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Benzo(a)pyrene control and transport processes in smoked meat products

Tesis doctoral por

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Que mejor manera de comenzar esta memoria que haciendo mención a todas las personas que han participado en ella, mi ilusión. Y digo bien, a todas las personas.

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el bienestar y la salud humana.

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RESUMEN

El incremento del consumo de carne y productos cárnicos en el mundo, así como su proyección en los países en desarrollo y desarrollados, ha estimulado la investigación de su impacto en la salud humana. Los productos cárnicos son componentes importantes de una dieta saludable y balanceada, destacando su elevado contenido proteico. El ahumado es una de las técnicas más antiguas para prolongar la vida útil de los alimentos, siendo utilizado con este objetivo en algunos países en desarrollo. Además confiere buenas propiedades organolépticas a los productos, por lo que todavía es aplicado en el 60% de los productos, en algunos países desarrollados. Las investigaciones se han focalizado en el aporte de sustancias cancerígenas a los productos cárnicos durante su procesado, destacando la contaminación por hidrocarburos aromáticos policíclicos (HAP) durante el ahumado directo. En este contexto, el Codex Alimentarius (CAC/RCP 68/2009) ofrece una guía del proceso de ahumado, y el contenido máximo de benzo(a)pireno (BaP) (un marcador de la presencia y efecto de HAP en alimentos), se fijó en 5 µg/kg, y se redujo a 2 µg/kg desde el 1/9/2014, por los reglamentos europeos No.1881/2006 y 835/2011, respectivamente. En esta tesis doctoral, se aborda el estudio de los procesos de transporte y contaminación por BaP, durante el ahumado directo de uno de los productos cárnicos españoles más conocidos, el chorizo, elaborado en una zona no estudiada antes. Además, se investigan las posibles causas para su prevención.

Para ello, se ha desarrollado un método analítico basado en la combinación de las técnicas de pretratamiento de muestra, sonicación y extracción en fase sólida (SPE), y la determinación mediante cromatografía de gases/espectrometría de masas (GC/MS). Este método ha sido aplicado, en primer lugar, en chorizos ahumados elaborados por 16 empresas del Principado de Asturias, confirmando que 5 sobrepasan el límite reglamentario de BaP. La falta de correlación con su humedad, revela la necesidad de optimizar el ahumado directo como método de secado.

En segundo lugar, se determinó la influencia del tiempo de ahumado directo, variable recomendada por el Codex Alimentarius, en la contaminación de chorizo por BaP, y se estudio por primera vez, la penetración de BaP en distintas profundidades del chorizo ahumado. Se demostró que el incremento del tiempo de ahumado, produce efectos contrarios entre el contenido de BaP y la humedad del chorizo. El contenido de humedad decrece, y el contenido de

BaP aumenta, y finalmente se estabiliza entre los 5 y 7 días de ahumado, con un valor por debajo del límite legal europeo. El contenido de BaP del chorizo disminuye desde la tripa, donde supera 10 veces el límite legal, hasta el interior del chorizo.

Posteriormente, en el tercer trabajo, se estudió la influencia del uso de distintos tipos de tripa, natural y sintética (de colágeno), en la penetración de BaP en chorizo ahumado, encontrando grandes diferencias. Para explicarlas, se diseñaron sistemas novedosos, que permiten la caracterización de las propiedades físicas de las tripas, mediante porosimetría, y se estudió la evolución de estos chorizos durante el ahumado, mediante imágenes tomadas con estereomicroscopio de fluorescencia óptica, y microscopio electrónico de barrido (SEM). Esta tesis evidencia, y explica por primera vez, la capacidad de las tripas sintéticas para prevenir la penetración de BaP dentro de chorizo ahumado. Los resultados encontrados permitieron proponer, por primera vez, un mecanismo que explica la contaminación por BaP de los productos cárnicos embutidos en tripa natural, durante el ahumado directo.

Finalmente, en un cuarto estudio, se compararon las propiedades, textura, color y humedad, de los chorizos embutidos en los 2 tipos de tripa, durante el ahumado. Esta tesis demuestra que ambos tipos, confieren buenas propiedades al chorizo, pero la tripa de colágeno permite reducir el tiempo de procesado, garantiza la estandarización, y previene la contaminación por BaP en el interior del producto, si es ahumado. Esta tesis doctoral recoge un análisis detallado bibliográfico de los alimentos ahumados, el proceso tecnológico de ahumado y los beneficios y desventajas que confiere, destacando la contaminación por HAP. Finalmente, se describen e identifican las variables del proceso más importantes a controlar para prevenir la contaminación de los productos cárnicos por HAP durante el ahumado.

La presente tesis doctoral ofrece datos científicos relevantes, basados en el control de variables del ahumado recomendadas por el Codex Alimentarius, que permiten a los fabricantes minimizar el contenido de BaP en chorizo ahumado a modo directo, con el objetivo de fabricar productos cárnicos más saludables, y respetar el nuevo límite de BaP fijado por la normativa europea.

ABSTRACT

The increase of global meat and meat products consumption, and its projection in developing and developed countries has stimulated research concerning its impact to human health. Meat products are an important component of a healthy and well balanced diet, mainly because of its high level protein content. The smoking process is one of the oldest techniques for prolonging food shelf life. It is applied with this aim in some developing countries. Moreover it is still applied to 60% of the meat products in some developed countries because of the special organoleptic profile that it confers. Research is focused in the addition of carcinogenic compounds to meat products during processing, standing out the contamination by polycyclic aromatic hydrocarbons (PAH) during direct smoking. Within this context, Codex Alimentarius (CAC/RCP 68/2009) provides guideline of smoking process, and the maximum permissible content of benzo(a)pyrene (BaP) (a marker for the occurrence and effect of PAH in foods) in smoked meat products was fixed in 5 µg/kg, and reduced to 2 µg/kg from 1/9/2014 on, by European Regulations No. 1881/2006 and No. 835/2011, respectively. This PhD dissertation is focused in the research of the transport processes and contamination by BaP during direct smoking process of a well known Spanish meat product called chorizo, manufactured in an as-yet unstudied region. Moreover the possible causes are studied, in order to prevent it.

An analytical method was developed for this purpose, consisting of PAH extraction assisted by sonication, followed by solid-phase extraction (SPE) sample clean-up, and analytical determination using Gas Chromatography/Mass Spectrometry (GC/MS). Firstly, this method was applied to smoked chorizos made by 16 different producers from the Principality of Asturias. It was found that 5 of the samples exceeded the BaP legal limit. As not correlation between moisture and BaP content was found, the necessity of the optimization of direct smoking process was demonstrated.

Secondly, the influence of direct smoking time, variable advised by Codex Alimentarius, in the BaP contamination of chorizo was determined, and the penetration of BaP in different depths in the smoked chorizo was studied for the first time. It was proved that an increase in smoking time produces opposite effects on the moisture and the BaP content of chorizo. While the moisture content decreases, the BaP content increases finally becoming stabilized after 5 and 7 days of smoking. Then, a concentration below the legal limit was found. The BaP content

decreases from the casing, where a value 10 times over the legal limit was found, towards the inside of the chorizo.

Afterwards, in the third work, the influence of casing types, natural and synthetic (collagen), in the BaP penetration in smoked chorizo was studied, finding large differences. In order to find the reasons, new systems were developed, allowing the physical characterization of the casings by porosimetry, and the evolution of chorizos during smoking was studied by means of Fluorescence Stereo Microscope and scanning electron microscopy (SEM) images. The capacity of synthetic casings to prevent the BaP penetration into smoked chorizo was shown, and explained by first time. According with the findings, a new mechanism explaining the BaP contamination of chorizo stuffed in natural casing during direct smoking was proposed by first time.

Finally, in the fourth work, the texture, color and humidity properties of chorizos stuffed in both types of casing during smoking time were compared. This thesis proves that both types of casings give good properties to chorizo, but synthetic casing allows the reduction of processing time, enables the standardization, and prevents the BaP contamination into the chorizo, in case of smoking application. This thesis includes detailed bibliographic reviews about smoked food, the smoking process and the benefits and disadvantages that it confers, standing out the contamination of food by PAH. Finally, the most important variables affecting the smoking process are defined and identified, in order to prevent the PAH contamination of meat products during smoking process.

The present dissertation provides relevant scientific dates, based on the control of smoking process variables advised by Codex Alimentarius, allowing producers to minimize the BaP content of direct smoked chorizo, with the aim of produce healthier meat products, and respect the new BaP legal limit fixed by the European regulation.

1

Introducción

1. INTRODUCCIÓN

1.1 INTRODUCCIÓN

La carne y los productos cárnicos han tenido un rol crucial en la evolución humana y son componentes importantes de una dieta saludable y balanceada (De Castro Cardoso Pereira & Dos Reis Baltazar Vicente, 2013). Desde el punto de vista nutricional, su importancia deriva de su elevado contenido en proteína, siendo una alimento que contiene aminoácidos esenciales, así como minerales, vitaminas, micronutrientes como el hierro, selenio, zinc y vitamina B12 (De Castro Cardoso Pereira & Dos Reis Baltazar Vicente, 2013; FAO, 2014a; OMS/FAO, 2003; USDA/HHS, 2010). Las vísceras como el hígado son además fuentes cruciales de vitamina A y ácido fólico (Biesalski, 2005).

Dada su importancia para la evolución humana, estos productos han adquirido un elevado interés económico, siendo actualmente los alimentos pecuarios de mayor valor. Tal y como refleja la figura 1, en España, la industria cárnica, con 22.167 millones de euros, lidera el sector de la Alimentación. Representa el 25,4% de éste, primera actividad económica del sector industrial, y el 3,94% del total de la industria (INE, 2014).

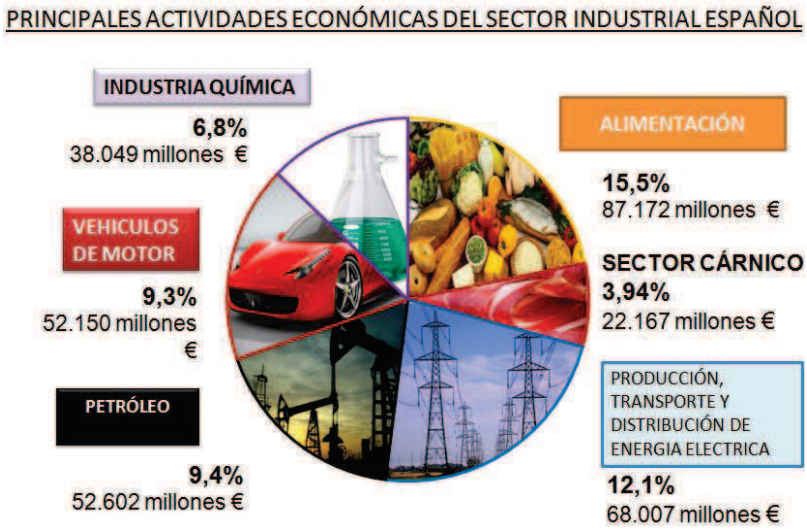

Figura 1.1. Elaboración propia con datos de "Encuesta Industrial de Empresas 2013" (INE, 2014).

Sin embargo, la industria cárnica se caracteriza por su elevada atomización, estando formada por un elevado número de empresas de pequeño tamaño. En España hay 4.036 empresas cárnicas (14% del número total de empresas alimentarias), de las cuales el 70,8% tiene menos de 9 asalariados y el 23,7% entre 10 y 49, generando en total el 22,90% de empleo de la industria alimentaria. Entre todas ellas, tan solo 10 empresas generan el 43% de las ventas totales del sector cárnico (MAGRAMA, 2014a). Esto hace que la gran mayoría de empresas cárnicas no puedan actualizar sus instalaciones con modernos y costosos sistemas de producción. Se proyecta que el consumo de carne en el mundo se duplique en el año 2050 (FAO, 2014a). Con un consumo per cápita medio de 15,3 kg/año, los productos cárnicos de cerdo serán en 2015 los más consumidos a nivel mundial (FAO, 2014b), habiéndose comercializado 110.270 miles de toneladas mundiales en 2011 (FAO, 2014c). España es la cuarta potencia productora de ellos (después de China, EEUU, y Alemania) (ANICE, 2013; MAGRAMA, 2013).

Figura 1.2. Chorizo.

Uno de los productos cárnicos embutidos españoles, elaborados entre otras materias primas con carne de cerdo, más importante y conocido es el chorizo. En el año 2013, se consumieron en España 53.011,54 toneladas de chorizos, generando 435,81 millones de euros para las empresas productoras (MAGRAMA, 2014b). Existen varias tipologías de chorizos tradicionales y denominaciones desarrolladas por el uso, reguladas recientemente por la nueva norma de calidad de productos cárnicos. Entre ellas se encuentran el chorizo criollo, chorizo cular, chorizo de cebolla, chorizo de entraña, chorizo de Pamplona, chorizo de Teror, chorizo palmero, chorizo de perro, chorizo rondeño y chorizo sabadiego (BOE, 2014). Además existen 2 chorizos con Indicaciones Geográficas Protegidas (IGP), el chorizo de Cantimpalos y el chorizo Riojano (MAGRAMA, 2014c). El chorizo asturiano está protegido y diferenciado bajo "marca colectiva" gracias al esfuerzo de entidades como el Centro Tecnológico Agroalimentario "Asociación de Industrias Cárnicas del Principado de Asturias" (ASINCAR), en colaboración con la Consejería de Agroganadería y Recursos Autóctonos (ASINCAR, 2015a). En el Principado de Asturias hay unas 73 empresas cárnicas (ASINCAR, 2015b), y se consumen 2.283,30 toneladas de chorizos al año (MAGRAMA, 2014b). Los chorizos elaborados en el Principado de Asturias, además de contener magro y panceta de cerdo, magro de vacuno ocasionalmente, sal, pimentón, ajo y otras especias, se caracterizan principalmente por sus cualidades organolépticas derivadas

de la exposición al ahumado. El ahumado es aplicado al modo tradicional en Asturias y otras regiones y países. Entre ellos destacan las zonas en desarrollo como África y Asia, donde la cadena de refrigeración no ha sido todavía establecida (FAO-Thiaroye, 2015; Ogbadu, 2014; Vaz-Velho, 2003). La técnica de ahumado optimizada ha sido implantada en algunos países desarrollados. El ahumado es muy aplicado en los productos cárnicos a nivel mundial. Por ejemplo, en Alemania se ahúman el 60% de los productos cárnicos (Frede, 2006).

El ahumado es una de las técnicas más antiguas de conservación de los alimentos, siendo probablemente los productos cárnicos los primeros alimentos ahumados por el hombre (Šimko, 2002, 2009). El humo está compuesto por numerosas sustancias químicas, habiéndose identificado más de 1100 compuestos (Wilms, 2000). Estos compuestos pueden clasificarse en muchos grupos, pero ejercen principalmente 2 efectos en productos cárnicos, efectos deseados y no deseados. Entre los efectos deseados destacan la prolongación de la vida útil y mejora de las propiedades organolépticas de los alimentos. Entre los compuestos que ayudan a prolongar la vida útil se encuentran algunos ácidos orgánicos y carbonilos (Milly, Toledo, & Chen, 2008; Montazeri, Himelbloom, Oliveira, Leigh, & Crapo, 2013), algunos compuestos fenólicos, como el fenol o el isoeugenol (Suñen, 1998; Young & Foegeding, 1993) que ejercen actividad antimicrobiológica frente a Listeria monocytogenes, otros compuestos bactericidas frente a Salmonella Typhimurium (Kim, Kang, Park, Nam, & Friedman, 2012), Escherichia coli (Van Loo, Babu, Crandall, & Ricke, 2012), Staphylococcus aureus y enterotoxinas estafilocócicas (Taormina & Bartholomew, 2005), y otros muchos compuestos con actividad biocida y fungicida (Möhler, 1978). El efecto de estas sustancias junto con el descenso de la actividad de agua de los alimentos durante el secado producen el efecto preservativo conocido del ahumado (Vaz-Velho, 2003). Además el ahumado mejora las propiedades organolépticas, color, textura y flavor de los alimentos (Adhikari, Heymann, & Huff, 2003; Ahmad, 2003; Birkeland, Bencze Rørå, Skåra, & Bjerkeng, 2004; Bozkurt & Bayram, 2006; Cardinal et al., 2001; Kim et al., 2014; Kostyra & Baryłko-Pikielna, 2006; Möhler, 1978; Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013a; Prändl, Fischer, Schmidhofer, & Sinell, 1994; Šimko, 2002, 2009; Vaz-Velho, 2003; Woods, 2003). Entre las sustancias que provocan el flavor y olor típico de los alimentos ahumados destacan los compuestos fenólicos, fenol, p-cresol y o-cresol, y los carbonilos, cicloten y 3-methylociclopenteno (Kostyra & Baryłko-Pikielna, 2006).

