{aA}	0.251±0.011^{aA}	0.280±0.045^{aA}	0.130±0.014^{aB}	0.127±0.014^{aB}	0.131±0.010^{aB}	ns	***
z^{NaCl}	0.056±0.004^{aA}	0.069±0.003^{aA}	0.082±0.008^{bA}	0.055±0.009^{aB}	0.054±0.006^{aB}	0.062±0.007^{bB}	**	**
a_w	0.962±0.004^{aA}	0.939±0.002^{bA}	0.931±0.006^{cA}	0.959±0.006^{aB}	0.960±0.003^{bB}	0.950±0.009^{cB}	***	***
x^{f}	0.002±0.001^{aA}	0.002±0.001^{aA}	0.002±0.001^{aA}	0.090±0.004^{aB}	0.104±0.005^{aB}	0.094±0.002^{aB}	ns	**
Exudate (w/w)	0.019±0.007^{a}	0.027±0.003^{b}	0.045±0.0018^{c}	-	-	-	***	-
Weight changes on wet basis								
ΔM_t^o	-0.120±0.033^{aA}	-0.116±0.024^{aA}	-0.125±0.020^{aA}	-0.070±0.013^{aB}	-0.085±0.008^{aB}	-0.085±0.012^{aB}	ns	**
ΔM_t^w	-0.147±0.028^{aA}	-0.154±0.015^{abA}	-0.157±0.012^{bA}	-0.071±0.014^{aB}	-0.096±0.009^{abB}	-0.102±0.012^{bB}	ns	***
ΔM_t^{NaCl}	0.034±0.005^{aA}	0.045±0.001^{aA}	0.055±0.006^{bA}	0.031±0.005^{aB}	0.030±0.004^{aB}	0.035±0.010^{bB}	**	***
Weight changes on fat free basis								
$\Delta M_t^o ff$	-0.120±0.033^{aA}	-0.116±0.024^{aA}	-0.126±0.020^{aA}	-0.083±0.015^{aA}	-0.119±0.016^{aA}	-0.106±0.015^{aA}	ns	ns
$\Delta M_t^w ff$	-0.147±0.028^{aA}	-0.153±0.015^{abA}	-0.157±0.012^{bA}	-0.074±0.015^{aB}	-0.109±0.011^{abB}	-0.108±0.012^{bB}	ns	***
$\Delta M_t^{NaCl} ff$	0.034±0.005^{aA}	0.045±0.001^{aA}	0.055±0.006^{bA}	0.032±0.005^{aB}	0.030±0.003^{aB}	0.036±0.003^{bB}	**	***

*Data obtained from a previous study (Rizo et al., 2015a). Different lower-case letters indicate significant differences for salt dose factor (S). Different capital letters indicate significant differences for fish species (F). ns: no significant, * p <0.05, ** p <0.01, *** p <0.001

The smoking-salting process led to a significant reduction in moisture and an increase in NaCl content, which lowered the initial a_w values. For each tested condition, smoke-flavoured cod showed higher salt and moisture contents than the salmon samples. In this sense, when using the same procedure conditions to achieve a similar a_w to salmon, a higher salt concentration is required with cod, given its higher moisture content. The statistical analysis indicated significant differences between cod and salmon concerning z^{NaCl}, and a_w values; however, at the lowest salt dose (4 g/100 g fresh fish) these parameters were similar.

When the total, water and salt weight changes in cod and salmon were compared, the most important factor was fish type. The ΔM_t^o, ΔM_t^w, and ΔM_t^{NaCl} values were always higher for the smoke-flavoured cod than for salmon with all the salt doses. This result indicates the influence of fish characteristics on the smoking-salting process. The most marked difference between both species was lipid content, which was higher in salmon than in cod (Table 2). In the literature it is well-known that lipid content in fish is a limiting factor during salting and drying processes, which acts as a physical barrier for water diffusion and replaces the aqueous part that serves as a vector for transfer during salting (Cardinal et al., 2001). Different studies have reported the strong effect of the lipid phase during fish drying or brine salting (Collignan et al., 2001; Czerner et al., 2013; Gallart-Jornet et al., 2007). During the salting process, the main mass transfer fluxes are water and soluble solids, which occur in the fish aqueous phase (Barat et al., 2003). Accordingly, fat acts as an inert component in the mass transfer. Therefore, weight changes (total, water and salt weight changes) would be lower in those fish species with a higher lipid content. This fact could explain why the ΔM_t^o, ΔM_t^w and ΔM_t^{NaCl} values were higher in the cod samples than in the salmon ones, and independently of the salt dose tested. However when these parameters were expressed on a fat-free basis, the difference between the cod

and salmon samples significantly reduced. The statistical analysis still reported significant differences between both fish species, a behaviour that has been previously described by other authors (Gallart-Jornet et al., 2007). Similar results were obtained by Aursand et al. (2008) when comparing water dynamics during brine salting of cod and salmon. These authors showed that throughout the salting process, WHC was lower and the NaCl gain was higher in cod than in salmon.

After finishing the smoking-salting process, the liquid released by fish into WP bags was collected and weighed. The exudate was composed mainly of water, unabsorbed salt and soluble proteins (Barat et al., 2003). No exudate was observed in the smoke-flavoured salmon samples, whereas a certain amount of exudate was collected from the smoke-flavoured cod samples, which became higher with increasing amounts of the salt dose used. Erikson et al. (2004) found differences in the ability to retain water between frozen-thawed and fresh cod. These differences were attributed to the fact that water-protein associations in fresh fish are partly replaced by protein-protein interactions during frozen storage. After salting, these authors did not found differences in WHC between frozen-thawed and fresh raw material. In this sense, it could be established that, in our study, the differences observed on the exudate formation could be attributed to muscle composition, more than to freezing-thawing before processing.

Zugarramurdi and Lupin (1980) developed models to predict sodium chloride uptake and water loss. The capacity of these models has been proven by different authors for several fish species (Bellagha et al., 2007; Boudhrioua et al., 2009; Sobukola and Olatunde, 2011). In dry salting studies, Bellagha et al. (2007) observed that water content decreased rapidly and the highest amount of exudate was released during the first 24 hours.

According to the models of Zugarramurdi and Lupin (1980), water loss is dependent on the initial and final moisture contents, which explains

the larger amount of exudate collected in WP bags after cod processing compared with salmon. The exudation rate was lower for the salmon samples, and the processing conditions (RH, temperature and processing time) were suitable for evaporating all the exudate. In contrast, the same conditions were inappropriate for evaporating all the exudate formed during cod processing with WP bags. It is kwon that drying rate is directly related to air temperature, air velocity and RH; therefore by modifying properly these parameters, it will be possible to evaporate water release by fish muscle. However, drying fish at very low RH can lead to hardening on the surface and a lower drying rate. Case hardening is the progressive formation of a thick crust of salt and protein on the surface. The crust prevents migration of water and the centre of the fish can become spoiled, even though it looks dried (Andrés et al., 2007). Crust formation was observed by Rizo et al. (2015a) by the same smoking-salting procedure at 50% RH, therefore 60% RH was fixed as a minimum value. Nevertheless, it is well-known that the temperature increase accelerates moisture and salt diffusion (Bellagha et al., 2007; Boudhrioua et al., 2009). Accordingly, increasing the chamber temperature slightly would result in a drop in humidity, which would enhance the drying rate (Rørå et al., 2005)

Water activity (a_w) is a decisive parameter to ensure smoked fish safety since it is directly related with the microbiological load of food products. With salted products, a high correlation exists between a_w and NaCl concentration in the liquid phase (z^{NaCl}) (Fuentes et al., 2008). By taking the a_w parameter as a reference to establish the processing conditions, a 4% salt dose allowed a smoke-flavoured cod product to be obtained with a similar a_w value to the target (a_w = 0.965, average values of both brands) (Table 1). However, the samples processed under these conditions showed a higher moisture and salt content than the reference. None of the smoke-flavoured cod samples obtained in this phase of the study achieved the reference moisture and salt content. For this reason, processing conditions should be

adjusted to obtain a smoke-flavoured cod product that has less moisture while maintaining the proposed salt level. As previously mentioned, more intense moisture loss can be achieved by raising the processing temperature, and by also prolonging the processing time. In order to avoid crust formation on cod samples, 60% and 70% RH were established in the drying chamber for smoking-salting process optimisation purposes.

3.3. Phase II. Optimisation of the smoking-salting process for cod

According to the results in Phase I, optimisation of the smoking-salting cod procedure entailed reducing salt dosage, modifying temperature and RH in the drying chamber, and prolonging the process.

Moisture of the smoke-flavoured cod samples significantly lowered with higher salt doses, a lower RH and longer processing times (Fig. 3 and Table 4). The samples processed with the highest salt dose (4%), the lowest RH (60%) and for a longer time gave the lowest moisture values. The samples submitted to a smoking-salting process that lasted less than 96 h did not achieve the reference moisture content, independently of the salt dose level or RH.

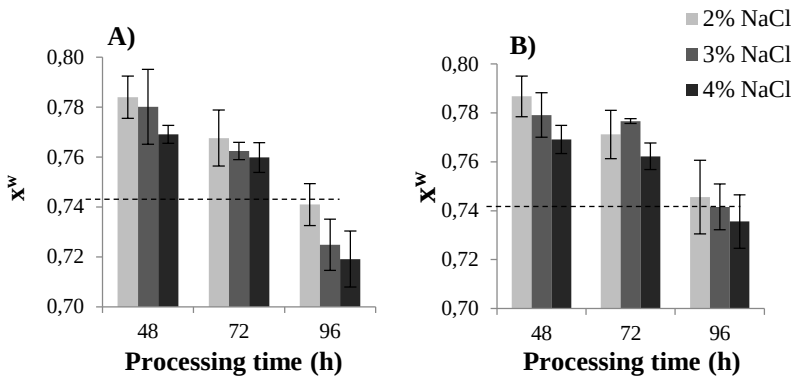

Fig. 3. Moisture values of the smoke-flavoured cod samples obtained using different relative humidity 60% RH (a) and 70% RH (b), salt doses (2, 3, and 4% NaCl), and processing times (48, 72, 96 h). Mean values ± SD (n=3). Bars represent the standard deviation from triplicate determination. The dashed line represents the reference values.

Salt concentration significantly increased with salt dose, RH and processing time (Fig. 4). Salt content was slightly lower in the samples processed at 70% RH compared with those processed at 60% RH, due to the lower dehydration undergone by these samples. This effect was stronger as the processing time increased. Likewise for the same salt dose, a longer processing time yielded higher salt content. The samples processed with the highest salt dose gave a very high final salt content. This is in contrast to food industry trends, which focus on reducing the amount of salt in processed food. Indeed, the EU has established a common framework to reduce salt intake in the general population (European Commission, 2009). The European approach to salt reduction focuses on a limited number of food categories, which include fish products. To achieve this goal, the European Commission supports research into reducing sodium in foods to the lowest

possible level, while maintaining food safety and consumer acceptance. Therefore, it would be interesting to select processing conditions that allow a smoked fish product to be obtained with the lowest salt content and an a_w value that ensures product safety.

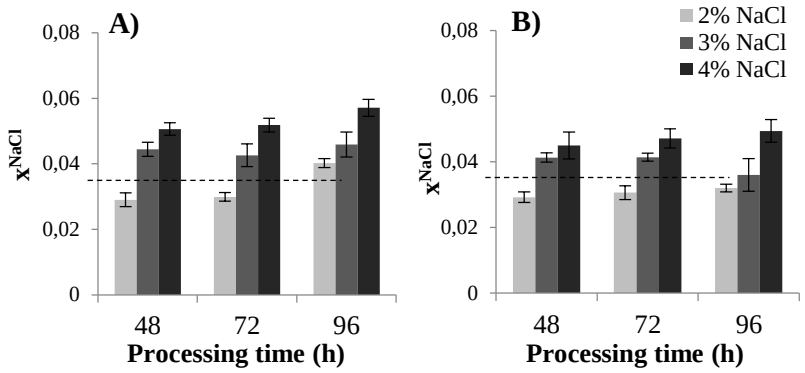

Fig. 4. Salt content values of the smoke-flavoured cod samples obtained using different relative humidity 60% RH (a) and 70% RH (b), salt doses (2, 3, and 4% NaCl), and processing times (48, 72, 96 h). Mean values ± SD (n =3). Bars represent the standard deviation from triplicate determination. The dashed line represents the reference values.

The effect of salt dose, RH and processing time on the a_w parameter was similar to that observed for moisture. Thus when salt dose increased and/or RH decreased, lower a_w values were detected (Figure 5).

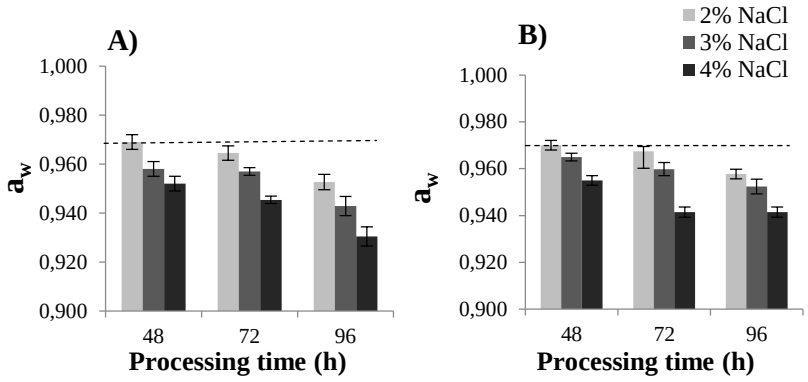

Fig. 5. Water activity values of the smoke-flavoured cod samples obtained using different relative humidity 60% RH (a) and 70% RH (b), salt doses (2, 3, and 4% NaCl), and processing times (48, 72, 96 h). Mean values ± SD (n=3). Bars represent the standard deviation from triplicate determination. The dashed line represents the reference values.

The relative mass changes and physico-chemical parameters in the smoke-flavoured cod obtained by the new smoking-salting process are shown in Table 3.

Table 3. Relative mass changes and physico-chemical parameters in smoke-flavoured cod obtained using different salt doses (S), relative humidity (RH), and processing times (t). Mean values ± SD (n = 3).

S (g salt/100g)	RH (%)	t (h)	ΔM_t^0	ΔM_t^W	ΔM_t^{NaCl}	X^{NaCl}	z^{NaCl}	Exudate (g/g)
2		48	-0.240±0.051	-0.253±0.043	0.016±0.001	0.134±0.005	0.036±0.003	0.001±0.0005
3	**60**	48	-0.219±0.012	-0.226±0.018	0.029±0.002	0.203±0.017	0.054±0.002	0.003±0.002
4		48	-0.230±0.034	-0.244±0.028	0.034±0.003	0.219±0.011	0.062±0.002	0.008±0.002
2		72	-0.276±0.015	-0.280±0.023	0.016±0.001	0.128±0.005	0.037±0.002	-
3	**60**	72	-0.271±0.041	-0.280±0.031	0.025±0.002	0.180±0.005	0.053±0.002	-
4		72	-0.260±0.019	-0.273±0.012	0.033±0.002	0.216±0.014	0.064±0.002	-
2		96	-0.371±0.032	-0.381±0.023	0.018±0.001	0.150±0.004	0.052±0.003	-
3	**60**	96	-0.374±0.013	-0.382±0.014	0.023±0.003	0.167±0.018	0.059±0.004	-
4		96	-0.375±0.009	-0.386±0.012	0.032±0.003	0.204±0.012	0.074±0.003	-
2		48	-0.187±0.040	-0.213±0.036	0.018±0.001	0.137±0.003	0.036±0.002	0.013±0.002
3	**70**	48	-0.184±0.019	-0.200±0.015	0.029±0.002	0.187±0.010	0.052±0.002	0.013±0.003
4		48	-0.184±0.021	-0.213±0.025	0.031±0.003	0.196±0.020	0.056±0.007	0.020±0.002
2		72	-0.221±0.010	-0.235±0.010	0.017±0.002	0.134±0.010	0.038±0.003	-
3	**70**	72	-0.219±0.024	-0.229±0.020	0.027±0.001	0.185±0.004	0.051±0.002	-
4		72	-0.211±0.030	-0.234±0.020	0.034±0.001	0.197±0.018	0.058±0.003	-
2		96	-0.303±0.020	-0.316±0.012	0.015±0.002	0.130±0.004	0.041±0.001	-
3	**70**	96	-0.284±0.034	-0.304±0.033	0.020±0.002	0.140±0.018	0.046±0.006	-
4		96	-0.276±0.049	-0.303±0.036	0.031±0.002	0.198±0.005	0.064±0.002	-

The longer the processing time, the more marked the total and water weight changes were (ΔM_t^o and ΔM_t^w) in higher dehydration. Regardless of salt dose, ΔM_t^o and ΔM_t^w were higher in the samples processed at 60% RH than the 70% RH samples. Total weight changes can be considered a combination of both weight changes (water and NaCl). These data were consistent with the moisture values recorded for the same conditions (Fig. 3). The modifications introduced into the processing parameters allowed the moisture and a_w of the fish samples to lower, but had very little impact on the salt content calculated on a dry basis (Table 3). No tendency to case hardening was detected in the samples, which indicates that using 60% or 70% RH and raising the temperature from 5 to 10ºC were adequate for the smoking-salting process.

It should be noted that no exudate was obtained after the smoking-salting in the samples processed for 72 h and 96 h under all the processing conditions studied. WP bags allowed the complete evaporation of the water released by muscle during processing, as observed in the smoking-salting of salmon with WP (Rizo et al., 2015a). These results confirm the effectiveness of WP bags, and that salting, drying and smoking stages can be carried out in a single step to thus reduce handling operations and processing steps compared with traditional methods. Evaporation of residual brine and a controlled salt dose reduce the final volume of brine wastes generated by the process. This could be a great advantage as these brines are highly polluting and require expensive treatment and waste disposal. Furthermore, smoking-salting inside a bag enables fish processing to take place under more controlled conditions than traditional methods (unpacked) (Rizo et al., 2015b).

The statistical analysis showed that all the factors strongly influenced each evaluated parameter (Table 4). The effect of salt dose and processing time was stronger compared to RH for all the considered

parameters. Salt dose was the factor with the most marked effect on the salt content-related variables measured (x^{NaCl}, X^{NaCl}, z^{NaCl}, and ΔM_t^{NaCl}), as confirmed by the F-ratio results obtained in the statistical analysis (Table 4). The same behaviour was observed for the a_w parameter, whereas processing time had a stronger effect on moisture, weight loss and water weight changes. These effects can be explained by the fact that the fish packaged in WP bags continued to lose moisture throughout the process, while the increase in salt content ended when muscle absorbed all the dosed salt. Some interactions were also detected between factors, but were generally non-significant or had a minor effect on the studied parameters compared with the independently analysed factors.

Table 4. F-ratio values and significance levels obtained in multifactor ANOVA for the physico-chemical parameters according to the factors: salt dose (S), relative humidity (RH), processing time (t) and their respective two-way interactions.

	S	**RH**	**t**	**S x RH**	**S x t**	**RH x t**
$\mathbf{x^w}$	10.19^{**}	7.25^{*}	111.85^{***}	0.58^{ns}	0.67^{ns}	1.85^{ns}
$\mathbf{a_w}$	238.99^{***}	33.22^{***}	153.17^{***}	2.44^{ns}	2.75^{*}	10.18^{***}
$\mathbf{x^{NaCl}}$	215.57^{***}	35.67^{***}	8.68^{***}	2.31^{ns}	5.81^{*}	8.39^{*}
$\mathbf{X^{NaCl}}$	113.45^{***}	11.73^{**}	5.89^{**}	1.96^{ns}	5.70^{**}	2.33^{ns}
$\mathbf{z^{NaCl}}$	228.18^{***}	41.35^{***}	22.94^{***}	2.04^{ns}	4.81^{**}	11.22^{***}
$\mathbf{\Delta M_t^0}$	1.02^{ns}	76.09^{***}	109.63^{***}	0.12^{ns}	3.76^{*}	0.16^{ns}
$\mathbf{\Delta M_t^w}$	1.44^{ns}	9.03^{**}	17.41^{***}	2.93^{ns}	0.20^{ns}	7.12^{**}
$\mathbf{\Delta M_t^{NaCl}}$	235.01^{***}	0.63^{ns}	8.90^{***}	0.13^{ns}	3.45^{*}	1.71^{ns}
Exudate (w/w)	0.86^{ns}	12.99^{***}	49.40^{***}	0.68^{ns}	0.86^{ns}	12.68^{***}

ns: no significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4. Conclusions

Smoke-flavoured cod samples showed higher salt and moisture contents than the salmon samples under the same processing conditions. In the cod samples, a higher salt concentration was required to achieve similar a_w values to those of salmon, which highlights the influence of lipid content during smoking-salting. The processing conditions which led to complete exudate evaporation through WP bags during salmon processing were insufficient to evaporate all the water released by fish muscle when the process was applied to cod. Optimising processing conditions allowed exudate evaporation when the process was prolonged 72 and 96 h.

According to the obtained results, the combination of WP bags and a 2% NaCl dose, 60% RH and a 96-hour processing time were the optimal conditions. These processing parameters enabled us to obtain a smoke-flavoured cod product with similar a_w, moisture and salt content to the reference, but with the lowest salt dose. Nevertheless, the suitability of the smoking-salting process applied to cod should be confirmed by shelf-life studies and sensory evaluations.

These results indicate that this new methodology can be applied to obtain smoke-flavoured products from different fish types by adapting processing parameters to the specific features of each fish species. This new smoking-salting is a suitable alternative to traditional cold-smoking procedures since it minimises brine wastes, cuts processing steps and facilitates fish handling during chain production, which makes the process more hygienic, simpler and faster.

Acknowledgements

The authors gratefully acknowledge Tub-Ex Aps (Taars, Denmark) for the supply of the water vapour permeable bags and for providing all the necessary information about their use. Author A. Rizo is grateful to Universitat Politècnica de Valencia for a FPI grant.

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CAPITULO 2

EVALUACIÓN DURANTE EL ALMACENAMIENTO DE LOS PRODUCTOS AHUMADOS

ARTÍCULO 3

PHYSICOCHEMICAL AND MICROBIAL CHANGES DURING STORAGE OF SMOKE-FLAVOURED SALMON OBTAINED BY A NEW METHOD

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Food Control 56 (2015) 195-201

Versión adaptada para la tesis doctoral

Abstract

The aim of this study was to evaluate the effect of a new smoking-salting method employing water vapour permeable (WP) bags on the physicochemical and microbial quality of smoke-flavoured salmon in refrigerated storage. Fresh salmon was subjected to a smoking process in the WP at 5ºC. Physicochemical and microbiological analyses were periodically carried out during the subsequent 40 days of refrigerated storage of the product. The WP bags enabled the evaporation of the exudate during the smoking-salting stage, enabling the drying of the product to take place at the same time. A slight increase of trimethylamine nitrogen (TMA-N) and total volatile basic nitrogen (TVB-N) contents was observed over the storage period. The concentration of TVB-N ranged from 14.26 to 21.48 mg N/100 g of fish, values far below the upper limits of acceptability. The low values of the thiobarbituric acid (TBA) index (final level of 0.71 mg MDA/kg) indicate that the lipid oxidation in the smoke-flavoured salmon was limited throughout the period studied. The initial K_1-value was high and only a slight increase was observed during storage. Counts of mesophilic, psychrotrophic, *Enterobacteriaceae* and lactic acid bacteria were low throughout the study. Given the changes observed for the physicochemical and microbiological parameters, it can be said that no spoilage took place in the smoke-flavoured salmon during the 40 days of storage. This new method could be of interest to producers as it enables smoke-flavoured salmon to be produced to a good standard of hygiene, minimizing handling and reducing processing steps and brine wastes.

Keywords: Smoke-flavoured salmon; water vapour permeable bags; quality changes; storage.

1. Introduction

Atlantic salmon farming has experienced significant growth over recent decades. World production of this fish increased from 299,000 tonnes in 1990 to 1.9 million tonnes in 2010 (FAO, 2012). The most popular processed product made from Atlantic salmon is smoked salmon. European production of smoked salmon was 160,000 tonnes in 2012, representing 28% of the total Atlantic salmon farmed (Marine Harvest, 2014).

Smoking is an ancient method of fish preservation. The smoking process preserves fish by means of the synergistic action of different factors, such as salt incorporation, the preservative effect of smoke compounds, and the dehydration to which the fish is subjected during processing. These changes delay the microbiological and oxidative changes that lead to spoilage, extending the shelf-life of the processed fish. However, there is a high variability in the characteristics of the raw material, as well as in the different processing techniques used for cold smoking of salmon. This fact leads to a wide range of product characteristics available on the market that directly influences the product´s shelf-life. Furthermore, with lightly preserved products, such as cold-smoked fish, the way in which they are handled and the sanitary conditions in the smokehouse may contribute to the microflora present on the final product and to the growth of foodborne pathogens (FDA, 2001). For these reasons, some studies have found high variability in the shelf-life of cold smoked salmon, which can range between 1-8 weeks, (Cardinal et al., 2004; Jørgensen, Dalgaard, & Huss, 2000; Leroi, Joffraud, Chevalier, & Cardinal, 2001). To achieve adequate hygienic quality and ensure consumer safety, strict control over the processing and handling conditions is necessary. In this regard, the development of new processing techniques that enable the improvement of hygienic quality is of great interest to the fish sector.

In recent years, novel materials made with highly water vapour permeable polymers have been introduced into the market. Such materials enable the drying of the product to take place while maintaining hygienic quality. This facilitates the handling and transport of the product during processing. Up to now, little research has been carried out into their use in food processing, being this focused exclusively on the dry aging of meat products (Ahnström, Seyfert, Hunt, & Johnson, 2006; Li, Babol, Wallby, & Lundström, 2013). A recent study has described a new method to produce smoke-flavoured salmon, based on the use of water vapour permeable bags (Rizo, Mañes, Fuentes, Fernández-Segovia, & Barat, 2015). This method consists of a controlled smoking-salting procedure performed inside these novel bags and processing takes place under established temperature and humidity conditions, applying a precise quantity of salt to each fish in order to achieve the optimal salt concentration. The use of smoke flavourings also provides the typical smoke flavour to the product so that smoking and salting can be performed in a single stage. The results obtained in this study showed that the water vapour permeable bags were suitable for the production of smoke-flavoured salmon with similar sensory characteristics to the smoked salmon currently available on the market. Therefore, this procedure could be an attractive alternative to traditional methods, as it does not affect the physicochemical properties or consumer acceptance, it minimizes product handling and brine wastes from the excessive use of salt, and it reduces processing steps. However, it is essential to evaluate the quality of this new product in order to determine its marketing period.

The aim of this study was to evaluate the effect of this new smoking-salting method employing water vapour permeable bags on the physicochemical and microbial quality of smoke-flavoured salmon stored at 4ºC.

2. Material and methods

2.1. Materials

Aquacultured salmon (*Salmo salar*) from a Norwegian farm (Hallvard Leroy AS, Bergen, Norway) was used as raw material. The fish were purchased in a local supermarket in Valencia (Spain). Three fish of commercial size weighing 2-3 kg, were used for the study. The specimens were headed, gutted and filleted, and two fillets were obtained from each fish. On arrival at the laboratory, the fillets were trimmed to remove bones, fins and visible adipose tissue. Initial microbial and physicochemical characterization of the raw material was carried out.

The salt used for the smoking-salting process was supplied by Panreac Química, S.A. (Barcelona, Spain). Natural liquid smoke (HARDWOOD AFS 10, Amcan Ingrédients Ltd., Le Chesnay, France) was applied to the samples. The water vapour permeable bags (WP) were supplied by TUB-EX ApS (Taars, Denmark) (polyamide mix; size: 200×300×0.04 mm; water vapour transmission rate: 5,000g/50μ/m²/24h (38°C/50% relative humidity)

2.2. Experimental design

The salmon fillets were cut into 4 cm portions, obtaining a total of 30 samples (5 portions per salmon fillet), which were subjected to a simultaneous smoking-salting procedure (Fig. 1), described in a previous study (Rizo et al., 2015). Liquid smoke previously diluted in distilled water (60 mL/100 mL solution) was applied to the fish portions by spraying the fish surface for 30 s. The samples were salted by means of a controlled process, dosing a previously established precise amount of salt over the fish muscle

surface (8 g salt/100 g fresh salmon), based on previous studies (Fuentes, Fernández-Segovia, Barat, & Serra, 2010a; Rizo et al., 2015). The salmon portions with the salt were vacuum packaged (Tecnotrip mod. EV-25-CD, Barcelona, Spain) in highly water vapour permeable bags (WP). It should be noted that vacuum packaging was used only to ensure a good contact between the fillet and the bag, since the vacuum could not be maintained inside the bags for a long time, due to the nature of the bag. The smoking-salting of the salmon was carried out in a chamber with controlled humidity (60% relative humidity) and temperature (5ºC) (Binder mod. KBF. Tuttlingen, Germany) for 24 h.

At the end of the established smoking-salting time, the salmon samples were removed from the bags. They were then placed in saturated brine while stirring constantly for 30 s to remove any traces of salt attached to the surface, dried with absorbent paper and weighed. Finally the fillets were vacuum packaged using high barrier bags and stored at 4ºC for 40 days. These conditions were selected as being the most commonly used in industry to store this type of products in their marketing period. The smoke-flavoured salmon was then characterized by means of analyses of moisture, lipid content, NaCl content, pH and a_w. Physicochemical and microbiological analyses of the samples were performed on days 0, 4, 7, 11, 14, 18, 22, 27, 32 and 40 of refrigerated storage. Three samples were taken on each sampling day (n=3). The analyses were performed in duplicate on each sample, except for pH, which was measured in quintuplicate.

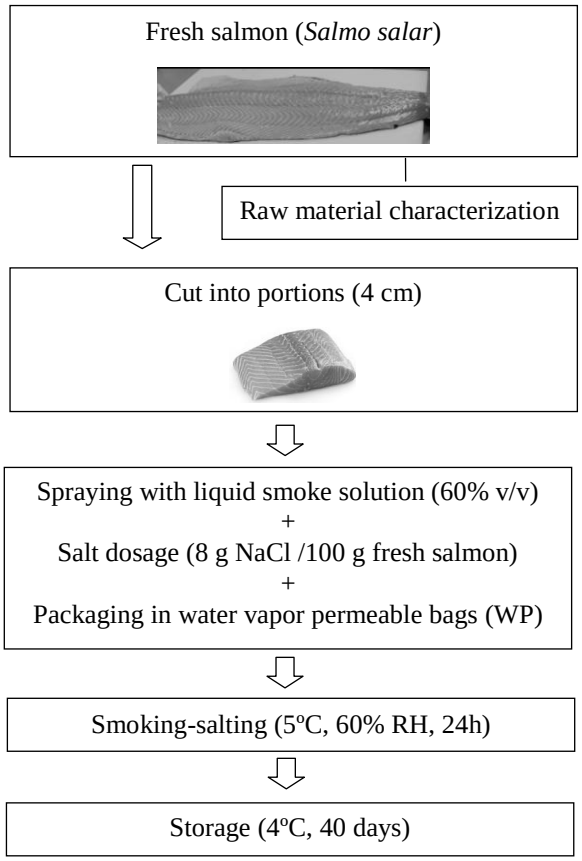

Fig. 1. Experimental design of the smoking-salting of salmon

2.3. Analytical determinations

2.3.1. Physicochemical analysis

Moisture content was determined by oven drying until constant weight at 105°C in accordance with AOAC method 950.46 (AOAC, 1997). The lipid content of the samples was determined by Soxhlet extraction using

petroleum ether in accordance with AOAC method 991.36 (AOAC, 1997). Sodium chloride content was determined in accordance with the procedure described by Fuentes, Fernández-Segovia, Serra, and Barat (2010b) using an automatic Sherwood Chloride Analyzer Model 926 (Sherwood Scientific Ltd., Cambridge, UK). Sodium chloride concentration in the liquid phase (z^{NaCl}) was calculated as described by Rizo, Fuentes, Fernández-Segovia, Masot, and Alcañiz (2013). The pH measurements were carried out using a micropH 2001 digital pH-meter (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) in five different locations on the sample. Water activity (a_w) was measured in minced samples with a fast water activity-meter (GBX scientific FA-st/1, Cédex, France). The total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents were determined by steam distillation according to the method described by Malle and Tao (1987). The thiobarbituric acid (TBA) index was measured using a spectrophotometric method with some minor modifications (Tarladgis, Watts, Younathan, & Dugan, 1960) to evaluate oxidation stability during chilled storage.

2.3.2. ATP-related compounds and K_1-values

HPLC was used to determine the ATP-related compounds, consisting of inosine-5'-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx), following the method described by Barat et al. (2008), with some minor modifications. The extraction method used was similar to that described by Burns and Kee (1985). The analysis was conducted on a Hitachi LaChrom Elite liquid chromatograph (Hitachi Ltd., Tokyo, Japan) with a pump (model L-2130), an auto-sampler (model L-2200) and a UV detector (model L-2400). Data acquisition was performed with the EZChrom Elite software (Agillent Technologies, Palo Alto, CA, USA). Separation was carried out on a reverse-phase Kinetex C18 column, 150 x 4.6 mm, with an

internal particle diameter of 5 µm (Phenomenex, Torrance, CA, USA). A guard column containing the same C18 packing as above was used to protect the analytical column from contamination. Compounds were identified by using a retention time comparison of the unknowns with those of the standards, and by means of standard addition or ‘‘spiking’’. IMP, Ino, and Hx were quantified according to the external standard method, using calibration curves of the peak area of compound versus the compound concentration under identical chromatographic conditions. K_1-values were calculated in accordance with Eq. (1):

$$K_1(\%) = \frac{[Ino] + [Hx]}{[IMP] + [Ino] + [Hx]} \times 100 \quad (1)$$

where IMP is inosine 5´-monophosphate; Ino, inosine; Hx, hypoxanthine.

2.3.3. Colour determinations

Colour determination was performed on the surface of the salmon fillets. A Minolta CM-700-d photocolorimeter (Minolta, Osaka, Japan) was used, with a 10° observer and illuminant D65. The sample was covered with low reflectance optical glass CR-A5/1829-752M to prevent deterioration of the integrating sphere. Using the CIE L*a*b* coordinates (where L* is lightness, a* deviation towards red or green and b* deviation towards yellow or blue), the psychophysical magnitudes of hue (h_{ab}*) and chroma (C_{ab}*) were calculated using Eqs. (2) and (3), respectively.

$$C_{ab}^* = \sqrt{a^{*2}+b^{*2}} \quad (2)$$

$$h_{ab}^* = \text{arctg}\ (b^*/a^*) \quad (3)$$

Furthermore, the overall colour differences (ΔE) of the smoke-flavoured samples over the storage period with respect to the recently smoke-flavoured samples (day 0) were calculated, according to Eq. (4).

$$\Delta E = \sqrt{(\Delta a^*)^2+(\Delta b^*)^2+(\Delta L^*)^2} \quad (4)$$

2.4. Microbiological analyses

Mesophilic bacteria, psychrotrophic bacteria, *Enterobacteriaceae* and lactic acid bacteria were determined according to the methods given by the following ISO standards: 4833:2003, 17410:2001, 21528-2:2004, and 15214:1998, respectively. All the analyses were performed in duplicate and the results were expressed as log cfu/g. All the culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain).

2.5. Statistical analysis

Data are reported as mean ± standard deviation. Statistical treatment of the data was performed using the Statgraphics Centurion software (Statpoint Technologies, Inc., Warrenton, VA, USA). The differences

between fresh and recently smoke-flavoured salmon were determined using Student's *t*-test. An analysis of variance (One-Way ANOVA) was conducted for each parameter to determine whether there were significant differences throughout the storage. All the physicochemical and microbiological parameters were considered as dependent variables and the storage time was the factor in these analyses. The LSD procedure (least significant difference) was used to test for differences between means at a significance level of 5%.

3. Results and discussion

3.1. Effect of the new smoking process on the physicochemical parameters

The values of the physicochemical parameters in the fresh salmon used as raw material and in the recently smoke-flavoured salmon are shown in Table 1. Moisture and lipid content for the raw material were similar to those reported by other authors for fresh salmon (Gallart-Jornet, Rustad, Barat, Fito, & Escriche, 2007). The high a_w value of the fresh salmon indicates the high susceptibility of this type of product to microbial spoilage, as is also the case with other fish species (Fernández-Salguero, Gómez, & Carmona, 1993). As expected, the smoking-salting process caused a significant reduction in the water content and an increase in the NaCl concentration, and consequently an important decrease in a_w values, as compared with fresh salmon. These changes are due to dehydration and NaCl absorption into the muscle.

The smoking process led to a significant decrease in pH compared with the values observed in the fresh salmon used as raw material, explained by the higher ionic strength of the internal solution in fish muscle cells, as described by Leroi and Joffraud (2000).

The values for moisture, a_w and pH of the smoke-flavoured product obtained by the new method were similar to those found for commercially available smoked salmon (Fuentes et al., 2010a; Rizo et al., 2015). The NaCl content was within the range reported in other studies on smoked salmon (Cardinal et al., 2004; Jørgensen et al., 2000). The product obtained fulfilled the Codex standard for smoked fish, smoke-flavoured fish and smoked dried fish (Codex, 2013), which establishes a minimum content of salt in the aqueous phase of 5% (z^{NaCl} = 0.05) for smoke-flavoured fish where the smoke flavour is provided by artificial flavour blends in order to provide complete protection against *Clostridium botulinum* at temperatures between 3ºC and 10ºC.

Table 1. Physicochemical parameters of fresh and recently smoked salmon. z^{NaCl}: NaCl concentration in liquid phase; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen; TBA: thiobarbituric acid; MDA: Malonaldehyde; IMP: inosine-5'-monophosphate; Ino: inosine; Hx: hypoxanthine. Mean values ± SD (n = 3).

	Fresh salmon	**Smoke-flavoured salmon**	**α**
Moisture (g H_2O/100 g)	71.38 ± 0.38[a]	58.94 ± 1.68[b]	***
Lipid content (g fat/100 g)	7.41 ± 0.41[a]	12.60 ± 0.54[b]	***
z^{NaCl} (g NaCl/mL)	0.003±0.001[a]	0.077±0.006[b]	***
pH	6.42 ± 0.01[a]	6.19 ± 0.03[b]	**
a_w	0.985 ± 0.004[a]	0.936 ± 0.009[b]	***
TVB-N (mg N/100 g)	13.54 ± 0.23[a]	14.26 ± 0.27[a]	ns
TMA-N (mg N/100 g)	2.66 ± 0.71[a]	3.75 ± 0.58[a]	ns
TBA index (mg MDA/kg)	nd	0.14 ± 0.02	
IMP (µmol/g)	1.40 ± 0.21[a]	1.57 ± 0.88[a]	ns
Ino (µmol/g)	4.62 ± 0.47[a]	4.43 ± 0.41[a]	ns
Hx (µmol/g)	2.81 ± 0.19[a]	2.46 ± 0.31[a]	ns
K_1-value (%)	85.11 ± 0.48[a]	81.63 ± 3.30[a]	ns

Different letters in the same row indicate significant differences.
MDA: malonaldehide. ns: non significant, ** p <0.01, *** p <0.001. nd: not detected

In the present study no sensory analysis was carried out. However, in a previous study it was demonstrated that the sensory attributes of smoke-flavoured salmon obtained by this new methodology were perceived with the same degree of acceptance as the commercial smoked salmon (Rizo et al., 2015).

In freshly caught fish, the content of total volatile basic nitrogen is typically between 5-20 mg N/100 g of fresh fish (Huss, 1995). The TVB-N value of the raw material used for this study was 13.54 mg N/100 g of fish, in agreement with the values reported by Fernández-Segovia et al. (2012) for fresh salmon. The smoking-salting process did not produce a significant change in the TVB-N value when compared with the raw material (Table 1).

The TMA-N concentration in the raw material was low (2.66 mg N/100 g). The smoking process led to a slight increase in this value, as has been reported in other studies on fish smoking (Fuentes, Fernández-Segovia, Barat, & Serra, 2011). The low values found for TVB-N and TMA-N indicate that the raw material is of an adequate hygienic quality.

The thiobarbituric acid index is a measurement of secondary oxidation products, such as carbonylic compounds that contribute to the unpleasant aroma and flavors of food whose fat had oxidised. In fish, the secondary oxidation products mainly come from the degradation of polyunsaturated fatty acid. No malonaldehyde (MDA) was detected in the fresh salmon, which means that no oxidation had taken place. In the recently smoke-flavoured salmon, the TBA index value was 0.14 (Table 1), lower than those reported in other studies on smoked fish (Bugueño, Escriche, Martínez-Navarrete, Camacho, & Chiralt, 2003; Fuentes et al., 2011). It should be noted that in the traditional smoking process the TBA value could be high because some compounds present in the smoke can react with the thiobarbituric acid. In addition to this, the high temperatures used during traditional smoking processes greatly influence the formation of secondary

oxidation compounds. The low TBA index values found in this study could be attributed to the low temperature (5ºC) used throughout the process.

Three ATP derivatives were identified and quantified: inosine 5'-monophosphate, inosine and hypoxanthine (Table 1). IMP concentrations were lower than the Ino content in fresh samples, which demonstrates that IMP degradation had occurred to some extent prior to the study. The smoking process did not affect the values of the three compounds.

Another widely used parameter in studies on fish quality and freshness is the K_1-value, which measures the extent of IMP degradation. The K_1-value found in the raw material was high due to the low IMP values, as mentioned above. The smoking process did not affect this parameter (Table 1).

It is important to note that no exudate was collected from the bags after the process. This means that, during the smoking-salting process, carried out in one single step, the complete evaporation of the water released by the fish muscle took place, indicating the effectiveness of the water vapour permeable bags. This result demonstrates the main advantages of this new method. These are: the reduction of processing steps, since the smoking, salting and drying are carried out in one single stage; the use of a low temperature (5ºC) during the whole process, which may improve the microbial quality of the smoke-flavoured product; and the fact that less brine waste is produced.

3.2. Changes in physicochemical quality

3.2.1. pH, TVB-N, TMA-N and TBA index

The evolution of pH, TVB-N and TMA-N and TBA index values in the smoke-flavoured salmon are shown in Table 2. Slight differences in the pH of the smoke-flavoured salmon were observed between day 0 and 40.

Table 2. Changes in pH, TVB-N (total volatile basic nitrogen), TMA-N (trimethylamine nitrogen), and TBA (thiobarbituric acid) index in samples of smoke-flavoured salmon during 40 days of storage at 4ºC. (Means and standard deviations, *n=3*). Different letters indicate significant differences.

Storage time (days)	pH	TVB-N (mg N/100 g)	TMA-N (mg N/100 g)	TBA index (mg MDA/kg)
0	6.19 ± 0.03^{a}	14.26 ± 0.27^{a}	3.67 ± 0.22^{a}	0.14 ± 0.02^{a}
4	6.05 ± 0.06^{bc}	16.35 ± 0.74^{ab}	4.68 ± 2.70^{abc}	0.04 ± 0.01^{ab}
7	5.98 ± 0.02^{c}	16.85 ± 0.86^{abc}	5.71 ± 0.41^{abcd}	0.14 ± 0.06^{a}
11	6.09 ± 0.07^{b}	16.48 ± 2.47^{ab}	5.37 ± 0.09^{abc}	0.28 ± 0.01^{b}
14	6.06 ± 0.07^{b}	17.41 ± 1.21^{bcd}	4.40 ± 1.24^{ab}	0.28 ± 0.00^{b}
18	6.02 ± 0.06^{bc}	19.62 ± 2.78^{de}	6.28 ± 0.73^{bcde}	0.31 ± 0.02^{bc}
22	6.05 ± 0.03^{bc}	19.40 ± 0.90^{cde}	7.95 ± 2.34^{e}	0.34 ± 0.03^{bc}
27	6.03 ± 0.00^{bc}	20.36 ± 0.82^{de}	6.17 ± 1.34^{bcde}	0.42 ± 0.07^{c}
32	6.10 ± 0.00^{b}	19.75 ± 0.06^{cde}	6.58 ± 0.72^{cde}	0.59 ± 0.15^{d}
40	$6.05 \pm 0.01b^{c}$	21.48 ± 1.59^{e}	7.31 ± 0.81^{de}	0.71 ± 0.07^{e}
α	*	***	*	**

MDA: malonaldehide
* p <0.05, ** p <0.01, *** p <0.001
Different letters in the same column indicate significant differences.

TVB-N is commonly used as an indicator of fish spoilage. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Fernández-Segovia et al., 2012; Özyurt, Kuley, Özkütük, & Özogul, 2009). There is a great variation in TVB-N acceptability limits in fresh fish. This variation is even higher in processed and semi-processed fish,

since the treatment to which the fish is subjected affects the TVB-N content in the final product (Fuentes et al., 2011). The acceptability limits proposed for smoked fish range between 30-40 mg N/100 g (Chilean fishing authority Sernapesca, 1996; cited by Dondero, Cisternas, Carvajas, & Simpson, 2004; Connell, 1995). In this study, the concentration of TVB-N in the smoke-flavoured samples gradually increased throughout the storage period (Table 2). The values ranged from 14.26 to 21.48 mg N/100 g of fish, in agreement with other studies of cold smoked salmon (Leroi et al., 2001; Bugueño et al., 2003). Therefore, TVB-N values remained far below the upper limits of acceptability throughout the period studied.

Regarding TMA-N, a slight increase was observed over the storage period, reaching a value of 7.31 mg/100 g fish at the end of the study (Table 2). For this parameter, no legal limits have been established for smoked fish; however, a sensory rejection limit of 10 mg N/100 g of fish has been proposed for smoked salmon (Truelstrup-Hansen, Gill, & Huss, 1995). No sample in this study reached this limit.

Given the low values obtained for TVB-N and TMA-N, it can be said that no spoilage took place in the smoke-flavoured salmon during the 40 days of storage.

The TBA values progressively increased throughout the study, reaching a final level of 0.71 mg MDA/kg. Different values for acceptability limits have been reported for this index. According to Connell (1995), values of 1–2 mg MDA/kg of fish flesh are usually regarded as the limit beyond which fish will normally develop an objectionable odor. Ruiz-Capillas and Moral (2001) established that the minimum value of TBA index detectable by panelists was 1.44 mg MDA/kg. However, Nunes, Cardinal, Mendes, Campos, Bandarra, and Lourenço (1992) observed that levels of 5–8 mg MDA/kg were generally regarded as the upper limit of acceptability for fish stored on ice. In this study, no sample had TBA values higher than 0.71 mg

MDA/kg. Therefore, the lipid oxidation in the smoke-flavoured salmon was limited throughout the period studied.

3.2.2. ATP related compounds and K_1-value

Changes in the levels of major adenine nucleotides and their related compounds are correlated with the freshness of the salmon (Fernández-Segovia et al., 2012). The catabolism of ATP to Hx has been reported to be caused essentially by endogenous enzymes. However, the hydrolysis of Ino and the formation of Hx may also result from the action of bacterial enzymes (Dalgaard, 2000). Table 3 shows the changes observed of IMP, Ino and Hx, as well as the K_1-value of the smoke-flavoured salmon during the 40 days of storage. A decrease in IMP content was observed, along with a slight increase in those of Ino and Hx. This means that IMP was transformed into Ino, and some Ino to Hx, but the Ino values remained higher than Hx throughout the study, showing that the degradation of Ino to Hx was relatively small. This is an indication of low microbiological activity, which agrees with the results found for bacterial growth, mentioned below. Truelstrup-Hansen et al. (1995) proposed a limit of sensory acceptability for Hx in smoked salmon of 5-7 µmol/g. In this study, Hx values in the smoke-flavoured samples reached a maximum value of 4.42 µmol/g during storage, being therefore, lower than the upper acceptability limit. These results confirm that the smoke-flavoured salmon remained of good quality for the 40 days of study.

Regarding the K_1-value, the initial values were high, as mentioned above, so only a slight increase was observed during storage.

Table 3. Changes in inosine-5'-monophosphate (IMP), inosine (Ino), hypoxanthine (Hx), and K_1-value in samples of smoke-flavoured salmon during 40 days of storage at 4ºC. (Means and standard deviations, *n=3*). Different letters indicate significant differences.

Storage time (days)	IMP (μmol/g)	Ino (μmol/g)	Hx (μmol/g)	K_1-value (%)
0	1.57 ± 0.88^{a}	4.43 ± 0.41^{ab}	2.46 ± 0.31^{a}	81.63 ± 3.34^{a}
4	1.69 ± 0.52^{a}	4.91 ± 0.14^{b}	2.98 ± 0.16^{ab}	84.84 ± 2.64^{ab}
7	0.82 ± 0.02^{b}	4.50 ± 0.30^{ab}	2.94 ± 0.13^{abc}	89.97 ± 0.45^{bc}
11	0.77 ± 0.24^{b}	4.13 ± 0.36^{ab}	3.18 ± 0.19^{abc}	90.57 ± 2.50^{c}
14	0.86 ± 0.27^{b}	4.29 ± 0.79^{ab}	3.10 ± 0.79^{abc}	89.75 ± 0.57^{c}
18	0.67 ± 0.10^{b}	3.74 ± 0.64^{a}	3.91 ± 0.41^{cd}	92.03 ± 0.41^{c}
22	0.66 ± 0.12^{b}	4.54 ± 0.54^{ab}	3.20 ± 0.64^{abc}	92.19 ± 0.44^{c}
27	0.60 ± 0.05^{b}	4.05 ± 0.30^{ab}	3.67 ± 0.75^{bcd}	92.84 ± 0.90^{c}
32	0.58 ± 0.13^{b}	4.68 ± 0.66^{ab}	3.77 ± 0.74^{cd}	93.65 ± 0.32^{c}
40	0.54 ± 0.16^{b}	4.89 ± 1.18^{ab}	4.42 ± 0.73^{d}	94.31 ± 0.56^{c}
α	*	***	*	**

Different letters in the same column indicate significant differences.
ns: non significant; * p<0.05; ** p<0.01; ***p<0.001

3.2.3. Colour parameters

The values obtained in the colour determinations (L*, a*, b*, hue (h_{ab}*), chroma (C_{ab}*) and colour differences (ΔE*)) are shown in Table 4. During storage all the parameters significantly increased, mainly during the last days of storage, except for h_{ab}*, for which differences were non-significant. The increase in lightness could be attributed to the water loss produced during storage, which would lead to greater water deposits on the fish surface, as a result of liquid retention between the film covering the samples, as has been reported in other studies of smoked fish (Fuentes, Fernández-Segovia, Serra, & Barat, 2012). These results entail an increase of the ΔE* at the end of the storage time.

Table 4. Changes in color parameters L* (lightness), a* (redness), b* (yellowness), C* (chroma), h* (hue) and ΔE* (color differences) in samples of smoke-flavoured salmon during 40 days of storage at 4ºC. (Means and standard deviations, *n=3*). Different letters indicate significant differences.

Storage time (days)	L*	a*	b*	C_{ab}*	h_{ab}*	ΔE*
0	33.76 ± 1.66^{a}	10.05 ± 1.21^{a}	10.44 ± 1.83^{a}	14.54 ± 1.86^{a}	0.800 ± 0.08^{abc}	-
4	35.54 ± 2.76^{ab}	11.00 ± 0.92^{ab}	10.86 ± 2.40^{ab}	15.51 ± 2.25^{ab}	0.769 ± 0.08^{a}	3.84 ± 1.71^{a}
7	38.33 ± 1.13^{bc}	11.20 ± 1.19^{ab}	12.17 ± 1.90^{ab}	16.57 ± 1.90^{abc}	0.823 ± 0.07^{abc}	5.40 ± 1.42^{ab}
11	36.70 ± 2.56^{ab}	11.16 ± 1.72^{ab}	13.15 ± 1.64^{bcd}	17.28 ± 2.11^{bcd}	0.869 ± 0.06^{bc}	4.90 ± 2.20^{ab}
14	38.35 ± 2.41^{bc}	11.34 ± 0.95^{abc}	12.48 ± 1.60^{ab}	16.89 ± 1.48^{bc}	0.831 ± 0.06^{abc}	5.75 ± 1.59^{ab}
18	37.77 ± 2.16^{bc}	11.60 ± 1.45^{bcd}	12.98 ± 1.95^{bcd}	17.42 ± 2.34^{bcd}	0.840 ± 0.03^{abc}	5.38 ± 2.49^{ab}
22	37.61 ± 1.49^{bc}	12.58 ± 1.76^{cd}	12.62 ± 2.13^{abc}	17.85 ± 2.50^{cd}	0.785 ± 0.06^{ab}	5.67 ± 1.77^{ab}
27	37.93 ± 1.15^{bc}	14.34 ± 0.88^{e}	15.23 ± 2.36^{cd}	20.97 ± 2.04^{e}	0.810 ± 0.07^{abc}	7.89 ± 1.92^{bc}
32	38.06 ± 1.97^{bc}	12.89 ± 1.37^{cd}	13.11 ± 1.75^{abcd}	18.42 ± 1.84^{cde}	0.792 ± 0.06^{abc}	6.37 ± 1.05^{abc}
40	40.63 ± 1.57^{c}	12.30 ± 1.39^{bcd}	15.03 ± 1.58^{d}	19.44 ± 1.82^{de}	0.885 ± 0.05^{c}	8.91 ± 0.52^{c}
α	*	***	*	**	ns	*

Different letters in the same column indicate significant differences.
ns: non significant; * p<0.05; ** p<0.01; ***p<0.001

No visual differences in colour were observed while the analyses were performed; however, sensory analyses should be conducted in order to determine whether the differences in colour detected by instrumental techniques at the end of the study could also be detected by consumers.

3.4. Microbiological analyses

The counts for mesophilic, psychrotrophic, lactic acid bacteria and *Enterobacteriaceae* are shown in Fig. 2.

The values for mesophilic and psychrotrophic bacteria did not vary significantly during the storage period. Limits of 6-7 log cfu g^{-1} of mesophilic bacteria in fresh water and marine species which are fit for human consumption have been established (Arashisar, Hisar, Kaya, & Yanik, 2004; Özogul, Polat, & Özogul, 2004). Mesophilic counts were low in all samples, ranging from 3.5-4.2 log cfu g^{-1} (Fig. 2.a). In cold smoked salmon, the microflora present at the time of spoilage is highly variable, with lactic acid bacteria and *Enterobacteriaceae* among the predominant microorganisms (Truelstrup-Hansen, Røntved, & Huss, 1998). In this study, counts of *Enterobacteriaceae* and lactic acid bacteria were low throughout the study. Although there were significant differences in the counts of both groups of microorganisms, it has to be borne in mind that the limit of quantification for these microorganisms is 1 log cfu g^{-1}, so that the differences found in these bacteria counts are still minimal. No samples reached the limit of 3 log cycles established for *Enterobacteriaceae* (Fuentes et al., 2011).

It should be noted that the use of WP bags enables the processing of the fish to take place under more controlled conditions, which entails a lower risk of microbiological contamination during the smoking-salting, as bags of this type present a barrier to bacteria. This attribute, combined with the use of

low temperature (5ºC) during the whole process, has the potential to improve the hygienic quality in lightly preserved fish, such as the smoke-flavoured salmon developed.

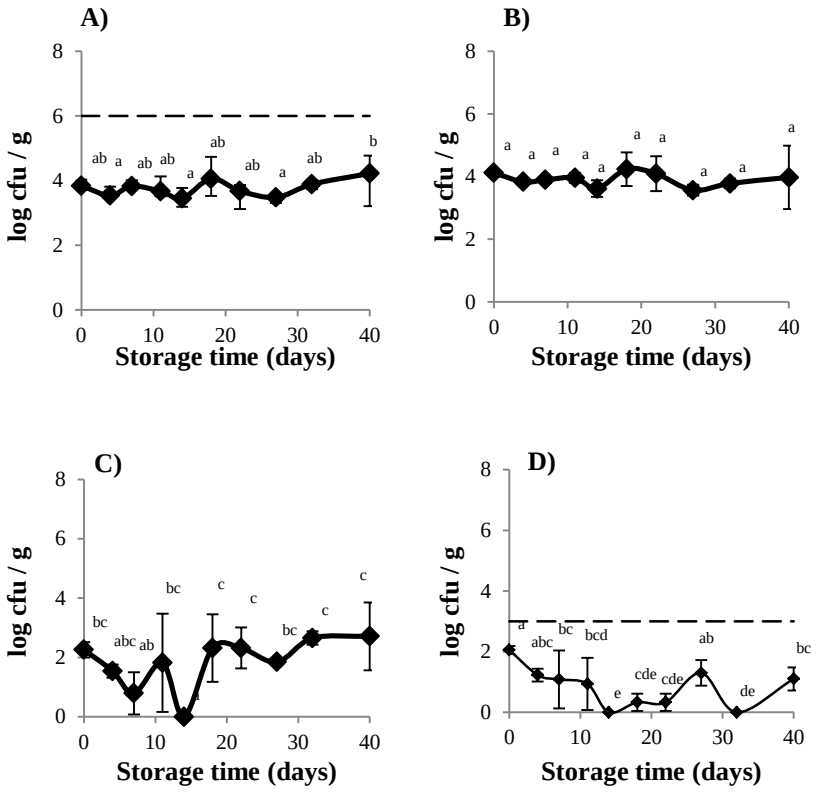

Fig. 2. Changes in mesophilic bacteria (A), psychrotrophic bacteria (B), lactic acid bacteria (C) and *Enterobacteriaceae* (D) in samples of smoke-flavoured salmon during 40 days of storage at 4ºC. (Means and standard deviations, *n=2*). Different letters indicate significant differences. The broken horizontal lines represent unacceptable levels in each figure.

According to these results, the smoke-flavoured salmon obtained by the new method presented here had an adequate microbial quality for the whole storage period. Therefore, this product would have been suitable for human consumption throughout the 40-day period studied.

4. Conclusions

The smoking-salting process caused a significant reduction in the water content, an increase in NaCl concentration, as well as a decrease in a_w and pH when compared with fresh salmon. The low degree of oxidation observed in the smoke-flavoured salmon could be attributed to the low temperature (5ºC) used throughout the smoking process. The water vapour permeable bags enabled the evaporation of the exudate during the smoking-salting process.

Given the changes observed of the TVB-N, TMA-N, TBA index and ATP-related compounds, it could be stated that no spoilage took place in the smoke-flavoured salmon during the 40 days in refrigerated storage. In general, the values obtained in the colour determinations slightly increased throughout the storage time. Although no visual differences of colour were found while the analyses were performed, sensory analyses should be conducted in order to determine whether the differences in the colour detected by the instrumental measurements could be detected by consumers. No significant changes were found during the storage time with regard to the microorganisms studied. The smoke-flavoured salmon did not exceed the acceptability limits proposed for mesophilic bacteria and *Enterobacteriaceae* at any time in the study. These results indicate that the shelf-life of smoke-flavoured salmon obtained using water vapour permeable bags and stored at 4ºC in vacuum packaging was over 40 days. This new method could be of interest to producers as it enables smoke-flavoured salmon to be produced to a good standard of hygiene, minimizing handling, and reducing processing steps and brine wastes.