Por otro lado el ahumado produce efectos no deseados en los alimentos, entre los que destaca la contaminación por sustancias tóxicas y cancerígenas, como los hidrocarburos aromáticos policíclicos (HAP) (CCA, 2009), las n-nitrosaminas (Herrmann, Duedahl-Olesen, & Granby, 2015; Yurchenko, & Mölder, 2007), las aminas aromáticas heterocíclicas (Naccari et al., 2009; Simon, De la Calle, Palme, Meier, & Anklam, 2005), y los β-carbonilos (Papavergou & Herraiz, 2003; Sen, Seaman, Lau, Weber & Lewis, 1995). Entre todas ellas, las investigaciones se han focalizado en los HAP, debido a su elevada actividad cancerígena (FAO/OMS, 2006; SCF, 2002). La principal fuente de contaminación por HAP para el ser humano es la dieta (Falcó, Domingo, Llobet, Teixidó, Casas, & Müller, 2003; Ibáñez et al., 2005; Lodovici, Dolara, Casalini, Ciappellano, & Testolin, 1995; Phillips, 1999), contribuyendo al 70% de exposición en los no fumadores (Gilbert, 1994; McGrath, Wooten, Geoffrey Chan, & Hajaligol, 2007). Se han reportado niveles elevados de HAP en numerosos alimentos ahumados y también no ahumados, probablemente debido a contaminación medioambiental (CCA, 2009; Rodríguez-Acuña, Pérez-Camino, Cert, & Moreda, 2008). De entre todos los alimentos, desde una perspectiva global, los mayores contribuyentes de HAP son los cereales y los aceites y grasas vegetales (CCA, 2009), debido al elevado nivel de consumo a nivel mundial. Sin embargo, algunas investigaciones indican que los mayores contribuyentes de HAP en la dieta son los productos cárnicos ahumados (Martorell et al., 2010), ya que en ellos se encontraron los niveles más elevados de HAP (Gomaa, Gray, Rabie, López-Bote, & Booren, 1993; Karl & Leinemann, 1996; Larsson, Pyysalo, & Sauri, 1988; Martorell et al., 2010).

Así mismo, se ha asociado el consumo de carne y productos cárnicos con un aumento en el riesgo de contraer ciertos tipos de enfermedades crónicas, destacando el cáncer colorectal (CCR) (Alexander et al., 2010; Alexander et al., 2011; Aune et al., 2013; Biesalski, 2005; Corpet, 2011; Demeyer et al., 2008; Ferguson, 2010; McNeill & Van Elswyk, 2012; OMS/FAO, 2003; WCRF/AICR, 2007; Wyness et al., 2011), primera causa de cáncer en la población Europea (con acerca de 28,2 casos por 100.000 habitantes al año), segunda en mujeres a nivel mundial (614,000 casos, 9,2% del total) y tercera en hombres a nivel mundial (746,000 cases, 10,0% del total) (IARC, 2012). Esta relación ha sido rechazada recientemente (McAfee et al., 2010), ya que existe alguna inconsistencia entre los datos observacionales y experimentales entre carne y cáncer (Dragsted et al., 2014). Se concluyó que se precisa una mayor revisión (Kim et al., 2013). Por todo ello, es necesario establecer medidas para la prevención del aumento de la actividad

carcinogénica de los productos cárnicos derivada de su procesado, en particular del ahumado directo.

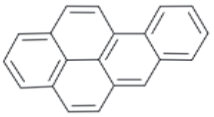

Figura 1.3. Benzo(a)pireno.

Para proteger a los consumidores en la ingesta de HAP, el contenido máximo permitido de estas sustancias en algunos alimentos fue regulado por el reglamento europeo N° 1881/2006, estableciendo al benzo(a)pireno (BaP) como el marcador de la presencia y efecto de los HAP en los alimentos, incluidos la carne ahumada y los productos cárnicos ahumados. Estudios previos indican que el BaP es el HAP más peligroso (Šimko, 2002). Recientemente (Septiembre, 2014) este contenido máximo ha sido reducido hasta 2 µg/kg por el reglamento (EU) N° 835/2011, añadiendo también como control la suma de 4 sustancias (HAP4) (BaP, benzo(a)antraceno, benzo(b)fluoranteno y criseno), manteniendo siempre un control separado para el BaP, para poder comparar con los datos previos y futuros. Un estudio reciente (Lorenzo et al., 2011) realizado para comprobar su idoneidad, concluye que el BaP es el marcador idóneo de la presencia de los 16 (reglamentación europea) o 7 (US EPA) HAP carcinogénicos reconocidos por la reglamentación, en concreto en chorizo gallego, muy similar al asturiano.

La contaminación por HAP de los productos cárnicos durante el ahumado puede ser controlada, para mantener los buenos efectos y prevenir los malos. En particular, la Comisión del Códex Alimentarius (CCA, 2009) proporciona las 10 variables que deben ser controladas para minimizar y prevenir la contaminación de los productos cárnicos por los carcinogénicos HAP durante el ahumado. El proceso tecnológico de ahumado es muy antiguo y ha sido sustituido en los países desarrollados por otras técnicas de conservación y procesado que permiten prolongar la vida útil (Zhou, Xu, & Liu, 2010), y prevenir la contaminación por HAP de los productos cárnicos, como por ejemplo sistemas de ahumado indirecto (Pöhlmann et al., 2013b). Sin embargo, la implantación de estos procesos es muy costosa e imposible para las comunidades de los países en desarrollo (FAO-Thiaroye, 2015; Ogbadu, 2014; Vaz-Velho, 2003), y las pequeñas empresas de la atomizada industria cárnica de los países desarrollados.

La prevención de la contaminación por HAP de chorizo ahumado a modo directo es un tema de elevada importancia para garantizar la seguridad alimentaria e inocuidad de estos alimentos, y en definitiva proteger la salud humana.

Figura 1.4. Chorizo ahumando.

1.2 OBJETIVOS

Así pues, a la vista de la necesidad de profundizar en los problemas de seguridad alimentaria derivados del consumo de productos cárnicos ahumados, se ha planteado como **Objetivo General (O.G)** de esta tesis doctoral **definir y determinar la contaminación por benzo(a)pireno (BaP) de chorizos ahumados del Principado de Asturias y sus mecanismos de transporte, proponiendo métodos para su prevención.**

Para ello se plantean los siguientes **objetivos específicos (O.E.)**:

O.E.1 Revisar los estudios que la comunidad científica ha desarrollado en relación a la contaminación de productos cárnicos ahumados por hidrocarburos aromáticos policíclicos (HAP), y analizar los métodos propuestos para su prevención.

O.E.2 Poner a punto un método analítico para la determinación de BaP en chorizo ahumado.

O.E.3 Determinar la presencia de BaP en chorizos ahumados, fabricados por diferentes empresas del Principado de Asturias, y analizar su contenido en humedad para evaluar el proceso de secado.

O.E.4 Evaluar la influencia de los siguientes parámetros del proceso de ahumado directo en la presencia de BaP en chorizo del Principado de Asturias:

a) El tiempo de ahumado.

b) El tipo de tripa: natural y sintética.

O.E.5 Estudiar la penetración de BaP en distintas profundidades de chorizo ahumado.

O.E.6 Caracterizar las diferencias físicas entre las tripas naturales y sintéticas y su influencia en la penetración de BaP en chorizo ahumado.

O.E.7 Proponer un mecanismo que explique la contaminación de chorizo por BaP durante el ahumado.

O.E.8 Caracterizar la influencia de las tripas natural y sintética en las cualidades organolépticas, color y textura, de chorizo ahumado.

1.3 ESTRUCTURA DE LA MEMORIA

La estructura de la presente memoria de tesis doctoral se presenta como convenio de publicaciones llevadas a cabo en la línea de investigación "contaminación por benzo(a)pireno de chorizos ahumados del Principado de Asturias y las posibles causas para su prevención". Cada una de las publicaciones tiene una estructura común, de acuerdo al esquema tradicional, constando de un resumen, una introducción, la descripción de los materiales y metodología empleados, la presentación y discusión de los resultados obtenidos, las conclusiones, los agradecimientos y las referencias utilizadas. Tres de los artículos presentados han sido ya publicados en revisas científicas incluidas en el Science Citation Index, incluyendo además otras que han sido enviadas. La estructura de la memoria presentada, está formada por 8 capítulos subdivididos en sus correspondientes apartados.

En el **capítulo 1** se expone la introducción, en la que se justifica la unidad temática de la presente tesis doctoral y la bibliografía de apoyo. La introducción (subapartado 1.1) enmarca la presente tesis doctoral en los ámbitos donde cobra interés, la industria alimentaria y la salud, define la problemática a resolver, y justifica el interés e importancia de los trabajos realizados. De acuerdo a ello, se exponen los objetivos propuestos (subapartado 1.2), y se describe la estructura de la memoria (subapartado 1.3). El **capítulo 2**, consideraciones teóricas, que formará parte de un volumen de una enciclopedia, aborda una revisión bibliográfica exhaustiva sobre los alimentos ahumados. En este capítulo el lector podrá encontrar información extensa sobre la historia de los alimentos ahumados, su rol en la dieta humana, su evolución en los distintos mercados de la economía mundial, los distintos tipos de alimentos ahumados y tecnologías de ahumado, la composición química del humo y los efectos deseados y no deseados que provoca en los alimentos. Entre los efectos deseados se detallan la prolongación de la vida útil y mejora del perfil organoléptico de los distintos alimentos. Entre los efectos no deseados el capítulo describe la contaminación de los alimentos por diversas sustancias tóxicas como las nitrosaminas, las aminas aromáticas heterocíclas y los β-carbonilos, y se enfoca en la contaminación por HAP.

En el **capítulo 3**, se describe de forma general, la metodología experimental, y las diferentes técnicas analíticas utilizadas en la presente tesis doctoral. La metodología utilizada en cada trabajo se detalla específicamente en el artículo científico correspondiente.

El **capítulo 4**, resultados, es la parte central de la presente memoria de tesis doctoral. En él se presentan los resultados obtenidos, organizados en 5 artículos científicos que abordan el tema principal de la tesis, "el estudio de la contaminación por benzo(a)pireno de chorizos ahumados del Principado de Asturias y las posibles causas para su prevención". En el capítulo 4.1 se expone la puesta a punto de un método analítico para la determinación de BaP en chorizo ahumado, y su aplicación en chorizos ahumados elaborados en el Principado de Asturias. En el resto de capítulos, se exponen los resultados del estudio de las causas, o variables de proceso, por las cuales el chorizo se contamina por BaP, durante el ahumado. Así, el subapartado 4.2 se enfoca en las variables "tiempo de ahumado y penetración del compuesto en distintas profundidades del producto", y los subapartados 4.3 y 4.4 en la influencia y caracterización del uso de distintos tipos de tripa, natural y de colágeno, en el mecanismo de contaminación por BaP (subapartado 4.3), y en la evolución de las propiedades, textura, color y humedad, y contaminación por partículas de humo (subapartado 4.4), del chorizo durante el ahumado. Finalmente, el subapartado 4.5, consta de un artículo, que aborda una revisión bibliográfica exhaustiva sobre el proceso tecnológico de ahumado de productos cárnicos, y el estado del arte en el control de la contaminación por HAP durante el mismo. En esta revisión, el lector podrá encontrar información y conceptos, tales como las referencias históricas, el avance y las modalidades de la tecnología de ahumado de productos cárnicos, el objetivo del ahumado, la formación química de HAP durante el mismo, la evolución de la regulación internacional sobre HAP en productos cárnicos, y los métodos analíticos utilizados por la comunidad científica para su determinación. En la parte central, encontrará una revisión de la presencia de BaP y HAP en productos cárnicos ahumados de diversos países, y su adecuación a la normativa, y por otro lado, estudios científicos sobre las variables del proceso de ahumado que afectan a la contaminación de los productos cárnicos por HAP (de acuerdo al Codex Alimentarius). El artículo concluye con la selección de las variables más importantes a controlar para la prevención de la contaminación por HAP de cárnicos ahumados.

El **capítulo 5** expone una discusión general sobre los resultados más importantes encontrados en la presente tesis doctoral, el avance que éstos suponen en el conocimiento científico-técnico y en su aplicación industrial. En el **capítulo 6** se exponen las principales conclusiones, que resumen los hallazgos más importantes encontrados en esta tesis doctoral.

El **capítulo 7** detalla el listado de referencias científicas y bibliografía utilizada en la presente memoria de tesis doctoral, omitiendo, las referencias asociadas a cada capítulo de resultados, las cuales se detallan en el apartado "referencias" de cada publicación correspondiente.

Finalmente, el **capítulo 8**, titulado "anexos", expone la difusión de la presente tesis doctoral mediante artículos científicos y comunicaciones a congresos, y el informe sobre el factor de impacto de las revistas científicas de los artículos publicados.

2

Consideraciones teóricas

2. CONSIDERACIONES TEÓRICAS

En este capítulo de la memoria se describen los alimentos ahumados. El capítulo introduce la historia del ahumado de alimentos, su rol en la dieta humana y su evolución en los mercados económicos. El capítulo presenta una revisión bibliográfica sobre los las diferentes tecnologías de ahumado y los principales tipos de alimentos ahumados, carnes, pescados y mariscos, quesos, bebidas y especias. Así mismo, se describe la composición del humo y se detallan los efectos deseados y no deseados que presenta en los productos. Entre los efectos deseados se describe la prolongación de la vida útil y la mejora del perfil organoléptico, el color, la textura y el flavor de distintos tipos de alimentos. Por otro lado, se desarrolla en detalle el principal efecto no deseado del ahumado, la contaminación de los alimentos por sustancias tóxicas y cancerígenas. Entre ellas se hace una introducción a las nitrosaminas, las aminas aromáticas heterocíclicas y los β-carbonilos, y se realiza una revisión detallada de los hidrocarburos aromáticos policlíclicos. Se describe la contaminación por PAH de varios tipos de alimentos ahumados, su toxicidad, aspectos legales sobre su regulación, y mecanismos generales para su prevención. El capítulo concluye caracterizando la necesidad y posibilidad de la prevención de los efectos no deseados, manteniendo las buenas propiedades de los alimentos ahumados.

Publicación: Smoked Food. Situación: Pendiente de envío para su revisión.

SMOKED FOOD

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ABSTRACT

This chapter is focused on smoked food. It starts by presenting an overview of the history of smoked food, its role on human diet and evolution in economic markets. Then the main types of smoked food and smoking methods are widely described. Next, the composition of smoke and its desirable and non desirable effects on smoked food are characterized in detail. Among the desirable effects there are food preservation and the improvement of the organoleptic profile, colour, texture and flavor of different types of food. On the other hand, the main undesirable effect of smoking is defined in detail. This is the contamination of food by toxic and carcinogenic compounds, standing out polycyclic aromatic hydrocarbons, and others, such as n-nitrosamines, heterocyclic aromatic amines and β-carbolines. Finally, the main conclusions are summarized, showing the necessity and possibility of preventing the non desirable effects and maintaining the good properties of smoked food.

Keywords: Smoked food, smoking methods, food preservation, food flavouring, polycyclic aromatic hydrocarbons, n-nitrosamines, heterocyclic aromatic amines, β-carbolines, food contamination, food control.

INDEX

1. History

Smoked food have had an important historical and economical role in human diet, and are still highly consumed and requested in modern markets and in some developing countries. Food smoking is one of the oldest technological processes which mankind has used since the beginning of time (Tóth, 1982). Probably meat products were the first smoked food produced by humans. It is possible that first humans hung the food over the fire as a protection method against canines, then the preservative action of smoke to prolong food shelf life was probably found (Šimko, 2002, 2009). The first proof of smoking as technological process dates back to 90.000 years ago. The oldest smoking house was discovered by archeologists in a stone age colony located in Zwierzymec, near Krakow in Poland (Möhler, 1978). Smoking process has been used like preservation method along the different ages of men. Food smoking was described in roman cultures in The book of Marcus Cato, 160 B.C, De Agri Cultura, (Leroy, Geyzen, Janssens, De Vuyst, & Scholliers, 2013; Mateo, Caro, Figueira, Ramos, & Zumalacarregui, 2009; Möhler, 1978; Zeuthen, 2007), and in middle age in the book of Marx Rumpolt, 1581 (Möhler, 1978). Romans probably learnt meat smoking methods from the Gauls and Celts. Europeans emigrants export this technique worldwide, including to the Americas, South Africa, and Australia (Leroy et al., 2013), but the smoking process was probably applied in other countries before the colonization. In the age of discovery, smoked food were very useful in long travels by boats. Smokehouses began very popular in every farm and dwelling of USA, and proliferated in the states such as Georgia, Carolinas, Virginia, Tennessee, Missouri and Kentucky. Only the introduction of refrigeration in the 1900´s has stopped smoking as preservation method, especially in meat products (Marianski, Marianski, & Marianski, 2009). Since the 19th century, when rail transport reduced the time from production to market, the production of smoked food decreased (McGee, 2004). Smoked food have had an important role in the diet of people from European countries during World Wars, when food supply was very low. That could explain that smoked meat products are more popular in Europe than in America (Marianski, et al., 2009).

Nowadays smoking is used in developed countries, mainly because of the specific organoleptic profile that it confers to food. Smoking improves food flavour, colour and smell, widely properties demanded by market (Lorenzo, Purriños, García Fontán, & Franco, 2010; Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013a; Šimko, 2002; Vaz-Velho, 2003). Smoked food have still a huge impact on the economy of countries. In fact, 30% of the meat products are

supposed to be smoked in USA (Marianski et al., 2009), while this figure is 60% in some European countries, like Germany (Frede, 2006). Smoking as preservation method has been replaced by modern methods like controlled drying chambers, liquid smoke (Kostyra & Baryłko-Pikielna, 2006; Lingbeck et al., 2014; Theobald et al., 2012) and other methodologies for fresh food preservation by means of refrigeration (like chilling, freezing, superchilling), ionising radiation, chemical preservatives and biopreservation, high hydrostatic pressure (HHP) and packaging methods like vacuum packaging, modified atmosphere packaging (MAP), active packaging (AP), antimicrobial packaging, and hurdle technology (HT) (Zhou, Xu, & Liu, 2010).

On the other hand, smoking is still used as preservation method in some developing countries, like Africa and Asia, where a refrigeration chain is not widely established (FAO-Thiaroye, 2015; Vaz-Velho, 2003). For instance, smoking is applied by the artisanal finishing population in Sub-Saharan Africa, which represents the 70% of the whole fishing entrepreneurs in this country. The bulk of all fish caught and all game hunted for food in this country, accounting 80% of the total, are smoke or heat preserved. More than 500 metric tons of smoked fish are exported from West Africa to the United Kingdom each year. This is valued at between 9.3 and 14.9 million United States dollars (Ogbadu, 2014).