Acknowledgements

The authors gratefully acknowledge the support of the company Tub-Ex Aps (Taars, Denmark) for the supply of the water vapour permeable bags and for providing all the necessary technical information. Arantxa Rizo would like to thank the Universitat Politècnica de València for the FPI grant. The proofreading of this paper was funded by the Universitat Politècnica de València, Spain.

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ARTÍCULO 4

FEASIBILITY OF PROCESSING TEMPERATURES ON THE QUALITY AND SHELF-LIFE OF SMOKE-FLAVOURED COD

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LWT - Food Science and Technology 69 (2016) 546-553

Versión adaptada para la tesis doctoral

Abstract

The feasibility of two processing temperatures on the quality and shelf-life of smoke-flavoured cod was studied. Cod was submitted to a smoke-flavouring process in water vapour permeable (WP) bags at 5 or 10ºC. Physicochemical and microbiological analyses were run for 40 days of cold storage. The WP bags allowed the exudate to evaporate during the smoke-flavouring process, which enabled salting, drying and smoking to be done in a single step. Processing temperature did not bring about major changes in the moisture, pH, a_w and colour of the smoke-flavoured cod. However, processing at 10°C increased volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) from the start of the study, whereas the samples obtained at 5°C maintained low TVB-N and TMA-N values, but only until day 21. The samples processed at 10ºC gave the highest Hx values due to degradation of IMP into Ino, and Ino into Hx. Microbiological counts were higher for the samples processed at 10°C compared to samples processed at 5°C, which did not reach the acceptability limits until day 40. Overall, the results provided of this study highlight the potential of smoke-flavouring process and in particular, the benefits of the use of refrigeration temperatures of 5°C.

Keywords: Smoke-flavouring; salting; cod; water vapour permeable bags; quality attributes.

1. Introduction

Smoking has been applied since ancient times to extend the shelf-life of fish products. Traditionally, smoking process involves different stages: salting, drying and smoking. Fish preservation has been achieved by the synergetic action of salt uptake, the partial dehydration of the tissues that occurs throughout various process stages and the preservative action of smoke compounds (Goulas & Kontominas, 2005).

Largely, refrigeration combined with new packaging technologies has lessened the necessity for high contents of salt and smoke components to preserve fish (Birkeland, Rørå, Skåra, & Bjerkeng, 2004). In this context, smoke flavourings offer several advantages compared with traditional smoking, such as the possibility of avoiding harmful components for human health and environment, lower investments in acquiring the required equipment, and the fact that use, dose and handling are much easier, less expensive and less time-consuming (Muratore, Mazzaglia, Lanza, & Licciardello, 2007). For these reasons, the possibility of extending applications of smoke flavourings compared to conventional smoking has to be taken into account in the fish industry, where smoke-flavoured fish appears to be a good alternative to traditional smoked products. In line with this, Rizo, Mañes, Fuentes, Fernández-Segovia and Barat (2015a) proposed a new process to obtain smoke-flavoured fish based on the application of water vapour permeable materials. This methodology consists of a simultaneous smoking-salting step, performed in water vapour permeable (WP) bags, under the established temperature and relative humidity conditions (RH) to facilitate product dehydration control. The use of smoke flavourings provides the typical smoked flavour to the product, and smoking and salting steps can be performed in a single stage. This procedure enabled us to obtain smoke-flavoured salmon with similar physicochemical characteristics and sensory acceptance to the smoked salmon product currently available on the market,

with good hygienic quality, under cold storage (Rizo et al., 2015a; Rizo, Mañes, Fuentes, Fernández-Segovia & Barat, 2015b). The use of this methodology could be an interesting alternative to traditional cold smoking of fish since the physicochemical properties, consumer acceptance and safety of the final product are not affected, it minimises product handling and brine waste, and cuts processing steps. This methodology has been tested in cod by adapting the processing parameters to the specific features of this fish species (Rizo, Fuentes, Fernández-Segovia & Barat, 2014; McDonagh, 2014).

The quality and shelf-life of smoked products depend on raw material characteristics, hygienic practices during handling, the salting and smoking method, and processing conditions, such as the salt dose, duration and temperature of the process (Rørå et al., 2005). Processing temperature is critical as regards maintaining hygienic quality. The effects of smoking and drying temperature on fish quality have been studied (Goulas & Kontominas, 2005; Rørå et al., 2005), as well as the effect of salting temperature (Birkeland & Bjerkeng, 2005). In addition, refrigeration temperatures within the 3-12ºC range have been recommended for preventing microbial growth during salting. However, temperatures used for drying and cold smoking are usually between 16 and 30ºC (Codex, 2003). Although other researchers have evidenced processing temperature effects on quality of smoked fish accomplished by traditional techniques, how processing temperatures can affect the quality of smoke-flavoured fish products, in which salting and drying take place simultaneously, remains unknown. Therefore, the effect of two smoke-flavouring temperatures (5 and 10ºC) using WP bags on the quality and shelf-life of smoke-flavoured cod was studied.

2. Materials and methods

2.1. Materials

Fillets of frozen Atlantic cod (Gadus morhua) obtained from Alimentos Friorizados, S.A. (Barcelona, Spain), commercial size of 1.2-1.4 kg, were employed as raw material. Before processing, fillets (n=10) were thawed at 4°C for 24 h. Then cod fillets were trimmed to remove bones and cut into 4-cm portions to obtain approx. five portions per fillet (48 samples were obtained in all). The average weight of fish portions was 136±23 g, and thickness was 2-3 cm. The initial microbial and physicochemical characterisation of the raw material was carried out.

The salt used for the smoke-flavouring process was supplied by Panreac Química, S.A. (Barcelona, Spain). Natural liquid smoke (HARDWOOD AFS 10, Amcan Ingrédients Ltd., Le Chesnay, France) consisting of a natural water-soluble condensate from the pyrolisis of walnut, maple, and other hardwoods, was applied to samples. Water vapour permeable (WP) bags were supplied by TUB-EX ApS (Taars, Denmark) (polyamide mix; size 200×300×0.04 mm; water vapour transmission rate 5,000g/50μ/m²/24 h (38°C/50% RH)).

2.2. Experimental design

Cod samples were subjected to a simultaneous smoke-flavouring procedure (Fig. 1) according to the method of Rizo et al. (2015a) to obtain smoke-flavoured fish. Liquid smoke, previously diluted in distilled water (60 mL/100 mL solution), was applied to surface of fish portions by spraying for 30 s. Portions were salted by dosing an amount of salt (2 g salt/100 g cod), according to previous studies (Rizo et al., 2014), to achieve similar a_w, salt

and moisture content values to those of commercial smoked cod (Kárasková et al., 2011; Rizo et al., 2014). Cod portions were vacuum-packed (Tecnotrip mod. EV-25-CD, Barcelona, Spain) in highly water vapour permeable bags. It should be noted that vacuum packing was used just to ensure initial contact between fish and the WP bag, since vacuum conditions cannot be maintained in these bags for a long time. Then cod samples were divided into two batches, which were processed at different temperatures (batch 1 at 5ºC and batch 2 at 10ºC). The smoke-flavouring of cod was carried out in a drying chamber (Binder mod. KBF. Tuttlingen, Germany) at 60% RH for 96 h.

At the end of the smoke-flavouring time, cod samples were removed from the bags. They were then placed in saturated brine with constant stirring for 30 s to remove any traces of salt attached to the surface, and were dried with absorbent paper and weighed. Finally, smoke-flavoured fillets were vacuum-packed in high barrier bags and stored at 4ºC for 40 days. These conditions were selected given its wide use in industry to store such products during their marketing period. The obtained smoke-flavoured cod was characterised by analyses of moisture, NaCl content, pH and a_w at day 0. Physicochemical and microbiological analyses of samples were performed at days 0, 7, 14, 21, 28, 35 and 40. Three samples were taken on each sampling day (n=3). Analyses were performed on each sample in duplicate, except for pH, which was measured in quintuplicate.

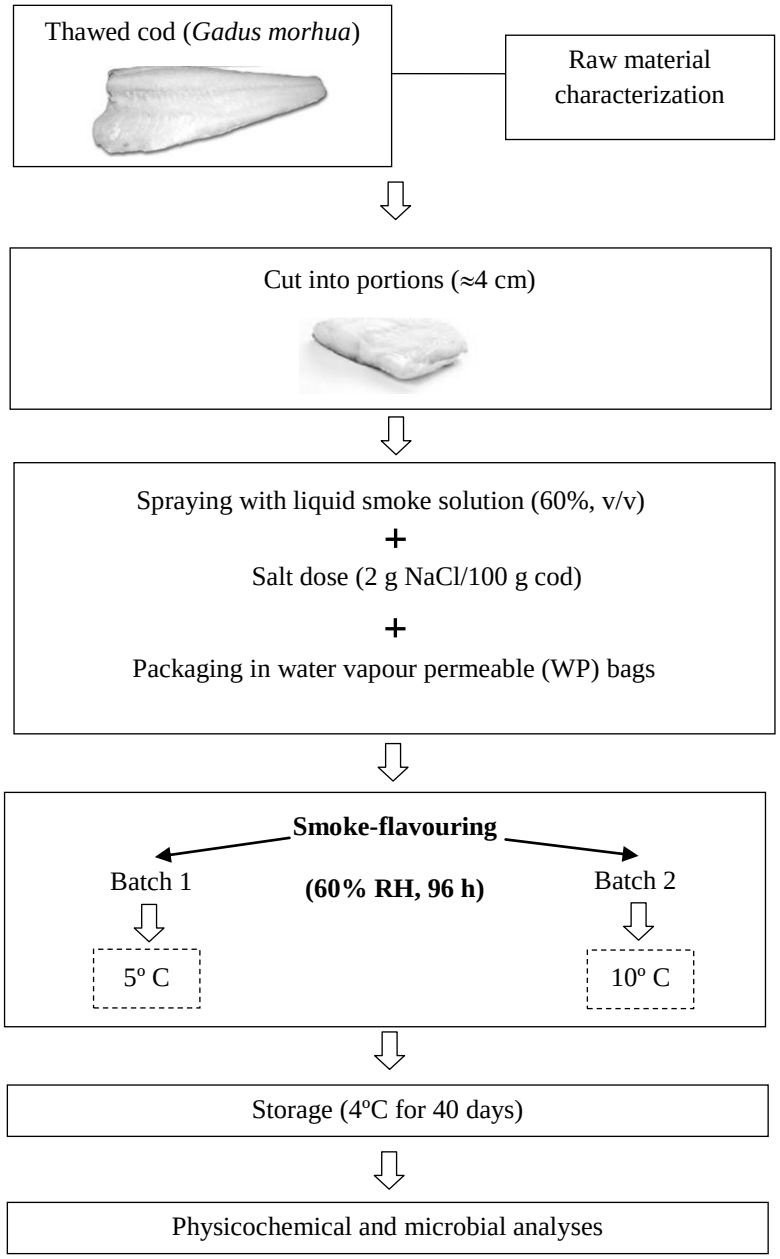

Fig. 1. Schematic representation of the smoke-flavouring process. RH: Relative Humidity.

2.3. Analytical determinations

2.3.1. Physicochemical analysis

Moisture contents (g/100 g) were determined in accordance with AOAC method 950.46 (1997). Sodium chloride content in the liquid phase (Z^{NaCl} (g NaCl/mL)) was measured in accordance with the procedure described by Fuentes, Fernández-Segovia, Serra and Barat (2010). pH measurements were taken with a micropH 2001 digital pH-meter (Crison Instruments, S.A., Barcelona, Spain) and a puncture electrode (Crison 5231) at five different sample locations. Water activity (a_w) was measured in minced samples with a fast water activity-meter (Aqualab dew point hygrometer model 4TE, Decagon Devices, Inc., Washington, USA). Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents (mg N/100 g) were determined according to the method described by Malle and Tao (1987). Weight changes in fish samples (ΔMt) were calculated according to Eq. (1).

$$\Delta M_t = \left(\frac{M_t - M_0}{M_0}\right) \quad (1)$$

where M_t is the sample weight at time t (g) and M_0 is the initial sample weight (g)

HPLC was used to determine the ATP-related compounds, consisting of inosine-50-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx), following the method described by Rizo et al. (2015b). K1-value was calculated in accordance with Eq. (2):

$$K_1(\%) = \frac{[Ino] + [Hx]}{[IMP] + [Ino] + [Hx]} \times 100 \quad (2)$$

where IMP is inosine 5´-monophosphate, Ino is inosine and Hx is hypoxanthine (µmol/g).

2.3.2. Colour determination

Colour determination was performed on the surface of the cod fillets. A Minolta CM-700-d photocolorimeter (Minolta, Osaka, Japan) was used, with a 10° observer and illuminant D65. The sample was covered with low reflectance optical glass CR-A5/1829-752M to prevent any deterioration to the integrating sphere. Using the CIE L*a*b* coordinates (where L* is lightness, a* deviation towards red or green, and b* deviation towards yellow or blue), the psychophysical magnitudes of hue (hab*) and chroma (Cab*) were calculated using Eqs. (3) and (4), respectively.

$$C_{ab}^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$h_{ab}^* = \text{arctg}\ (b^*/a^*) \quad (4)$$

2.4. Microbiological analyses

Mesophilic bacteria and Enterobacteriaceae were determined according to the methods in Standard 4833:2003 and 21528-2:2004, respectively. The results were expressed as log cfu/g. All the culture media were purchased from Scharlau Chemie, S.A. (Barcelona, Spain).

2.5. Statistical analysis

One-way analysis of variance (ANOVA) was conducted to establish significant differences between the fresh samples and those recently smoked at 5 and 10ºC. Physicochemical and microbiological parameters were analysed by a multifactor ANOVA to evaluate the effect of processing temperature and storage time. The least significant difference (LSD) procedure was used to test for differences between averages at the 5% significance level. Statistical treatment was performed using Statgraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA).

3. Results and discussion

3.1. Effect of the smoke-flavouring process

The physicochemical, microbiological and colour parameters analysed in the fresh cod used as raw material and in the recently smoke-flavoured cod are shown in Table 1.

Compared with fresh cod, the smoke-flavouring process noticeably reduced water content, increased the NaCl concentration and lowered the a_w values probably due to NaCl uptake and dehydration.

Table 1. Physicochemical, microbiological and colour parameters of fresh and smoke-flavoured cod at day 0. (Means and standard deviations, *n=3*).

	Fresh cod	**Smoke-flavoured cod**		**α**
Processing temperature		**5ºC**	**10ºC**	
Moisture (g H_2O/100 g)	82.93±0.90^a	71.21 ± 1.41^b	72.86 ± 1.87^b	***
Z^{NaCl} (g NaCl/mL)	0.005±0.001^a	0.055±0.003^b	0.053±0.005^b	***
a_w	0.994±0.001^a	0.955 ± 0.009^b	0.955 ± 0.004^b	***
ΔM_t	-	-0.368 ± 0.034^a	-0.345 ± 0.040^a	ns
pH	6.76 ± 0.06^a	6.32 ± 0.17^b	6.54 ± 0.34^b	**
TVB-N (mg N/100 g)	10.60 ± 4.27^a	20.95 ± 2.42^b	39.38 ± 9.09^c	**
TMA-N (mg N/100 g)	2.26 ± 0.80^a	7.19 ± 0.06^a	25.53 ± 2.42^b	***
Mesophilic bacteria (log cfu/g)	4.17 ± 0.27^a	4.58 ± 0.25^b	5.94 ± 0.13^c	***
***Enterobacteriaceae* (log cfu/g)**	1.45 ± 0.41^a	1.54 ± 0.09^a	3.00 ± 0.58^b	**
IMP (µmol/g)	3.36±0.03^a	3.98±0.88^a	0.57±0.19^b	**
Ino (µmol/g)	1.87±0.31^a	2.05±0.18^a	1.92±0.81^a	ns
Hx (µmol/g)	0.24±0.02^a	0.94±0.10^b	3.41±0.25^c	***
K_1-value	38.42±3.54^a	49.04±2.74^b	89.33±2.67^c	***
L*	56.55 ± 6.22^a	49.88 ± 2.61^b	48.87 ± 4.04^b	*
a*	-4.24 ± 0.30^a	-2.14 ± 1.29^b	-3.28 ± 0.93^{ab}	*
b*	-2.74± 2.24^a	10.71 ± 1.44^b	6.12 ± 2.39^b	**
C_{ab}*	5.44 ± 0.80^a	11.02 ± 1.21^b	7.44 ± 0.39^{ab}	*
h_{ab}*	0.66 ± 0.18^a	178.85 ± 0.75^b	179.07 ± 3.52^b	***

z^{NaCl}: NaCl concentration in liquid phase; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen; IMP: inosine-5'-monophosphate; Ino: inosine; Hx: hypoxanthine. Mean values ± SD (n = 3). Different letters indicate significant differences.
ns: no significant * p <0.05, ** p <0.01, *** p <0.001

The smoke-flavouring process led to a drop in pH, compared with the raw material values possibly due to the greater ionic strength of the internal solution in fish muscle cells associated with salt uptake (Leroi & Joffraud, 2000). The obtained smoke-flavoured product fulfilled the Codex standard for smoked fish, smoke-flavoured fish and smoked dried fish (Codex, 2013), which requires a minimum salt content of 5% (z^{NaCl} = 0.05) for smoke-flavoured fish, where smoke flavour is provided by artificial flavour blends in order to prevent the growth of Clostridium botulinum at storage temperatures of 3-10ºC. For both processing temperatures, the recorded moisture, a_w, pH and NaCl of smoke-flavoured product fell within

the range of those reported elsewhere for commercially available smoked cod. Moreover, processing temperature did not significantly affect these parameters.

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) are widely used as indicators of fish spoilage (Okpala, Choo, & Dykes, 2014; Özoğul, & Balikci, 2013). In fresh fish of good quality, total volatile basic nitrogen content is typically less than 20 mg N/100 g (Cardinal et al., 2004). Critical limits of 35 mg TVB-N/100 g have been established by the European Union for unprocessed fishery products species of Gadidae family such as cod (CEE, 2005), although these values can vary widely depending on whether the fish is fresh or processed (Siriskar, Khedkar & Lior, 2013).

The TVB-N and TMA-N values of the raw material were 10.60 and 2.26 mg N/100 g of fish, respectively, which are in agreement with values for fresh cod reported by Ruiz-Rico et al. (2013), who found TVB-N levels ranging between 10-14 mg N/100 g. A significant increase in these parameters was observed after the smoke-flavouring process, which could be partially explained by the dehydration that the smoke-flavouring process causes, and the subsequent concentration of TVB-N and TMA-N, as observed in other studies (Goulas & Kontominas, 2005). However, this increase was significantly higher ($p<0.05$) for the smoke-flavoured cod processed at 10ºC (39.38 mg N/100 g) as it almost doubled that recorded for the cod processed at 5ºC (20.95 mg N/100 g). These results suggest a higher level of spoilage in the smoke-flavoured cod at 10ºC, which correlated with the microbial growth described below.

Counts of mesophilic bacteria and Enterobacteriaceae in the samples that had been smoked at 5ºC appeared stable. However, when the processing temperature was 10ºC, higher levels of mesophilic bacteria and Enterobacteriaceae were found, which came close to the upper limits of

acceptability (7 log cfu g^{-1} and 3 log cfu g^{-1}, respectively) set in other studies (Fuentes, Fernández-Segovia, Barat & Serra, 2011; ICMSF, 1986).

The low values obtained for TVB-N, TMA-N, mesophilic bacteria and Enterobacteriaceae in fresh cod indicated that the raw material used in the present study exhibited good hygienic quality.

Regarding colour, the smoke-flavoured cod samples obtained lower L* values and higher a*and b* values compared with the fresh cod samples (Table 1). The reduction in lightness may be caused by the dehydration and protein denaturation that probably occurred in the fish during smoking processes, similarly to that reported by Birkeland et al., (2004). The increase in coordinates a* and b* can be associated with the application of liquid smoke to the fillets' surface, and also to the dehydration that causes yellowing on salted cod (Oliveira, Pedro, Nunes, Costa & Vaz-Pires, 2012). Processing temperature had no significant effect on the colour of cod samples.

The inosine 5'-monophosphate, inosine and hypoxanthine values of the fresh cod and smoke-flavoured fish at day 0 are shown in Table 1. The raw material showed values for these parameters consistent with those found in another study of fresh cod (Ruiz-Rico et al., 2013). The smoke-flavouring process conducted at 5ºC did not significantly affect the IMP and Ino concentrations. However, when smoke-flavouring was conducted at 10ºC, the IMP value noticeably dropped, probably due to the higher spoilage at this temperature. The samples processed at 10ºC gave the highest Hx values due to degradation of IMP into Ino, and Ino into Hx. Likewise, the K1-values were significantly affected by processing temperature. The initial K1-value of samples processed at 5ºC was much lower than the value of the samples processed at 10ºC.

No exudate was collected from the bags after the process; the WP bags were permeable enough to allow the water released by fish muscle to largely evaporate, thus less brine waste would be generated. As previously highlighted herein, the salting, drying and smoking stages can be carried out in a single step with this process, which would reduce processing steps compared to traditional methods, where salting, drying and/or smoking are conducted separately. Furthermore, smoke-flavouring inside a bag enables fish processing to take place under more controlled conditions, which entails a lower risk of microbiological contamination during smoke-flavouring as bags of this type offer a barrier to bacteria (Rizo et al., 2015b).

3.2. Physicochemical quality evolution during storage

3.2.1. pH, TVB-N and TMA-N

The pH, TVB-N and TMA-N changes during the storage of smoke-flavoured cod are shown in Fig. 2.

pH values remained constant throughout storage as only minor fluctuations were noted, which is in agreement with the results obtained by other authors (Goulas & Kontominas, 2005). This parameter was not affected by processing temperature during storage. This revealed that pH was not useful for monitoring fish quality changes, which is consistent with other studies (Arkoudelos, Stamatis & Samaras, 2007; Li, Li, Zhu, Wang, Fu & Xuan, 2011).

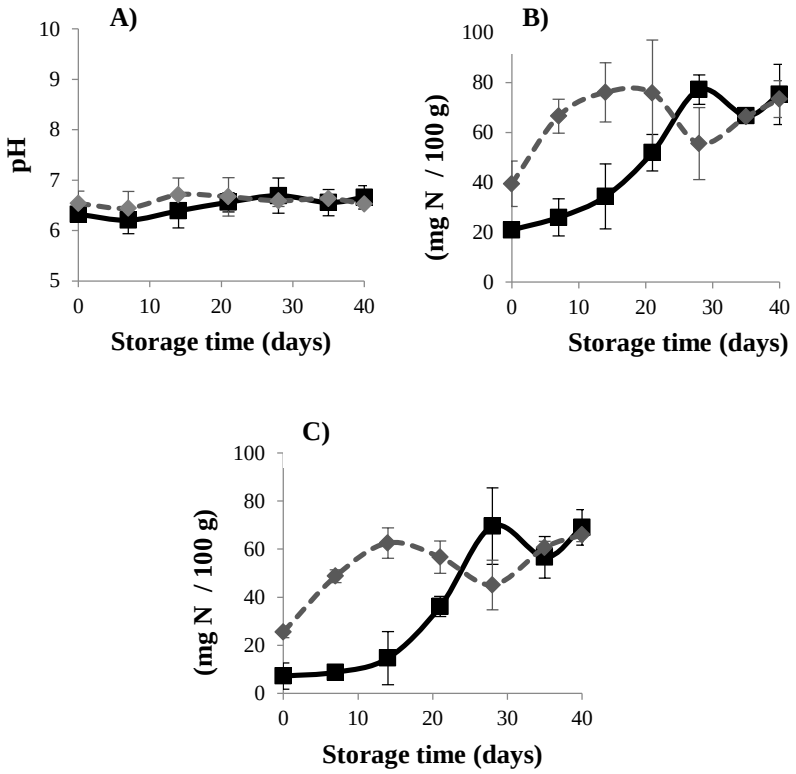

Fig. 2. Evolution of pH (A), TVB-N (B), and TMA-N (C) in smoke-flavoured cod samples processed at different temperatures (5ºC(■) and 10ºC (♦)) during 40 days of storage at 4ºC. (Means and standard deviations, *n=3*).

As previously mentioned, TVB-N is a common indicator of spoilage for many fish species. The limits of acceptability proposed by some authors for smoked fish fall within 30-40 mg N/100 g (Dalgaard, 2000). However, higher limits of acceptability have been given for salted and dried fish. Values higher than 75 mg N/100 g have been found in sugar-salted herring with acceptable sensory quality (Dalgaard, 2000), 50-110 mg N/100 g fish in dried-salted tuna products (Gallart-Jornet, Escriche, Chilet & Fito, 2005) and

57 mg N/100 g in salted anchovies (Oetterer et al., 2003). Herein, TVB-N values of cod samples steadily increased with storage (Fig. 2). The samples obtained at 10ºC showed significantly higher TVB-N values compared to those processed at 5ºC during the first weeks of storage. From day 28, the values of this parameter were similar for both sample types.

The TMA-N values obtained in the smoke-flavoured cod samples displayed a similar pattern to TVB-N during storage. As with TVB-N, a wide variability in the limits of acceptability was seen for TMA-N. In general, 10-15 mg/100 g could be considered the upper limit for this parameter (Connell, 1995). Although TMA-N is believed to be generated by the action of spoilage bacteria, the correlation found with bacterial numbers is not often very good (Huss, 1995), consistent with the present study, as reported in other studies of smoked fish (Joffraud et al., 2006; Fuentes et al., 2011).

According to the limits of acceptability proposed for TVB-N and TMA-N, the smoke-flavoured cod processed at 10ºC showed spoilage from the beginning of the study. In contrast, the smoke-flavoured cod obtained at 5ºC did not exceed these values until storage day 21. Nevertheless, some studies have suggested that a single biochemical or microbiological parameter could not be used alone to precisely evaluate fish shelf-life (Leroi, Joffraud, Chevalier & Cardinal, 2001; Jørgensen Dalgaard & Huss, 2000). For this reason, the TVB-N and TMA-N values should be compared with the other quality parameters.

3.2.2. ATP-related compounds and K_1-value

IMP, Ino and Hx contents, as well as the K1-value, of the cod kept under cold storage of 40 days are shown in Fig. 3.

The breakdown of IMP into Ino, and Ino into Hx, is caused by endogenous enzymes. However, the hydrolysis of Ino and Hx formation may also result from the action of bacterial enzymes (Fernández-Segovia, Escriche & Serra, 2008).

A progressive lowering of IMP contents, along with an increase in Hx contents, was observed for both sample types throughout storage. During the first weeks, higher IMP values and lower Hx values were obtained for the samples processed at 5ºC compared with those processed at 10ºC. However, similar values were found for both samples from day 21.

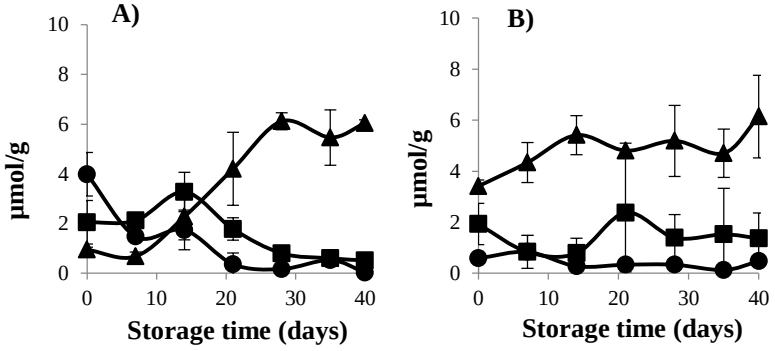

Fig. 3. Evolution of inosine-5'-monophosphate (IMP (●)), inosine (Ino (■) and hypoxanthine (Hx (▲)) in the smoke-flavoured cod samples processed at different temperatures (5ºC (A) and 10ºC (B)), during 40 days of storage at 4ºC. (Means and standard deviations, *n=3*).

The K1-value quantifies the extent of IMP degradation (Fig. 4). The initial K1-values of the samples obtained at 5ºC were low (49%), but

increased sharply to reach values over 93% by the end of the study. Higher values were recorded for the smoke-flavoured cod processed at 10ºC, which varied slightly during the study (from 89 to 98%) as IMP degradation occurred almost completely at the beginning of storage. These results correlate with the loss of freshness in cod observed by other authors (Fernández-Segovia et al., 2008; Ruiz-Rico et al., 2013).

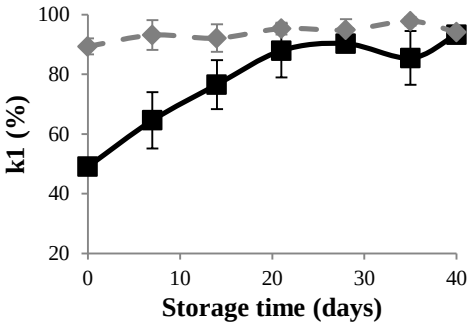

Fig. 4. Evolution of the K_1-value in the smoke-flavoured cod samples processed at different temperatures (5ºC (■) (A) and 10ºC (♦) (B)) during 40 days of storage at 4ºC. (Means and standard deviations, *n=3*).

3.2.3. Colour parameters

The changes in the colour parameters of the smoke-flavoured cod during storage are shown in Table 2.

Neither processing temperature nor storage time had a significant effect on the colour parameters ($p>0.05$). These results differ from those obtained in a previous work, in which significant colour differences were

reported during storage in smoke-flavoured salmon obtained by the same method (Rizo et al., 2015b). In addition, different studies have highlighted the influence of the smoking temperature in the colour of smoked fish (Birkeland et al., 2005). However, it seems that smoke-flavouring temperature does not influence these parameters.

Table 2. Changes in colour parameters L* (lightness), a* (redness), b* (yellowness), C* (chroma), h* (hue) in the smoke-flavoured cod samples processed at different temperatures (5 and 10ºC) during 40 days of storage at 4ºC. (Means and standard deviations, *n=3*).

t (days)	T (ºC)	L*	a*	b*	C_{ab}*	h_{ab}*
0	5	49.88±2.61^{aA}	-2.14±1.29^{aA}	10.71±1.44^{aA}	11.03±1.20^{aA}	178.85±0.75^{abA}
	10	48.87±4.04^{aA}	-3.28±0.93^{aA}	6.12±4.39^{aA}	7.44±3.52^{aA}	179.07±0.39^{abA}
7	5	53.40±3.09^{aA}	-2.60±0.53^{aA}	10.83±1.65^{abA}	11.17±1.51^{abcA}	178.67±0.07^{aA}
	10	50.62±3.39^{aA}	-2.83±1.28^{aA}	10.74±4.12^{abA}	11.34±3.59^{abcA}	178.75±0.24^{aA}
14	5	52.97±1.88^{aA}	-1.38±1.16^{aA}	12.60±2.89^{abA}	12.78±2.61^{abcA}	178.57±0.158^{aA}
	10	50.31±5.33^{aA}	-2.93±0.85^{aA}	7.79±3.41^{abA}	8.67±2.47^{abcA}	178.88±0.39^{aA}
21	5	50.33±2.34^{aA}	-3.02±0.91^{aA}	8.96±2.11^{abA}	9.55±1.86^{abA}	178.77±0.14^{aA}
	10	49.16±4.93^{aA}	-1.51±0.92^{aA}	11.13±2.80^{abA}	11.27±2.78^{abA}	178.57±0.09^{aA}
28	5	49.14±2.43^{aA}	-2.23±0.76^{aA}	11.80±1.71^{bA}	12.05±1.60^{bcA}	178.83±0.76^{abA}
	10	52.62±4.59^{aA}	-1.77±1.59^{aA}	12.71±4.01^{bA}	12.99±3.80^{bcA}	178.82±0.76^{abA}
35	5	50.66±2.14^{aA}	-1.00±0.76^{aA}	12.73±2.28^{bA}	12.80±2.25^{cA}	178.83±0.96^{aA}
	10	53.42±2.64^{aA}	-3.12±1.38^{aA}	12.13±3.01^{bA}	12.65±2.69^{cA}	178.70±0.15^{aA}
40	5	48.32±2.39^{aA}	-2.18±1.17^{aA}	11.97±1.31^{bA}	12.24±1.17^{bcA}	178.84±0.7^{bA}
	10	53.38±4.32^{aA}	-1.73±2.36^{aA}	11.97±1.31^{bA}	12.92±4.84^{bcA}	179.31±0.47^{bA}

Different lower-case letters indicate significant differences for processing temperature factor (T). Different capital letters indicate significant differences for processing temperature (t). ($p < 0.05$).

3.4. Microbiological analyses

The evolution of mesophilic and Enterobacteriaceae throughout the 40 days of storage is shown in Fig. 5.

During storage, mesophilic bacteria increased sharply for both processing temperatures. The samples obtained at 10ºC exhibited greater microbial growth for mesophilic bacteria, which exceeded the upper limit of acceptability (7 log cfu g^{-1}) from storage day 7, whereas the samples obtained at 5ºC did not reach this limit during the study.

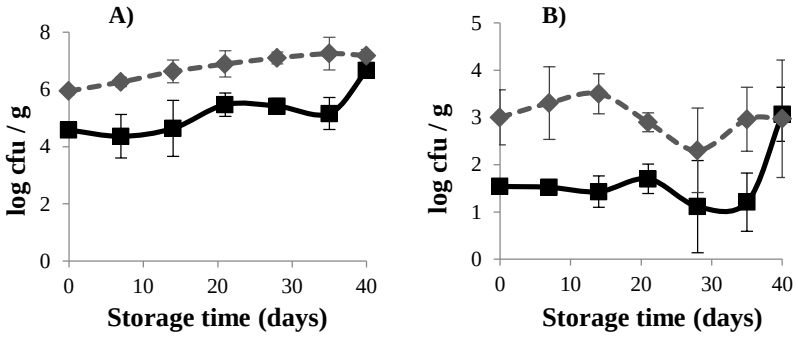

Fig. 5. Changes in mesophilic bacteria (A) and *Enterobacteriaceae* (B) in the smoke-flavoured cod samples processed at different temperatures (5ºC (■) (A) and 10ºC (♦) (B)) for 40 storage days at 4ºC. (Means and standard deviations, *n=3*). Different letters indicate significant differences.

In cold smoked fish, the microbial flora present at the time of spoilage is variable and complex, with lactic acid bacteria and Enterobacteriaceae among the dominant microorganisms (Løvdal, 2015;

Joffraud et al., 2006). In this sense, some studies have found high levels of mesophilic and lactic acid bacteria in cold smoked salmon (10^7-10^8 cfu g^{-1}) weeks before signs of spoilage became apparent, which sometimes make them unreliable quality indicators of cold smoked fish (Gram & Huss, 1996; Løvdal, 2015; Joffraud et al., 2006). In contrast, the presence of large numbers of Enterobacteriaceae is often associated with fish spoilage (Gram & Huss, 1996). Leroi et al. (2001) observed that shelf-life in smoked salmon was highly variable (1-6 weeks) and was related to the initial Enterobacteriaceae counts, which depends on the hygienic conditions during handling to a great extent. In this study, the initial Enterobacteriaceae counts were strongly affected by processing temperature. In the samples smoked at 10ºC, the above-cited limit of 3 log cfu g^{-1} was exceeded from the beginning of the study (day 0), but this limit was not reached until storage day 40 in the samples obtained at 5ºC. The lack of correlation between the values of the volatile bases (TVB-N and TMA-N) and the microbiological counts should be noted, especially for the cod processed at 5ºC.

According to the values obtained for TVB-N and TMA-N, the shelf-life of the smoke-flavoured fish processed at 5ºC would be shorter than 21 days. However, taking into account the results of Enterobacteriaceae the fish would not show spoilage until day 40. With the cod obtained at 10ºC, fish came close to the limit of acceptance from the beginning of the study, which indicates that the smoke-flavouring process at 10ºC was not adequate to obtain smoke-flavoured cod.

3.5. Statistical analysis

The multifactor ANOVA results obtained for each analysed parameter are shown in Table 3.

Table 3. F-ratio values and significance levels obtained in the multifactor ANOVA for the physicochemical and microbiological parameters according to factors storage time (t) and processing temperature, (T) and their interaction (t x T).

	t	**T**	**t x T**
pH	2.25^{ns}	1.26^{ns}	1.73^{ns}
TVB-N	12.68^{***}	14.12^{***}	7.50^{***}
TMA-N	29.05^{***}	32.26^{***}	11.26^{***}
IMP	13.85^{***}	29.94^{***}	9.12^{***}
Ino	1.96^{ns}	0.03^{ns}	2.97^{*}
Hx	13.91^{***}	7.29^{*}	7.04^{***}
K_1-value	26.17^{***}	104.41^{***}	15.16^{***}
Mesophilic bacteria	9.40^{***}	87.30^{***}	2.37^{ns}
Enterobacteriaceae	2.21^{ns}	40.98^{***}	1.75^{ns}

ns: no significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The analysed data revealed that storage time strongly influenced the TVB-N, TMA-N, IMP, Hx, K1-value and mesophilic bacteria values. Processing temperature also showed significant effects on all the considered variables, with the exception of pH and Ino. In general, the interactions between storage time and processing temperature were less marked than when the factors were considered individually.

These results highlight the importance of conducting the smoke-flavouring process at refrigeration temperatures below 5°C. The salting stage is especially critical in safety and quality terms as fish are more susceptible to spoilage when salt is not totally absorbed by muscle.

4. Conclusions

The water permeable (WP) bags allowed the exudate to completely evaporate during the smoke-flavouring process, which enabled salting, drying and smoking to be done in a single step. Processing temperature did not have effect on moisture, a_w, pH, colour or NaCl of the smoke-flavoured cod, but affected the initial values of TVB-N, TMA-N and microbiological counts. The smoke-flavoured cod recently obtained (day 0) at 10ºC came close to the microbiological and chemical limits of acceptance. In contrast, the smoke-flavoured cod processed at 5ºC exhibited an optimal initial microbiological quality. Only the smoke-flavouring process conducted at 5ºC enabled smoke-flavoured cod to be produced with an adequate degree of hygiene and with a shelf-life longer than 35 days in cold storage.

This methodology facilitates handling during chain production, and cuts processing steps and brine waste, which make the smoke-flavouring process simple and fast.

Acknowledgements

The authors gratefully acknowledge the support of the company Tub-Ex Aps (Taars, Denmark) for supplying the water vapour permeable bags and for providing all the necessary technical information. Arantxa Rizo would like to thank the Universitat Politècnica de València for the FPI grant.

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CAPITULO 3

DESARROLLO DE PRODUCTOS DE PESCADO AHUMADO CON CONTENIDO REDUCIDO DE SODIO

ARTÍCULO 5

DEVELOPMENT OF A NOVEL SMOKE-FLAVOURED SALMON PRODUCT WITH REDUCED SODIUM CONTENT USING WATER VAPOUR PERMEABLE BAGS

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Journal of food engineering (enviado)

Versión adaptada para la tesis doctoral

Abstract

The aim of this study was to evaluate the use of sodium-free (SF) salt and KCl to develop a novel smoke-flavoured salmon product with reduced sodium content. Fifty percent of NaCl was replaced with either 50% of sodium-free (SF) salt or 50% KCl in the salmon smoke-flavouring process. Triangle tests showed that samples with NaCl replacement with either SF salt or KCl were statistically similar to the control samples (100% NaCl). No sensorial advantage in using SF salt was found compared with using KCl. The changes of physicochemical and microbial parameters in smoke-flavoured salmon salted with the selected formulation (50%KCl-50%NaCl) and the control samples during 42 days at 4ºC showed that partial replacement of NaCl with KCl did not affect the quality and shelf-life of smoke-flavoured salmon, which was over 42 days. Smoke-flavoured salmon with 37% sodium reduction was developed without affecting sensory features and shelf-life.

Keywords: Smoke-flavouring; salmon; sodium reduction; KCl; water vapour permeable bags; shelf-life.

1. Introduction

Smoking has been applied since ancient times to preserve fish. The shelf life of fish is prolonged by means of salt uptake, dehydration, and smoke absorption that fish undergo in the different smoking process stages (salting, drying and smoking).

In particular, salt plays a vital role to protect fish from spoilage in cold-smoked products. However, high salt levels go against current trends to reduce the amount of sodium in processed food in line with public health demands. Indeed, WHO Member States have agreed on a voluntary global target to achieve a dietary salt reduction in the mean population by 30%, which involves a salt intake lower than 5 g per day (approx. 2 g of sodium) by 2025 (WHO, 2014). Likewise, the EU has established a common framework to reduce salt intake in the general population (EC, 2009). The European approach towards reducing salt focuses on a limited number of food categories, which include fish products. To achieve this goal, the European Commission supports research and work programmes with industry on sodium reduction in foods up to the lowest possible level, while maintaining food safety and consumer acceptance. For this reason, some manufacturers are reformulating recipes to reduce the salt content of their products, and many studies focus on salt reduction and salt replacement in different food types (Webster Trieu, Dunford & Hawkes, 2014; Toldrá & Barat, 2012).

To date, the partial substitution of NaCl for KCl seems the best alternative to reduce sodium content as potassium intake has not been linked to the development of hypertension and cardiovascular diseases. KCl has similar properties as NaCl and can be used to produce low-sodium food products without affecting their functionality. Furthermore, KCl is listed as being generally recognised as safe (GRAS) and appears to have a similar antimicrobial effect as NaCl against foodborne pathogens (Bidlas & Lambert,

2008), such as *Listeria monocytogenes* (Boziaris, Skandamis, Anastasiadi & Nychas, 2007) and *Clostridium botulinum* type E (Pelroy, Scherer, Peterson, Paranjpye & Eklund, 1985). The problem with potassium is that it can impart bitter and metallic flavour to foods when used at high levels (Toldrá & Barat, 2012). To overcome this limitation, application of flavour enhancers and bitter inhibitors to increase the saltiness perception, and to mask the aftertaste associated with potassium, are being studied (Dos Santos, Campagnol, Morgano & Pollonio, 2014). In recent years, commercial salt substitutes have been introduced into the market to fully or partially replace sodium chloride in various products (Pietrasik & Gaudette, 2014). Many are KCl-based salt formulations, which contain flavour enhancers such as glutamic acid derivatives, like monosodium glutamate (MSG), often combined with 5´-ribonucleotides (disodium guanylate and disodium inosinate), as well as bitter inhibitors like amino acids lysine and taurine. The development of reduced-sodium fish products that do not affect product quality and safety is relevant as many governments attempt to promote fish consumption for its good nutritional characteristics. However, sodium replacement in fish products has not yet been extensively researched, and very few studies on salted cod (Rodrigues, Ho, López-Caballero, Bandarra, & Nunes, 2005), surimi (Tahergorabi & Jaczynski, 2012) smoked sea bass (Fuentes, Fernández-Segovia, Serra & Barat, 2010b) and smoked salmon (Almli & Hersleth, 2012) are available.

The aim of this work was to assess the use of KCl and a KCl-based salt substitute to develop a reduced sodium smoke-flavoured salmon product with similar sensory features and shelf-life to smoke-flavoured salmon salted with 100% NaCl.

2. Material and methods

2.1. Materials

The fish employed as raw material was aquacultured salmon (Salmo salar) from Norway (Marine Harvest, Bergen, Norway). It was purchased from a local market in the city of Valencia (Spain) and its commercial size was 2-3 kg. Salmons were headed, gutted and filleted, and two fillets per fish were obtained. Before processing, fillets were trimmed to remove bones and cut into 4-cm portions, which provided five portions per fillet. The average fish portion weight was 136±23 g and thickness was 2-3 cm.

In this study, a KCl-based salt substitute and two salt types (KCl and NaCl) were employed for salmon smoke-flavouring. The salt substitute was commercial sodium-free (SF) salt from the company Navarro e Hijos, S.A. (Alicante, Spain). Its composition included potassium chloride, l-lysine monohydrochloride, glutamic acid, potassium tartrate and silicon dioxide. NaCl and KCl salts were supplied by Panreac Química, S.A. (Barcelona, Spain). Natural liquid smoke (HARDWOOD AFS 10) was provided by Amcan Ingrédients Ltd. (Le Chesnay, France) and consisted in a natural water-soluble condensate from the pyrolisis of walnut, maple, and other hardwoods. The water vapour permeable (WP) bags used for smoke-flavouring were supplied by TUB-EX ApS (Taars, Denmark) (polyamide mix; size: 200×300×0.04 mm; water vapour transmission rate: 5,000g/50μ/m²/24 h; (38°C/50% RH).

2.2. Experimental design

2.2.1. Phase I: Evaluation of partial NaCl replacement with SF salt and KCl in smoke-flavoured salmon.

Salmon portions were subjected to a simultaneous smoking-salting procedure in which water vapour permeable (WP) bags were used following the method developed by Rizo, Mañes, Fuentes, Fernández-Segovia and Barat (2015a) to obtain smoke-flavoured fish (Fig. 1). Liquid smoke, previously diluted in distilled water (60 mL/100 mL solution), was applied to the fish portions by spraying the fish surface for 30 s. Samples were randomly divided into three groups to be salted: the first with 100% NaCl (batch I); the second with the formulation that contained SF salt and NaCl at 50% (w/w) (batch II); the third with the formulation III that contained KCl and NaCl at 50% (w/w) (batch III). The NaCl substitution percentage was selected according to the results obtained in a previous work done in our laboratory, which concluded that NaCl could be replaced with up to 50% of SF salt without affecting the sensory and physicochemical traits of smoke-flavoured salmon. Portions were salted by means of a controlled process, dosing an amount of salt (7 g salt /100 g fresh salmon) to achieve similar a_w, chloride content and moisture values to those of commercial smoked salmon (Rizo et al., 2015a). Salt content was expressed as the chloride concentration in the liquid phase (z^{Cl-}) to properly compare all the experimental data, regardless of whether samples contained NaCl or mixtures of NaCl and SF salt or KCl.

Then, the salmon portions were vacuum-packaged (Tecnotrip mod. EV-25-CD, Barcelona, Spain) in highly water vapour permeable bags (WP). It should be noted that vacuum packaging was used just to ensure the initial contact between fish and the WP bag since vacuum conditions cannot be maintained for a long periods of time. The smoke-flavouring process was carried out in a drying chamber (Binder mod. KBF. Tuttlingen, Germany) for

24 h at 60% RH. When the processing time ended, salmon samples were removed from bags. They were then placed in saturated brine with constant stirring for 30 s to remove any traces of salt attached to the surface, dried with absorbent paper and weighed. The obtained raw material and smoke-flavoured salmon were characterised by analyses of moisture, chloride, sodium and potassium contents, a_w, and weight loss (ΔMt). Moreover, sensory evaluations were carried out.

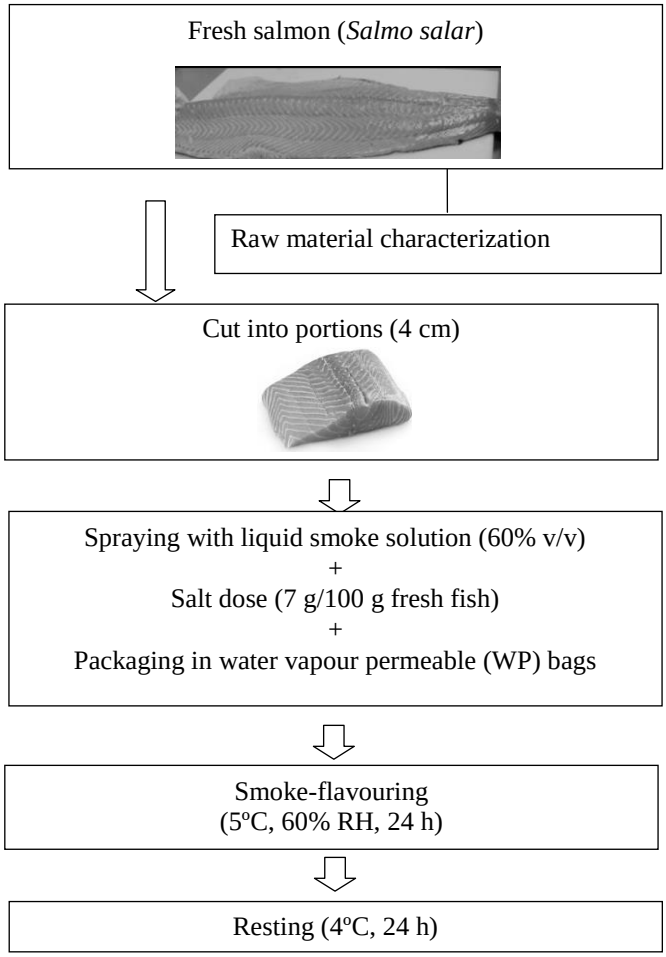

Fig. 1. Smoke-flavouring process of salmon.

2.2.2. Phase II: Quality and shelf-life assessment of the smoke-flavoured salmon product

The objective of this second phase was to evaluate the quality and shelf-life of the smoke-flavoured salmon salted with 50% KCl-50%NaCl (selected in Phase I) during cold storage. Control samples were also prepared using 100% NaCl. The smoke-flavoured salmon samples, obtained according to the process illustrated in Fig. 1, were vacuum-packaged and stored for 42 days at 4ºC. The packaging and storage conditions were selected as being the most commonly used in industry for such products during their marketing period. Physicochemical and microbiological analyses were performed on the raw material on day 0 and on the smoke-flavoured product obtained on cold storage days 0, 7, 14, 21, 28, 35 and 42. Three samples of each salt type were taken (n=3) on each sampling day. Analyses were performed in duplicate on each sample, except for pH, which was measured in quintuplicate.

2.3 Sensory analyses

Two triangle tests (ISO standard 4120, 2004) were carried out to test for similarity between the smoke-flavoured samples with NaCl replacement and the control samples. Sensory evaluations were made during two sessions with 66 untrained panellists. The first test was conducted with the samples salted by the 50%SF-50%NaCl formulation and the control samples (100% NaCl). During the second session, the smoke-flavoured salmon salted with 50% KCl-50% NaCl and the control samples were evaluated.

All the samples were obtained by the previously described smoke-flavouring procedure. Then they were filleted, vacuum-packaged and kept at 4ºC until the sensory evaluation was made (approx. 24 h after the whole process finished).

Assessors received a set of three samples and were informed that two of the samples were alike and one was different. Assessors were asked to report which sample they believed was different, even if the selection was based only on guesswork. Samples were randomly served on the same dish at room temperature and coded with a 3-digit random number.

2.4. Analytical determinations

2.4.1. Physicochemical analyses

Moisture content was determined by oven drying until constant weight at 105°C in accordance with AOAC method 950.46 (1997). The lipid content of samples was determined by Shoxlet extraction using petroleum ether in accordance with AOAC method 991.36 (AOAC, 1997).

Chloride content was determined in accordance with the procedure described by Fuentes et al. (2010b) after sample homogenisation in distilled water using an automatic Sherwood Chloride Analyser, Model 926 (Sherwood Scientific Ltd., Cambridge, UK). The same extract was used to analyse sodium. Potassium was analysed by absorption spectrophotometry in a Perkin-Elmer spectrophotometer model 3100 (Norwalk, CT, USA).

pH measurements were taken with a micropH 2001 digital pH-meter (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) at five different sample locations. Water activity (a_w) was measured in minced samples with a fast water activity-meter (Aqualab dew point hygrometer model 4TE, Decagon Devices, Inc., Washington, USA).

Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents were determined by steam distillation, following the

method described by Malle and Tao (1987). The thiobarbituric acid (TBA) index was measured by a spectrophotometric method with minor modifications (Tarladgis, Watts, Younathan & Dugan, 1960) to evaluate oxidation stability during chilled storage.

Weight changes in fish samples (ΔMt) were calculated according to Eq. 1.

$$\Delta M_t = \left(\frac{M_t - M_0}{M_0}\right) \quad (1)$$

where M_t is the sample weight at time t (g) and M_0 is the initial sample weight (g)

HPLC was used to determine the ATP-related compounds, which consisted in inosine-5´-monophosphate (IMP), inosine (Ino), and hypoxanthine (Hx), following the method described by Rizo, Mañes, Fernández-Segovia and Barat (2015b). The K1-values were calculated by Eq. 2.

$$K_1(\%) = \frac{[Ino] + [Hx]}{[IMP] + [Ino] + [Hx]} \times 100 \quad (2)$$

where IMP is inosine 5´-monophosphate, Ino is inosine and Hx is hypoxanthine.

The shear force test was performed with a Texture Analyser TA.XT2® (Stable Micro Systems, Surrey, UK), equipped with an HDP/BS Warner-Bratzler test cell, which sliced samples perpendicularly to muscle orientation at a constant speed of 1 mm/s with a 90º angle-inverted knife. Samples were obtained by cutting out parallelepiped pieces (3 × 2 cm) from

the same fish part. Shear force was determined by the maximum recorded force (N).

2.4.2. Microbiological analyses

Mesophilic bacteria and Enterobacteriaceae were determined according to the methods provided by ISO standard 4833:2003 and 21528-2:2004, respectively. All the analyses were performed in duplicate and the results were expressed as log cfu/g. All the culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain).

2.5. Statistical analyses

A one-way ANOVA was conducted with the data of the physicochemical and microbial analyses of Phases I and II to test whether there were significant differences between the fresh and recently smoke-flavoured salmons obtained with the different salt formulations. The triangle test results were analysed using the corresponding table of triangle tests for similarity according to ISO 4120:2004.

During the storage study, the data on each parameter were analysed by a multifactor ANOVA to evaluate the effect of salt formulation, storage time and their interactions. All the physicochemical and microbiological parameters were considered dependent variables. Salt formulation and storage time were taken as factors in these analyses. The least significant difference procedure was used to test for differences between averages at the 5% significance level. Data are reported as mean ± standard deviation.

Statistical data processing was performed with the Statgraphics Centurion software (Statpoint Technologies, Inc., Warrenton, VA, USA).

3. Results and discussion

3.1. Phase I: Evaluation of partial NaCl replacement with SF salt and KCl in smoke-flavoured salmon.

3.1.1 Physicochemical analyses

Table 1 provides the results from the physicochemical characterisation of the smoke-flavoured samples obtained with 100% NaCl and by partial NaCl replacement.

The smoke-flavouring process led to a significant reduction in moisture and an increase in chloride content, which reduced a_w compared with the raw material. These changes were due to dehydration and salt absorption in muscle, which were related directly to shelf-life and sensory characteristics in smoked fish. The moisture, chloride content and a_w of the different smoke-flavoured salmon samples were similar to those found for commercially available smoked salmon (Fuentes, Fernández-Segovia, Barat & Serra, 2010a; Rizo et al., 2015a). According to the Codex standard (Codex, 2013), smoke-flavoured fish requires a minimum NaCl content of 5% (z^{NaCl} =0.05) to prevent Clostridium botulinum from growing at storage temperatures between 3-10ºC, when the smoke flavour is provided by artificial flavour blends. To fulfil the Codex standard, the minimum chloride content value in this study should be at least 3% ($z^{Cl^-} \geq 0.03$) considering that NaCl is made up of 60% Cl^-. By taking into account these data, the products obtained with the different salt formulations fulfilled this requirement (Table 1).

Table 1. Physicochemical parameters of the smoke-flavoured salmon samples obtained with 100% NaCl and by partial NaCl replacement (50% SF-50% NaCl and 50% KCl-50% NaCl). (Means and standard deviations, *n=3*).

	Fresh salmon	Smoke-flavoured salmon			
		NaCl	SF-NaCl	KCl-NaCl	α
Moisture (g H_2O/100 g)	68.7±0.2^{a}	59±4^{b}	61±1^{b}	61±4^{b}	**
z^{Cl-} (g Cl^-/mL)	-	0.037±0.003^{a}	0.034±0.004^{a}	0.034±0.004^{a}	ns
Na^+ (mg/100 g)	59±7^{a}	1529±100^{c}	910±50^{b}	964±87^{b}	***
K^+ (mg/100 g)	299±13^{a}	289±42^{a}	1535±117^{b}	1625±299^{b}	***
a_w	0.990±0.003^{a}	0.945±0.003^{b}	0.953±0.003^{b}	0.949±0.009^{b}	***
ΔMt	-	-0.110±0.009^{a}	-0.10±0.03^{bc}	-0.10±0.01^{b}	*

z^{Cl-}: Cl^- concentration in liquid phase; ΔMt: weight loss
Different letters in the same row indicate significant differences.
ns: no significant $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

The antimicrobial effect of KCl, compared to NaCl, with a view to NaCl replacement in food products has been confirmed by different studies (Bidlas & Lambert, 2008; Boziaris et al., 2007). These studies have reported that NaCl and KCl perform the same action against foodborne pathogens when present in food in equimolar KCl:NaCl mixtures, or when its concentration leads to an equivalent a_w in the product. In line with this, Pelroy et al. (1985) demonstrated that it is feasible to substitute 50% NaCl for KCl in hot smoked fish without it affecting the inhibition of the Clostridium botulinum type E toxin formation. Thus, KCl is a safe alternative for reducing NaCl in smoked fish products. Partial NaCl replacement with SF salt and KCl significantly reduced the sodium content and increased the potassium content of smoke-flavoured salmon. The moisture and a_w values

were similar in the three sample types and no significant differences among them were found. Weight loss was also similar in all these samples.

3.1.2 Sensory analyses

Two triangle tests for similarity were carried out to test if the samples salted with 50% SF-50% NaCl or 50% KCl-50%NaCl were perceived as being similar to the control samples (100% NaCl).

The first triangle test results revealed that no meaningful differences were perceptible between the samples salted with SF-NaCl and the control samples as the assessors correctly identified the odd sample in 22 cases of 66, unlike the tabulated value at $p<0.05$, which corresponded to 28 (ISO 4120:2004). Similar results were obtained in the second triangle test carried out with the KCl-NaCl and control samples, in which the panel correctly identified the different sample in 24 cases. These results show that there is no advantage in using SF salt to replace NaCl to produce smoke-flavoured salmon compared with employing KCl. It also appeared that smoke-flavoured salmon was potentially not as sensitive to NaCl replacement with KCl as other food types, probably because of the smoky flavour (Mitchell, Brunton & Wilkinson, 2011; Pietrasik & Gaudette, 2014). By taking into account the lower price of KCl compared with SF, and that sodium-free salt ingredients (l-lysine monohydrochloride and glutamic acid) do not offer any advantage in terms of masking the potassium aftertaste, the KCl-NaCl samples were selected for the next study phase.