2. Types of smoked food

A great number of smoked food have traditionally been produced, and can still be found in the market. Table 1 shows the main groups of smoked food, that are described in this section.

Table 1. Some types of smoked food.

Smoked food	Origin/ main use	Smoked food varieties
Smoked meat products		
Sausages	Austria	Vienna sausage
	Croacia and Serbia	Kulen
	France	Andouille, morteau sausage
	Hungary	Hungarian sausage, winter salami
	Germany	Ahle wurst, amsterdam ossenworst, bierwurst, bockwurst, debrecener, knackwurst, knipp, kochwurst, kohlwurst, liverwurst, braunschweiger, mettwurst, pinkel, teewurst
	Greek	Loukaniko
	Italy	Bologna sausage, ciauscolo, lucanica, salami
	Lithuanian	Skilandis
	Netherlands	Rookworst
	Pennsylvania	Lebanon bologna
	Poland	Kielbasa, krakowska, linguiça
	Portugual	Farinheira, chouriço, paínho,paio tradicional, chouriço mouro, cacholeira and morcela.
	Romania	Nădlac sausage
	Serbia	Cajna sausage and sremska sausage, sremska kobasica, Petrovská klobása.
	Spain	Androlla, botillo, chistorra, chorizo, farinato, longaniza, morcilla, morcón, sabadiego, salchichón, sobrasada, smoked dry cured sausage and smoked potato sausage.
	Sweden	Isterband
	Turkey	Sucuk
	USA	Breakfast sausage, half-smoke
Ham	Bulgaria	Elenski but
	Croatia	Pršut
	England	Yule ham
	France	Jambon de Bayonne
	Germany	Ammerländer schinken, black forest ham, eisbein, schwarzwälder schinken, westphalian ham.
	Italy	Smoked prosciutto
	Portugal	Jamón ahumado
	Romania	Jambon
	Spain	Jamón ahumado
	Sweden	Swedish ham smoked
	USA	Country ham, tasso ham

Table 1. (continued).

Smoked food	Origin/ main use	Smoked food varieties
Smoked meat products		
Other varieties of smoked meat products	Africa	Smoked pork from Cape Town
	Brasil	Smoked bacon, loin, turkey.
	Bosnia and Sebia	Suho meso
	Canada	Bacon, oreilles de crises, montreal-style smoked meat
	China	Zhangcha duck
	Estonia	Smoked meat
	France	Brési
	Germany	Bacon, dutch meatloaf, flurgönder, kassler, schäufele
	Hungary	Szalonna
	Iceland	Grjúpán, hangikjöt
	Indonesia	Se'i
	Irland	Bacon
	Italia	Pitina
	Japon	Bacon
	Kazakhstan	Qarta, zhal
	Korea	Jeju black pig
	Kuwait	Smoked meat
	Malaysia	Beef satay
	Norway	Smalahove
	Pennsylvania	Pennsylvania dutch meatloaf
	Spain	Cecina, chosco, smoked tenderloin, smoked jowl.
	Taiwan	Smoked chicken
	United Kingdom	Bacon, gammon, speck, turkey bacon
	USA	Bacon, burnt ends, chaudin, dutch loaf, picnic ham" shoulder, pork jowl
	Various countries	Jerky, meatloaf, pastrami, pickled pigs feet, pork tail, salo

Table 1. (continued).

Smoked food	Origin/ main use	Smoked food varieties
Smoked fish		
Smoked fish products	Africa	African longfin eel, bokkoms, Ethmalosa fimbriata, smoked Sardinella sp. and anchovies (Anchou guineensis)
	Iceland	Smoked Salmo Salar
	Indonesia	Cakalang fufu, Ambon's smoked tuna
	Japan	Katsuobushi (smoked skipjack tuna)
	Korea	Gwamegi
	Norway	Smoked Salmo Salar
	Philippine	Tinapa
	Scandinavia and Finlandia	Smoked cod roe, smörgåskaviar
	Spain	Atlantic salmon (Salmo salar) treated with liquid smoke flavourings
	Sweden	Buckling, Lysekil caviar
	United Kingdom	Arbroath smokie, Finnan haddie, Traditional Grimsby smoked fish (cod and haddock)
	USA	Lox
	Various countries over the world	Atlantic mackerel, Bückling, eel, cod, haddock, halibut, goldeye, herring (Bloater herring, blueback herring, kipper, craster kipper), mackerel, mussels, oyster, pollock, salmon, sardines, tilapia, scallo, sprats, trout.
Smoked cheese		
	Ireland	Ardrahan cheese, Corleggy cheeses, Burren Gold (Gouda style), Gubbeen farmhouse cheese
	India	Bandel cheese
	Romania	Brânză de coșuleț
	France	Brie
	Armenia	Chechil
	Russia	Chechil
	England	Cheddar cheese, Lincolnshire Poacher cheese, Wensleydale cheese, Orkney (cheddar)
	Circassia	Circassian smoked cheese
	Spain	Gamonéu cheese, Herreno cheese, Palmero cheese, Peña Pelada smoked cheese, Idiazábal cheese, San Simón Da Costa cheese, Liébana cheese, Campoveja cheese, Pría smoked cheese
	Netherlands	Gouda cheese

Table 1. (continued).

Smoked food	Origin/ main use	Smoked food varieties
Smoked cheese		
	Slovakia	Korbáčik
	South Africa	Kwaito cheese
	Greece	Metsovone, Kashkaval
	Italy	Smoked mozzarella, Provola, Provolone, Ricotta, Scamorza, Caciocavallo
	Poland	Oscypek
	Slovakia	Oštiepok, parenica
	Serbia	Pule
	Turkey	Circassian cheese, Kashar cheese
	Germany	Rauchkäse, reichkäse, caramakase
	USA	Boerkäse
	Brasil	Queijo prato
	Norwey	Smokelet
	Hungary	Szekeley
	Iran	Seretpanir
Smoked beverage		
Whisky	UK (Scotland),Irland	Whisky
Tea	China	Lapsang souchong, Assam smoked oolong.
	Taiwan	Lapsang souchong (Tarry souchong)
	India	Mattha (Mate), Earl Grey smoked tea
	South America	Mate
Beer	Germany	Rauchbier
Other	China	Suanmeitang, grappa (Spanish aguardiente de orujo)
Smoked spices		
	America, Hungary, Turkey and Spain	Paprika
	Various	Liquid smoke (inventor: Fessmann Gerhard)
	Other countries	Smoked salt, smoked garlic, merquén and chipote

2.1 Smoked meat products

Smoked meat products are one of the most important and produced smoked food. They probably were the first smoked food produced by humans. Meat products are an important component of a healthy and well balanced diet (De Castro Cardoso Pereira & Dos Reis Baltazar Vicente, 2013), mainly due to its high protein content (De Castro Cardoso Pereira & Dos Reis Baltazar Vicente, 2013; FAO, 2014a; USDA/HHS, 2010; WHO/FAO, 2003). They are the most valuable livestock products. Thereby, world meat production is projected to double by 2050. In 2015, the average world meat per capita consumption is expected to be 41.3 kg/year. This consumption will be 31.6 kg/year and 95.7 kg/year in developing and industrial countries respectively (FAO, 2013, 2014a). Smoking was an important way to preserve this type of food in the history. Thereby, the most typical smoked meat products are produced in the traditional way.

The table 1 shows some smoked meat products from different countries around the world, that have been studied by researchers (Chiu, Lin, & Chen, 1997; Djinovic, Popovic, & Jira, 2008; Fretheim, 1976; García-Falcón & Simal-Gándara, 2005; Gomes, Santos, Almeida, Elias, & Roseiro, 2013; Hitzel, Pöhlmann, Schwägele, Speer, & Jira, 2013; Jahurul et al., 2013; Ledesma, Rendueles, & Díaz, 2014, 2015a,b; Lorenzo et al., 2010; Lorenzo et al., 2011; Marianski et al., 2009; Martin & Ruiz, 2007; Roseiro, Gomes, Patarata, & Santos, 2012; Santos, Gomes, & Roseiro, 2011; Pöhlmann et al., 2012, 2013a,b; Purcaro, Moret, & Conte, 2009; Reinik et al., 2007; Šimko, 2002; Škaljac et al. 2014; Węgrzyn, Grześkiewicz, Popławska, & Głód, 2006; Wretling, Eriksson, Eskhult, & Larsson, 2010; Yabiku, Martins, & Takahashi, 1993). As the table 1 shows, the most known smoked meat products can be classified in 3 types, sausages, hams and the rest of smoked meat products. Most of the types of smoked meat sausages and hams are produced by Germany, Spain, Portugal and Italia.

2.2 Fish and sea food

Fish production and consumption is growing in the world. Global fish production was 158 million of tones in 2012. The human consumption increase from 9.9 to 19.9 kg per capita, from 1960 to 2012. The 12% of this fish was commercialized smoked, dried, cured or salted, and the rest was used fresh and refrigerated (46%), frozen (29%) and transformed or canned (13%) (FAO, 2014b). The smoking sector is of considerable economic importance to the fish product market,

accounting for a profit of over US$1,110,000,000 (FAO, 2008). The world production of smoked fish is near 160,000 tons (FAO, 2008).

Table 1 shows some typical kinds of smoked fish from different countries around the world, that have been studied by several researchers (Adekunle & Akinyemi, 2004; Birkeland, Rørå, Skåra, & Bjerkeng, 2004; Brett, Short, McLauchlin, 1998; Cardinal et al., 2001; Gómez-Estaca, Montero, Giménez, & Gómez-Guillén, 2007; Hayward & Mosse, 2012; Ikutegbe & Sikokib, 2014; Ishizaki, Saito, Hanioka, Narimatsu, & Kataoka, 2010; Martinez, Salmerón, Guillén, & Casas, 2007; Montiel, De Alba, Bravo, Gaya, & Medina, 2010; Plahar, Nerquaye-Tetteh, & Annan, 1999; Stołyhwo, Kołodziejska, & Sikorski, 2006; Yanar, Çelik, & Akamca, 2006).

Salmon and herring are the most important smoked species with a production of around 51,000 and 15,000 tons respectively in 2006. Other important types of smoked fish are cod, tuna, mackerel or trout (Fuentes, Fernández-Segovia, Serra, & Barat, 2010). Fish can be smoked by direct methods, such as traditional smoking, or indirect methods, such as liquid smoke. The main producers of fish smoked in a traditional way are Africa and Asia (FAO, 2014b). As it has been previously said, 80% of the fish captured and processed in Africa is smoked or heat preserved (Ogbadu, 2014). The lost and deterioration of smoked fish are important problems in this country. An special technique of smoking, known as “technique FAO-Thiaroye” has recently been designed in the city of Senegal, thanks to a project of USA Red Cross and FAO (FAO-Thiaroye, 2015). In Europe, the main producers of smoked fish are the central and oriental Europe countries, especially Poland and the Baltic states (FAO, 2014b). Both direct and indirect smoking methods are applied in these countries.

2.3 Cheeses

Cheese is a dairy product that is available in a wide range of types. Cheese types are produced by using different types of milk, from cow, cattle, sheep, goat, she-ass, etc., and manufacturing processes, including different types of rennets, molds, repining degrees, and smoking processes (Naccari et al., 2009). Cheese has been traditionally smoked in order to prolong its shelf life. The shelf life of milk is short, and smoking became a way to provide good nutrients, as calcium, during all year, when refrigeration chain was not established yet. Nowadays smoking is applied to cheese in order to achieve differentiation (Shakeel-ur-Rehman, Farkye, & Drake, 2003). Cheese can be smoked either by using smoke flavorings or by traditional smoking.

However the use of smoke flavorings for the smoking of cheese is not allowed in some countries, such as Spain (Guillén, Palencia, Ibargoitia, Fresno & Sopelana, 2011). Table 1 (Aydinol & Ozcan, 2013; Adhikari, Heymann, & Huff, 2003; Esposito et al., 2015; Garabal, Rodríguez-Alonso, Franco, & Centeno, 2010; Guillén, Ibargoitia, Sopelana, Palencia, & Fresno, 2004; Musullugil & Koca, 2012; Naccari et al., 2009; Palencia, Ibargoitia, Fresno, Sopelana, & Guillén, 2014; Shakeel-ur-Rehman et al., 2003) shows different types of smoked cheeses according to its origin. As table 1 shows, different types of smoked cheeses are produced all over the world. The most common varieties of smoked cheeses are Seretpanir (Iran), Caramakase (Germany), Bandal (India), and Provolone (Italy) (Shakeel-ur-Rehman et al., 2003).

2.4 Beverages

Opposite to other food, the aim of smoking in beverages production has not traditionally been prolong shelf life. The main aim of smoking in the beverages process was flavouring. The main smoked beverages are whisky, tea and beer. Table 1 (Bringhurst & Brosnan, 2014; Buglass & Caven-Quantrill, 2012; Harrison, 2012; Pavsler & Buiatti, 2009; Pincemaille, Schummer, Heinen, & Moris, 2014) shows some types of these smoked beverages.

Whisky is the most famous alcoholic smoked beverage. The most known smoked whisky is produced in Scotch whisky distilleries, especially those located in Islay. The smoking process is applied to whisky during a step often named peating (Bringhurst & Brosnan, 2014). The location from which the peat is extracted is an important factor to give distinctive characteristics to the spirit. By burning of peat, malted barley is dried and some flavouring characteristics are produced, such us peaty, phenolic, smoky, burnt, and medicinal tastes and smells (Bringhurst & Brosnan, 2014). The degree of thermal degradation and decomposition of lignin and other polyphenol components from the plant, have a great influence in the flavour composition of the peat smoke (Harrison, 2012).

Tea (Camellia sinensis) is the most widely consumed beverage in the world, after water (FAO, 2015; Pincemaille et al., 2014). The most known smoked tea is the black tea, in particular Lapsang Souchong. Lapsang Souchong comes from south-eastern China and Taiwan, where is often known as Tarry Souchong. Pine, spruce and bamboo are typical plants used for the smoking of tea. They give a heavy smoky taste and smell to the tea leaves and its infusions (Pincemaille et al., 2014). Other smoked teas are Mate, typical of South American countries such as Paraguay,

Uruguay, Argentina, Bolivian Chaco and southern Brazil, and Mattha tea, from India. During the manufacturing process for the herbal infusion yerba mate (Ilex paraguariensis), the leaves are dried slowly often using wood smoke (Heck & De Mejia, 2007). The special processing gives mate its typical bitter aromatic and slightly smoky flavor (Schulz, Fritz, & Ruthenschrör, 2014). Mattha may be smoked before serving for flavouring proposes.

Beer is one of the oldest and most popular beverages all over the world (Wunderlich & Back, 2009). There are more than 100 varieties of beer. Depending on the process used, a first classification can be made according to the fermentation process, in top and bottom fermentation beers. Bottom fermented beers are commonly named Lager. Lager is the dominant style in almost all countries, and represents more than 90% of the beer produced worldwide. Top fermentation beers are commonly named Ale, and are very popular in Britain, Germany, Canada ' s eastern provinces, United States and Belgium (Pavsler & Buiatti, 2009). The most popular type of smoked beer is a top fermentation beer named Rauchbier. This smoked beer is produced in a town of Germany called Bamberg. An historic brewpub named Schlenkerla is especially well known for its smoked Aecht Schlenkerla Rauchbier. Thereby the production of this beer is not very important compared to the whole beer sector. During the production of Rauchbie an aromatic smoke is produced by means of burning beech-wood logs. A smoky bacon flavour and aroma are achieved by exposing the malt to this intense, aromatic smoke. After mixing it with premium-class hops in the brew, it matures in 700–year–old cellars. The percentage of alcohol of this smoked beer ranges between 4.8% and 6.0% (Buglass & Caven-Quantrill, 2012; Pavsler & Buiatti, 2009).

Finally, other smoked beverages can be found in the world, such as grappa or "aguardiente de orujo", which smoking process of marc has been studied (Da Porto, 2012; Da Porto, Moret, & Soldera, 2006), Jamaican rum, the traditional Chinese beverage Suanmeitang, that is made of sour plums (specifically, smoked Chinese plums), rock sugar, and other ingredients such as sweet osmanthus.

2.5 Spices and flavourings

Some types of smoked spices are shown in table 1 (Doymaz & Pala, 2002; Gallardo-Guerrero, Pérez-Gálvez, Aranda, Mínguez-Mosquera, & Hornero-Méndeza, 2010; Lingbeck et al., 2014; Mateo, Aguirrezábal, Domínguez, & Zumalacárregui, 1997; Ramesh, Wolf, Tevini, & Jung,

2001; Turhan, Turhan, & Sahbaz, 1997). The most known and studied smoked spices and flavourings are paprika and liquid smoke. Other less known types of smoked spices are smoked salt, smoked garlic and smoked chili peppers, like merquén and chipote.

2.5.1 Paprika

Paprika is a red powder made from grinding the dried pepper pods of some varieties of *Capsicum annuum* L (Mateo et al., 1994; Reineccius, 1994). This natural food product is commonly used as spice and natural colorant to provide redness to other food, such us meat products and commercial sauces (Attokaran, 2011). Although paprika is original from America, it is also produced in Europe, particularly in Hungary, Turkey and Spain (Palacios-Morillo, Jurado, Alcázar, & de Pablos, 2014). Some kinds of paprika, such as the Spanish "pimentón de la Vera", are dried by traditional smoking process. During smoking, logs are burnt and the ripe red fruits of the pepper are slowly dehydrated by means of the heat and smoke. Smoking time ranges between 7 to 10 days (Gallardo-Guerrero, et al., 2010) or 12 to 15 days (Mateo et al., 1997). Temperature in the chamber is about 40ºC during the first 5 days, and 60ºC from 5 days to the end of the process. The final water content of the product is below 15% (Mateo et al., 1997). Drying process has an important role on the composition of final paprika, in its carotenoids content and non-enzymatic browning (Lee & Kim, 1989), colour, l-ascorbic acid and sugar retention (Sigge, Hansmann, & Joubet, 1999) or colour (Carbonell et al., 1986; Doymaz & Pala, 2002). The dehydration and drying kinetics of red pepper under different pretreatments and drying conditions have been previously studied (Doymaz & Pala, 2002; Ramesh et al., 2001; Turhan et al., 1997). Therefore, smoking process should be optimized in all cases, in order to obtain good quality characteristics of the final paprika.