Different results relating these ingredients have been reported in other studies. Dos Santos et al. (2014) observed that using mixtures of KCl, lysine and monosodium glutamate sufficed to remove the defects caused by a 60-75% replacement of NaCl with KCl without affecting quality in fermented

cooked sausages. Mitchell et al. (2011) replaced 60% of NaCl in chilli con carne ready meals with a commercial mixture of KCl and L-lysine, and found no sensory differences in salty taste between the control and the low-salt samples that contained this salt substitute, nor any bitter or metallic flavours. However, when salting is applied to products whose initial structure must be preserved, such as smoked fish, salts and other ingredients must diffuse from the point of entry to the whole product, which means that transport by diffusion plays a vital role in the process (Barat, Pérez-Esteve, Aristoy & Toldrá, 2013). The SF salt used herein was intended to be used as table salt. Hence the optimisation of the physical form of its ingredients to allow them to better penetrate inside the muscle and to interact with each other, may improve the masking effect of lysine and glutamic acid on potassium-bitterness, when KCl is used at high levels.

3.2. Phase II: Quality and shelf-life assessment of the smoke-flavoured salmon product

3.2.1 Effect of the smoke-flavouring process

The results of the parameters analysed in the fresh salmon used as raw material and in the recently smoke-flavoured salmon are shown in Table 2.

Table 2. Physicochemical and microbiological parameters of fresh and recently smoke-flavoured salmon (day 0) salted with 100% NaCl and by 50% KCl-50% NaCl. (Means and standard deviations, *n=3*).

	Fresh salmon	**Smoke-flavoured salmon**		**α**
		NaCl	**KCl-NaCl**	
Moisture (g H_2O/100 g)	72±1[a]	61±2[b]	61±2[b]	***
z^{Cl-}(g Cl^-/mL)	-	0.037± 0.003[a]	0.038±0.006 [a]	ns
Na^+ (mg/100 g)	45±25[a]	1654±265[c]	1040±253[b]	***
K^+ (mg/100 g)	294±12[a]	238±101[a]	1493±318[b]	***
pH	6.58±0.02[a]	6.27±0.06[b]	6.25±0.08[b]	***
a_w	0.991±0.003[a]	0.934±0.010[b]	0.936±0.010[b]	***
ΔM_t	-	-0.09±0.01[a]	-0.08±0.05[a]	ns
Lipid (g/100 g)	9.81±0.03[a]	12±1[a]	12±4[a]	ns
TBA (mg MDA/kg)	nd	0.08±0.06[a]	0.07±0.03[a]	ns
TVB-N (mg N/100 g)	12.6±0.5[a]	13.4±0.9[ab]	15±1[b]	ns
TMA-N (mg N/100 g)	4.84±0.06[a]	6.0±0.4[b]	5.6±0.1[ab]	ns
IMP (µmol/g)	0.74±0.09[a]	0.9±0.2[a]	1.1±0.6[a]	ns
Ino (µmol/g)	7±1[a]	7±1[a]	8±1[a]	ns
Hx (µmol/g)	1.3±0.1[a]	1.2±0.1[a]	1.4±0.1[a]	ns
K_1- value (%)	91.5±0.2[a]	90.9±0.9[a]	90.9±0.9[a]	ns
Mesophilic (log cfu/g)	4.50±0.03[a]	2.6±0.5[b]	2.9±0.2[b]	***
Enterobacteriaceae (log cfu/g)	2.1±0.2	nd	nd	

z^{Cl-}: Cl^- concentration in the liquid phase; ΔMt: weight loss; TBA: thiobarbituric acid index; MDA: malonaldehyde; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen; IMP: inosine-5'-monophosphate; Ino: inosine; Hx: hypoxanthine.
Different letters in the same row indicate significant differences. nd: not detected.
ns: not significant, *** $p < 0.001$

As expected, the smoking-salting process reduced water content and the a_w values due to salt uptake and dehydration. The moisture, a_w and chloride content values of the obtained smoke-flavoured products fell within the range of values reported for commercially available smoked salmon (Fuentes et al., 2010a; Rizo et al., 2015a,b). No differences in these parameters, nor in pH and ΔMt, were observed according to the employed

salt formulation. Replacing NaCl partially by KCl implied a reduction in sodium content of approximately 37%.

The TVB-N and TMA-N values of the raw material were 12.60 and 4.84 mg N/100 g of fish, respectively, which agrees with the values reported by other authors for fresh salmon (Rizo et al., 2015b; Fernández-Segovia, Fuentes, Aliño, Masot, Alcañiz & Barat, 2012). The smoke-flavouring process slightly increased these parameters compared with the raw material, but no statistical significance was found. No differences were observed between the samples obtained with KCl-NaCl and the control samples.

The TBA index was used to evaluate the secondary products of lipid oxidation, which produce characteristic and undesirable off-odours (Sohn & Ohshima, 2010). In fish, these products come mainly from polyunsaturated fatty acid degradation. No malonaldehyde was detected in fresh salmon, so lipid oxidation was not considered to take place. Low values were recorded for the recently smoke-flavoured samples (Table 2). No significant differences were found between salt types for this parameter.

The contents of inosine 5'-monophosphate, inosine, hypoxanthine and K1-value of the fresh salmon and the recently smoke-flavoured fish are shown in Table 2. Neither the smoke-flavouring process nor salt formulation had a significant effect on any of these parameters.

The smoke-flavouring process reduced the growth of mesophilic and Enterobacteriaceae bacteria compared with fresh fish. According to the low values found for volatile bases and microbial growth, the raw material and the recently smoke-flavoured salmon used in this study exhibited adequate hygienic quality.

No exudate was observed in the bags after the process. The liquid released by samples completely evaporated through the WP bags during the

process as if there was no packaging. As demonstrated in previous studies, this method is a suitable alternative to traditional cold-smoking procedures since it enables good quality smoke-flavoured salmon to be obtained, while reducing product handling, brine waste and processing steps (Rizo et al., 2015a,b).

3.2.2 Changes in physicochemical and microbiological quality during storage

The results of the pH, TBA index, TVB-N, TMA-N and shear force analyses in the smoke-flavoured salmon salted with KCl-NaCl and the control samples are shown in Fig 2.

The pH values were not affected by salt formulation and remained nearly constant during storage, as observed in other studies into smoked fish (Goulas & Kontominas, 2005).

The TBA values progressively increased throughout storage for both sample types, which ranged from 0.08 to 0.46 mg MDA/kg fish. These values were below the limit reported by Connell (1995) of 1-2 mg MDA/kg fish. Values above this limit can entail rancid flavour and odour.

TVB-N is a common indicator of spoilage for many fish species, which quantifies mainly ammonia, trimethylamine (TMA) and dimethylamine (DMA). The TVB-N value greatly depends on storage conditions, hygienic practices, processing types, etc. (Fernández-Segovia et al., 2012; Fuentes, Fernández-Segovia, Barat & Serra, 2011). The upper acceptability limits of spoilage for smoked fish fell within 30-40 mg N/100 g (Dalgaard, 2000).

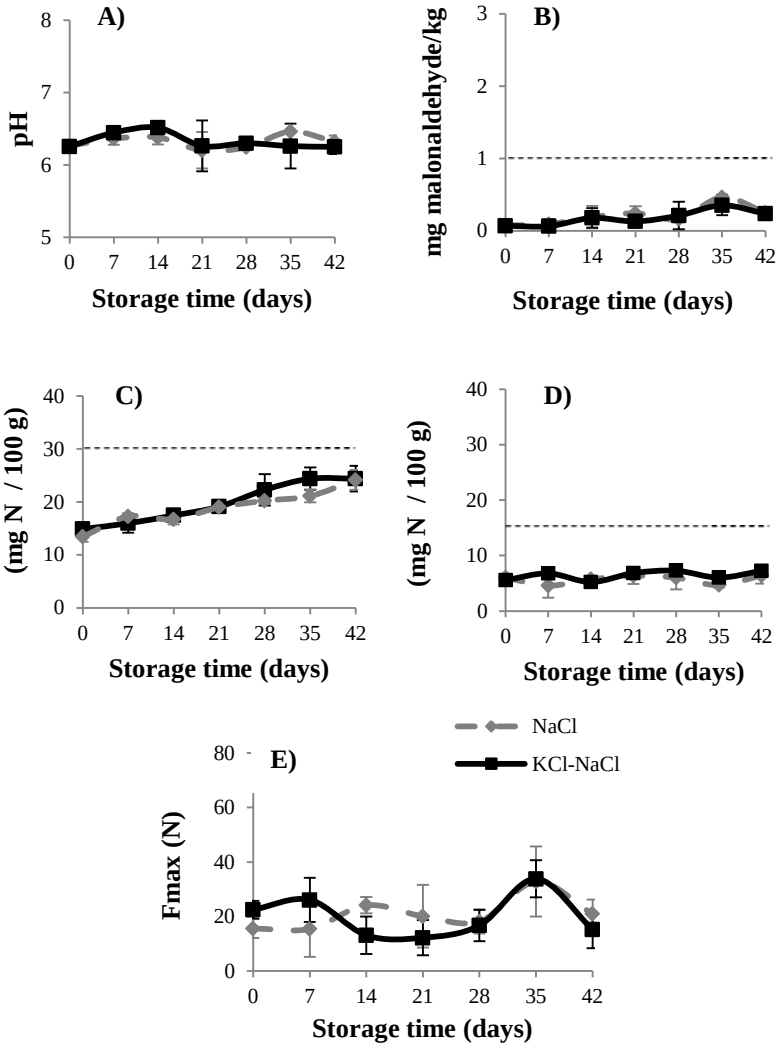

Fig. 2. Evolution of pH (A), TBA index (B), TVB-N (C), TMA-N (D) and shear force (E) in samples of smoke-flavoured salmon obtained with different salt formulations (100% NaCl and 50% KCl-50% NaCl) during 42 days of storage at 4 ºC. (Means and standard deviations, *n=3*). Bars indicate the standard deviation. The dashed line represents unacceptable levels in each figure.

In this study, the TVB-N concentration progressively increased throughout the storage period from 13.4 to 24.4 mg N/100 g of fish (Fig. 2C.), which agrees with other studies into cold-smoked salmon (Rizo et al., 2015b). In contrast, the TMA-N values remained nearly constant throughout the study. In general, 10-15 mg/100 g can be considered the upper limit for this parameter (Connell, 1995; Huss, 1995). No sample reached the limits of acceptability proposed for TVB-N and TMA-N, so no spoilage took place in the smoke-flavoured salmon, regardless of salt type.

Figure 2 shows the shear force test results of smoke-flavoured salmon during cold storage. Although the F max values oscillated during the study, no clear trend for this parameter was observed throughout storage, possibly due to the variability in thickness and/or lipid distribution among the fresh fish portions employed. Lipid content was typically distributed heterogeneously in the fish fillets, which affects texture to a great extent (Ginés, Valdimarsdottir, Sveinsdottir & Thorarensen, 2004; Katikou, Hughes & Robb, 2001). Minor differences were observed between salt types, but were not significant.

Changes in IMP, Ino and Hx of smoke-flavoured salmon for 42 cold storage days are shown in Fig 3.

ATP degradation compounds provide information about the chemical changes that occur in fish flesh during storage, which have been used extensively as a freshness index (Fernández-Segovia, Escriche & Serra, 2008). A progressive drop in IMP content and an increase in Hx were observed for both sample types during storage. The maximum recorded Hx value was 3.47 µmol/g, which was below the limit of sensory rejection of 5-7 µmol/g set for smoked salmon according to Truelstrup-Hansen, Gill and Huss (1995). The Ino values were higher than Hx throughout the study, and showed that the degradation of Ino to Hx was limited. According to many authors (Dalgaard, 2000; Fernández-Segovia et al., 2008), degradation of

IMP to Ino is attributed to autolytic enzymes, but accumulation of Hx in fish muscle is also connected to microbial spoilage. These results (no degradation of Ino to Hx) suggest limited microbiological activity, which is in agreement with the bacterial growth described below.

The K1-value is a freshness ratio, obtained from concentrations of ATP breakdown products, that quantifies the extent of IMP degradation. The K_1-value increased moderately from 90.9 for both samples to 96% by the end of the study (data not shown). These high values were caused by the low IMP levels recorded from the beginning of the study as IMP degradation had occurred almost completely in the raw material. Similar K_1-value results have been reported for smoke-flavoured salmon by Rizo et al. (2015b). The partial replacement of NaCl with KCl did not affect the values of this parameter.

Spoilage flora in smoked fish is variable and complex, and often dominated by lactic acid bacteria and Enterobacteriaceae (Løvdal, 2015; Joffraud et al., 2006). Mesophilic bacteria significantly increased in both samples during storage (Fig 3C), without reaching the value established as the upper tolerable limit for cold-smoked fish (7 log cfu g^{-1}) (ICMSF, 1986). High levels of Enterobacteriaceae are related to poor hygienic practices during handling, and can determine the product's shelf-life (Gram & Huss, 1996; Leroi, Joffraud, Chevalier & Cardinal, 2001).Counts of Enterobacteriaceae were below the limits of quantification for these microorganisms (1 log cfu g^{-1}) almost throughout the study (Fig. 3D), which indicates a good hygiene level during smoke-flavouring. No differences in the evolution of these microorganisms were noted according to the salt used. These results agree with those of Fuentes et al. (2011), who found no differences for Enterobacteriaceae growth when smoked sea bass was salted with the 50% NaCl-50% KCl mixture or with 100% NaCl.

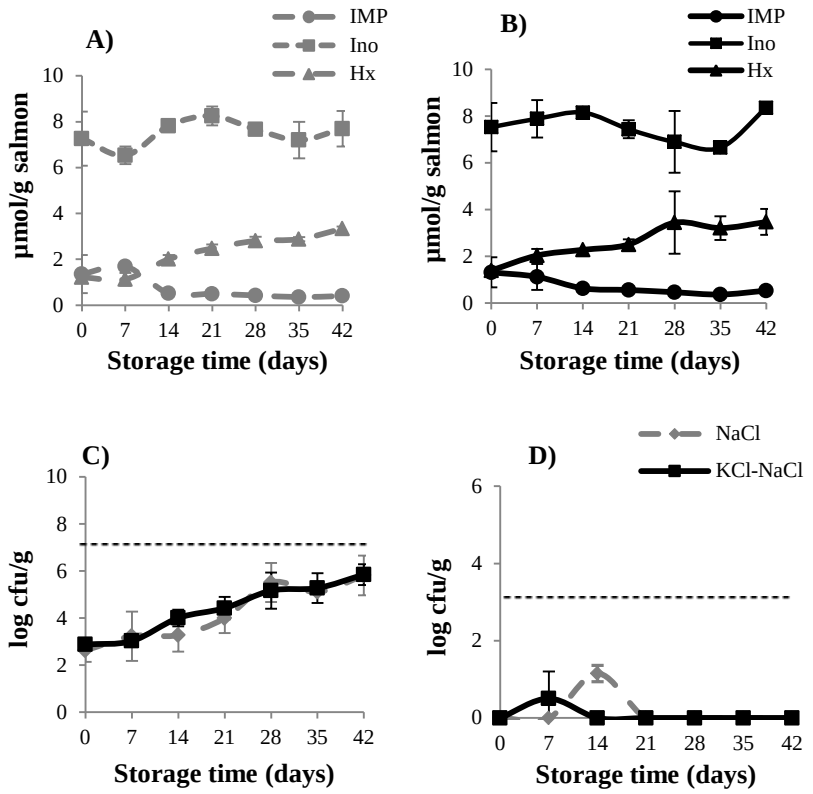

Fig. 3. Evolution of inosine-5'-monophosphate (IMP), inosine (Ino) hypoxanthine (Hx), mesophilic bacteria (C) and *Enterobacteriaceae* (D) in samples of smoke-flavoured salmon obtained with different salt formulations (100% NaCl (A) and 50% KCl-50% NaCl (B)) during 42 days of storage at 4 ºC. (Means and standard deviations, *n=3*). Bars indicate the standard deviation. The dashed line represents unacceptable levels in each figure.

No differences were observed depending on sample type, which also occurred with the physicochemical parameters described above.

According to these results, the smoke-flavoured salmon obtained with the 50% KCl-50%NaCl mixture maintained good microbial and

physicochemical quality throughout storage time. Therefore, it is suitable for human consumption for the studied 42-day period.

3.3. Multifactor analysis

The results obtained in the multifactor ANOVA done for each analysed parameter are shown in Table 3.

Table 3. F-ratio values and significance levels obtained in multifactor ANOVA for the microbiological and physicochemical parameters according to the factors: salt type (S), storage time (t) and their interaction (S x t).

	S	**t**	**S x t**
pH	0.44^{ns}	4.33^{*}	0.31^{ns}
TBA	0.90^{ns}	7.56^{***}	0.49^{ns}
TVB-N	2.96^{ns}	27.02^{***}	0.39^{ns}
TMA-N	4.26^{ns}	1.65^{ns}	0.44^{ns}
IMP	0.05^{ns}	5.66^{**}	0.31^{ns}
Ino	0.05^{ns}	1.30^{ns}	1.06^{ns}
Hx	3.81^{ns}	11.89^{***}	0.36^{ns}
K_1-value	0.35^{ns}	21.74^{***}	2.07^{ns}
Shear force	0.21^{ns}	3.42^{*}	1.50^{ns}
Mesophilic bacteria	0.52^{ns}	19.43^{***}	0.41^{ns}
Enterobacteriaceae	0.66^{ns}	2.21^{ns}	2.84^{*}

TBA: thiobarbituric acid index; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen; IMP: inosine-5'-monophosphate; Ino: inosine; Hx: hypoxanthine.
ns: not significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The statistical analysis confirmed that storage time strongly influenced all the analysed parameters except for TMA-N, Ino and Enterobacteriaceae. In contrast, salt type did not affect any of the considered variables. In general, the interactions between factors were non-significant.

4. Conclusions

The maximum NaCl proportion that can be replaced with sodium-free (SF) salt was 50%. The smoke-flavoured salmon obtained by replacing 50% NaCl with either 50% SF salt or 50% KCl was perceived as being similar to the samples processed with 100% NaCl by the panellists. As using SF salt to replace NaCl offered no sensory advantage compared with using pure KCl, and as the price of SF salt is higher than KCl, it can be concluded that KCl was a better choice for replacing NaCl than SF salt.

During the storage study, no lipid oxidation was recorded in the smoke-flavoured samples, regardless of the salt type employed. N-BVT, N-TMA, mesophilic and Enterobacteriaceae increased similarly for both salt formulations, without exceeding the acceptance limits at any time of the study. The 50% replacement of NaCl with KCl did not cause major changes in the physicochemical parameters and shelf-life of smoke-flavoured salmon, which was over 42 days. The 50% NaCl replacement with KCl implied an approximate 37% reduction in sodium content in the smoke-flavoured salmon. This is an interesting option for reducing sodium content in such products to help meet the needs set by both health authorities and consumers.

Acknowledgements

The authors gratefully acknowledge the support of Tub-Ex Aps (Taars, Denmark) for suppling the water vapour permeable bags and for providing all the necessary technical information. Arantxa Rizo would like to thank the Universitat Politècnica de València for the FPI grant.

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ARTÍCULO 6

DEVELOPMENT OF SMOKE-FLAVOURED TROUT: AN APPROACH TO SODIUM REDUCTION AND SHELF LIFE ASSESSMENT

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LWT- Food Science and Technology (en revisión)

Versión adaptada para la tesis doctoral

Abstract

This work aimed to develop a reduced sodium smoke-flavoured trout product with similar physico-chemical traits and sensory quality to commercial smoked trout. In a first phase, a reduced sodium smoke-flavoured trout product was developed by a novel smoke-flavouring process using water vapour permeable (WP) bags. In a second phase, the obtained product's microbial and physico-chemical quality was evaluated for 42 cold storage days. A smoke-flavoured trout product with similar physico-chemical characteristics and sensory acceptance to commercial smoked trout was achieved through smoke-flavouring with WP bags. Partial substitution of NaCl for KCl led to a 42% sodium reduction in the smoke-flavoured trout and did not affect its physico-chemical traits, sensory attributes and hygienic quality during storage. Smoke flavouring with WP bags is a suitable process for obtaining good quality reduced sodium smoke-flavoured trout with similar characteristics to commercial smoked trout.

Keywords: Smoke flavouring; trout; NaCl; KCl; water vapour permeable bags; shelf life.

1. Introduction

Fish smoking techniques involve a salting step prior to smoking, which is essential in preservation, texture and product flavour terms. However, processed foods like "ready-to-eat" fish products are considered important contributors to dietary salt intake, which is linked to increased risk of cardiovascular disease. Some countries have implemented control measures, such as mandatory labelling for such products as "highly salted" to promote consumer awareness (WHO, 2009). Health authorities' efforts to encourage low-sodium diets and increase fish intake render the development of less salty fish products is a relevant issue.

To achieve this goal, the food industry is attempting to reformulate recipes to reduce the sodium of its products, while maintaining food safety and consumer acceptance. The main strategy to adopt in order to reduce the sodium in these foodstuffs consists in the partial replacement of NaCl with other salts (KCl, $CaCl_2$, $MgCl_2$, K-lactate, etc.). Partially substituting NaCl for KCl is the best alternative to reduce sodium content, but the main limitation of using KCl is the bitter and metallic flavour that it confers foods if used at high levels (Toldrá & Barat, 2012), and 50:50 NaCl/KCl blends are the common practical industrial limit. However, this limit can vary depending on the type of food and presence of other ingredients, such as spices or smoke flavours, which can mask the residual flavours associated with using KCl. Hence the sodium substitution level in smoked products could be higher than in other kinds of food matrices (Fuentes, Fernández-Segovia, Serra & Barat, 2012; Mitchell, Brunton & Wilkinson, 2011).

In this context, we developed a new methodology to obtain smoke-flavoured salmon based on a controlled salted process and the use of water vapour permeable (WP) bags (Rizo et al., 2015a). The process was found to effectively reduce handling, brine waste and processing steps without affecting the smoke-flavoured fish's sensory acceptance and the physico-

chemical quality (Rizo, Mañes, Fuentes, Fernández-Segovia & Barat, 2015a, b; Rizo, Fuentes, Fernández-Segovia & Barat, 2016a, b).

Rainbow trout (*Oncorhynchus mykiss*) is one of the most produced aquacultured fish in Europe (FAO, 2014). Lower stable market prices, and its smaller whole "easy-to-handle" fillets, make trout a more profitable raw material for smoking than Atlantic salmon, especially given the close resemblance between the commercial smoked products of both species (Salán, Galvão & Oetterer, 2006).

Thus we considered that a combined approach that would integrate partial NaCl replacement into the described smoke-flavouring process would provide high added value to smoke-flavoured trout products, which could meet the needs of both consumers and producers, who demand healthier fish products and improved process yields.

The objectives of this study were to: (a) develop a reduced sodium smoke-flavoured trout product by the new smoke-flavouring process; (b) evaluate the obtained product's physico-chemical and microbial quality during storage.

2. Material and methods

2.1. Materials

Aquacultured trout (*Oncorhynchus mykiss*) (Piscifactorias Andaluzas, S.A, Granada, Spain), of commercial weight 300-700 g, were purchased from a local market in the city of Valencia (Spain). Trout were headed and gutted, and fillets were trimmed to remove bones before

processing. Eighty-six trout fillets (average weight 111±25g) were employed for the complete test (38 for the first phase and 48 for the second).

NaCl and KCl salts were supplied by Panreac Química, S.A. (Barcelona, Spain) and natural liquid smoke HARDWOOD AFS 10 was provided by Amcan Ingrédients Ltd., Le Chesnay, France). The water vapour permeable bags (WP) used for smoking-salting were supplied by TUB-EX ApS (Taars, Denmark) (polyamide mix; size: 200×300×0.04 mm; water vapour transmission rate: 5,000g/50µ/m²/24 h (38°C/50% RH).

Two smoked trout batches of three different brands were analysed to establish the target smoke-flavoured trout's physico-chemical parameters (moisture, salt content and a_w). The raw material of these products was aquacultured rainbow trout, processed according to traditional cold-smoking techniques in a smoking chamber.

All the reagents and culture media were provided by Scharlau Chemie, S.A. (Barcelona, Spain).

2.2. Experimental design

2.2.1. Phase I: Developing a reduced sodium smoke-flavoured trout product

2.2.1.1 Smoking-salting process optimisation

The optimal conditions for obtaining smoke-flavoured trout were established by studying the effect of the amount of salt doses and processing time on the final product physico-chemical properties. These conditions were set to obtain smoke-flavoured trout with similar characteristics to currently marketed products. The values considered as reference were obtained from the analysed commercial products.

Trout fillets were subjected to a simultaneous smoking-salting procedure based on the use of water vapour permeable (WP) bags (Fig. 1a) following the method developed by Rizo et al. (2015a). Diluted liquid smoke was applied to fish by spraying fish surface for 30 s. In this phase, three salt dose concentrations were considered, 2, 4, and 6 g salt/100 g fresh trout, as were two processing times, 12 h and 24 h. Then trout samples were vacuum-packaged (Tecnotrip mod. EV-25-CD, Barcelona, Spain) in highly water vapour permeable (WP) bags. The smoke-flavouring process was carried out at 60% relative humidity (RH) and 5ºC in a drying chamber (Binder mod. KBF. Tuttlingen, Germany). After the processing time, trout samples were removed from the bags and were placed in saturated brine under constant stirring for 30 s to remove any traces of salt attached to surfaces. Finally, fillets were dried with absorbent paper and weighed. The obtained smoke-flavoured trout was characterised by analyses of moisture, chloride content, a_w and weight loss (ΔMt). The sensory acceptance of the obtained products was also evaluated.

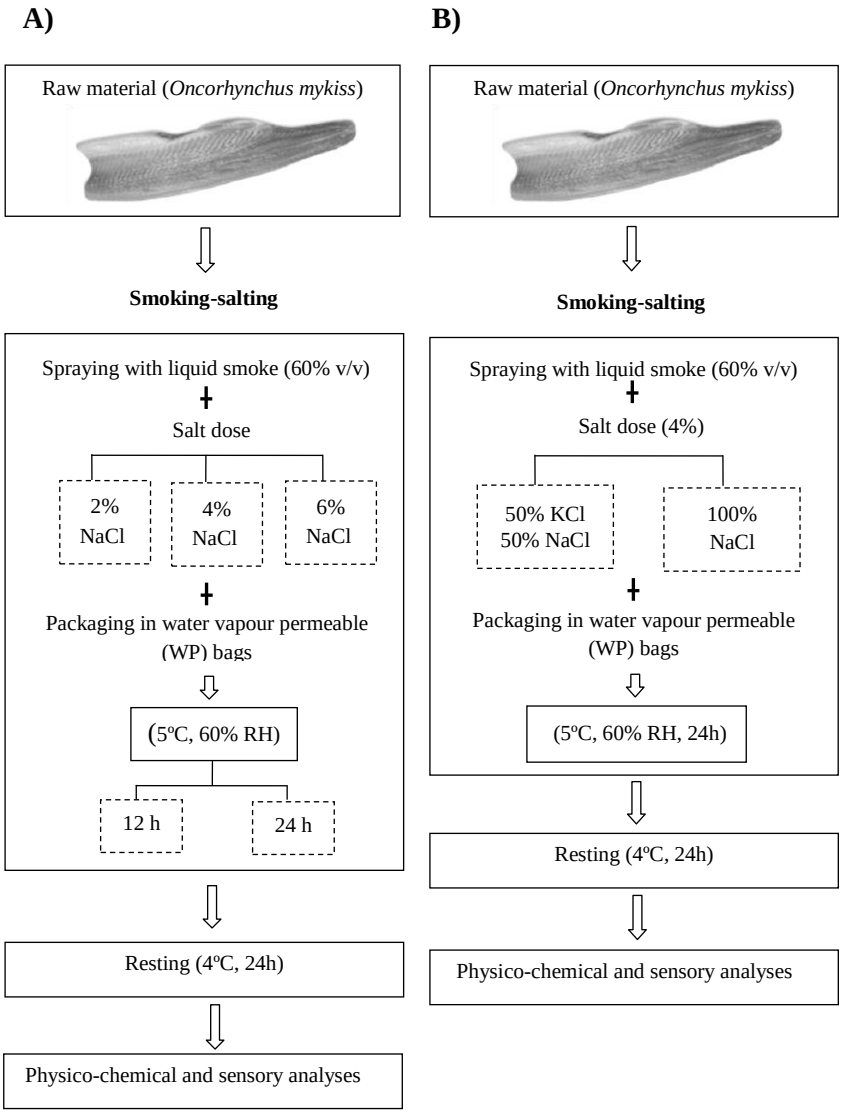

Fig 1. Trout smoking-salting (phase I): process optimisation (a). Developing the reduced sodium product (b)

2.2.1.2. Developing a reduced sodium product

After establishing the appropriate processing conditions (4% of salt dose, 24 h), the sodium reduction approach was applied (Fig. 1b). Trout fillets were processed by using a salt mixture of 50% KCl-50% NaCl (w/w) and 100% NaCl (control samples). The percentage of substitution was selected according to the results obtained in a previous work (Fuentes, Fernández-Segovia, Barat & Serra, 2011), which concluded that NaCl can be replaced with up to 50% KCl without affecting the smoke-flavoured fish sensory and physico-chemical traits. The obtained samples were characterised by physico-chemical and microbiological analyses, and a sensory test was conducted.

2.2.2. Phase II: Physico-chemical and microbial quality during storage

The objective of the second phase was to evaluate the quality and shelf life of the novel reduced sodium smoke-flavoured trout fillets obtained in Phase I. For this purpose, samples were vacuum-packaged and stored for 42 days at 4ºC. The physico-chemical and microbiological analyses were performed on the smoke-flavoured products (reduced-sodium and control) on cold storage days 0, 7, 14, 21, 28, 35 and 42. On each sampling day, three bags were analysed by salt formulation (n=3). Duplicate analyses were performed on each sample, except for pH, which was measured in quintuplicate.

2.3. Analytical determinations

2.3.1. Physico-chemical analyses

Moisture and lipid content were determined in accordance with AOAC methods 950.46 and 991.36, respectively (AOAC, 1997). Chloride content was determined after sample homogenisation in distilled water using an automatic Sherwood Chloride Analyser Model 926 (Sherwood Scientific Ltd., Cambridge, UK). The same extract was used to determine sodium and potassium contents by absorption spectrophotometry using a Perkin-Elmer spectrophotometer, model 3100 (Norwalk, CT, USA). pH measurements were taken by a micropH 2001 digital pH-meter (Crison Instruments, S.A., Barcelona, Spain) with a puncture electrode (Crison 5231) at five different locations on the fish fillets. Water activity (a_w) was measured with an Aqualab dew point hygrometer model 4TE (Decagon Devices, Inc., Washington, USA). Total volatile basic nitrogen (TVB-N) and trimethylamine nitrogen (TMA-N) contents were determined by steam distillation according to the method described by Malle and Tao (1987). The thiobarbituric acid (TBA) index was measured by a spectrophotometric method with some minor modifications (Tarladgis, Watts, Younathan & Dugan, 1960).

2.3.2. Texture measurements

A texture profile analysis (TPA) and a shear force test were performed on the smoke-flavoured trout fillets with a Texture Analyser TA.XT2® (Stable Micro Systems, Surrey, UK) equipped with a load cell of 250 N. Previously skinned fillets were cut to obtain parallelepiped pieces (3 x 2 cm) from the dorsal part of fillets. Measurements were taken of the samples at room temperature.

For the TPA analysis, a flat-ended cylindrical plunger (7.5mm diameter) was employed. This plunger was pressed into the sample at a constant speed of 1 mm/s until it reached 50% of sample height. Force-distance curves were processed to obtain hardness, chewiness, adhesiveness, springiness, cohesiveness and resilience parameters.

For the shear force test a HDP/BS Warner-Bratzler cell was used, which sliced the samples perpendicularly to the muscle orientation at a constant speed of 1 mm/s using a 90º angle inverted knife. Shear force was determined by the maximum force (N) recorded.

2.3.3. Colour determinations

Colour determination was performed in the flesh of trout fillets. A Minolta CM-700-d photocolorimeter (Minolta, Osaka, Japan) was used, equipped with a 10° observer and illuminant D65. Using the CIE L*a*b* coordinates the overall colour differences (ΔE) of the recently smoke-flavoured samples (day 0) compared with each storage study sampling day were determined. The psychophysical magnitudes of hue (hab*) and chroma (Cab*) were calculated.

2.4. Microbiological analyses

Mesophilic bacteria and Enterobacteriaceae were determined according to the methods provided by ISO standard 4833:2003 and 21528-2:2004, respectively.

2.5 Sensory analyses

A sensory assessment was made to determine the smoke-flavoured trout product sensory acceptance. This test was conducted with the smoke-flavoured trout samples obtained under different processing conditions (salt dose: 2%, 4%, 6% NaCl; processing time: 12 h and 24 h) and with a commercial smoked trout sample. Attributes like appearance, colour, odour, smoke odour, taste, saltiness and global acceptance were evaluated. Tests were done on semi-structured scales with 8 cm lines and three anchor points (0 = unpleasant, 4 = acceptable, and 8 = pleasant) for all the attributes, except for smoke odour and saltiness, where the anchors corresponded to insufficient, optimum and excessive (0, 4, and 8, respectively). A selected trained panel of seven assessors with experience in smoked fish assessment performed the sensory evaluation. Two sessions (one per processing time) were conducted, during which panellists were served four randomised samples on the same dish (3 smoke-flavoured trout samples and 1 commercial sample).

A triangle test (ISO standard 4120: 2004) was carried out to test for similarity between the reduced smoke-flavoured trout and the control samples (100% NaCl). Test sensitivity, given by α and β-risk was established as 0.05. The sensory assessment was made by 66 untrained panellists, who received a set of three samples and were informed that two were alike and one was different. They were asked to report which sample they believed to be different.

2.6. Statistical analysis

Statistical treatment of the data was performed using the Statgraphics Centurion software (Statpoint Technologies, Inc., Warrenton,

VA, USA). In Phase I, a one-way ANOVA was conducted to discriminate among means. The least significant difference (LSD) procedure was used to test for the differences between averages at the 5% significance level. During the storage study, physico-chemical and microbiological data were analysed with a multifactor ANOVA to evaluate the effect of salt formulation and storage time. Tukey´s test procedure was used to test for any differences between means ($p<0.05$).

3. Results and discussion

3.1 Phase I: developing a reduced sodium smoke-flavoured trout product

3.1.1. Smoking-salting process optimisation

The physico-chemical parameters of the commercial smoked trout of three different brands were used to establish the reference values for the smoke-flavoured trout product (Table 1).

Table 1. Moisture, lipid, chloride content, and a_w of commercial smoked trout (brand 1, 2 and 3). Mean values±SD (n=3).

Brand	Moisture (g/100g)	Lipid (g/100g)	NaCl (g/100g)	a_w
1	54.6 ± 0.4^{a}	19.8 ± 0.1^{a}	3.27 ± 0.03^{a}	0.951 ± 0.001^{a}
2	68.3 ± 0.2^{c}	6.7 ± 0.3^{b}	3.34 ± 0.05^{a}	0.968 ± 0.006^{b}
3	61.09 ± 0.01^{b}	8.2 ± 6.1^{b}	3.51 ± 0.02^{b}	0.951 ± 0.001^{a}
α	***	***	*	***

**p<0.05; ***p<0.001. Different letters in the same column indicate significant differences*

Wide variability was found among the physico-chemical parameters of all three brands, which occurred with other studies reported for smoked fish (Cornu et al., 2006; Fuentes, Fernández-Segovia, Barat & Serra, 2010). Given the differences found among brands, the average of the three brands was established as a reference value (62 g H_2O/100 g, 3.4 g NaCl/100 g, a_w =0.957).

To achieve a smoke-flavoured trout product with similar physico-chemical traits to those established as a reference of commercial products, three salt doses (2%, 4% and 6% NaCl) and two processing times (12 h and 24 h) were tested.

As expected, the smoke-flavouring process significantly lowered the moisture and a_w values, and salt concentration increased compared with fresh trout (Fig. 2). The longer the processing time and the higher the salt dose, the lower the moisture and water activity. Salt content in the smoke-flavoured samples increased with salt dose, but a longer processing time did not significantly affect salt concentration. In contrast, weight loss was affected by processing time, but not by salt dose, which agrees with previous results from a similar study done with smoke-flavoured cod (Rizo et al., 2016a).

After the process, no exudate was collected from the bags of any samples as the WP bags were permeable enough to allow all the water released by fish muscle to completely evaporate, which thus reduced brine waste. Similar results have been reported for salmon and cod obtained by smoke flavouring with WP (Rizo et al., 2015a; Rizo et al., 2016a). These results confirmed that trout can be salted, dried and smoked in a single step inside WP bags by a controlled process. This implies reducing not only brine waste, but also the handling and processing steps, compared with traditional methods in which salting, drying and/or smoking are carried out separately.

Of all the tested conditions, salting with 4% NaCl for 24 h produced a smoked trout product that had the closest physico-chemical values to the reference ones.

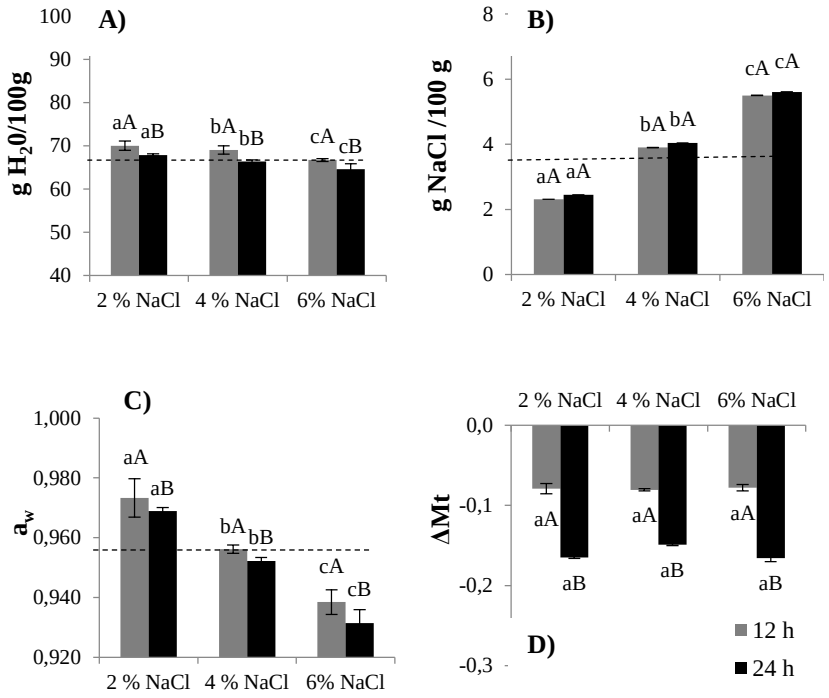

Fig. 2. Moisture (a), NaCl content (b), water activity (c) and weight loss (ΔMt) of the smoke-flavoured trout samples obtained by using different salt doses (2%, 4%, and 6% NaCl) and different processing times (12 h and 24 h). Mean values±SD (n=3). Bars indicate standard deviation. The dashed line represents the reference value. Different lower case and capital letters indicate significant differences.

The NaCl content of these samples corresponded to a sodium chloride content of 5.7% in the liquid phase (z^{NaCl} = 0.057). This value fulfilled the Codex standard for smoked fish, smoke-flavoured fish and

smoked dried fish (Codex, 2013), in which a minimum 5% content of NaCl (z^{NaCl} = 0.05) is required to ensure complete protection against Clostridium botulinum at storage temperatures between 3°C and 10°C.

An acceptance test was carried out to check the acceptability of the obtained products. The smoke-flavoured trout samples obtained by the novel methodology and commercial smoked trout (brand 3) as a control sample were evaluated (Table 2).

Table 2: Score average for the attributes evaluated in the smoked trout samples using different salt dose (2, 4 and 6 g/100g fresh fish) and processed during 12 and 24 h.

Processing time	Attributes	Commercial	Salt dose g/100g		
			2	4	6
12h	**Appearance***	5.94±1.11	6.14±1.61	6.70±0.95	6.67±1.06
	Colour*	6.10±1.19	6.76±0.99	6.97±0.93	6.90±0.83
	Odour*	6.60±0.91	5.80±1.10	6.23±0.71	5.68±1.32
	Smoke odour **	4.04±0.80	3.91±0.38	3.94±0.89	4.80±0.85
	Taste*	5.69±1.13	5.34±1.31	5.87±1.45	6.23±1.35
	Texture*	6.36±0.50	5.60±0.81	6.23±1.20	5.87±1.04
	Saltiness **	4.91±0.91	3.73±0.84	4.39±0.72	4.77±0.78
	Global acceptance*	6.37±0.74	5.44±0.69	5.91±1.22	5.94±1.48
24h	**Appearance***	6.13±1.38	6.61±0.60	6.87±0.50	6.44±0.96
	Colour*	6.09±1.58	6.67±0.68	6.60±0.73	6.76±0.64
	Odour*	6.31±1.00	6.36±0.53	6.11±1.38	6.83±0.74
	Smoke odour **	4.16±0.84	5.16±1.13	3.70±1.39	4.10±0.82
	Taste*	4.16±0.83	6.30±1.05	6.24±1.15	6.17±1.80
	Texture*	5.36±1.66	6.40±1.21	6.19±1.69	5.91±1.67
	Saltiness **	5.91±1.69	4.24±1.16	4.63±1.11	5.24±1.06
	Global acceptance*	4.73±1.05	6.19±0.96	5.73±1.62	5.81±1.29

* (0 = unpleasant, 4 = acceptable, and 8 = pleasant).
** (0 = insufficient 4 = optimum, and 8 = excessive).

All the samples obtained scores above 4 for all the evaluated attributes, which indicates that sensory acceptance was satisfactory. The smoke-flavoured samples generally obtained a higher score for appearance, colour and odour than the commercial samples, regardless of processing time and salt dose. Regarding saltiness, the samples processed with a 6% salt dose

and the commercial samples scored furthest from the optimal value for all the processing times. The panellists considered that the saltiness of these samples was excessive. For taste and global acceptance, the samples processed for 24 h scored higher than the commercial samples, but the samples processed for 12 h obtained lower acceptability scores. This evaluation revealed that the smoke-flavoured trout sensory attributes were perceived with the same degree of acceptance as the commercial smoked trout. This finding indicates that this methodology is suitable for obtaining a smoke-flavoured trout products with adequate sensory quality. These results are consistent with those reported for the smoke-flavoured salmon obtained by the same technique (Rizo et al., 2015a).

According to the sensory and physico-chemical results, a processing time of 24 h and a salt dose of 4 g/100 g were selected to develop reduced sodium smoke-flavoured trout.

3.1.2. Developing reduced sodium products

Table 3 shows the recently analysed parameters in the smoke-flavoured trout in which NaCl was replaced with KCl and the control samples were salted with 100% NaCl.

The moisture, chloride content and a_w of the obtained smoke-flavoured products were similar to the reference values established in Phase I, and fulfilled the minimum salt content (z^{NaCl}=0.05) expressed as chloride content (z^{Cl^-}= 0.03), as set out by the above-mentioned standard for smoked fish, smoke-flavoured fish and smoked dried fish (Codex, 2013). No significant differences in these parameters, or in ΔMt and pH, were observed according to the salt formulation used. The obtained results showed that the

mixture of salts employed allowed an approximate 42% reduction of sodium content compared with the control samples.

Table 3. Physico-chemical and microbiological parameters of the recently smoke-flavoured trout (day 0). (Means±SD, n=3).

	Smoke-flavoured trout		α
	KCl-NaCl	**NaCl**	
Moisture (g H_2O/100 g)	66±1[a]	67±1[a]	ns
Z^{Cl-} (g Cl^-/mL)	0.034±0.002[a]	0.036±0.001[a]	ns
NaCl (g/100g)	2.4±0.2[a]	4.0±0.2[b]	***
a_w	0.955±0.003[a]	0.952±0.003[a]	ns
ΔM_t	-0.17±0.04[a]	-0.18±0.03[a]	ns
pH	6.04±0.05[a]	6.0±0.1[a]	ns
Na^+ (mg/100g)	945±193[a]	1610±218[b]	***
K^+ (mg/100g)	1129±119[a]	334±49[b]	***
Lipid (g/100 g)	6.2±0.3[a]	6.4±0.7[a]	ns
TBA (mg MDA/kg)	0.12±0.03[a]	0.12±0.07[a]	ns
TVB-N (mg N/100 g)	15±3[a]	15±2[a]	ns
TMA-N (mg N/100 g)	1.8±0.6[a]	1.5±0.4[a]	ns
Mesophilic bacteria (log cfu/g)	4.1±0.3[a]	4,0±0.2[a]	ns
***Enterobacteriaceae* (log cfu/g)**	1.3±0.6[a]	1.9±0.4[a]	ns
Textural parameters			
TPA test			
Hardness (N)	61.2±0.6[a]	52±5[a]	ns
Chewiness (N)	30.7±0.5[a]	22±1[b]	*
Adhesiveness (g/s)	-0.14±0.07[a]	-0.2±0.1[a]	ns
Springiness	0.71±0.05[a]	0.69±0.02[a]	ns
Cohesiveness	0.66±0.05[a]	0.60±0.02[a]	ns
Resilience	0.41±0.07[a]	0.38±0.03[a]	ns
Shear test			
F_{max} (N)	18±7[a]	21±5[a]	ns
Colour parameters			
L*	33±3[a]	33±2[a]	ns
a*	11±5[a]	10±3[a]	ns
b*	15±4[a]	13±3[a]	ns
C_{ab}*	19±6[a]	16±4[a]	ns
h_{ab}*	55±9[a]	51±5[a]	ns

z^{Cl-}: Cl^- concentration in liquid phase; ΔMt: weight loss; TBA: thiobarbituric acid index; MDA: malonaldehyde; TVB-N: total volatile basic nitrogen; TMA-N: trimethylamine nitrogen.
Different letters indicate significant differences, ns: no significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The TBA index was used to evaluate the secondary lipid oxidation products which produce characteristic and undesirable off-odours. Similar values were recorded for both sample types (0.12 mg MDA/kg), which were lower than those reported in other studies into smoked fish (Bugueño, Escriche, Martínez-Navarrete, Camacho & Chiralt, 2003; Fuentes et al., 2011). These lower values could be related to the refrigeration temperatures employed throughout the process (5ºC) as processing temperatures influence the formation of secondary oxidation compounds (Espe, Nortvedt, Lie & Hafsteinsson, 2002; Goulas & Kontominas, 2005)

As with the TBA index, no differences were observed in the TVB-N and TMA-N values, mesophilic bacteria and Enterobacteriaceae between the samples obtained with KCl-NaCl and the control samples. According to these results, the recently trout smoke-flavoured product offered adequate hygienic quality. Salt replacement did not affect trout texture, except for chewiness which obtained higher values for the KCl-NaCl samples (Table 3). Regarding colour, partial NaCl substitution had no significant effect on the recently smoke-flavoured samples. Liquid smoke application on fish, and also the initial variability among the fresh fish fillets employed, reduce the possible differences that using different salts could have (Fuentes et al., 2012). No exudate was observed in any of the bags because, as described above, the liquid released by samples evaporated completely through the WP bags during the process.

A triangle test for similarity was carried out to check if there were any perceptible differences between the samples salted with KCl-NaCl and the control samples. According to the results obtained (23 correct responses of 66 evaluations), no more than 20% of the consumers were able to detect differences between the samples with a confidence level of 0.05 (α and β-risk). The use of high replacement levels of NaCl with KCl above 40-50% can diminish flavour intensity and produce bitter tastes, but the replacement

level varies according to food product type, and presence of significant levels of smoke flavours and spices can help mask the bitter taste conferred by K^+ (Mitchell et al., 2011).

3.2. Phase II: physico-chemical and microbial quality during storage

Figure 3 illustrates the evolution of the TBA index, TVB-N, TMA-N, mesophilic bacteria and Enterobacteriaceae in the smoke-flavoured samples.

The TBA values increased for both sample types throughout storage. Samples obtained with KCl-NaCl displayed lower lipid oxidation than control samples. The values of both sample types remained generally lower than the limits proposed by Connell (1995) of 1-2 mg MDA/kg of fish flesh, at which fish can develop an objectionable odour. So shelf life was not limited by lipid oxidation.

TVB-N is a common indicator of spoilage for many fish species. No sample exceeded the upper limit of acceptability of spoilage established for smoked fish of 30-40 mg N/100 g (Dalgaard, 2000). In this study, the TVB-N concentration increased throughout the storage period from 14 to 23 mg N/100 g of fish (Fig. 3), which agrees with the results reported by Alçiçek et al. (2011) for liquid-smoked trout stored under vacuum conditions. The same tendency was found for TMA-N, for which 10-15 mg/100 g was the upper limit for this parameter (Connell, 1995). No differences were found between salt formulations in the TVB-N and TMA-N values, which remained far below the limits of acceptability throughout the study period.

Mesophilic bacteria significantly increased for all the samples during storage, but did not reach the value established as the upper tolerable

limit for cold-smoked fish (7 log cfu g^{-1}) (ICMSF, 1986) until day 35. Some studies have found high mesophilic bacteria levels in cold-smoked salmon (10^7-10^8 cfu g^{-1}) before signs of spoilage became apparent, which sometimes make them unreliable as quality indicators of cold-smoked fish (Joffraud et al., 2006; Løvdal et al., 2015).

High levels of Enterobacteriaceae are related to poor hygiene practices during handling and can determine the shelf-life of the product (González-Rodríguez, Sanz, Santos, Otero & García-López, 2002). Enterobacteriaceae counts remained below the limit of acceptability for these microorganisms (3 log cfu g^{-1}) throughout the study, which indicates a good level of hygiene during smoking-salting. No differences in the evolution of these microorganisms during storage were recorded according to the salt used. This could suggest that partial sodium replacement did not affect microbial fish spoilage, which agrees with Fuentes et al. (2011), who found no differences for mesophilic bacteria and Enterobacteriaceae growth when liquid-smoked sea bass was salted with a 50% NaCl:50% KCl mixture and by 100% NaCl. Several studies have confirmed similar antimicrobial activity of KCl and NaCl at an equivalent a_w (Bidlas & Lambert, 2008; Boziaris, Skandamis, Anastasiadi & Nychas, 2007). According to these results, the shelf life of smoke-flavoured trout would be around 1 month, regardless of salt formulation.

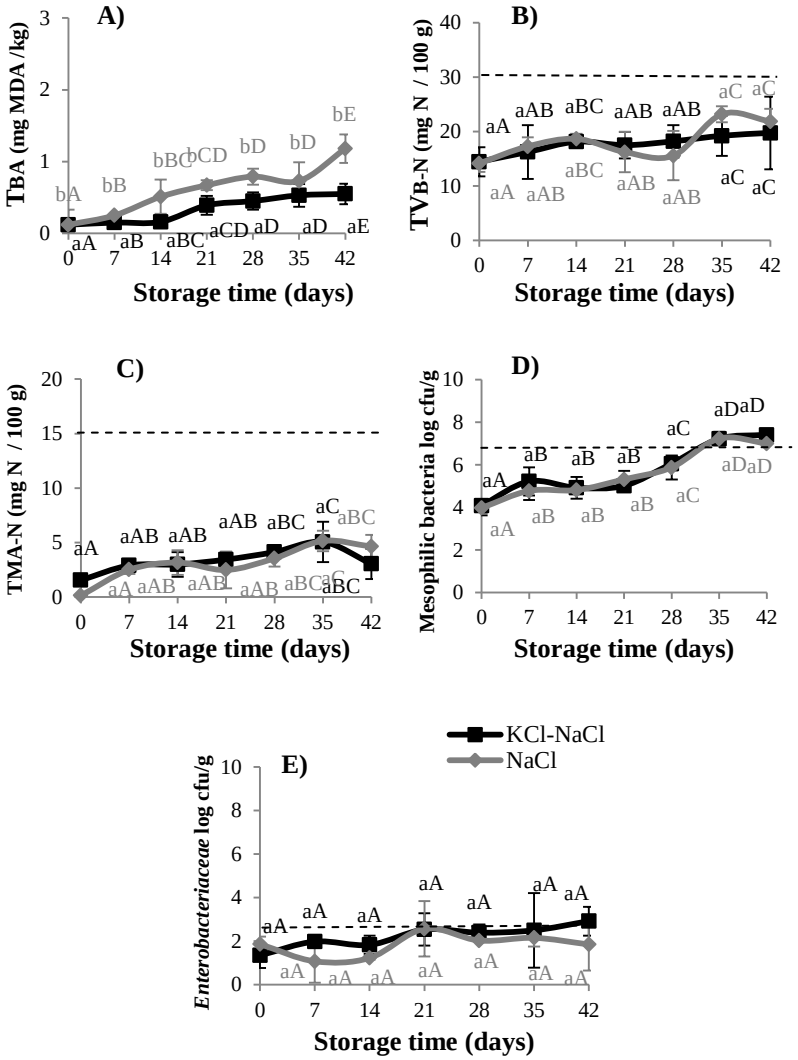

Fig. 3. Evolution of the TBA index (a), TVB-N (b), and TMA-N (c), mesophilic bacteria (d), *Enterobacteriaceae* (e) of the smoke-flavoured trout samples obtained with different salt formulations (KCl-NaCl and NaCl) for 42 storage days at 4ºC. Bars indicate standard deviation. The dashed line represents unacceptable levels in each figure. Different lower case and capital

letters indicate significant differences for the salt type (S) and storage times (T) factors, respectively ($p < 0.05$).

Partial NaCl replacement and storage time did not result in any significant changes in the colour of samples, except for lightness (table 4). The increase in L* coordinate could be attributed to water loss from samples during storage, which is retained in the plastic that covered the samples, as reported in other studies into smoked fish (Fuentes et al., 2012; Rizo et al., 2015b).

For the TPA test and shear force, no differences according to salt formulation were found, except for adhesiveness and springiness, whose values were slightly higher in the control samples (table 4).

Table 4. Changes in colour and texture parameters of smoke-flavoured trout samples obtained with different salt formulations (KCl-NaCl and NaCl) during 42 days of storage at 4 ºC. (Means±SD, n=3).

	Salt type	Storage time (days)						
		0	7	14	21	28	35	42
Colour								
L*	**KCl-NaCl**	33±3^{aA}	35±2^{aA}	35±3^{aAB}	37±2^{aBC}	38±2^{aCD}	41±2^{aCD}	41±3^{aD}
	NaCl	33±2^{aA}	32±2^{aA}	35±2^{aAB}	38±2^{aBC}	39±3^{aCD}	39±2^{aCD}	41±2^{aD}
a*	**KCl-NaCl**	11±5^{aA}	11±4^{aA}	10±2^{aA}	14±2^{aA}	13±2^{aA}	12±2^{aA}	11±3^{aA}
	NaCl	10±3^{aA}	13±3^{aA}	11±3^{aA}	10±3^{aA}	11±3^{aA}	11±2^{aA}	13±3^{aA}
b*	**KCl-NaCl**	15±4^{aA}	13±2^{aA}	12±2^{aA}	14±1^{aA}	13±2^{aA}	14±2^{aA}	13±2^{aA}
	NaCl	13±3^{aA}	14±2^{aA}	14±2^{aA}	12±2^{aA}	13±3^{aA}	13±2^{aA}	13±3^{aA}
C_{ab}*	**KCl-NaCl**	19±6^{aA}	17±3^{aA}	16±3^{aA}	20±3^{aA}	18±3^{aA}	18±3^{aA}	17±3^{aA}
	NaCl	16±4^{aA}	19±3^{aA}	19±3^{aA}	16±3^{aA}	17±4^{aA}	16±2^{aA}	18±4^{aA}
h_{ab}*	**KCl-NaCl**	55±9^{aA}	52±10^{aA}	51±5^{aA}	44±5^{aA}	46±5^{aA}	50±4^{aA}	50±5^{aA}
	NaCl	51±5^{aA}	49±5^{aA}	52±5^{aA}	50±7^{aA}	49±5^{aA}	50±3^{aA}	46±5^{aA}
ΔE*	**KCl-NaCl**	-	7±4^{aA}	8±3^{aA}	6±2^{aA}	8±3^{aA}	9±2^{aA}	10±4^{aA}
	NaCl	-	5±2^{aA}	6±2^{aA}	9±3^{aA}	9±5^{aA}	9±3^{aA}	10±2^{aA}
Texture								
TPA test								
Hardness (N)	**KCl-NaCl**	61.2±0.6^{aA}	42±9^{aA}	43±12^{aA}	55±20^{aA}	42±5^{aA}	53±6^{aA}	68±14^{aA}
	NaCl	52±5^{aA}	60±27^{aA}	39.7±0.1^{aA}	57±2^{aA}	73±13^{aA}	77±25^{aA}	68±15^{aA}
Chewiness (N)	**KCl-NaCl**	30.7±0.5^{aA}	21±7^{aA}	18±4^{aA}	23±5^{aA}	17±3^{aA}	22±5^{aA}	26±7^{aA}
	NaCl	22±1^{aA}	26±12^{aA}	19±7^{aA}	24±2^{aA}	36±8^{aA}	32±11^{aA}	24±4^{aA}
Adhesiveness (g/s)	**KCl-NaCl**	-0.16±0.07^{aA}	-0.29±0.11^{aA}	-0.25±0.14^{aA}	-0.28±0.12^{aA}	-0.18±0.04^{aA}	-0.21±0.05^{aA}	-0.31±0.05^{aA}
	NaCl	-0.21±0.14^{bA}	-0.34±0.10^{bA}	-0.15±0.03^{bA}	-0.39±0.07^{bA}	-0.46±0.09^{bA}	-0.47±0.25^{bA}	-0.25±009^{bA}
Springiness	**KCl-NaCl**	0.71±0.05^{aA}	0.70±0.09^{aA}	0.60±0.12^{aA}	0.66±0.01^{aA}	0.60±0.07^{aA}	0.66±0.11^{aA}	0.66±0.05^{aA}
	NaCl	0.70±0.01^{bA}	0.71±0.02^{bA}	0.72±0.05^{bA}	0.66±0.03^{bA}	0.73±0.04^{bA}	0.70±0.04^{bA}	0.62±0.01^{bA}
Cohesiveness	**KCl-NaCl**	0.66±0.02^{aA}	0.67±0.05^{aA}	0.68±0.08^{aA}	0.65±0.09^{aA}	0.67±0.02^{aA}	0.63±0.08^{aA}	0.57±0.01^{aA}
	NaCl	0.60±0.05^{aA}	0.62±0.06^{aA}	0.67±0.05^{aA}	0.64±0.01^{aA}	0.66±0.05^{aA}	0.60±0.04^{aA}	0.58±0.06^{aA}
Resilience	**KCl-NaCl**	0.41±0.07^{aA}	0.42±0.05^{aA}	0.41±0.03^{aA}	0.41±0.04^{aA}	0.41±0.01^{aA}	0.38±0.06^{aA}	0.33±0.01^{aA}
	NaCl	0.40±0.06^{aA}	0.39±0.04^{aA}	0.44±0.03^{aA}	0.39±0.04^{aA}	0.42±0.02^{aA}	0.37±0.04^{aA}	0.36±0.05^{aA}
Shear test								
F max (N)	**KCl-NaCl**	24±1^{aAB}	20±9^{aA}	29±3^{aAB}	31±6^{aB}	31±4^{aB}	26±10^{aAB}	27±1^{aAB}
	NaCl	21±5^{aA}	20±2^{aA}	21±7^{aAB}	32±3^{aB}	33±5^{aB}	31±7^{aAB}	21±1^{aAB}

Different lower-case letters indicate significant differences for salt dose factor. Different capital letters indicate significant differences for storage time factor. ($p < 0.05$)

4. Conclusions

Smoke-flavoured trout was achieved with similar physico-chemical characteristics and sensory acceptance to commercial smoked trout by means of the novel smoke-flavouring process using WP bags. Partial substitution of NaCl with KCl led to an approximate 42% sodium reduction of smoke-flavoured trout, and affected neither its physico-chemical characteristics nor sensory features.