2.5.2 Liquid smoke

Liquid smoke is a more actual type of food flavouring. Liquid smoke is mainly applied to meat products, fish and poultry, but it is also applied to non-meat food such as cheese, tofu and even pet food (Lingbeck et al., 2014). Liquid smoke is highly used in marinades, sauces or brines (Lingbeck et al., 2014; Rozum, 2009). In addition, liquid smoke exhibits antimicrobial activity against *Listeria* (Gedela, Gamble, Macwana, Escoubas, & Mariana, 2007; Martin et al., 2010; Messina, Ahmad, Marchello, Gerba, & Paquette, 1988), *Escherichia coli* (Van Loo, Babu, Crandall, & Ricke, 2012), *Staphylococcus aureus* and staphylococcal enterotoxins (Lingbeck et al., 2014;

Taormina & Bartholomew, 2005). During its production, liquid smoke is filtered and subjected to fractionation and purification processes to remove toxic and carcinogenic particles and compounds. Therefore, its use is generally considered to be of less health concern than the traditional smoking process. However, the possibility of broader applications of smoke flavorings compared to conventional smoking has to be taken into account in safety assessments (EC No 2065/2003; Lingbeck et al., 2014). Liquid smoke is more environmentally friendly, faster, more specific and easier of application than traditional smoking, and allows good reproducibility of desired characteristics in the end product (Lingbeck et al., 2014; Suñen, Fernandez-Galian, & Aristimuño, 2001).

3. Food smoking methods

The traditional and uncontrolled method of food smoking by means of biomass fires has been improved, and introduced in some modern developed countries. However, the modern techniques are still very extensive to be used in some sectors and developing countries.

Food smoking methods and techniques have been classified and described in several works (Ahmad, 2003; Möhler, 1978; Šimko, 2009; Vaz-Velho, 2003; Woods, 2003), based on the temperature of the smoke, the location of smoke generation with respect to the position of the foodstuff, and the device used for generating smoke. In this chapter, the different smoking methods are classified in two main groups: direct and indirect smoking.

3.1 Direct smoking methods

During direct smoking, smoke is produced in the same chamber where the meat is processed (CAC/RCP 68/2009). Direct smoking methods mainly comprise traditional techniques. The traditional smoking method consists of direct thermal degradation of wood to produce smoke (Ahmad, 2003). This definition can be extended, as others kinds of fuels were erroneously used in the past all over the world. In fact, humans used to burn any kind of waste in bonfires to produce smoke, such as food waste (coconut shells, ears of corn, fruit stones, etc.) and even newspapers or furniture (wood treated with paint) (CAC/RCP 68/2009). Various unhealthy compounds are formed if the correct fuel is not used, especially PAH. Direct smoking methods can be classified according to the temperature of the smoke.

3.1.1 Traditional cold smoking

During cold smoking, wood is burned and smoke is produced. Meat products are hung from shelves placed above the hearth, located in a grilled floor through which the smoke passes. Smoking chambers are usually large. When burning finishes, the fire is not poked and the smoke cools. According to different authors, the required temperature in a smoking chamber to achieve cold smoking conditions should be below 20°C (Möhler, 1978), between 15 -25°C (Šimko, 2009; Woods, 2003), or below 30°C (Ahmad, 2003). Raw hams (Möhler, 1978; Šimko, 2009), heat fermented untreated products like salami (Šimko, 2009) and other stuffed meat products, like chorizo, are usually produced by cold smoking.

3.1.2 Traditional hot smoking

During traditional hot smoking, the chamber is heated by the burning of wood in a similar process to a typical old baking oven. Once placed inside the chamber, the meat is heated and dried by embers of burnt wood. Sawdust is then introduced into the chamber and the fire is stoked with the aim of producing a large amount of smoke (Möhler, 1978). Temperatures of 130°C in the smoke and 80°C in the meat are needed in hot smoking (Ahmad, 2003; Möhler, 1978), although some authors specify lower temperatures, between 55 and 80°C (Woods, 2003).

3.2 Indirect smoking methods

Indirect smoking comprises a number of new methods that help reduce PAH contamination of meat products. These are summarized below.

3.2.1 Smoke produced by a friction generator

Primitive tribes produced smoke during the discovery of fire by means of the uninterrupted friction of wood on wood. This may well have provided the inspiration for friction smoke generation methods. The first development of this technique for producing smoke was first developed as a modern technology by Rasmussen and Rasmussen (1961) and was protected by US patent 3.001, 879. Since then, new models have been introduced. Nowadays, smoking chambers are designed to control all the processing steps, including preheating and reddening, friction smoke generation, smoke evacuation and drying. These steps are repeated in cycles, the number and duration of which depend on the type of meat product. Typical smoke generation

values are 20-second intervals of continuous friction of a gearwheel with wood followed by a pause of between 70 and 175 seconds. During this process, a temperature range between 180 and 380°C is reported by Varlet et al. (2007). The process, adapted for the production of a typical Spanish meat product called chorizo, includes 5 cycles of 30 minutes of preheating (150 minutes), reducing the temperature from 18 to 2°C and the humidity from 95 to 90%, 6 hours of drying at 25°C and 90% humidity, 41 cycles, consisting of 10 minutes of smoking, 3 minutes of smoke evacuation, and 20 minutes of drying at 25°C and 90% humidity, and a final step of 3 cycles of 8 hours of drying (24 hours) at 80% humidity, decreasing the temperature from 23 to 18°C. According to Pöhlmann et al. (2013a), Frankfurter-type sausages are exposed to reddening for 10 min at 52°C, drying for 12 min at 56°C and friction smoking for 26 to 40 hours, followed by a final step of scalding at 75°C for 25 min. With friction smoke generation, operation time and wood requirements are reduced and production is controlled and optimized. Furthermore, meat industry safety is increased, as PAH production is very low (Pöhlmann et al., 2013a), workers' health is improved by preventing fire hazards, product weight losses are reduced, product flavor is enhanced by avoiding the concealing of the taste of the ingredients, product shelf life and quality are suitable and product standardization and homogenization is made possible.

3.2.2 Liquid smoke production

The development of smoke flavourings dates back to the late 19th century (Fessmann, 1972; Miler & Kozlowski, 1969). It was developed with the aim of replace the traditional smoking process (Theobald et al., 2012). The flow diagram of typical liquid smoke production has been recently described by Lingbeck et al. (2014). Nowadays, liquid smoke is produced by condensing wood smoke formed by the controlled, minimal oxygen pyrolysis of sawdust or wood chips. The wood is placed in large retorts where intense heat is applied, causing the wood to smolder (not burn), releasing the gases seen in ordinary smoke. These gases are quickly chilled in condensers, thus liquefying the smoke. The liquid smoke is then forced through refining vats and subsequently filtered to remove toxic and carcinogenic impurities containing PAH. Finally, the liquid is aged for mellowness (Lingbeck et al., 2014). An outline scheme for the preparation of a PAH-free liquid smoke was described by Ahmad (2003). The use of liquid smoke is considered to be healthier than the use of smoke obtained in the traditional way. However, the possibility of broader applications of smoke flavorings compared to conventional smoking has to be taken into account in safety assessments (EC No 2065/2003; Lingbeck et al., 2014).

3.2.3 Electrostatic smoking

In this type of smoking, the product is positioned in a continuous tunnel between live electrical wires that are charged to between 20 and 60 kV. Smoke passing through this system is charged according to its phase (smoke is a two-phase system, particulate and vapor), and smoke components can precipitate on the oppositely charged food surface (Vaz-Velho, 2003; Woods, 2003). The movement of gas and liquid in smoking chambers has barely been studied (Pinilla, Díaz, & Coca, 1984). In order to ensure sedimentation of the smoke components on the surface of the product, the smoking step is usually followed by infrared irradiation (Möhler, 1978). This process helps to avoid PAH contamination of foodstuffs.

3.2.4 Other smoke generation technologies

Other well-known smoke generation technologies include steam, fluidization, touch and smoldering smoke generators. Steam smoke is produced by passing superheated steam through chopped wood, inducing pyrolysis. The resulting smoke passes through the smoking chamber, being cooled to 80°C (Prändl, 1994; Tóth & Potthast, 1984). According to Müller (1982), the steam smoke generation temperature varies between 450 and 650°C. A fluidization smoke generator allows pyrolysis of wood shavings suspended in air that has been previously heated to 300-400°C. Pyrolysis is carried out within a reaction chamber and smoke and solid particles are separated on passing through a cyclone refiner (Klettner, 1979; Nicol, 1960; Prändl, 1994; Tóth & Potthast, 1984). The schematic representations of the different smoking generators are reported by Prändl (1994). Smoldering, steam and touch smoke generation systems for the production of Frankfurter-type sausages are described in detail by Pöhlmann et al. (2012, 2013a). In these technologies, smoking conditions are highly controlled and optimized, including different smoke densities (light, medium, and intensive), temperatures of smoke generation (ranging between 300 and 520°C), ventilator speeds (750, 1500, and 3000 rpm) and exposure times to smoke (from 3 to 40 minutes). The control of smoking conditions in these smoke generation technologies allows the prevention of final contamination of meat products with PAHs (Pöhlmann et al., 2013a).

4. Effect of smoking in food

4.1 Composition of smoke

The effect of smoking in food is defined by its composition. Smoke used in food production consists of a suspension of solid particles in a gaseous phase of air, carbon oxide, carbon dioxide, water vapor, methane, and other gases, making up an aerosol (Ahmad, 2003; CAC/RCP 68/2009; Šimko, 2009; Woods, 2003). The size of these particles generally ranges between 0.2 µm and 0.4 µm, and the total range is 0.05 µm-1 µm (CAC/RCP 68/2009). The main factors affecting smoke composition are the type of wood, the wood combustion temperature, the amount of water vapor available (or smoke house humidity), the amount of oxygen present, the effect of air flow rate, and the time of smoking (Woods, 2003). Temperature is the most important factor, and the constituents of wood, hemicellulose, cellulose and lignin, react at different temperatures (Ahmad, 2003; Woods, 2003). Table 2 summarizes the temperatures of decomposition of wood constituents (Ahmad, 2003; Woods, 2003).

Table 2. Temperatures of decomposition of wood constituents

Temperature (°C)	Thermochemical conversion processes of wood constituents
Up to 170	Drying
200-260	Pyrolysis of hemicelluloses
260–310	Pyrolysis of cellulose
310–500	Pyrolysis of lignin

Up to 1100 chemical compounds have been identified (Wilms, 2000). Wood smoke is composed by over 400 volatile components comprising 48 acids, 22 alcohols, 131 carbonyls, 22 esters, 46 furans, 16 lactones, 75 phenols, and some 50 miscellaneous compounds (Woods, 2003). Smoke compositions obtained from different types of wood have been described in detail (Ahmad, 2003; Hitzel et al., 2013; Stumpe-Vīksna et al., 2008).

Some main components of smoke condensates are shown in table 3 (Ahmad, 2003). Some different groups of smoke components are classified in table 4 (Ahmad, 2003; Guillén & Manzanos, 2002; Kostyra & Baryłko-Pikielna, 2006; Möhler, 1978; Ojeda, Barcenas, Pérez-Elortondo, Albisu, & Guillén, 2002; Woods, 2003).

Table 3. Composition of a smoke condensate (Ahmad, 2003).

Components	Percentage (%)
Phenols	0.07
Formaldehyde	0.12
Formic acid	0.38
Aldehydes of higher molecular weight	0.57
Ketones	0.67
Methanol	0.96
Acetic acidand acids of higher molecular Weight	1.71
Extracts from activated charcoal	4.08
Residue	4.21
Tar	4.81
Water	82.42
Total	100%

As table 4 shows, smoke contains around 20 groups of chemical compounds. However, these can be divided in 2 main groups depending on their desirable or non-desirable effects in the end product. The desirable compounds are responsible of good food preservation and flavouring, while the non desirable compounds are responsible of concerning about smoked food toxicity. These effects of smoking are described in the next sections.

Table 4. Chemical composition of smoke and technological effect.

MAIN GROUPS OF COMPOUNDS IN SMOKE		EFFECT ON MEAT PRODUCTS
Group 1 **CH series compounds**	Saturated and unsaturated aliphatic hydrocarbons (paraffin and olefins).	Low effect due to their low reactivity
Group 2 **CH series compounds**	Aromatic hydrocarbons (benzol, polyphenol).	Negative effects: Bad taste
Group 3 **COH_2 series compounds:** **Aliphatic compounds with one hydroxyl, alcohol and ethers:**	Methyl alcohol: precursor of formaldehyde and formic acid. Ethyl alcohol, propyl alcohol and iso propyl alcohol: oxidation to produce carbonyls and acids. Butyl alcohol and amyl alcohol. Fenyl alcohols (benzyl alcohols, phenethyl alcohol)	Non-desirable, toxic Desirable Desirable (oil aroma) Desirable (smell of roses)
Group 4 **COH_2 series compounds:** **Aromatic compounds with one hydroxyl, phenol**	Guaiacol, methylguaiacol, cresol and xinelone. Phenol Phenol derivates: Orthocresol, meta cresol, para-cresol Xylenols Thymol	Desirable, preservatives and flavoring Non desirable, bad taste Desirable in low quantities Good effects in preservation, smoke smell and color Strong antimicrobial attributes, biocide Fungicide effect Preservative Thyme aroma
Group 5 **COH compounds (carbonyls): Aliphatic aldehydes**	Formaldehyde	Desirable Hardening of natural casing Connective tissue Bactericide Food preservative
Group 6 **COH compounds (carbonyls): Aromatic aldehydes**	Benzaldehyde	Desirable, Bitter almond aroma
Group 7 **CO compounds: Aliphatic ketones**	Acetone and unsaturated long-chain compounds.	Undesirable aroma
Group 8 **CO compounds:** **Aromatic ketones**	Acetophenone Hydrindenes	Hay smell Soft aroma
Group 9 **COO Compounds: Alyphatic carboxylic acids**	Formic acid and acetic acid. Butyric acid, valerianic acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid. Unsaturated carboxylic acid (crotonic and tiglic)	Desirable for: Color setting, Good smell.Bactericidal effect Smell Desirable for color No desirable for taste

Table 4. (continued).

MAIN GROUPS OF COMPOUNDS IN SMOKE		EFFECT ON MEAT PRODUCTS
Group 10 **COO compounds: Aromatic carboxylic acids**	Benzoic acid Salycilic acid, gallic acid, toluic acid, phthalic acid, isophthalic acid, terephthalic acid	Specific action against the aerobic sporadic germ of the genus bacillus which contaminates meat products
Group 11: **Aromatic polyvalent hydroxy compounds**	Dihydroxybenzole Guayacol	
Group 12: **Hydroxy-oxo- compounds:** **Aliphatic hydroxyaldehydes and hydroxy ketones.**	Acetol	Desirable Reaction with meat proteins to develop the characteristic color of smoke
Group 13: **Hydroxy-oxo- compounds:** **Aromatic compounds, phenol aldehydes and phenol ketones**	Salicylaldehyde (2-hydroxybenzaldehyde), 4-Anisaldehyde, Vanillin and coniferaldehyde	Desirable: intense aroma and flavor of vanilla and conifers
Group 14: **Compounds with various oxo-groups:** **Di aldehydes and Di ketones**	Glyoxal Diacetyl	Casing hardening Margarine and bread color and smell
Group 15 **Compounds with 2 or more carboxyl groups:** **Saturated and unsaturated dicarboxylic acids**	Maleic acid	Desirable for color: pigment
Group 16 **Oxo- carboxy compounds : Keto acids**	Pyruvic acid, levulinic acid	
Group 17 **Nitrogenous organic compounds**	Pyrrole, pyrazine, indole, carbazole	Darkening of color
Group 18 **Non-aromatic cyclic compounds of C**	Cyclotene	Food aroma
Group 19 **Heterocyclic compounds**	Furan Lactone (Maltol)	Polymerization to obtain dark pigments. Good aroma. Flavor enhancer
Group 20 **Polycyclic aromatic hydrocarbons (PAH)**	Benzo(a)pyreno, PAH 4 (ΣPAH: Benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene.	Non-desirable in food science: Toxic, carcinogenic

4.2 Food preservation

Preservation has been the main objective of food smoking during several years, when the refrigeration chain was not established yet (Marianski et al., 2009), and it is still the main aim in some developing countries (FAO-Thiaroye, 2015; Vaz-Velho, 2003). As table 3 shows, the main component of smoke is water, so the reason of the preservation quality of smoking is not only this high component of smoke (Möhler, 1978). The main ways of smoking for food preservation are drying and the presence of some desirable components in smoke. During drying the water activity of the food decrease. Besides some components, such as thymol, formaldehyde, formic, acetic and benzoic acids, orthocresol, meta-cresol, para-cresol, guaiacol, methylguaiacol, cresol and xinelone have a desirable bactericidal, antimicrobial, biocidal, fungicidal and preservative effects. Both properties limit the growth of some types of microorganisms (Vaz-Velho, 2003). The antimicrobial activity of the different components of liquid smoke has been reviewed (Lingbeck et al., 2014). Some phenolic compounds, especially isoeugenol, in addition with the acids, have an antimicrobial activity on L. monocytogenes (Suñen, 1998; Young & Foegeding, 1993). Not all the phenols have the same property, because phenol concentration is not indicative of the antimicrobial activity against L. monocytogenes (Suñen et al., 2001). On the other hand, organic acids and carbonyls have been found to have more antilisterial and bacteriostatic properties than phenols (Milly, Toledo, & Chen, 2008; Montazeri, Himelbloom, Oliveira, Leigh, & Crapo, 2013). Liquid smoke has demonstrated to be bactericidal against Salmonella Typhimurium (Kim, Kang, Park, Nam, & Friedman, 2012) and E. coli (Van Loo et al., 2012). Liquid smoke has also been found to reduce Staphylococcus aureus and staphylococcal enterotoxins in food (Taormina & Bartholomew, 2005).

4.3 Food flavouring

Nowadays food flavouring is the main aim of smoking in the developed countries. A controlled process of smoking has a desirable effect in the food colour, texture, smell and taste.

4.3.1 Colour

Smoking is applied to improve the colour of the food. Several studies are focused on the changes on the colour parameters, lightness (L*), redness (a*), yellowness (b*), browning index (BI), hue angle, chroma, and total colour change (ΔE), of different food during smoking, even with

different smoke generation methods (Pöhlmann et al., 2013a). These studies include, smoked meat products, such as chorizo (Gimeno, Ansorena, Astiasarán, & Bello, 2000), a turkish dry-fermented sausage named Sucuk (Bozkurt & Bayram, 2006), Frankfurter-type sausages (Pöhlmann et al., 2013) or smoked blood sausages (Silva et al., 2013), smoked fish products, such as smoked Atlantic salmon (Salmo salar L.) (Birkeland, Bencze Rørå, Skåra, & Bjerkeng, 2004; Cardinal et al., 2001) and smoked cheeses, such as smoked Cheddar and Swiss cheeses (Hendrick, Bratzler, & Trout, 1960; Riha & Wendorff, 1993). The common characteristic of any smoked food is a decrease in L* (lightness). The main changes in the colour of any type of food are produced in their external surfaces. It has been found that the greatest number of soot particles is deposited in the external surface of the smoked food (Ledesma et al., 2015b). This fact explains the lightness decrease during food smoking.

4.3.2 Texture

Smoking has an important effect on food texture. Softness or hardness can be considered a good or a bad attribute, depending on the type smoked food. For instance, softness or tenderness can be a positive attribute in some types of chorizo (like "chorizo asturiano"), while in others (like ''chorizo de Pamplona") it can be considered a defect (Gimeno, Astiasarán, & Bello, 1999; Spaziani, Del Torre, & Stecchini, 2009). The final texture of the food can be defined by controlling the smoking time and other parameters of the manufacturing process. Some studies have been conducted with this purpose, and in different types of smoked food, mainly in meat products (Kim et al., 2014), fishes (Birkeland et al., 2004; Cardinal et al., 2001), and cheeses (Adhikari et al., 2003). The main determinants of smoked food texture are the extent and rate of water loss, the fat content of the product and its distribution, the extent of denaturation of structural and connective tissue protein, and the extent of autolysis, particularly proteolysis (Woods, 2003). Temperature of smoking also has an important role in the texture of the final product. The hardness of the food generally increases with the smoking time and the temperature. European cold-smoked products generally have a soft and tender texture, while the equivalent hot-smoked products have a hard dry surface with softer interior (Woods, 2003).

4.3.3 Flavour

Flavour is a sensory impression perceived by means of at least three different senses: aroma perception in the olfactory epithelium, taste perception predominantly on the tongue and

chemesthesis of 'irritants' in the areas of the nose, eyes and mouth (Methven, 2015). Smoke components and smoking temperature, time and technique have an important effect on food flavour.

Among all the groups of compounds produced during wood combustion, some flavouring compounds (Möhler, 1978) are cited in table 4. These include butyl alcohol and amyl alcohol (oil aroma), fenyl alcohols, like nenzyl alcohols and phenethyl alcohol (smell of roses) and benzaldehyde (bitter almond aroma) (Möhler, 1978). Several studies about the volatile compounds in smoke flavourings, its sensory characterization and descriptors have been done (Kostyra & Baryłko-Pikielna, 2006; Ojeda et al., 2002; Pino, 2014). The aroma associated with a specific compound produced during smoking is cited in some works, such as Möhler, 1978. However, while the single flavouring volatile compounds can be separated, identified and quantified by means of instrumental analysis, its sensory analysis and characterization is more problematic. The aroma, taste or smell of a smoke flavouring cannot be related only with one compound (Kostyra & Baryłko-Pikielna, 2006). However, some compounds seem to have greatest effect, and have been selected as sensory descriptors and standard references of the smoke flavourings (Ojeda et al., 2002), such as nerolidol for fruity, ethylbenzene for combustible, propionic acid for sharp, geraniol for floral, cyclotene for caramellic, maltol for sweet, isobutyric acid for pungent, acetic acid for acidic, thymol for wood, guaiacol for medicinal, eugenol for spice/aromatic herb, and 1-Octen-3-ol for musty flavors (Ojeda et al., 2002). Carbonyls contribute significantly to smoke-curing aroma, but phenols have been confirmed as the main (but not exclusive) contributor to that specific flavor. Phenol, p-cresol and ocresol (phenolic compounds) and cycloten and 3-methylocyclopenten (carbonyls) seem to play the most important role in smoke-curing aroma. Syringol and its derivatives do not contribute to the odour identified as typical for freshly smoked product (Kostyra & Baryłko-Pikielna, 2006). Besides, the amount of the compound must be enough to reach the flavor threshold of the consumer (Woods, 2003).

The flavor and smell of smoked food is affected by the smoking temperature, time and technique. The smoky flavor and smell are common characteristics of all smoked food. However, specific organoleptic profiles of different types of smoked food have been described. Among the smoked meat products, interesting works have been done in the sensory evaluation of Frankfurter-type sausages. The odour, flavor and complete sensory evaluation of Frankfurter-type sausages smoked by means of smouldering, steam, friction, and touch smoking methods

increases with smoking density (from lightly to intensively and medium) and time (from less than 30 minutes to more than 30 minutes) (Pöhlmann et al., 2012, 2013a). The ventilator velocity or the moisture of the beech wood chips have not significant influence on the sausages flavor (Pöhlmann et al., 2012). Temperatures bellow 500ºC and higher than 750ºC produce excessive and insufficient smoke flavours in sausages, respectively (Pöhlmann et al., 2012). Interesting studies about the organoleptic profile of smoked fish have been done. The intensity of smoke odour and flavor of smoked salmon is affected by the smoking temperature or technique. A higher temperature increases the deposit of smoke compounds, and the smoky flavor. Special toasted bread odour and low flavour intensity is produced when this fish is smoked by the electrostatic technique (Cardinal et al., 2001). A model to define the parameters affecting cold-smoked salmon odour and flavour intensity has been proposed (Cardinal et al., 2004). Among all the selected parameters, including remaining time, phenol, lipid, total volatile basic nitrogen, trimethylamine, and salt contents, phenol content has the major influence on smoke odour and flavor of cold-smoked salmon (Cardinal et al., 2004). Several studies about smoked cheeses and beverages flavour have been done. Smoked Cheddar cheeses are characterized by specific smokey and skunky flavors, compared to non smoked Cheddar cheeses. Non smoked Cheddar cheeses exhibited higher intensities of “cooked,” “whey,” “milkfat,” “sweet” and “nutty” flavors than the smoked cheeses (Shakeel-Ur-Rehman et al., 2003). Distinctive smoky and medicinal aromas are conferred to the whisky when the malt is smoked (Bringhurst & Brosnan, 2014; Jack, 2012).

5. Smoked food toxicology

Smoking improves food color, texture, flavor and final value, prices can be reduced and shelf life can be extended (FAO, 2014a; Ledesma et al., 2015b). However, non-desirable substances can be produced during smoking, damaging the final composition of food. The main toxic compounds on smoked food are discussed in this section.

5.1 Polycyclic aromatic hydrocarbons

5.1.1 Toxicity

Polycyclic aromatic hydrocarbons (PAH) are the best known toxic substances on smoked food. Research about PAH is focused on its carcinogenic activity. The first evidence of PAH carcinogenic activity was discovered in 1775 by Percival Pott of St Bartholomew's Hospital in London. He noticed that sweeps who cleaned the chimneys removing soot developed cancer (Šimko, 2002). Since that moment several studies have been done, and the carcinogenic, mutagenic and bioaccumulative capacities of PAH have been reported by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), (WHO, 2006), the International Agency for Research on Cancer (IARC), the European Scientific Committee on Food (SCF), (SCF, 2002), the European Food Safety Authority (EFSA) and the US Environmental Protection Agency (EPA) and the Department of Health and Human Services (ATSDR, 1995; ATSDR, 2009).

5.1.2 PAH contamination mechanism

The mechanism of food contamination by PAH during smoking process has been described in detail (Ledesma et al., 2015b). Smoked food can be PAH contaminated by three ways: "Pre-contamination" of raw materials due to atmospheric pollution (CAC/RCP 68/2009), food technology processes other than smoking, such as packaging, drying, grilling, roasting (Chung et al., 2011), baking, barbecuing (Kazerouni, Sinha, Hsu, Greenberg, & Rothman, 2001) and frying, and mainly during the smoking process. During smoking the biomass conversion takes place. About 750°C tar aerosols containing PAH are produced (Basu, 2010; Li et al., 2009; Liu et al., 2013; McGrath, et al., 2001; Simoneit, 2002). Then PAH are transported through the smoking chamber and reach products contaminating food (CAC/RCP 68/2009; Ledesma et al., 2015b). Moreover, the fat content of the food falls down onto the fire, thereby increasing the PAH content of the smoke (Chung et al., 2011; Janoszka, Warzecha, Blaszczyk, & Bodzek, 2004). On the other hand, some fat already contaminated falls onto other food, increasing its final PAH content (CAC/RCP 68/2009; Viegas, Novo, Pinto, Pinho, & Ferreira, 2012). The greatest amount of soot particles and PAH is deposited in the external surfaces of food, such as the rind of cheese (Guillén & Sopelana, 2004) or the casing of meat products (Ledesma et al., 2014, 2015b; Santos et al., 2011), then the PAH migrate into the food. Natural casing morphology and physical

characteristics (Ledesma et al., 2015b) facilitate PAH contamination and penetration into the smoked meat products.

5.1.3 Regulation

To protect consumers against PAH intake from diet, the European Commission (EC) has adopted several regulations over the last 10 years. The names, abbreviations, relative molecular weights and chemical structures of the 16 priority polycyclic aromatic hydrocarbons (PAH) regulated food products by the European Union are cited in the table 5.

In accordance with the European Scientific Committee on Food (SCF) (SCF, 2002), PAH levels in food have been regulated via EC Regulation No 208/2005 and European Union (EU) Commission Regulations No 1881/2006 and No 835/2011. In accordance with EU regulation No 835/2011 (table 6) two dates must be considered as regards PAH contamination in smoked food since 1/9/2014, a separate level for benzo(a)pyrene (BaP), the main traditional marker, and a new maximum level for the sum of four substances known as "PAH4", BaP, benzo(a)anthracene, benzo(b)fluoranthene and chrysene (EC, No 835/2011). The regulation should continue be reviewed. A recent study by Lorenzo et al. (2011) reports that BaP is still a good marker for the sum of 15 PAH as well as for 7 PAH classified as probable human carcinogenics by the USEPA in 'chorizo gallego'. There is not a European PAH limit for some smoked food, such as cheese, beer, whisky and some spices, such as paprika. The Spanish law established a BaP limit of 10 µg/kg in the smoked cheese rind (BOE, 1985; Guillén, Palencia, Sopelana, & Ibargoitia, 2007), and the US EPA established a BaP limit of 0.2 µg/kg in ambient water (ATSDR, 2009; EPA, 2013). On the other hand, the regulation establishes PAH limits for non smoked food, such as oils (including coconut oil) and fats, cocoa beans and derived products, processed cereal-based food and baby food for infants and young children, and infant formulae and follow-on formulae, including infant milk and follow-on milk.

Table 5. Name, abbreviations, relative molecular weights and chemical structures of the 16 polycyclic aromatic hydrocarbons (PAH) regulated in meat products by the European Union.

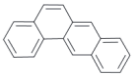
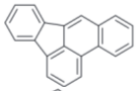
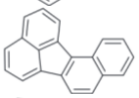
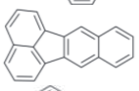
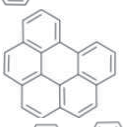
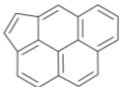
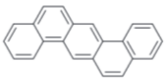
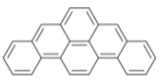
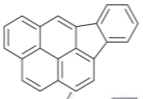
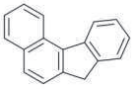
PAH compound	Abbreviation	Molecular weight	Chemical structure
Benzo(a)pyrene	BaP	252	
Benz(a)anthracene	BaA	228	
Benzo(b)fluoranthene	BbF	252	
Benzo(j)fluoranthene	BjF	252	
Benzo(k)fluoranthene	BkF	252	
Benzo(g,h,i)perylene	BghiP	276	
Chrysene	Ch	228	
Cyclopenta(c,d)pyrene	CPP	226	
Dibenz(a,h)anthracene	DBahA	278	
Dibenzo(a,e)pyrene	DBaeP	302	
Dibenzo(a,h)pyrene	DBahP	302	
Dibenzo(a,i)pyrene	DBaiP	302	
Dibenzo(a,l)pyrene	DBalP	302	
Indeno(1,2,3cd)pyrene	IP	276	
5-methylchrysene	5MeCh	242	
Benzo(c)fluorine	BcF	216	

Table 6. Maximun legal limits for benzo(a)pyrene and PAH4 in smoked foods fixed by EC Regulation 835/2011

Smoked food	Maximum levels (µg/kg)	
	Benzo(a)pyrene	PAH4[a]
Smoked meat and smoked meat products	5.0[b] 2.0[d]	30.0[c] 12.0[d]
Muscle meat of smoked fish and smoked fishery products *(The maximum level for smoked crustaceans applies to muscle meat from appendages and abdomen. In case of smoked crabs and crab-like crustaceans (Brachyura and Anomura) it applies to muscle meat from appendage)*	5.0[b] 2.0[d]	30.0[c] 12.0[d]
Smoked sprats and canned smoked sprats (Sprattus Sprattus). Bivalve molluscs (fresh, chilled or frozen) Heat treated meat and heat treated meat products sold to the final consumer	5.0	30.0
Smoked bivalve mollusks	6.0	35.0

[a] PAH4: Sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene
[b] until 31/8/2014
[c] from 1.9.2012 until 31.8.2014
[d] from 1/9/2014 on

5.1.4 PAH in smoked food

Humans are exposed to PAH due to environmental contamination, but the main source of PAH is diet (Falcó et al., 2003; Ibáñez et al., 2005; Lodovici, Dolara, Casalini, Ciappellano, & Testolin, 1995; Phillips, 1999), contributing to more than 70% of total exposure in nonsmokers (Gilbert, 1994; McGrath, Wooten, Geoffrey Chan, & Hajaligol, 2007). The PAH contamination of smoked food has been studied for a long time. Table 7 shows recent data about PAH in different types of smoked food around the world, including smoked meat products, fish, cheese and some teas.

Table 7. PAH in smoked food

World Smoked Food	PAH (µg/kg)				Reference
	BaP		PAH		
	Maximun	Minimiun	Maximun	Minimun	
Smoked meat products from					
Africa	10.02 smoked pork	<0.1 smoked beef and chicken			Olatunji et al., 2014
Estonia	31.20 smoked meat products	<0.3 various products	PAH[a]: 8.0 smoked ham	PAH[a]: 5.7 smoked chicken	Reinik et al., 2007
Italy	0.8 smoked Pitina	<0.05 smoked bacon, smoked pork and beef meats			Purcaro et al., 2009
Latvia	6.03 smoked pork	<0.05 various products	PAH4: 34.65 smoked pork speck	PAH4: 0.15 smoked pork	Rozentāle et al., 2015
Portugal	0.63 smoked Paínho	0.32 smoked morcela	PAH4: 6.94 smoked chouriço mouro	PAH4: 1.84 smoked morcela	Santos et al., 2011
Republic of Korea	0.08 smoked sausages	0.01 processed products	PAH[b]: 1.09 Bacon	PAH[b]: 0.15 processed products	Chung et al., 2011
Serbia	1.09 smoked beef ham	0.24 smoked cajna sausage	PAH[c]: 21.3 smoked beef ham	PAH[c]: 4.4 smoked cajna sausage	Djinovic et al., 2008
Spain	3.21 ± 0.12 smoked chorizo	0.38±0.08 smoked chorizo			Ledesma et al., 2015a
Sweden	36.9 smoked ham	n.d various smoked products	PAH4:209 smoked ham	PAH4: n.d various	Wretling et al., 2010

Table 7. (continued).