Sodium replacement did not modify physico-chemical and microbiological quality during storage. No sample exceeded the limits of acceptance proposed for these parameters at any time during this study, except for mesophilic bacteria, which limited the product shelf life to 1 month. In general, texture and colour were not affected by NaCl replacement throughout the study period.

The smoke-flavouring process with WP bags is a fast convenient option to obtain high quality, reduced sodium smoke-flavoured trout products with similar characteristics to commercial smoked trout.

Acknowledgements

The authors gratefully acknowledge the support of Tub-Ex Aps (Taars, Denmark) for the supply of the water vapour permeable bags and for providing all the necessary technical information. Arantxa Rizo would like to thank the Universitat Politècnica de València for the FPI grant.

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CAPITULO 4

CONTROL DEL PROCESO DE SALADO-AHUMADO MEDIANTE ESPECTROSCOPÍA DE IMPEDANCIA

ARTÍCULO 7

DEVELOPMENT OF A NEW SALMON SALTING-SMOKING METHOD AND PROCESS MONITORING BY IMPEDANCE SPECTROSCOPY

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LWT - Food Science and Technology 51 (2013) 218-224

Versión adaptada para la tesis doctoral

Abstract

In this work two objectives were proposed: (i) to optimize a new salmon salting-smoking method using vacuum packaging and (ii) to evaluate the application of impedance spectroscopy (IS) to the on-line monitoring of the process. Different processing conditions were evaluated (4 smoke flavoring (SF) salt concentrations, 3 salting times, salting in vacuum or in air). Physico-chemical analyses and IS measurements were performed with three different sensors during the process. Salting with 16 g SF salt/100 g fish in vacuum packaging provided smoked salmon similar to products currently available on the market. This new method has the advantages of reducing processing times and waste. IS measurements were carried out by three different electrodes. The most appropriate sensor for process monitoring was a needle electrode, with which robust prediction models for NaCl content, moisture and a_w during the salting-smoking process were obtained. The results showed the potential of IS as a rapid on-line monitoring method of the salmon salting-smoking process.

Keywords: Salmon; vacuum salting-smoking; impedance spectroscopy; process monitoring.

1. Introduction

Smoking is one of the oldest methods of fish preservation. The preservative effect of smoking is due to a combination of different factors, including the addition of salt, partial dehydration of the tissues that occurs throughout the different stages of the process, as well as the preservative action of the smoke components. The smoking process slows down the biological processes and oxidative damage and gives the final product sensory characteristics highly appreciated by consumers.

Improvements in the smoking process, including the reduction in processing times and the amount of brine wastes or the improvement of the hygienic quality would be of interest to this sector. To obtain high-quality smoked salmon with a long shelf life, optimization of the various stages that constitute the smoking process is essential. The salting step is especially critical. A salting process in which the exact amount of salt to be absorbed by the fish would be directly dosed, combined with vacuum packaging, could be an alternative to these techniques. With this new method both brine wastes and contamination would be reduced, since the lack of oxygen in vacuum packaging would delay microbial growth and lipid oxidation. The main disadvantage that could present this method is the growth of anaerobic microorganisms, such as Clostridium botulinum. Smoke flavoring salt could also be used and would provide salt and a smoky flavor to the product in a single stage, so that the total processing time would be significantly shortened.

It is well-known that certain physico-chemical parameters, such as a_w or salt content, directly affect the shelf life of smoked salmon. However, some studies have found high variability of these parameters within the same fish product (Cornu et al., 2006; Espe, Kiessling, Lunestad, Torrissen, & Røra, 2004; Fuentes, Fernández-Segovia, Barat & Serra, 2010a), which have implications for consumers' safety and also for the sensory characteristics of

the product. This is due to the fact that smoking processes are standardized for a certain fish species, without taking into account the effects of the initial characteristics (fat, moisture, fish size, freshness, etc.) of the raw material (Barat, Gallart-Jornet, Andrés, Akse, Carlehög, & Skjerdal, 2006). In this regard, the development of rapid non-destructive methods for on-line monitoring of the process, in order to detect when the product has reached optimum moisture, salt and/or a_w values would be of interest to producers.

Electronic sensors based on impedance spectroscopy (IS) could help to meet this objective. The relationship between sodium chloride content and impedance measurements has already been demonstrated (Guerrero et al. 2004; Karásková, Fuentes, Fernández-Segovia, Alcañiz, Masot, & Barat, 2011). In the IS technique an electrical sinusoidal stimulus is applied to the electrodes in order to measure the impedance of the sample at different frequencies. The module and phase of the impedance can vary significantly according to the charges present (free ions), types of microstructure and electrolytes, as well as texture, geometry and the electrodes used (Masot, 2010).

In this work two objectives were proposed. The first was to optimize a new salting-smoking method for salmon using vacuum packaging. The second was to evaluate the application of impedance spectroscopy in the on-line monitoring of the salting-smoking process.

2. Materials and methods

2.1. Sample preparation

Fillets of Atlantic salmon from a Norwegian farm (Hallvard Leroy AS) of commercial size 1.4-1.8 kg was used as raw material. The fillets were purchased in a local supermarket and transported to the laboratory under refrigeration.

Fourteen salmon fillets were employed for the complete test (8 for the first phase and 6 for the second). The fillets were cut transversally into 4 cm portions, obtaining 6 or 7 samples per fillet. Each sample was weighed.

Smoke flavoring (SF) salt (Salinera Española, SA) was used in the salting-smoking stage. Its composition included 50% refined salt, white sugar, baking soda, smoke flavoring and anti-caking agent (E-536).

2.2 Experimental design

2.2.1. Phase I: Optimization of salting-smoking process and selection of impedance spectroscopy electrode.

This phase of the study had two different aims. The first was to establish the appropriate salting conditions (correct amount of smoke flavoring salt, processing time and type of packaging) to obtain smoked salmon with similar characteristics to currently marketed products (60-63 g H_2O/100 g, 3.5-3.8 g NaCl/100g, a_w=0.963-0.965) (Cardinal, Gunnlaugsdottir, Bjoernevik, Ouisse, Vallet, & Leroi, 2004; Fuentes et al., 2010a) while generating the minimum of brine waste. The smoked salmon obtained by this new process is intended to be distributed vacuum packaged

under refrigeration. The second objective was to select the most suitable electrode to monitor the salting-smoking process.

A total of 48 portions of fresh salmon obtained as described above were randomly divided into 4 batches. Each batch was submitted to a salting-smoking process under different conditions (Fig. 1). Four concentrations of smoke flavoring salt were studied: 4, 6, 8 and 16 g SF salt/100 g fresh salmon. These concentrations were selected from previous studies (Fuentes, Pérez, Fernández-Segovia, & Barat, 2011). The weight of SF salt was spread over the fish muscle surface and the samples were individually placed inside plastic bags. Each batch was subdivided into 2 further groups, one was packaged in air and the other in vacuum (Fig. 1). Three processing times were also studied (12, 18 and 24 h). The salting-smoking process was carried out at 4 °C. At the end of the processing time, the samples were placed in saturated brine under constant stirring for 30 s to remove any traces of SF salt attached to the surface. Finally, the samples were dried with absorbent paper and re-weighed. Two samples were used for each condition (n=2).

Analysis of moisture, pH, NaCl content and a_w were carried out on the fresh salmon and the smoked samples at different times during the study. Impedance spectroscopy measurements were also carried out using the 3 different sensors (double electrode, arrow electrode and a coaxial needle) described below.

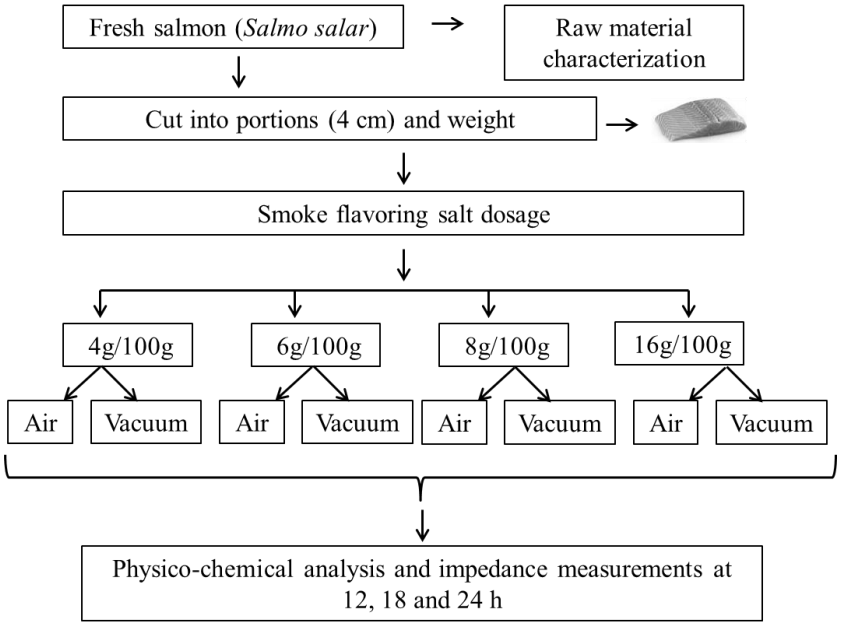


Fig. 1. Experimental design of Phase I.

2.2.2. Phase II: Monitoring of salting-smoking process using impedance spectroscopy

The objective of this second phase of the study was to evaluate the application of impedance spectroscopy to monitoring the salmon salting-smoking process.

The salting-smoking conditions that provided smoked salmon similar to currently available products were selected from the results obtained in the previous phase. This part of the study was repeated with 3 batches of fish consisting of 12 samples per batch. Each batch was purchased at intervals of 1 week.

The samples were salted-smoked with 16 g SF salt /100 g fresh salmon in vacuum packaging for 25 h at 4 °C. Analyses were carried out at 5 h-intervals, after rinsing the samples in brine and drying as described above. Physico-chemical analyses were carried out (moisture content, pH, NaCl content and a_w) as well as impedance spectroscopy measurements with the electrode selected in Phase I (needle electrode). Two samples were used (n=2 in each batch) for each salting time, including time 0, corresponding to fresh salmon.

2.3. Analytical determinations

The physico-chemical analysis and impedance spectroscopy measurements were performed in the centre of each fillet. The analyses were done in triplicate on each sample, except for pH, which was measured in quintuplicate.

2.3.1 Physico-chemical analyses

Moisture content was determined according to the AOAC method 950.46 (1997). The pH measurements were carried out using a digital pH-meter micropH 2001 (Crison Instruments, S.A., Barcelona, Spain) with puncture electrode (Crison 5231) in five different locations on the sample. Water activity (a_w) was measured in minced samples with a fast water activity-meter (GBX scientific FA-st/1, Cédex, France). Sodium chloride content was determined according to the procedure described by Fuentes, Fernández-Segovia, Barat, and Serra (2010b) using an automatic Sherwood Chloride Analyzer Model 926 (Sherwood Scientific Ltd., Cambridge, UK).

Changes in total mass, water and sodium chloride during the salting process were estimated by Eqs. (1), (2) and (3):

$$\Delta M_t^o = \left(\frac{M_t^o \cdot - M_0^o}{M_0^o} \right) \quad (1)$$

$$\Delta M_t^w = \left(\frac{M_t^o \cdot x_t^w - M_0^o \cdot x_0^w}{M_0^o} \right) \quad (2)$$

$$\Delta M_t^{NaCl} = \left(\frac{M_t^o \cdot x_t^{NaCl} - M_0^o \cdot x_0^{NaCl}}{M_0^o} \right) \quad (3)$$

(M_t^o and M_0^o are the salmon weight, x_t^w and x_0^w are the water weight fractions in the salmon, and x_t^{NaCl} and x_0^{NaCl} are the NaCl weight fraction in the salmon, at sampling times t and 0, respectively).

Sodium chloride concentration referred to the fish liquid phase (Z^{NaCl}) was estimated from the determinations of weight fractions of water (x^w) and sodium chloride (x^{NaCl}) according to Eq. (4), thus considering that nearly all the sodium chloride and water were free in the salmon muscle.

$$z^{NaCl} = \left(\frac{x^{NaCl}}{x^w + x^{NaCl}} \right) \quad (4)$$

2.3.2. Impedance spectroscopy measurements

A low-cost, flexible, light, non-destructive measurement system was developed by the Instituto de Reconocimiento Molecular y Desarrollo Tecnológico (IDM) at the Universitat Politècnica de València (UPV) (Masot et al. 2010). This impedance spectroscopy measurement system applies an electric signal to food products and measures the response in a frequency sweep between 1Hz and 1MHz.

Since the electrical response depends on the type of electrode used, three different electrodes were tested in this study. One was a double electrode (DE) composed of two stainless steel needles 1.5 cm long and 1 mm in diameter, separated by a distance of 1 cm in a non-conductive frame. This design keeps the separation between both needles constant during measurements.

The second electrode, known as the Arrowhead (AH), was designed using thick-film technology, which uses high-resolution screen-printing methods to deposit pastes or inks of different electrical characteristics (conductive, resistive and dielectric) on an insulating substrate, in order to form an electronic circuit. This electrode is designed with a pointed end to help it to penetrate through the sample.

The third sensor (needle electrode) consisted of a hollow needle with an internal isolated wire, so that a two-electrode system is configured. The external part of the needle is made of stainless steel and acts as the outer electrode. The internal wire is also made of stainless steel and acts as the inner electrode. Both electrodes are separated by dielectric material (epoxy resin). The hollow needle (TECAN 53156, Oxford-FEDELEC) has an outer diameter of 0.46 mm.

The impedance measurements were taken by inserting the sensors into the sample perpendicular to the muscle fibers of the fish. The penetration depth of the electrodes was constant in all the analyses (1.5 cm). All measurements were carried out at room temperature.

2.4. Statistical analysis

Data are reported as mean ± standard deviation. One-way ANOVA was conducted for each physico-chemical parameter evaluated in Phase II, to determine whether there were significant differences between the salting-smoking times. Statistical treatment of the data was performed using the Statgraphics Centurion XVI (Manugistics Inc., Rockville, MD, USA).

In order to assess the feasibility of the impedance spectroscopy technique to discriminate between different moisture, NaCl contents, and/or a_w levels, three Principal Component Analyses (PCAs) were carried out with data obtained from the DE, AH and needle electrodes. PCAs were performed using impedance module and phase data obtained by the equipment in the frequency range established for each sensor. A PCA was conducted in the same way for the needle electrode with data obtained in Phase II. Partial Least Squares (PLS) were also carried out to create predictive models of each physico-chemical parameter evaluated from the IS measurements. PLS prediction models were created using a set of experimental data (calibration set). The model was then validated with a new set of experimental data (validation set). All multivariate analyses were performed using MATLAB ® PLS Tool-box (Eigenvector Research, Inc.).

3. Results and discussion

3.1. Phase I: Optimization of the salting-smoking process and selection of the impedance spectroscopy electrode.

3.1.1. Physico-chemical analyses.

Moisture, pH, a_w and sodium chloride content values of raw material (t=0) and smoked salmon are shown in Table 1. The values obtained for the raw material are similar to those reported by other authors for fresh salmon (Fuentes, Fernández-Segovia, Masot, Alcañiz, & Barat, 2010c; Gallart-Jornet, Barat, Rustad, Erikson, Escriche, & Fito, 2007).

In all the experimental conditions the salting-smoking process caused a significant reduction in the water content and a_w values, as well as an increase in the NaCl concentration, as compared with fresh salmon. Reducing the a_w values lengthens smoked salmon shelf-life. These changes are due to dehydration and NaCl absorption into the muscle. It should be noted that the samples with higher sodium chloride levels showed a slight decrease in pH values, due to the higher ionic strength of the internal solution in fish muscle cells, as described by Leroi and Joffraud (2000).

In both types of packaging, the highest SF salt dosages (8 and 16 g SF salt/100 g fish) caused the largest increase in NaCl content, with the consequent reduction of a_w values as processing time advanced. However, for the 4 and 6% SF salt doses, the magnitude of these changes was smaller in samples packaged in air, being practically negligible in salmon processed in vacuum packaging (Table 1). This could be explained by the fact that in these last cases at 12 h almost all the SF salt dose had been absorbed, so that the changes during the rest of the processing time were minimal.

Regarding the type of packaging, vacuum packaging caused faster sodium chloride absorption and dehydration of the salmon than air packaging. This effect was only observed for the highest SF salt dose (16 g/100 g fish), with minimal differences between the two types of packaging for the rest of the studied dosages, since these low amounts of salt are easily dissolved and absorbed in the first hours of processing in both types of packaging.

Table 1. Physico-chemical parameters of raw material (S=0, t=0) and salmon submitted to salting-smoking with different smoke flavoring (SF) salt doses (S) (g SF salt/100 g fresh salmon), types of packaging (P) and processing times (t). Mean values ± SD (n=2).

S	P	t (h)	Moisture (g H_2O/100g)	pH	a_w	NaCl (g NaCl/100g)
0		**0**	70.39 ± 1.47	6.13 ± 0.02	0.992 ± 0.002	0.00
4	**Air**	**12**	67.34 ± 0.13	6.10 ± 0.01	0.980 ± 0.000	1.84 ± 0.09
		18	65.91 ± 0.18	6.08 ± 0.08	0.978 ± 0.000	1.71 ± 0.07
		24	67.81 ± 0.24	6.07 ± 0.06	0.972 ± 0.002	2.03 ± 0.04
	Vacuum	**12**	67.03 ± 0.12	6.09 ± 0.04	0.979 ± 0.001	1.78 ± 0.05
		18	67.13 ± 0.29	6.09 ± 0.02	0.980 ± 0.001	1.79 ± 0.01
		24	68.03 ± 0.01	6.09 ± 0.03	0.980 ± 0.000	1.68 ± 0.01
6	**Air**	**12**	66.11 ± 0.21	6.12 ± 0.02	0.978 ± 0.001	1.77 ± 0.06
		18	65.13 ± 0.30	6.10 ± 0.03	0.977 ± 0.000	2.04 ± 0.01
		24	65.70 ± 0.17	5.99 ±0.02	0.978 ± 0.001	2.54 ± 0.08
	Vacuum	**12**	65.52 ± 0.02	6.08 ± 0.04	0.978 ± 0.001	2.02 ± 0.02
		18	66.95 ± 0.18	6.15 ± 0.05	0.982 ± 0.001	1.67 ± 0.00
		24	67.23 ± 0.80	6.06 ± 0.05	0.976 ± 0.000	2.01 ± 0.02
8	**Air**	**12**	65.65 ± 0.20	6.14 ± 0.04	0.977 ± 0.000	2.16 ± 0.10
		18	65.52 ± 1.22	6.08 ± 0.02	0.976 ± 0.000	2.14 ± 0.05
		24	64.69 ± 0.35	6.10 ± 0.04	0.971 ± 0.000	2.58 ± 0.03
	Vacuum	**12**	65.15 ± 0.28	6.13 ± 0.03	0.978 ± 0.001	1.98 ± 0.09
		18	65.57 ± 0.17	6.16 ± 0.05	0.977 ± 0.000	2.13 ± 0.16
		24	64.20 ± 0.03	6.05 ± 0.02	0.965 ± 0.001	3.01 ± 0.00
16	**Air**	**12**	62.68 ± 0.28	6.15 ± 0.06	0.976 ± 0.002	1.79 ± 0.05
		18	62.34 ± 0.88	6.17 ± 0.02	0.978 ± 0.000	1.90 ± 0.14
		24	59.91 ± 0.06	5.99 ± 0.05	0.968 ± 0.001	3.40 ± 0.10
	Vacuum	**12**	60.53 ± 0.67	6.12 ± 0.06	0.969 ± 0.002	2.35 ± 0.22
		18	60.99 ± 0.26	6.01 ± 0.03	0.967 ± 0.000	3.12 ± 0.25
		24	62.45 ± 0.39	5.96 ± 0.01	0.963 ± 0.002	3.62 ± 0.02

Of all the conditions studied, only those samples salted-smoked with 16 g SF salt/100 g of fresh salmon for 24 h in vacuum reached the levels of moisture, NaCl and a_w, previously established (60-63 g H_2O/100 g, 3.5-3.8 g NaCl/100 g, a_w = 0.963-0.965). These were consequently the salting-smoking conditions selected for Phase II.

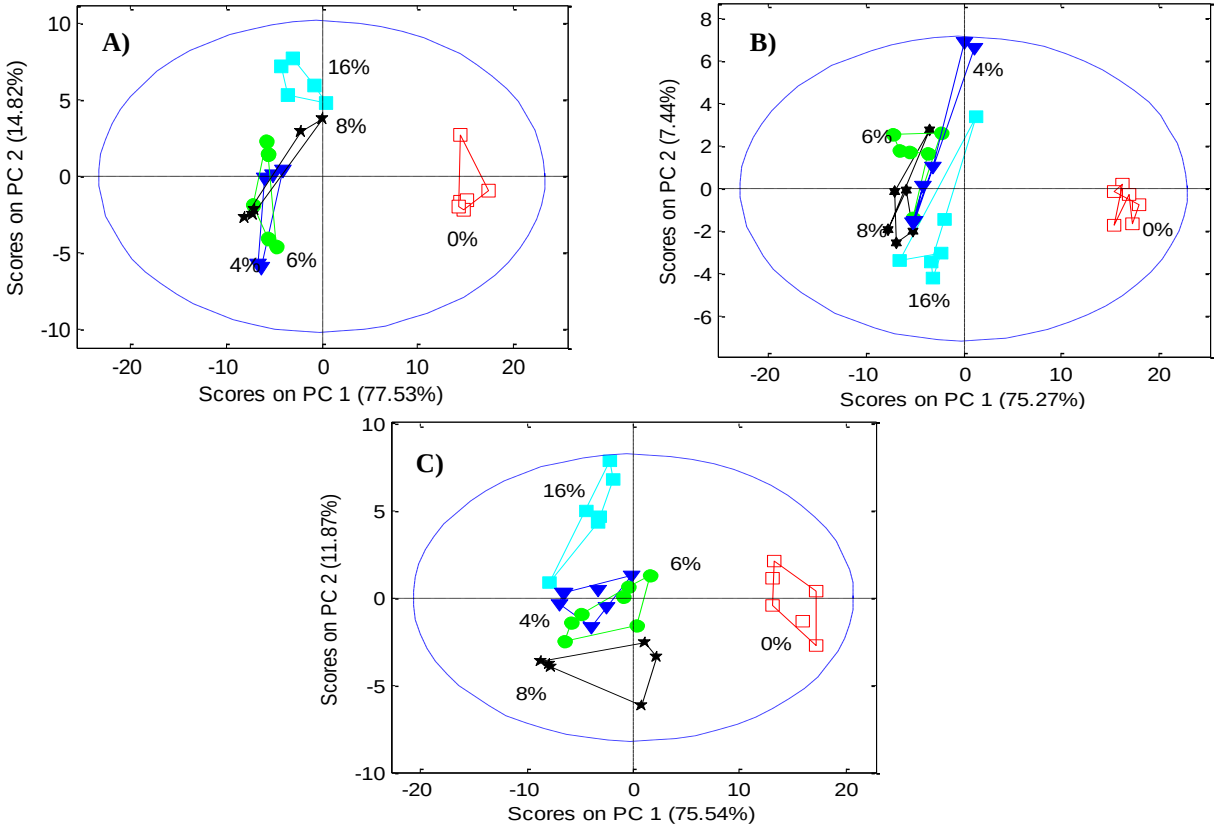
3.1.2. Impedance spectroscopy

Impedance spectroscopy was used to detect changes in the salmon muscle during the salting-smoking process. The impedance measurements of fresh salmon were compared with those of samples submitted to salting-smoking under the different conditions described above. In this phase, 3 different electrodes (DE, AH and needle) were studied as described in Section 2.3.2 on Materials and methods.

Impedance spectroscopy equipment generate 100 values for each measurement, corresponding to the modules and phases of the 50 frequencies analyzed. A Principal Component Analysis (PCA) was conducted for each electrode to determine whether impedance spectroscopy could discriminate between the different samples. The impedance data used in this analysis were from the samples processed for 24 h, since the highest differences in moisture, NaCl content and a_w, from the 4 SF salt levels were obtained for this time.

The results of the PCAs carried out on the DE, AH and needle electrodes are shown in Figs. 2.a, 2.b and 2.c, respectively.

Fig. 2: Principal component analysis (PCA) performed on the impedance spectroscopy measurements of samples in air and in vacuum with different smoke flavoring salt dosages (0 (□), 4 (▼), 6 (●), 8 (★) and 16 (■) g SF /100 g) for 24 h. (A) Double electrode, (B) Arrowhead electrode and (C) Needle electrode.



In all cases, a clear separation of the raw material (0%) from the rest of the samples was observed. The ED electrode could also discriminate samples processed with 16 g SF salt/100 g fish, while no discrimination was observed for the rest of the samples (Fig. 2.a). For the AH electrode, all samples subjected to the salting-smoking process were overlapped (Fig. 2.b). The needle electrode showed 4 clusters: fresh salmon (0%), samples with 16% SF salt, samples with 8% SF salt and a fourth group with samples with 6% and 4% SF salt (Fig. 2.c). The best sensor in discriminating the different levels of moisture, NaCl contents and/or a_w was therefore the needle electrode and was consequently selected for the next phase of the study. These results confirm the importance of the measuring sensor design (electrode geometry and characteristics).

3.2. Phase II: Monitoring of salting-smoking process using impedance spectroscopy

3.2.1. Physico-chemical analyses.

Values of moisture, pH and a_w of salmon submitted to the salting-smoking process (16% SF salt dosage, vacuum packaging) for 25 h are shown in Table 2. Moisture content progressively decreased during the salting process, with a higher rate at the beginning of the process, mainly due to the presence of salt crystals on the surface. The use of solid salt causes greater dehydration in the product at the beginning of the process. The water exiting from the muscle is needed to dissolve the salt on the surface and form brine at the interface, which enables the salt to later penetrate into the fish muscle.

The pH values showed a slight drop with processing time, although the differences were not significant. The a_w values decreased throughout the salting-smoking time, due to muscle dehydration and salt penetration.

Table 2. Physico-chemical parameters of raw material (t=0) and salmon submitted to salting-smoking process (16% smoke flavoring salt dosage, vacuum packaging) for different processing times. Mean values ± SD (n=6).

t (h)	Moisture (g H_2O/100g)	pH	a_w
0	70.97 ± 2.64^{a}	6.08 ± 0.04^{ab}	0.991 ± 0.002^{a}
5	66.45 ± 2.56^{b}	6.13 ± 0.04^{b}	0.972 ± 0.002^{b}
10	64.90 ± 1.94^{bc}	6.11 ± 0.07^{b}	0.968 ± 0.001^{b}
15	63.91 ± 2.32^{bc}	6.10 ± 0.07^{b}	0.963 ± 0.004^{c}
20	63.85 ± 2.84^{cb}	6.08 ± 0.02^{ab}	0.957 ± 0.008^{d}
25	62.42 ± 2.30^{c}	6.03 ± 0.06^{a}	0.957 ± 0.002^{d}
α	***	ns	***

Same letters in the same column indicate homogeneous group membership. Significance level (α): ns no significant difference; *** $p < 0.001$

Fig. 3 shows the evolution of sodium chloride content (x^{NaCl}, X^{NaCl} and Z^{NaCl}) during the salting-smoking process. The NaCl content of the samples increased progressively with processing time. The highest increase was observed in the case of sodium chloride concentration expressed on a dry basis (X^{NaCl}), because of the solute incorporation in fish muscle and osmotic dehydration that occurs during the process.

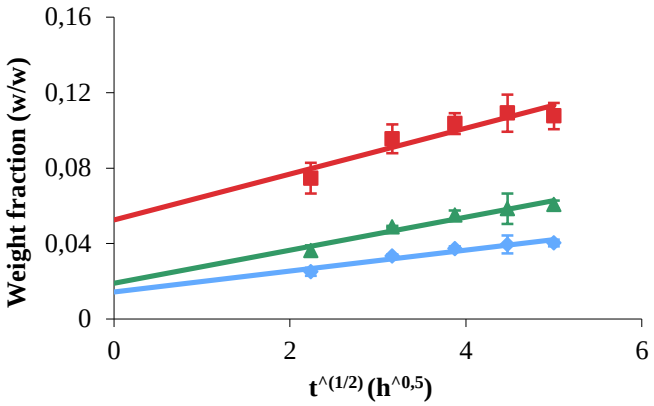


Fig. 3. NaCl weight fraction (x^{NaCl} (♦), y = 0.005x + 0.014, R^2 = 0.933), NaCl concentration on a dry basis (X^{NaCl} (■), y = 0.012x + 0.053, R^2 = 0.875) and NaCl weight fraction in the liquid phase of salmon (Z^{NaCl} (▲), y = 0.009x -+ 0.019, R^2 = 0.951) *versus* square root of the processing time ($t^{0.5}$).