World Smoked Food	PAH (µg/kg)				Reference
	BaP		PAH		
	Maximun	Minimiun	Maximun	Minimun	
Smoked fish from					
Africa (Republic of Ghana)	73.78 ± 11.44 (max) 52.00 ± 18.88 µg/kg (mean) smoked herrings	3.36 ± 4.52 (mean) smoked herrings	PAH[d]: 1461.79 smoked herrings	PAH[d]: 510.59 smoked herrings	Essumang et al., 2012
Denmark	11.4 smoked cod roe	<0.2 Various	PAH[e]: 78 smoked cod roe	PAH[e]: 1.1 smoked trout	Duedahl-Olesen et al., 2010
Iran	7.56 H. molitrix smoked fish	<LOQ	PAH4: 12 smoked fish	PAH4: 3 smoked fish	Mohammadi,Ghasemzadeh-Mohammadi, Haratian, Khaksar, & Chaichi, 2013
Nigeria	38.0 smoked mudfish	3.0 smoked Jackfish	PAH8[f]: 197.2 smoked mudfish	PAH8[f]: 44.1 smoked Jackfish	Akpambang et al., 2009
Sweden	14.4 smoked herring	n.d salmon	PAH4: 81.2 mackerel fillet	-	Wretling et al., 2010
CTUIR[*]	>35 smoked salmon	<2 smoked salmon	PAH[g]: 110 ± 89 smoked salmon	PAH[g]: 26 ± 17 smoked salmon	Forsberg et al., 2012
Smoked cheese					
Circassia/Turkey	1.52 smoked Circassian cheese	n.d smoked Circassian cheese	PAH[h]:3.51 smoked Circassian cheese	PAH[h]:0.11 smoked Circassian cheese	Gul et al., 2015
Italy	417.8 rind of mozzarella smoked with corrugated cardboard	n.d smoked mozzarella	PAH4: 1892.5 rind of mozzarella smoked with corrugated cardboard	PAH4: n.d	Esposito et al., 2015
Spain	0.52 ± 0.03 smoked sheep cheese rind	n.d smoked cheese	PAH[i]: 1,037.23	PAH[i]: 36.31	Guillén & Sopelana, 2004
Spain	0.51 smoked Palmero cheese rind	n.d smoked Palmero cheese	PAH[i]: 739.55 smoked Palmero cheese	PAH[i]: 29.21 smoked Palmero cheese	Guillén et al., 2007

Table 7. (continued).

World Smoked Food	PAH (µg/kg)				Reference
	BaP		PAH		
	Maximun	Minimiun	Maximun	Minimun	
Smoked beverage					
Tea from different manufactureres	n.d smoked black tea	n.d smoked black tea	PAH4: 2.7 smoked black tea	PAH4: <0.4 smoked black tea	Pincemaille, Schummer, Heinen, & Moris, 2014
Tea from different manufactureres	237 mate tea	0.8 black tea and herbal and fruit tea	PAH[j]: 1,997.9 mate tea	PAH[j]: 13.8 herbal and fruit tea	Ziegenhals, Jira, & Speer, 2008
Tea from different manufacturers	73.2 ± 8.5 black tea (Kayuaro)	n.d other types of tea	PAH[k]: 628.8 (smoked) Oolong tea	PAH[k]: 55.4 black tea (Darjeeling)	Ishizaki, Saito, Hanioka, Narimatsu, & Kataoka, 2010
Tea from different manufacturers	460 Lapsang souchong tea	n.d other types of tea	PAH4: 1700 Lapsang souchong tea	PAH4: n.d other types of tea	Schulz, Fritz & Ruthenschrör, 2015
Tea from different manufacturers	380 mate tea	n.d other herbal and fruit tea	PAH4: 1400 mate tea	PAH4: n.d other herbal & fruit tea	Schulz et al., 2015

a: ΣPAH: BaA, BbF, BjF, BkF, BghiP, BaP, Ch, DBahA, DBaeP, DBalP, IP, 5MeCh.
b: ΣPAH: PAH: CHR, BbF, BkF, BaP, DBahA, BghiP, IP
c: ΣPAH: BcL, BaA, CPP, CHR, 5MC, BbF, BjF, BkF, BaP, BgP, DhA, IcP, DeP, DhP, DiP, DlP
d: ΣPAH: Na,Ap,Ac,F, A, Pa, Fl,P,BaA,BkF,IP, BbF,Ch, BaP, BghiP, DBahA
e: ΣSCF: BaA, CHR, CPP, 5MeCHR, BbF, BjF, BkF, BaP, IND, DBahA, BghiP, DBalP, DBaeP, DBaiP, DBahP.
f: ΣPAH8: BaA,Ch,BbF, BkF, BaP,DBahA, BghiP, IP
g: ΣPAH: BaP and relative potency factor adjusted of Fl, BbF, BaA, Ch, BkF
h: ΣPAH5: BaA, BkF, BghiP, BaP, DBahA
i: ΣPAH: sum of more than 50PAH
j: ΣPAH: BcL BaA, CHR, TP, CPP, 5MC, BbF, BkF, BjF, BaP, IcP, DhA, BgP, DlP, DeP, DiP, DhP
k: ΣPAH: Na, Ac, Fl, Pa, A, Fl, P, BaA, Chr, BbF, BkF, BaP, DaHA, BghiP, IP
*The Confederated Tribes of the Umatilla Indian Reservation (CTUIR), Columbia River Basin Traditional Native American.

As table 7 shows, PAH have been found in all types of smoked food. Particular attention has traditionally been paid to smoked meat products, because the highest levels of total PAH have been detected in these food (Gomaa, Gray, Rabie, Lopez-Bote, & Booren, 1993; Karl and Leinemann, 1996; Larsson, Pyysalo, & Sauri, 1988; Martorell et al., 2010) and because they are the major contributors to PAH dietary intake in some developed locations (Martorell et al., 2010). However low values of below 1 µg/kg have recently been reported in smoked meat products from developed countries (Jira, Ziegenhals, & Speer, 2006; Purcaro et al., 2009; Roseiro et al., 2012; Santos et al., 2011). The legislation and some modern smoking techniques, such as liquid smoke or indirect smoking methods help to reduce the PAH content of smoked food in these countries.

From a global perspective, the Codex Alimentarius states that the major contributors to PAH intake are cereals and cereal products (owing to high consumption in diets) and vegetable fats and oils (due to higher concentrations of PAH in this food group) (Codex Alimentarius CAC/RCP 68/2009). As table 7 shows, special attention should be paid in the developing countries and some ancient societies, because of the high amounts of PAH that have being reported in smoked food from these locations, such as 73.78 ± 11.44 µg/kg of BaP in smoked herrings in Africa (Essumang, Dodoo, & Adjei, 2012), or more than 35 µg/kg in smoked salmon in traditional native American tribes (Forsberg et al., 2012). The National Training Centre for Fisheries and Aquaculture Technicians (CNFTPA) of Senegal in collaboration with FAO have designed and developed the FTT-Thiaroye technique. This technique and its diffusion help to minimize the PAH content of the smoked food in the developing countries (FAO-Thiaroye, 2015).

As table 10 shows, a high level of PAH has been found in some smoked teas, such as 237, 73.2 ± 8.5, and 460 µg/kg of BaP in mate (Ziegenhals et al., 2008), Kayuaro black tea (Ishizaki et al., 2010), and Lapsang souchong tea (Schulz et al., 2014), respectively. Smoked food are not the only contributors to PAH in the diet, PAH have been detected in roasted and toasted food, like coffee (Houessou, Delteil, & Camel, 2006), toasted bread (Rey-Salgueiro, García-Falcón, Martínez-Carballo, & Simal-Gándara, 2008), and non processed food, such as vegetables, eggs, oils, shellfish, fruits or milk (Martorell et al., 2010), probably because of environmental contamination (CAC/RCP 68/2009; Rodríguez-Acuña, Pérez-Camino, Cert, & Moreda, 2008).

5.1.5 Prevention

PAH contamination in smoked food can be prevented, maintaining the beneficial effects of smoking. The "Code of practice for the reduction of contamination of food with (PAH) from smoking and direct drying processes" (CAC/RCP 68/2009) provides the variables that need to be controlled to minimize the PAH contamination of smoked food. The variables have been studied by several researchers in different smoked food. They include the kind of fuel, studied in smoked meat (García-Falcon & Simal-Gándara, 2005; Hitzel et al., 2013; Pöhlmann et al., 2012; Stumpe-Vīksna et al., 2008) and smoked cheese (Conde, Ayala, Afonso, & González, 2005; Esposito et al., 2015; Guillén et al., 2007), smoking method, studied in smoked meat (Pöhlmann et al., 2012, 2013a; Škaljac et al., 2014), smoked fish (Duedahl-Olesen, Christensen, Højgård, Granby, & Timm-Heinrich, 2010; Hattula, Elfving, Mroueh, & Luoma, 2001; Karl & Leinemann, 1996; Varlet et al., 2007; Varlet, Serot, Monteau, Le Bizec, & Prost, 2007), and smoked cheese (Aydinol & Ozcan, 2013; Esposito et al., 2015), smoking duration, studied in smoked meat (Djinovic et al., 2008; Ledesma et al., 2014), and fish food (Essumang et al., 2013) fat content, studied in smoked meat products (Gomes et al., 2013; Pöhlmann et al., 2013b) and its evolution during processing (Ledesma et al., 2015b), distance and position between the food and the heat source, studied in smoked meat products (Pöhlmann et al., 2013b) and smoked cheeses (Guillén et al., 2011), cleanliness and maintenance of equipment, and the design of the smoking chamber and the equipment (Gomes et al., 2013; Pöhlmann et al., 2013a). The casing type is also an important and recently studied variable (García-Falcon & Simal-Gándara, 2005; Gomes et al., 2013; Ledesma et al., 2015b; Pöhlmann et al., 2013b; Škaljac et al., 2014).

Among all the variables recommended by CAC/RCP 68/2009, three seem to have a greater influence on preventing PAH contamination: the temperature of smoke generation, the type of casing and the smoking method (direct or indirect). Temperature is the most important variable to be considered (Pöhlmann et al., 2012). A smoke generation temperature below 600°C should be applied to prevent the formation of PAH (Pöhlmann et al., 2012). The use of synthetic instead of natural casings prevents PAH from penetrating inside smoked food (Garcia-Falcón & Simal-Gándara, 2005; Gomes et al., 2013; Ledesma et al., 2015b; Skaljac et al., 2014). Indirect smoking systems (e.g., friction or liquid smokes) can highly reduce the PAH content of food (Hattula et al., 2001; Pöhlmann et al., 2013a; Varlet et al., 2007). On the other hand, the control of other variables such as wood (never gasoline, treated woods or wasted oil), smoking time, fat

content, some types of pretreatment such as marinating (Farhadian, Jinap, Faridah, & Zaidul, 2012), microwave preheating, and aluminum wrapping (Farhadian, Jinap, Hanifah, & Zaidul, 2011), and the addition of onion and garlic as meat additives (Janoszka, 2011), can help to reduce the final PAH content of grilled and smoked food.

5.2 Other toxic compounds

During the food smoking process other toxic compounds are produced, such as formaldehyde, N-Nitrosamines, heterocyclic aromatic amines, β-carbolines and β-carbolines. These compounds have toxic effects on smoked food and could also contaminate the ambient air of the food factories.

5.2.1 Formaldehyde

Formaldehyde (CH_2O) is an important compound for the global economy, widely used in construction, wood processing, furniture, textiles, carpeting, and in the chemical industry (Tang et al., 2009). Formaldehyde is naturally present in the environment, some food and in the human body (IARC, 2006). However, some industrial processes and food processing methods such as smoking increase the formaldehyde content up to contaminant levels (IARC, 2006). Formaldehyde can be found in several non smoked food, including fruit, vegetables, milk, beverages or eggs because of environmental contamination of raw materials (Feron et al., 1991; IARC, 2006), but its concentration could be increased during cooking processes at high temperatures and because of the exposition to smoke from wood combustion (Herrmann, Granby, & Duedahl-Olesen, 2015). Formaldehyde has bacteriostatic and bactericidal effects on smoked food (Rørvik, 2000), that help to prolong the products shelf-life (Goulas & Kontominas, 2005). However, it is carcinogenic to humans (IARC, 2006; Vincent et al., 2005). Formaldehyde is added inappropriately in some food because of its preservative effect (Tang et al., 2009; Wang, Cui, & Fang, 2007; Zhao & Zhang, 2009). Formaldehyde as additive in food, in animal feeding, and as a contaminant in fermented alcoholic beverages is regulated by the U.S. Food and Drug Administration (FDA) (EPA, 1989, 2007), the European Union (EU, 2004/C 50/01) and China (MOH, 2005), respectively. A few research works have been focused on the formaldehyde contamination of smoked food. A high concentration of formaldehyde, up to 125 mg/kg wet weight, has been found in smoked meat products (Zhu, Peng, Wang, Wang, & Rui, 2012). A lower concentration of formaldehyde has been found in smoked fish, such as 855 ± 56

µg/kg in smoked salmon (Gosetti et al., 2011). The effect and toxic contamination of formaldehyde in smoked fish (Varlet, Prost, & Serot, 2007) and other smoked food should be further investigated.

5.2.2 N-Nitrosamines

N-Nitrosamines (NAs) are a group of compounds, of which many have been classified as genotoxic and probable human carcinogens (IARC, 1978). Several factors affect the NAs contamination of food. The most known factor is the addition of nitrite (E 249–E 250) or nitrate (E 251–E 252) during salting, and smoking processes (Herrmann, Duedahl-Olesen, Christensen, Olesen, & Granby, 2015). There is a positive though not necessarily linear correlation, between the amount of nitrite added and the amount of NAs formed (Drabik-Markiewicz et al., 2009, Drabik-Markiewicz et al., 2011; Yurchenko & Mölder, 2007). According to the European Food Safety Authority (EFSA) opinion, expressed on 26 November 2003, the maximun levels of nitrites and nitrates allowed to add to food products have been reduced by the Directive 2006/52/EC, in order to keep the level of nitrosamines as low as possible (EC, 2006/52). Other factors have an important, even more significant, role in the NA content of the final products, such as some precursors added via wood smoke, spices or other ingredients (Herrmann et al., 2015), and the heat applied during drying and smoking processes (Herrmann, Duedahl-Olesen, & Granby, 2015). Some nitrosamines, such as N-nitroso-thiazolidine-4-carboxylic acid (NTCA) and N-nitroso-2-methyl-thiazolidine 4-carboxylic acid (NMTCA) can be found in smoke (Massey et al., 1991) and its production increases by increasing the temperature during the smoking process (Herrmann et al., 2015). Several works have been focused in the determination of NAs in smoked food, such as smoked meat products (Yurchenko, & Mölder, 2007), smoked fish (Herrmann et al., 2015; Yurchenko, & Mölder, 2006), smoked beverages like Rauchbier beer (Mangino, 1981), even in smoked oysters (Lijinsky, 1999). A high concentration of various nitrosamines has been found in smoked food, such as 32 µg/kg of dimethylamine in smoked pickled fish, 10 µg/kg of pyrrolidine in smoked meat, 9 µg/kg of piperidine in spiced smoked meat, 2100 and 167 µg/kg of proline in smoked pork and smoked oyster, respectively (Lijinsky, 1999).

5.2.3 Heterocyclic aromatic amines

Heterocyclic aromatic amines (HAAs) are an important group of food mutagens in the Ames/salmonella assay. These compounds are potential dietary carcinogens in rodents and primates (Ka-Wing et al., 2006). The factors affecting the content of HAAs in food have recently been reviewed (Alaejos & Afonso, 2011). HAAs are formed during heating processes, thereby including food smoking process (Szterk & Waszkiewicz-Robak, 2014). Its formation mainly depends on the process temperature (Alaejos & Afonso, 2011), but other factors, such as cooking time and method (including smoking), concentration of precursors, presence of enhancers or inhibitors, amount of lipids or water and pH have also an important role (Bordas, Moyano, Puignou, & Galceran, 2004; Juhee & Ingolf, 2005; Ristic, Cichna, & Sontag, 2004; Skog, Eneroth, & Svanberg, 2003). Several studies have been focused on the formation and prevention of HAAs in grilled and roasted and grilled food (Alaejos & Afonso, 2011). A few studies are focused on the HAAs content of smoked food, such as smoked pork rib (Knize et al., 1998), smoked fish, such as salmon and flounder (Johansson & Jägerstad, 1994) and smoked cheeses, like Provola (Naccari et al., 2009). Like PAH (Guillén & Sopelana, 2004), highest levels of HAAs were found in the cheese rind, the part in direct contact with smoke during the smoking processes, and then could migrate via the fats to reach the interior part of the smoked cheese, where less concentration was found (Naccari et al., 2009). Some authors have studied the role of smoke flavourings in HAAs production and concentration (Solvakov et al., 1999; Stavric et al., 1997). Traditional smoking techniques contribute to the final HAAs content of the food (Naccari et al., 2009), while the use of filters or liquid smoke flavourings reduces the amount of HAAs in the final product by almost 100% (Naccari et al., 2009; Simon, De la Calle, Palme, Meier, & Anklam, 2005). Liquid smoke seems to be a safe technique to prevent HAAs contamination of smoked food. In fact, the composition of smoke flavor is regulated by the European Parliament and Council Regulation EC No 2065/2003, and no reference to HAs is mentioned (EC, 2065/2003; Naccari et al., 2009).

5.2.4 β-carbolines

β-carbolines are a group of compounds that lead to toxic activity on food. The genotoxic potential and co-mutagenic properties these compounds have been reviewed (De Meester, 1995). Some β-carbolines interact with various macromolecules and enzymatic systems and may modify and increase the genotoxic and toxic consequences of other toxic compounds cited here, such as PAH or n-nitrosamines (De Meester, 1995). The smoking process can increase the

concentration of these compounds in foodstuff (Papavergou & Clifford, 1992). Smoked products appear to be one of the foodstuffs with the highest amount of some β-carbolines, such as tetrahydro-b-carboline (Papavergou & Herraiz, 2003). Some studies (Papavergou & Herraiz, 2003; Sen, Seaman, Lau, Weber & Lewis, 1995) have been focused on the presence of some β-carbolines in smoked food. The main β-carboline in smoked food is 1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid, which concentration ranges between 0.03–12.2 μg/g, 0.07–6.06 μg/g, and 0.01–14.8 μg/g in smoked fish, smoked cheeses and smoked sausages and meats, respectively (Papavergou & Herraiz, 2003). Like PAH (Guillén & Sopelana, 2004) and HAAs (Naccari et al., 2009) the highest levels of this contaminant were found in the external part or the products, which is in direct contact with smoke during the smoking processes (Papavergou & Herraiz, 2003).

6. Conclusions

Smoked food are one of the most ancient preserved food and have had an important role on humans diet and economical markets. The main smoked food are smoked meat, fish and cheese, but smoking is also applied to some beverages and spices. Nowadays the smoking technique has been improved and can be replaced, in developed countries, by more controlled and optimized indirect smoking methods, such as liquid smoke and smoke produced by friction generation, and other methodologies for fresh food preservation. Several compounds are produced during smoking, conferring desirable and undesirable effects on the smoked food. The desirable effects are food preservation, which is still the main aimed effect in developing countries, and the improvement of food organoleptic profile, colour, texture and flavor, which is nowadays the main aimed effect in developed countries. The main undesirable effect of smoking is the contamination of food by toxic and carcinogenic compounds, such as PAH, n-nitrosamines, heterocyclic aromatic amines and β-carbolines. These compounds are produced in the traditional smoking processes and are mainly accumulated in the surface of the food, such as the meat product casing and the cheese rind. Thereby the external surface of smoked food should be removed before the consumption. The application of the modern smoking technologies and the direct smoking process in a controlled way, accordingly to Codex Alimentarius (CAC/RCP 68/2009), let maintain the desirable effects and reduce or prevent the contamination of smoked food by toxic compounds. This is especially important in developing countries, where direct smoking is still applied in the traditional way as the main food preservation method.

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bool.html%2526r%3D2%2526f%3DG%2526l%3D50%2526co1%3DAND%2526d%3DPALL%2526s1%3DRasmussen.INNM.%2526s2%3DSMOKE.TI.%2526OS%3DIN%2FRasmussen%252BAND%252BTTL%2FSMOKE%2526RS%3DIN%2FRasmussen%252BAND%252BTTL%2FSMOKE

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3

Materiales y métodos

3. MATERIALES Y MÉTODOS

3.1 OBTENCIÓN DE MUESTRAS

3.1.1 TRIPAS

Las tripas estudiadas en esta tesis han sido de dos tipos, natural (Viuda de Inocencio Fernández, España), y artificial (Fibrán, España). Todas fueron facilitadas por El Hórreo Healthy Food S.L (Noreña, España).

La tripa natural utilizada, procede de intestino delgado de cerdo, tiene un calibre dado en el rango 28/30 cm y se adquirieron presentadas en madejas. Estas tripas se conservan en refrigeración, antes de su uso. Las tripas naturales más comunes en el mercado son de origen porcino, vacuno, ovino, caprino y equino. Se comercializan en barriles, cubos de distintas capacidades, en bolsas (saladas o en salmuera), entubadas y en madejas. En el mercado se presentan tripas de distintos calibres, desde 26/28 cm hasta 44/46 cm, en intestino delgado de cerdo, y otros formatos procedentes del intestino grueso del cerdo, incluyendo las tripas de cerdo vuelto, semirizada, semicular, cular, ciegos y vejigas (Viuda de Inocencio Fernández S.A, 2015).

La tripa artificial utilizada, se clasifica en la categoría “colágeno”, y tiene un calibre de 26/28 cm. La ficha técnica de este producto, facilitada por el fabricante, clasifica el producto en la tipología S1. Su composición es la siguiente: 74,7% colágeno, 18,0% glicerina, 2,0% grasas, 0,3% cenizas y 5,0% agua. Entre sus especificaciones físicas se encuentran un grosor de 0,04-0,09 mm y una elasticidad del 15-20%. Estas tripas se almacenan en un lugar fresco y seco, protegido de la luz solar, antes de su refrigeración. Existen otras tipologías de tripas artificiales tanto comestibles como no comestibles, de celulosa, de fibra, textiles, de lino, de poliéster, cloruro de polivinilideno (PVDC), poliamida (nylon), y multicapas (nylon, poli-olefina, y otros polímeros), cuya clasificación es revisada por Savic (2012).

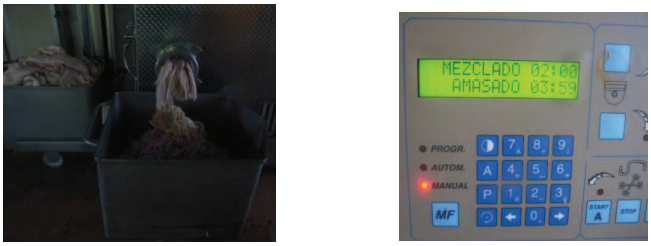
3.1.2 CHORIZO

3.1.2.1 Muestras de chorizo elaboradas

Todas las muestras de chorizo elaborado específicamente para este trabajo fueron fabricadas en la empresa El Hórreo Healthy Food S.L. Las muestras se pueden clasificar en 2 tipos, chorizo embutido en tripa natural (ChN) y chorizo embutido en tripa sintética (ChS). Las muestras de chorizo fueron tomadas en las instalaciones de la empresa durante días productivos, con el objetivo de reproducir las condiciones reales de fabricación. La cantidad de chorizo fabricada para cada estudio fue de 50kg, cantidad considerada como una producción a escala industrial.

Las proporciones de ingredientes seleccionados para la elaboración de los chorizos estudiados fueron: magro de cerdo (46,8%), panceta de cerdo (46,8%), sal (1,8%), ajo (1%), pimentón dulce (2%), y especias y aditivos (1,6%). La mezcla de especias y aditivos está formada por ingredientes para garantizar la estabilidad microbiológica y el control de calidad de los chorizos, como dextrina, dextrosa, citrato sódico (E-331iii), polifosfatos de sodio (E-451i), ascorbato sódico (E-301), nitrito sódico (E-250), y el colorante (E-120).

El magro de cerdo, y la panceta de cerdo, se picaron por separado en una picadora industrial (Taffo, España), y posteriormente se mezclaron, junto al resto de ingredientes, en una amasadora de vacío (Tec Maq, España).



a. Picado b. Amasado

Figura 3.1. Picado (a) y amasado (b) de los ingredientes para la fabricación de muestras de chorizo.

Del resultado del picado y mezclado de los ingredientes, se obtiene una masa, que se deja reposar durante 1 día. Este tiempo es necesario para permitir la maceración de la mezcla y que tengan lugar los primeros procesos de fermentación del producto. Una vez pasado este tiempo, la masa pasa a la embutidora (Handtmann, Alemania), donde es embutida en la tripa

estudiada, natural o artificial, previamente remojada en agua con sal. Las siguientes imágenes muestran el proceso de embutido.

Figura 3.2. Selección y remojado de la tripa.

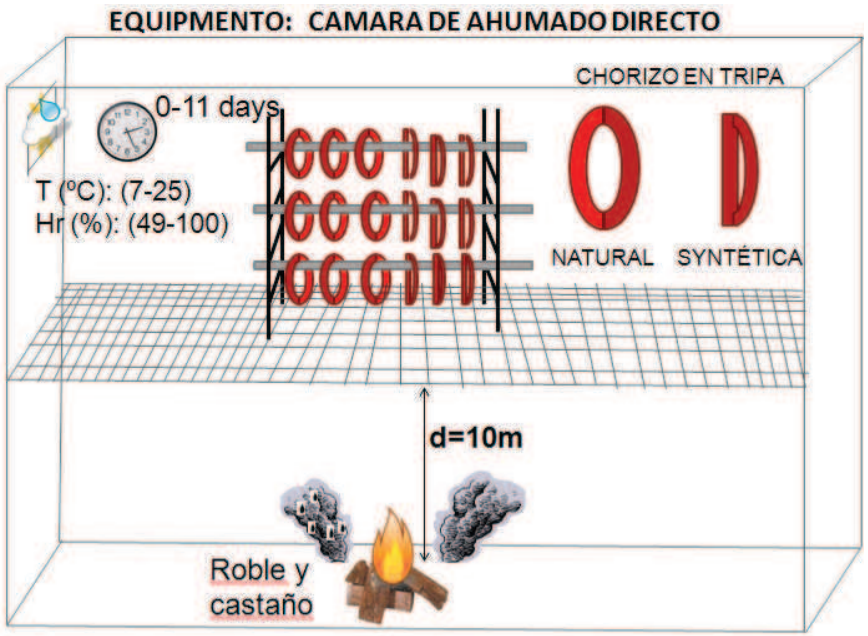
Figura 3.3. Proceso de embutido de la masa en la tripa.

Por cada 50 kg de producción se elaboraron unas 100 riestras de chorizos, conteniendo cada riestra 4 unidades de chorizo, las cuales fueron colgadas en barras y situadas en estanterías, tal y como muestra la siguiente figura.

Figura 3.4. Colocación de chorizo en estantería para llevar a la cámara de ahumado.

Una vez colgados los chorizos, se situó la estantería en la cámara de ahumado, la cual se encuentra a una distancia de 10 m respecto a la fuente de humo. Si una cantidad de chorizo menor es introducida en la cámara de ahumado, puede ser expuesta a una cantidad excesiva de humo, por lo que las muestras se realizaron con cantidades industriales de chorizo (50kg por estantería). Para el ahumado directo de las muestras se utilizaron maderas de roble y castaño, especificándose las proporciones es en cada trabajo. Las condiciones de humedad y temperatura de las muestras ahumadas al modo directo están definidas por las condiciones meteorológicas y se indican en cada estudio. El tiempo de exposición de los chorizos varía en función del estudio, desde los 0 a los 11 días de ahumado.

La siguiente figura esquematiza la cámara de ahumado tradicional utilizada.



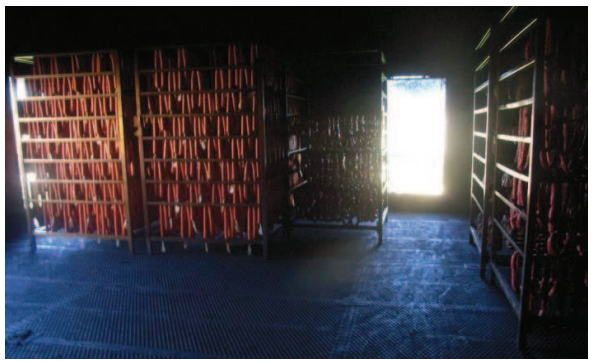


Figura 3.5. Cámara de ahumado directo industrial (tradicional) de El Hórreo Healthy Food S.L.

La siguiente figura resume el flujo del proceso de fabricación de chorizo.

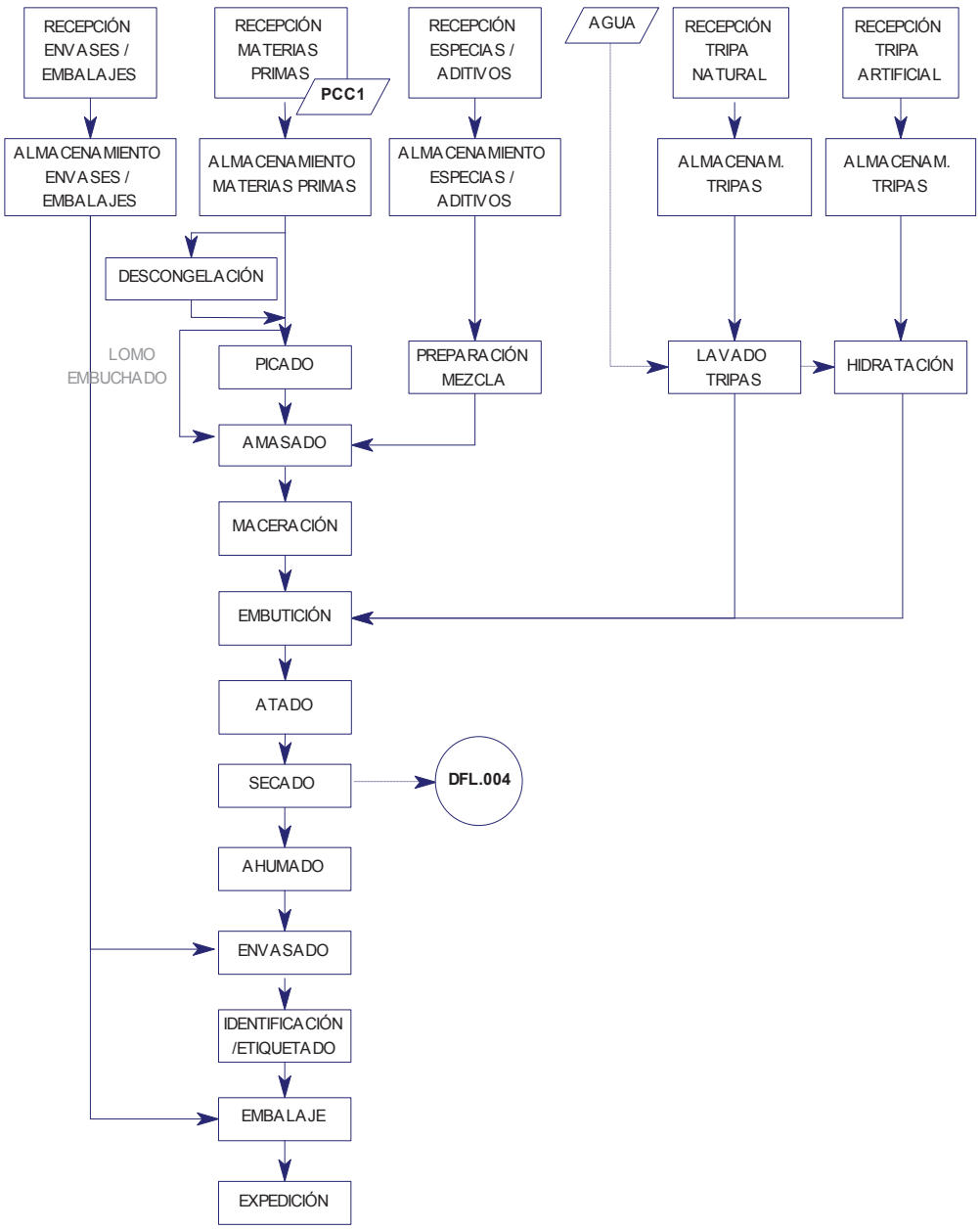


Figura 3.6. Diagrama de flujo del proceso de fabricación de muestras de chorizo.

3.1.2.2 Chorizos comerciales

Todas las muestras estudiadas en este trabajo, en cuya descripción no se indica haber sido elaboradas en las instalaciones de El Hórreo Healthy Food S.L son muestras de chorizo adquiridas en establecimientos comerciales. Entre esta tipología de muestras en total se estudiaron chorizos ahumados elaborados por 16 diferentes empresas de productos cárnicos del Principado de Asturias.

3.1.3 ACEITE

El aceite utilizado en este trabajo fue aceite de oliva extra virgen (EVOO) (Carbonell, España), adquirido en establecimientos comerciales. Los hidrocarburos aromáticos policíclicos son compuestos lipofílicos (Vázquez Troche, García Falcón, González Amigo, Lage Yusty, & Simal Lozano, 2000), que se disuelven en las grasas en estado líquido de manera uniforme. Para evaluar las diferencias de penetración de BaP a través de los distintos tipos de tripa, se diseñó un nuevo sistema ideal de dilución, formado por aceite contenido en los distintos tipos de tripa.

En primer lugar, se tomaron 40 cm de ambas tripas, natural y artificial y se remojaron en agua con sal durante 30 minutos, y seguidamente se llenaron las tripas con 400 mL de EVOO, tal y como muestra la figura 3.7.

a b c

Figura 3.7. Selección y medida (a), remojo (b) y llenado (c) de las tripas.

Se guardaron en la botella original 40 mL de aceite sin procesar, en la oscuridad, para su análisis (aceite sin ahumar). Las muestras se colgaron separadas 40 cm una de la otra en la misma barra de una estantería, tal y como muestra la figura 3.8.

Figura 3.8. Sistema de aceite en distintos tipos de tripa, colgado en la estantería.

La estantería se emplazó en la cámara de ahumado, de manera que ambos sistemas se encontraron en la misma posición y localización de la cámara de ahumado, a la misma distancia de la fuente generadora de humo, 10 m, y se ahumaron durante 7 días empleando una mezcla de roble (90%) y castaño (10%).

Una vez ahumadas, las muestras se colgaron en una barra situada dentro de una caja de cartón y fueron transportadas hasta el laboratorio de análisis, tal y como muestra la figura 3.9.

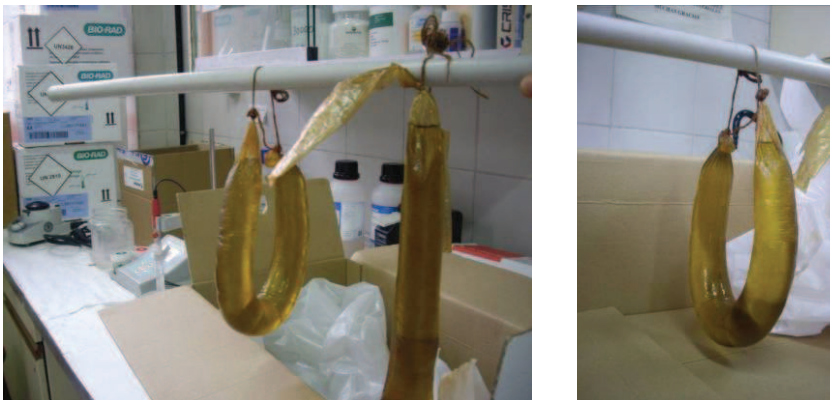


Figura 3.9. Transporte de sistemas de aceite en distintos tipos de tripa.

El contenido de aceite de las muestras se guardó en matraces individuales de cristal, a 5ºC en la oscuridad tal y como muestra la figura 3.10.

Finalmente, se analizaron 15 muestras, 3 por cada uno de los siguientes productos: aceite original (sin ahumar), aceite ahumado embutido en la tripa natural, aceite ahumado embutido en la tripa sintética, tripa natural ahumada y tripa sintética ahumada.