Total weight, water weight and sodium chloride weight changes are shown in Fig. 4. Moisture and sodium chloride variations showed opposing behavior throughout the salting, as mentioned above (Fig. 4).

Total weight changes could be considered a combination of both weight changes (water and NaCl). However, protein denaturation due to salt action would also contribute to the weight loss, as different authors have shown (Barat, Rodriguez-Barona, Andrés, & Fito, 2003; Ismail & Wootton, 1982). It can be assumed that there is pseudo-diffusional transport due to the strong dependence between the weight changes and the square root of time, as has been pointed out by other authors (Barat et al., 2006; Fuentes, Barat, Fernández-Segovia, & Serra, 2008; Gallart-Jornet et al., 2007).

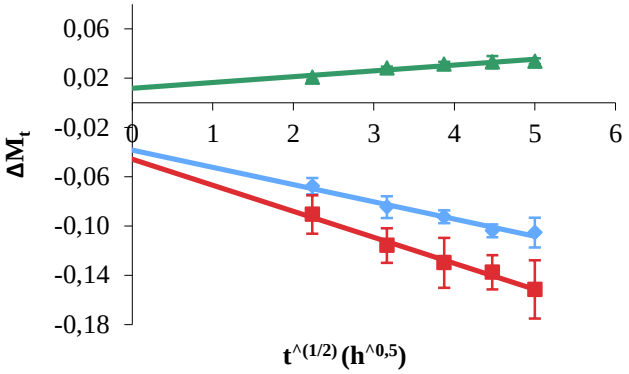


Fig. 4. Total weight changes (ΔM_t^0 (♦), y = -0.014x-0.038, R^2 = 0.974), water weight changes (ΔM_t^w (■), y = -0.021x-0.046) (R^2 = 0.987) and sodium chloride weight changes (ΔM_t^{NaCl} (▲), y = 0.005x + 0.012) (R^2 = 0.915) *versus* the square root of the processing time ($t^{0.5}$).

It should be noted that samples reached the selected NaCl and a_w levels after 15 h of processing, a shorter time than in the preliminary study (Phase I). This is due to the salting process depending directly on the composition and initial quality of the raw material (Barat et al., 2006). Different batches of fresh salmon were used in Phases I and II, so that the differences in the raw material could have been the cause of the differences found in the process kinetics.

The use of vacuum during processing and distribution of salmon could permit the growth of Clostridium botulinum type A, B and E, as well as toxin production. C. botulinum type E could grow in smoked salmon under vacuum at temperatures as low as 3.3 ºC, if a_w is higher than 0.966. To minimize the risk of C. botulinum growth, the exact control of a_w is of

outmost importance. This confirms the need for rapid monitoring methods that can be used on-line to determine the end of the salting process.

3.2.2 Impedance spectroscopy

A PCA was used to assess the feasibility of impedance spectroscopy for monitoring the salmon salting-smoking process, with the impedance spectroscopy values obtained at 5 h-intervals during the 25 h process.

Fig. 5 shows the results of the PCA performed with data obtained from the needle electrode according to processing time. The samples can be seen to be clearly separated according to processing time, except for 20 and 25 h, which are overlapped in the same graphic area. These two samples showed similar values for all the physico-chemical parameters studied, which justifies the behaviour observed in the PCA.

Since the PCA analysis showed that impedance spectroscopy with the needle electrode could discriminate between different levels of moisture, NaCl and/or a_w, a statistical tool (PLS) was used to predict the values of these parameters from the measurements of the impedance device. In this way, statistical models were established for all the parameters except for pH, whose evolution was not significant throughout the processing time.

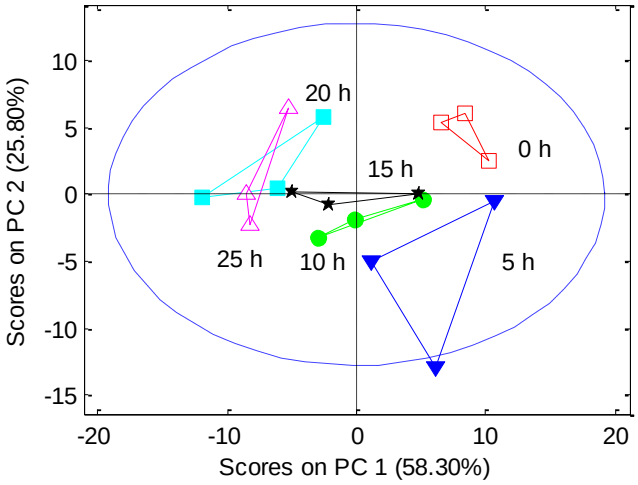


Fig. 5. Principal components analysis (PCA) performed with the impedance spectroscopy measurements (needle electrode) of salmon submitted to the salting-smoking process (16% smoke flavoring salt dosage, vacuum packaging) for different processing times (0 (□), 5 (▼), 10 (●), 15 (★), 20 (■) and 25 (Δ) h).

Sodium chloride, a_w and moisture experimental values versus values predicted by the PLS statistical models are shown in Figs. 6.a, 6.b and 6.c, respectively.

In all cases, the predicted values successfully fitted to the experimental values (RMSEP values of 0.685 for NaCl, 0.006 for a_w and 3.579 for moisture), especially in the case of a_w, for which the intercept was near to 0 and the slope near to 1. These results agree with other studies on the use of impedance spectroscopy in the characterization of commercial smoked salmon and cod, in which the best predictions were obtained for a_w (Karásková et al., 2011).

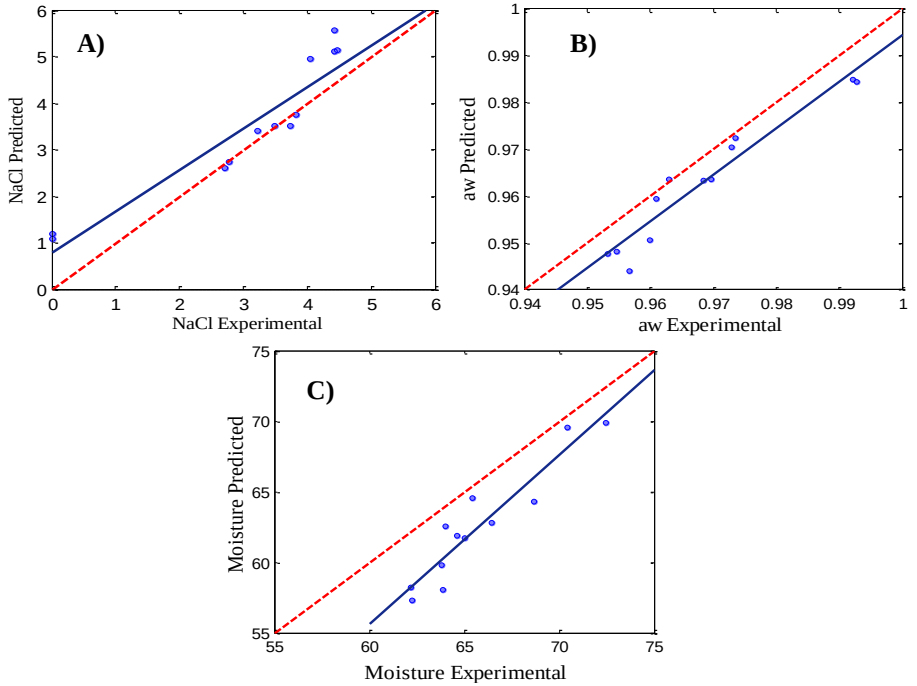


Fig. 6. Predicted *versus* experimental values by the PLS statistical model (—) and ideal behaviour (---). (a) NaCl (b) a_w and (c) moisture.

The results obtained from the PLS confirm the potential of impedance spectroscopy with needle electrode for monitoring the salmon salting-smoking process.

4. Conclusions

The results obtained from the physico-chemical analyses showed that packaging under vacuum speeded up the process of NaCl absorption and dehydration in salmon, although this effect was observed only for the highest dosage of smoke flavoring salt (16 g/100 g). The optimum processing conditions to obtain a similar product to the currently available smoked salmon on the market were 16 g SF salt/100 g salmon in vacuum packaging. This new method has the advantages of reducing processing times and waste. Further sensory evaluation and shelf-life studies should now be carried out to determine whether the sensory characteristics of smoked salmon obtained by the new method are comparable to currently marketed products and whether it has a similar or longer shelf-life.

Of the three electrodes used (double, arrowhead and needle electrode) in the IS measurements, the needle electrode was found to be the most appropriate for process monitoring. The increase in NaCl content and the reduction in moisture and a_w values with 16 g SF salt/100 g fish for 25 h in vacuum were detected by the EI technique using the needle electrode. This sensor was able to obtain robust NaCl content, moisture and a_w prediction models during the process. The best of these was the a_w prediction model, which is particularly interesting because of the relationship between this parameter and the shelf-life of smoked products. The results therefore showed that IS is a rapid on-line monitoring method for the salmon salting-smoking process and could provide an important tool to obtain products of uniform quality.

Acknowledgements

The authors gratefully acknowledge the financial support for the work reported here received from the Generalitat Valenciana (GV/2011/098) and the Universitat Politècnica de València (UPV) (PAID-06-09-2940). A. Fuentes would like to thank the Campus de Excelencia Internacional at the UPV for its support. The proof-reading of this paper was funded by the UPV, Spain.

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4. DISCUSIÓN GENERAL

DISCUSIÓN GENERAL

El trabajo efectuado en esta tesis doctoral se ha centrado en el desarrollo y optimización de un proceso de salado-ahumado simultáneo de pescado. Este nuevo procedimiento consiste en un salado controlado combinado con la utilización de humo líquido y bolsas permeables al vapor de agua (WP), para obtener productos de la pesca ahumados de calidad adecuada y estables microbiológicamente durante el almacenamiento, minimizando además el volumen de residuos de salmuera generados. Por otro lado, se propuso su mejora nutricional a través de la reducción del contenido en sodio, para hacer más saludables el consumo regular de estos productos.

A modo aclaratorio, se exponen a continuación las características que definen de forma general el proceso de ahumado tradicional:

El proceso comienza con una etapa de salado previa al ahumado. Estas etapas se realizan en cámaras independientes a diferentes condiciones de temperatura y humedad. El salado se realiza habitualmente a temperaturas de refrigeración, depositando un exceso de sal sólida en la superficie, la cual es absorbida paulatinamente por el músculo, provocando que el pescado expulse gran parte de su líquido de constitución mezclado con parte de la sal disuelta no absorbida. En la fase de ahumado, el pescado ya salado es introducido en cámaras especiales para tal fin, realizándose el proceso a temperaturas comprendidas entre 20 y 30ºC, para favorecer la absorción de los componentes del humo y cierto grado de deshidratación en el producto (Birkeland et al., 2004; Codex 2003; FDA, 2001; Løje, 2007).

En el nuevo proceso de salado-ahumado desarrollado en este trabajo, el uso de aromas de humo permite prescindir de la etapa de ahumado con humo natural, de forma que en una única etapa se consigue incorporar la sal y proporcionar aroma a humo. Por este motivo, mediante la aplicación del salado-ahumado simultáneo es posible realizar el proceso completo a bajas

temperaturas, en una sola etapa y una única cámara climática, proporcionando el nivel de secado que el producto requiere, mediante el manejo de las condiciones de humedad relativa y temperatura. En este sentido, la realización del proceso dentro de bolsas WP aporta múltiples ventajas sin interferir en el secado del producto. Su utilización permite mantener el pescado protegido facilitando su manipulación y transporte. Además, se consigue un proceso más higiénico al retener el exudado que se genera durante el mismo, evitando goteos de unas piezas sobre otras, antes de que se produzca su completa evaporación. Asimismo, la aplicación del salado controlado y la evaporación del exudado a través de las bolsas WP permiten disminuir el volumen de residuos de salmuera generados, lo que es de gran interés para las empresas productoras, dado el impacto ambiental de estos residuos, así como el elevado coste de su tratamiento. Desde el punto de vista económico, otra ventaja que ofrece este método, es la menor inversión en instalaciones que requiere, al no precisar del uso de cámaras separadas para realizar el salado y el ahumado, como sucede con los métodos tradicionales. En este sentido, la simplicidad de esta técnica facilita su implementación a escala industrial, permitiendo que el salado, ahumado y secado de las piezas de pescado envasadas pueda realizarse durante su distribución y transporte.

A continuación se describen los resultados más relevantes obtenidos durante el desarrollo de este trabajo, organizados por capítulos.

El primer capítulo consistió en el desarrollo de la nueva metodología para obtener productos de pescado ahumado. Inicialmente se determinaron los parámetros fisicoquímicos que caracterizan a los productos comerciales de pescado ahumado (contenido en grasa, humedad, NaCl y a_w). Se analizaron distintas marcas comerciales de varios tipos de pescado ahumado, para ser utilizados como valores de referencia en el desarrollo de los nuevos productos durante todo el estudio. Posteriormente, se estudió la viabilidad de esta nueva técnica para ser aplicada en distintas especies de pescado ahumado. El estudio se desarrolló inicialmente con salmón y se

completó con otras especies: bacalao y la trucha, por ser habituales en el mercado español y por presentar diferentes características en cuanto a su composición (contenido graso y humedad) y su calibre, que pueden afectar a su comportamiento frente al salado-ahumado.

Para la optimización de los procesos de salado-ahumado, se estudiaron distintas condiciones de procesado:

-Cantidad de sal dosificada.
-Humedad relativa (HR) en la cámara de secado.
-Tipo de envase.
-Tiempo de procesado.

Las condiciones ensayadas fueron escogidas en función de la materia prima y de las características del producto comercial de referencia, con la finalidad de obtener un producto con parámetros de calidad adecuados y estable microbiológicamente durante su almacenamiento. Otro de los objetivos de esta parte del estudio fue obtener la evaporación completa del líquido exudado por el pescado durante el salado-ahumado. De este modo, se consigue minimizar la generación de salmueras residuales derivadas del proceso, que como se ha comentado anteriormente, es una de las ventajas que aporta esta nueva metodología. Los productos de pescado ahumado obtenidos se evaluaron a través de análisis de contenido de humedad, grasa y NaCl, pH, a_w, variación de masa total (ΔMt) y porcentaje de líquido exudado en el salado-ahumado.

En el desarrollo del producto de salmón ahumado (artículo 1) se estudió la cantidad de sal dosificada, la humedad relativa en la cámara de secado y el tipo de envase (bolsas de alta barrera y bolsas WP). Las condiciones que permitieron obtener un producto de características fisicoquímicas y aceptación sensorial similares a los productos comerciales fueron: una dosis de 8% NaCl, 60% HR y 24 h. Estas condiciones

permitieron además la evaporación total del líquido exudado por el pescado durante el proceso, minimizando los residuos de salmuera generados.

En el caso del producto de bacalao ahumado (artículo 2), las condiciones de procesado estudiadas fueron:

-Cantidad de sal dosificada.
-HR en la cámara de secado.
-Tiempo de procesado.

Debido a los menores valores de contenido graso y mayor humedad del bacalao fresco, en comparación con el salmón, la dosis de sal adecuada para obtener una concentración óptima en el producto final y valores adecuados de humedad y a_w fue menor (2% NaCl). La HR seleccionada fue 60% siendo necesario prolongar el tiempo de procesado de 24 a 96 h para obtener un nivel de humedad adecuado y permitir la evaporación completa del exudado a través de las bolsas WP.

En el caso de la trucha con sabor a humo (artículo 6) se evaluaron 2 parámetros de procesado:

-Cantidad de sal dosificada.
-Tiempo de procesado.

En este caso, las condiciones óptimas de procesado para obtener un producto similar al comercial y de igual aceptación sensorial fueron 4% NaCl, durante 24 h. Estas condiciones fueron similares a las utilizadas para obtener salmón con sabor a humo, excepto en el caso de la dosis de sal que se redujo de un 8% NaCl a un 4% NaCl. Esto es debido al menor contenido de grasa y al grosor más reducido de los filetes de trucha utilizados, en comparación con el salmón. Estos resultados ponen de manifiesto que en los fenómenos asociados al proceso de salado-ahumado (penetración de la sal y secado) hay una gran influencia de parámetros relacionados con las

características iniciales de la materia prima, como el contenido en grasa, humedad y grosor de las piezas de pescado. De ahí, la importancia de adaptar las condiciones de procesado en función de las características del pescado de partida.

En la Tabla 1 se muestran los valores de los parámetros fisicoquímicos analizados en los productos ahumados desarrollados (bajo las condiciones de procesado consideradas óptimas) y de sus correspondientes materias primas.

Tabla 1. Parámetros fisicoquímicos de los productos de pescado ahumado desarrollados y sus respectivas materias primas. Valores promedio ± SD (n=3).

	Salmón		**Bacalao**		**Trucha**	
	Materia prima	**Producto ahumado (1)**	**Materia prima**	**Producto ahumado (2)**	**Materia prima**	**Producto ahumado (3)**
Humedad (g/100g)	65,4± 1,9	61,33±2,09	83,65±0,49	74,10±0,85	73.8±1.2	66,36±0.38
Grasa (g/100g)	12,5±3,5	9,39±1,88	0,16±0,09	-	7.1±0.4	6,35±0,65
NaCl (g/100g)	-	3,98±1,12	-	4,01±0,14	-	4,03±0,10
z^{NaCl}	-	0,062±0,007	-	0,052±0,003	-	0,057±0,001
pH	6,2±0,1	6,07±0,03	6,64±0,02	6,27±0,02	6,41±0,06	6,10±0,04
a_w	0,993±0,003	0,950±0.009	0,994±0,001	0,953±0,003	0.991±0.001	0,952±0,001
ΔMt	-	-0,085±0.01	-	-0,371±0.03	-	-0,149±0.04

(1) Condiciones de procesado: 8% NaCl, 60% HR, 24 h, 5ºC
(2) Condiciones de procesado: 2% NaCl, 60% HR, 96 h, 5ºC
(3) Condiciones de procesado: 4% NaCl, 60% HR, 24 h, 5ºC

Las materias primas de las distintas especies de pescado (salmón, bacalao y trucha fresca) mostraron diferencias importantes en sus valores de contenido en humedad y grasa, lo que justifica las diferentes condiciones de procesado seleccionadas, tal y como se ha comentado anteriormente. Los valores de humedad entre los distintos productos ahumados también mostraron gran variabilidad, a diferencia de los valores de NaCl y de a_w. En el caso del parámetro de a_w, sus valores fueron similares, no solo entre los

productos ahumados desarrollados, sino también entre las distintas especies de pescado fresco. Este parámetro se relaciona directamente con la estabilidad microbiológica de los productos salados y ahumados, lo que hace de ella una herramienta más orientativa para garantizar la seguridad y calidad de estos productos, que el contenido de humedad. Por otro lado, existe una correlación negativa entre la a_w y la concentración de NaCl en fase liquida (z^{NaCl}). En la norma para el pescado ahumado, pescado con sabor a humo y pescado secado con humo se contemplan valores mínimos recomendados de z^{NaCl} (Codex, 2013), para controlar la seguridad del pescado ahumado, minimizando la posible generación de toxinas de *Clostridium botulinum*. Concretamente, en los productos de pescado en los que el sabor a humo se imparte mediante mezclas de sabores artificiales, la normativa exige un mínimo de un 5% (z^{NaCl}=0,05). Los productos de pescado ahumado desarrollados en este trabajo alcanzaron esos valores mínimos. Puede considerarse que este parámetro, al igual que la $a_{w,}$ da una información más útil acerca de la seguridad y calidad de los productos salados y ahumados, que los valores de contenido de sal y humedad por separado.

En el **segundo capítulo** se evaluó la calidad y vida útil durante el almacenamiento en refrigeración de los productos obtenidos en la fase anterior.

En el estudio de almacenamiento del producto de salmón ahumado desarrollado (artículo 3), teniendo en cuenta los resultados obtenidos en la evaluación microbiológica y los diversos parámetros fisicoquímicos indicadores del deterioro, se concluyó que su vida útil en refrigeración fue superior a 40 días. Cabe destacar, que los niveles de oxidación lipídica en el producto fueron muy limitados, lo que podría ser atribuido a las bajas temperaturas de procesado utilizadas (5ºC) en comparación con los procesos tradicionales, donde se emplean habitualmente temperaturas de ahumado comprendidas entre 20 y 30 ºC.

En el estudio de calidad durante el almacenamiento del bacalao ahumado desarrollado (artículo 4) se evaluó, además, el efecto de la temperatura de procesado. El estudio fue realizado con muestras ahumadas a 5 y 10 ºC. La temperatura de procesado no afectó a los parámetros de humedad, NaCl, a_w y pH que caracterizan al producto, pero sí tuvo efecto en a los valores nitrógeno básico volátil total (N-BVT), nitrógeno de trimetilamina (N-TMA), compuestos de degradación del ATP y los recuentos microbianos, cuyos valores en las muestras procesadas a 10ºC superaron los límites de aceptabilidad desde el primer día de almacenamiento. Sin embargo, los parámetros indicadores de deterioro y los recuentos microbiológicos de las muestras procesadas a 5ºC mostraron una mayor estabilidad. La vida útil del bacalao obtenido a 5ºC fue superior a 35 días, por lo que puede considerarse que la temperatura de procesado es una variable crítica de control en el proceso.

En la evaluación de la calidad durante el almacenamiento de la trucha ahumada desarrollada (artículo 6), la vida útil estuvo delimitada únicamente por el crecimiento de aerobios mesófilos, el cual superó los límites de aceptabilidad el día 35 de almacenamiento.

El **tercer capítulo** aborda la mejora de los productos de salmón y trucha desarrollados, a través de la reducción de su contenido en sal. Además del desarrollo de nuevos procesos o la mejora de los ya existentes, la reducción del contenido en sodio de los productos salados y ahumados, es otro de los retos a los que se enfrenta el sector de elaboración de productos de la pesca. La disminución del contenido de sodio en este tipo de semiconservas no es simple, ya que la sal no solo juega un papel importante en el sabor del producto, sino que es imprescindible en términos de conservación. En este último capítulo, se propone la aplicación de la sustitución parcial de NaCl por otras sales alternativas en el nuevo método de salado-ahumado para el desarrollo de productos de salmón y trucha ahumada con contenido reducido de sodio.

En el artículo 5 se estudió el efecto del reemplazo parcial de NaCl por dos tipos de sales en los parámetros fisicoquímicos y sensoriales, así como en la calidad y vida útil durante el almacenamiento en refrigeración del salmón ahumado desarrollado en el capítulo 1. Las sales empleadas fueron KCl puro y sal sin sodio comercial, compuesta por KCl en su mayoría y saborizantes para enmascarar el sabor residual del KCl. Cabe destacar, que la dosificación de sal óptima para el salmón seleccionada en el capítulo 1 (8% NaCl), en este estudio se redujo a un 7% NaCl, dado el menor grosor de los filetes de salmón empleados como materia prima. La sustitución de un 50% de NaCl por sal sin sodio fue el máximo porcentaje admisible, para obtener un producto de salmón con sabor a humo sensorialmente aceptable y de características físico-químicas similares a los productos comerciales. Por otro lado, pruebas triangulares mostraron que el salmón con sabor a humo obtenido con sustitución parcial de un 50% de NaCl por sal sin sodio y por KCl no presentaba diferencias sensoriales perceptibles, respecto al salmón obtenido con 100% NaCl. Por ello, se consideró que el uso de la sal sin sodio no aportaba ninguna ventaja, desde el punto de vista sensorial respecto al uso de KCl puro. La sustitución de un 50% de NaCl por KCl permitió una reducción de un 37% de sodio respecto a las muestras control saladas con 100% NaCl. En el estudio de almacenamiento, se observó que la sustitución del 50% de NaCl por KCl, no tuvo implicaciones sobre la calidad y la vida útil del salmón con sabor a humo envasado a vacío y almacenado en refrigeración.

En el artículo 6, se estudió el efecto de la sustitución de un 50% de NaCl por KCl en los parámetros fisicoquímicos y sensoriales del producto de trucha ahumada con contenido en sodio reducido, así como su calidad y vida útil durante el almacenamiento en refrigeración. Al igual que en el caso del salmón, la sustitución de un 50% de NaCl por KCl no presentó ningún efecto sobre la calidad físico-química y microbiológica del producto recién procesado (día 0) y en este caso permitió una reducción de un 42% de sodio

respecto a las muestras control saladas con 100% NaCl. No obstante, las muestras saladas con 50% KCl-50% NaCl mostraron una menor oxidación lipídica que las muestras saladas con un 100% NaCl. De forma general, la sustitución parcial de NaCl por KCl no afectó a la textura y al color de las muestras. La vida útil del producto estuvo delimitada por el crecimiento de mesófilos y fue menor a 35 días, tanto en el caso de las muestras con reemplazo parcial de NaCl como en las saladas con 100% NaCl, lo que contrasta con los resultados del producto de salmón ahumado con contenido reducido en sodio en los que la vida útil fue de 42 días. Estos resultados podrían ser debidos a los menores valores de humedad y a_w que presentó el salmón, en comparación con los del producto de trucha ahumada.

En base a estos resultados, puede considerarse que la sustitución de un 50% de NaCl por KCl, para obtener productos de pescado ahumado aplicando la técnica de salado-ahumado simultáneo fue adecuada, ya que no afectó a los parámetros fisicoquímicos, microbiológicos, a la textura y al color. Por tanto, esta metodología supone una buena alternativa para reducir los riesgos asociados a la excesiva ingesta de sodio que conlleva el consumo regular de este tipo de productos.

En el **capítulo 4** se estudió la aplicación de la técnica de espectroscopía de impedancia (EI) como herramienta de monitorización on-line del proceso de salado-ahumado de salmón. En este trabajo se empleó un salado-ahumado alternativo empleando un sazonador con sabor a humo y bolsas de alta barrera. Cabe destacar, que éste es un estudio previo al desarrollo del salado-ahumado con las bolsas permeables al vapor de agua, por lo que las condiciones empleadas no coinciden con las descritas en los capítulos anteriores. En el estudio del proceso, se ensayaron distintas condiciones de procesado: (dosificación de sazonador, tiempo de procesado y tipo de envasado). En los productos obtenidos, se llevaron a cabo análisis fisicoquímicos de contenido de humedad, NaCl, pH y $a_{w.}$ Paralelamente se efectuaron mediciones con tres electrodos diferentes: electrodo doble, punta

de flecha y aguja, con el objetivo de evaluar la capacidad de los mismos para detectar los cambios que se producen en el músculo de salmón durante el proceso de salado-ahumado.

De los 3 sensores utilizados en las medidas de espectroscopía de impedancia, el electrodo de aguja fue el que mostró una mayor capacidad para discriminar entre las diferentes dosificaciones de sal de los productos de salmón ahumado, tal y como mostró el análisis estadístico multivariante de componentes principales (PCA). Posteriormente a partir de las condiciones de procesado seleccionadas y empleando el electrodo de aguja, se realizó un seguimiento del proceso de salado-ahumado durante 25 h de procesado, realizando mediciones con el electrodo de impedancia y los análisis fisicoquímicos descritos, a intervalos periódicos de 5 h. Durante el proceso de salado-ahumado a vacío con 16% de sazonador durante 25 h, se produjo un aumento en la concentración de sal y una reducción en los valores de humedad y a_w, cambios que fueron detectados mediante la técnica de EI con el electrodo de aguja. A partir de las medidas de impedancia se obtuvieron modelos robustos de predicción del contenido en sal, humedad y a_w. Los mejores ajustes correspondieron a la predicción de los valores de a_w, lo que resulta especialmente interesante, debido a la relación que existe entre este parámetro y la vida útil de los productos ahumados. Los resultados mostraron el potencial de la EI como método rápido de monitorización on-line del proceso de salado-ahumado de salmón. Este control contribuiría a ofrecer productos de calidad homogénea y garantizar un periodo de vida útil seguro para el consumidor.

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5. CONCLUSIONES GENERALES

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-El salado-ahumado simultáneo de pescado en bolsas WP aporta ventajas respecto a los procesos tradicionales de ahumado, como son la reducción de etapas de procesado, la disminución del volumen de residuos de salmuera y la protección del producto frente a contaminaciones durante el procesado.

-Mediante la adaptación de las condiciones de procesado de la nueva técnica en función de las características de la materia prima (contenido en humedad, grasa, a_w, grosor de las piezas…), es posible obtener productos de salmón, bacalao y trucha ahumada, de características similares a los productos comerciales, sin afectar a sus parámetros fisicoquímicos y a su aceptación por parte del consumidor.

-La vida útil de los productos de pescado ahumado desarrollados es similar a la de los productos de pescado ahumado que se comercializan actualmente en el mercado, superior a 40 días para el salmón ahumado, mayor a 35 días para el bacalao ahumado y superior a 28 días para la trucha ahumada.

- La sustitución de un 50% de NaCl por KCl no afecta a las características físico-químicas, sensoriales y microbiológicas en los productos de pescado ahumado y permite una reducción de un 37% y un 42% de sodio en el salmón y en la trucha ahumada, respectivamente. Del mismo modo, el reemplazo parcial no afecta a la estabilidad de los productos ahumados durante su almacenamiento en refrigeración.

- Se ha demostrado el potencial de la técnica de espectroscopía de impedancia en el seguimiento on-line de los parámetros fisicoquímicos que definen el salado-ahumado, como la humedad, el contenido en sal y la a_w, lo que la convierte en una herramienta muy interesante para el control de calidad de los productos de la pesca ahumados.