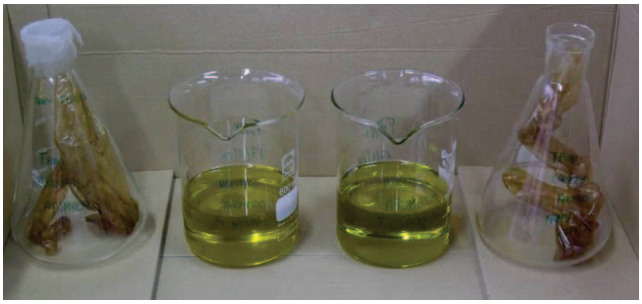


Figura 3.10. Preparación de sistema de aceite en tripa natural y colágeno, y toma de muestras.

3.2 DETERMINACIÓN DE BENZO(A)PIRENO

3.2.1 REACTIVOS Y PATRONES

La solución de patrón analítico Benzo(a)pireno (BaP) fue obtenida en Sigma-Aldrich (St. Gallen, Alemania), con una concentración de 100 µg/mL en ciclohexano y un volumen total de 2 mL. La solución del patrón interno Benzo(a)pireno (BaP d12, 98%), fue obtenida de Cambridge Isotope Laboratories (Andover, MA, EEUU), a una concentración de 200 µg/mL en isooctano y un volumen de 1,2 mL. Todos los disolventes para la preparación de muestras, hexano (ref. 32293), diclorometano (ref. 66740), ciclohexano (ref. 2893) e isooctano (2, 2, 4-Trimetilpentano) (ref. 59050), fueron suministrados por Sigma-Aldrich con una pureza de grado ACS≥ 99% (GC).

Los cartuchos de extracción de fase sólida (SPE) fueron suministrados por Varian (Palo Alto, California, Estados Unidos) y Agilent Technologies (Santa Clara, California, Estados Unidos).

Se emplearon cartuchos Mega Bond Elut, los cuales contienen fase adsorbente de sílice, con un tamaño de partícula de 40nm, una masa de 5g y un volumen de 20 mL. Se utilizaron matraces y viales de color ámbar (para proteger a los compuestos a determinar de la descomposición debida a la luz. Se utilizaron específicamente matraces de cristal ámbar de 15 mL (21 x 70 mm) adquiridos en Sigma-Aldrich para la recolección de los HAP durante la etapa SPE.

3.2.2 PRETRATAMIENTO DE MUESTRAS DE CHORIZO

Para llevar a cabo los análisis de contenido de BaP en las muestras, es necesario realizar un proceso de pretratamiento de las muestras para la extracción del analito de interés. En la siguiente figura 3.11, se esquematizan los pasos involucrados en los métodos de pretratamiento de muestras probados en el laboratorio. Seguidamente se detallan los pasos de la vía analítica finalmente seleccionada y utilizada en todos los experimentos de este trabajo, la cual consiste en la combinación de sonicación y extracción en fase sólida (SPE) (vía de la derecha).

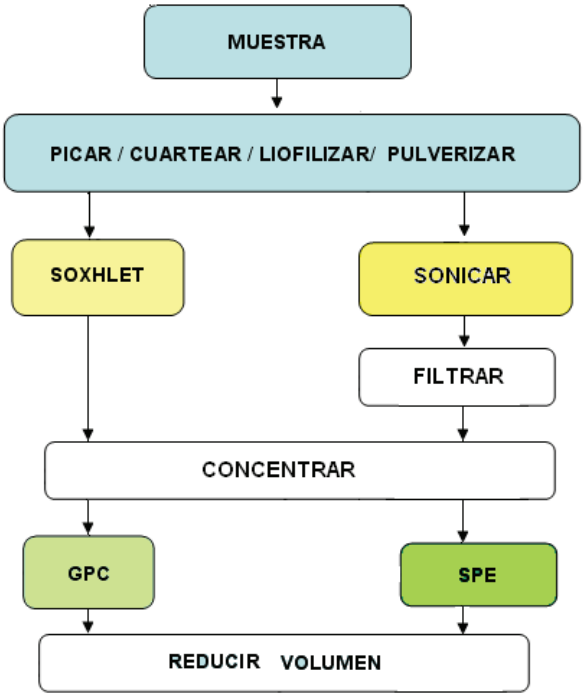


Figura 3.11. Pretratamiento.

3.2.2.1 Selección de la muestra y alícuotas a analizar

En primer lugar, se caracterizaron las muestras, tomando medidas de su longitud, diámetro e inspección visual. Seguidamente, se seleccionaron las alícuotas de las muestras a analizar. El procedimiento de pretratamiento de las muestras se realizó por triplicado (3 alícuotas de cada muestra) en todos los estudios. En función del tipo de estudio los métodos de toma de muestras utilizados se pueden clasificar en 2 tipos:

a) Toma de muestras del producto completo homogeneizado (sin la tripa).

b) Separación de producto en diferentes profundidades, y toma de muestras.

Figura 3.12. Selección y cuarteamiento de muestras.

3.2.2.2 Picado

Una vez seleccionadas las muestras, se picaron y homogeneizaron con ayuda de una picadora de carne (Minirobot D81 de Moulinex, France). También se utilizó en un molinillo de café (Moulinex, France) para pulverizar muestras, tal y como sugieren Purcaro, Moret, y Conte (2009).

3.2.2.3 Liofilización

Se tomaron 3 alícuotas de 20g de chorizo y se colocaron en platos individuales del liofilizador (Telstar-Cryodos, España), cuidadosamente cubiertos con papel de aluminio y se pesaron antes del proceso de liofilización. Cada muestra fue congelada a -80ºC durante 4 horas y liofilizada en un liofilizador durante 1 día. Finalmente se pesaron los platos, conteniendo las muestras secas.

Figura 3.13. Equipo de liofilización.

3.2.2.4 Sonicación

Se llevaron 2 g de chorizo liofilizado de cada plato a matraces de base ancha, de 250 mL. La cantidad sobrante de chorizo se guardó en un congelador. Seguidamente, se añadieron 20 mL de n-hexano y 200 µL de la solución estándar interno de benzo(a)pireno-d12 con una concentración de 100 µg/L (habiendo hecho las diluciones de la disolución madre previamente).

Estas disoluciones se mantuvieron bajo refrigeración durante 3 días para permitir que el patrón interno deuterado se adaptase a la matriz de la muestra. Este es el momento apropiado para añadir el estándar interno (y adiciones estándar para determinar la recuperación) pues es el primer momento en el que ambos compuestos se encuentran en las mismas condiciones y en el mismo medio de dilución (Purcaro et al., 2009; Stumpe-Vīksna, Bartkevics, Kukare, & Morozovs, 2008). Finalmente, las muestras fueron sonicadas durante 1 hora en un baño de ultrasonidos (Cod. 3000513, J.P. Selecta, S.A, Barcelona, España) a temperatura ambiente.

3.2.2.5 Filtración

Después de sonicar, las muestras fueron filtradas con un papel de filtro (número de referencia 1242, 11 cm, Albet) para separar el residuo sólido, que fue lavado 2 veces con 5mL de n-hexano. La disolución fue recogida en un matraz y el volumen fue llevado a 10 mL con ayuda de un rotavapor (Heidolph Laborota 4000 efficient, Alemania) y la adición de la cantidad necesaria de n-hexano.

Figura 3.14. Filtrado.

3.2.2.6 Extracción en fase sólida (SPE)

El extracto obtenido después de filtrar contiene una cantidad de grasa muy elevada (Purcaro et al., 2009). Para separar los compuestos de interés (benzo(a)pireno y benzo(a)pireno-d12 (BaP-d12)) se utilizó un procedimiento de extracción en fase sólida (SPE) en un equipo de vacío Supelco Visiprep TM SPE con 12 puertos (Sigma-Aldrich, USA), previamente utilizado para la determinación de HAP en aceites vegetales por Moret y Conte (2002), y comprobada su validez en productos cárnicos ahumados por Purcaro et al. (2009).

Figura 3.15. Equipo SPE.

En primer lugar se lava el cartucho SPE de 5g de sílice (Mega Bond Elut, 20 mL, Varian) con 20 mL de diclorometano durante 2 horas y se seca por completo al vacío. Posteriormente se acondiciona el cartucho con 20 mL de hexano durante 1 hora, evitando en todo momento que se seque. A continuación se carga lentamente (durante 30 minutos aproximadamente) 1mL de la muestra sonicada, diluida con 3 mL de hexano, evitando en todo momento que el cartucho llegue a sequedad.

Posteriormente, se eluye la fracción correspondiente a los hidrocarburos alifáticos mediante la adición en el cartucho de 8 mL de una mezcla de hexano y diclorometano 70:30 (v/v). Finalmente se eluye lentamente y hasta sequedad (durante 1 hora) la fracción de HAP, nuevamente con 8 mL de una mezcla de hexano y diclorometano 70:30 (v/v). Ambas fracciones se conservan en tubos especiales para SPE de color ámbar de 15 mL. Se expone a continuación un esquema del proceso.

PROCESO DE EXTRACCIÓN DE HAP MEDIANTE SPE

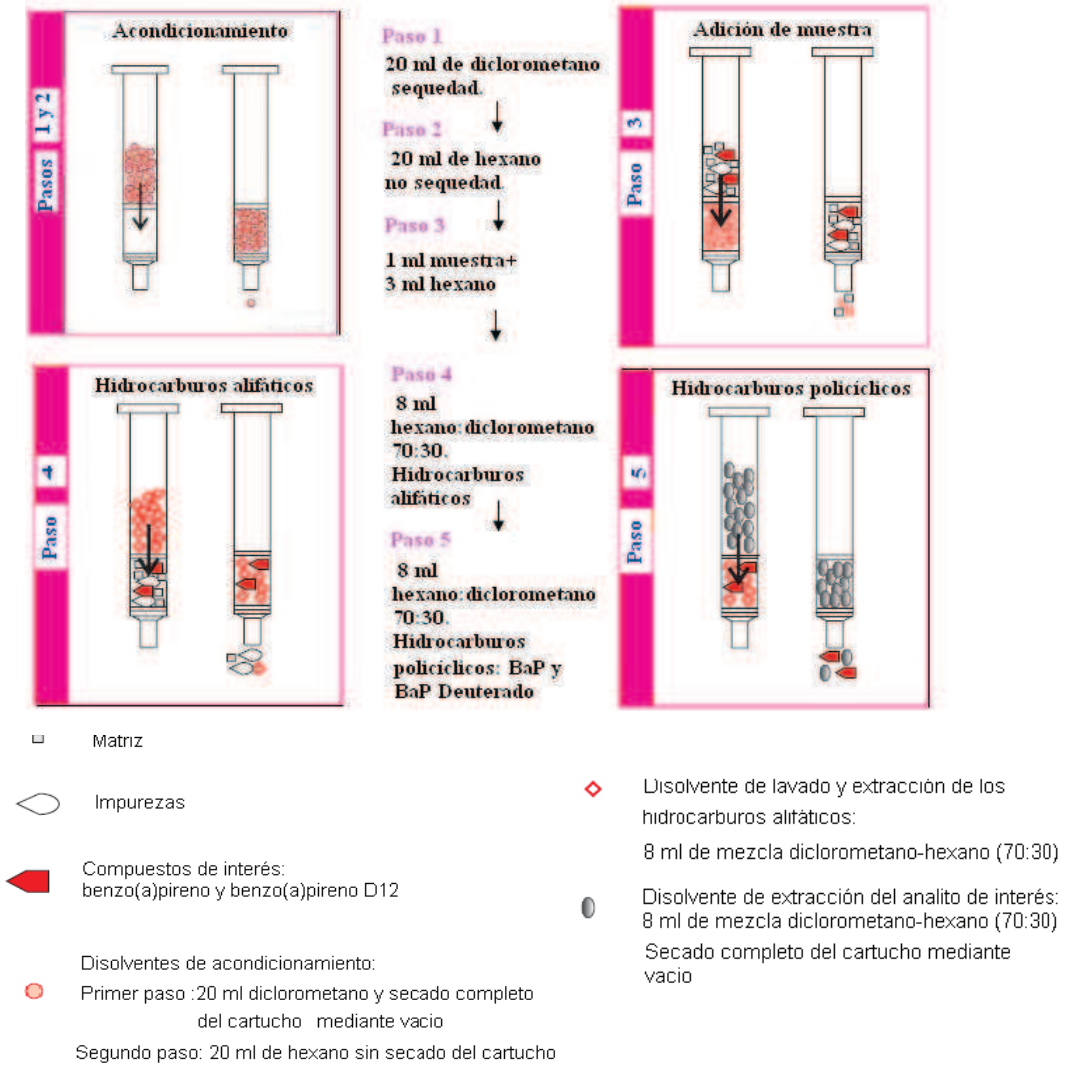


Figura 3.16. Proceso de extracción en fase sólida, de BaP en productos cárnicos ahumados.

3.2.2.7 Concentración

Se reduce el volumen de muestra (contenida en el vial ámbar de 15 mL procedente de SPE) con una corriente de nitrógeno (situado dentro de una campana de extracción de gases) hasta un volumen un poco inferior a 1 mL, durante aproximadamente 40 minutos. Finalmente se traspasa la muestra al vial de análisis de color ámbar y se ajusta el volumen hasta 1mL. Para comprobar que existe exactamente 1 mL de la mezcla de disolventes en el vial final de análisis, debe pesarse este vacío (antes de llenarlo) y finalmente lleno. Por diferencia de pesadas se comprueba el volumen en el vial. Se encontró que al finalizar el secado, solo permanece hexano en el vial. Por esta razón, para realizar la comprobación mediante pesada, se debe considerar la densidad del hexano (ρhexano a 20 ºC: 0,660 g/mL), en lugar de la densidad de la mezcla de hexano y diclorometano 70:30 (v/v) (ρmezcla=0,859 g/mL), la cual se calcula mediante la siguiente ecuación:

$$\frac{1}{\rho_M} = \frac{\hat{x}_{Hexane}}{\rho_{Hexane}} + \frac{\hat{x}_{Dichlorometane}}{\rho_{Dichloromethane}} \qquad \text{ec.(1)}$$

donde:

ρ_M = densidad de la mezcla.

ρ_{hexano} = densidad del hexano a 20 ºC: 0,660 g/mL.

$\rho_{diclorometano}$ = densidad del diclorometano a 20 ºC: 1,325 g/mL.

$\hat{x}_{hexano}$ = fracción en peso del hexano.

$\hat{x}_{diclorometano}$ = fracción en peso del diclorometano.

Figura 3.17. Vial.

3.2.3. PRETRATAMIENTO DE MUESTRAS DE ACEITE

En el pretratamiento de las muestras de aceite se utilizó un procedimiento de extracción en fase sólida (SPE) para extraer el benzo(a)pireno and benzo(a)pireno-d12 (BaP-d12), de acuerdo al descrito por Moret y Conte (2002), similar al previamente descrito para las muestras de chorizo. Se pesaron 2.5 g ± 0.001 g de aceite en un matraz volumétrico de 10 mL y se añadieron 200 µL de disolución de patrón interno benzo(a)pireno-d12 con una concentración de 100 µg/L y se añadió n-hexano hasta llevar el volumen hasta 10mL. Esta disolución fue guardada en refrigeración durante 3 días para permitir que el patrón interno se adaptase a la matriz de la muestra. Entonces, las muestras fueron sometidas a sonicación, durante 1 hora a temperatura ambiente, y 1 mL de la muestra fue sometido al procedimiento SPE, del mismo modo que el descrito en las muestras de producto cárnico.

3.2.4 PRETRATAMIENTO DE LAS TRIPAS

Las tripas fueron pesadas en matraces, se añadieron 20 mL de n-hexano y 200 µL de disolución de patrón interno benzo(a)pireno-d12 con una concentración de 100 µg/L. Esta disolución fue guardada en refrigeración durante 3 días para permitir que el patrón interno se adaptase a la matriz de la muestra. Las muestras fueron tratadas con los mismos pasos que los descritos para el chorizo desde la etapa de sonicación.

3.2.5 DETERMINACIÓN DE BAP MEDIANTE CROMATOGRAFÍA DE GASES/ ESPECTROMETRÍA DE MASAS (GS/MS)

3.2.5.1 Calibración GC/MS

La cuantificación de BaP fue realizada mediante la calibración del equipo GC/MS con disoluciones patrón de benzo(a)pireno y benzo(a)pireno-d12. Las curvas de calibrado fueron generadas inyectando por triplicado 6 disoluciones conteniendo los patrones BaP y BaP-d12, en ciclohexano. Las concentraciones de patrón BaP utilizado en las calibraciones están en el rango desde 0,05 hasta 10 ppb, en función de cada estudio. La concentración de BaP-d12 para todos los viales de calibrado fue siempre 2 ppb. El programa de MS (Agilent Technologies) compara la relación entre la señal analítica de ambos patrones (BaP y BaP-d12) para determinar la

concentración final. La identificación de los compuestos fue realizada comparando el tiempo de retención de los compuestos en las muestras con los patrones y confirmado verificando que la relación de los ratios de los iones (taget ion/ ion cualificador) estaba dentro del criterio ±20% respecto a la misma relación en los patrones. Un vial blanco (conteniendo solo disolvente), fue introducido entre todos los viales.

3.2.5.2 Cromatografía de Gases (GC)

La separación de BaP y BaP-d12 fue realizada con una columna, de referencia HP-5MS 19091S-433 y dimensiones (30m x 250 µm x 0.25 µm) de Agilent Technologies (Santa Clara, CA, EEUU) en un cromatógrafo de gases (GC) de Agilent Technologies, versión 6890. La temperatura y el volumen de inyección fueron 300 ºC y 1 µL (splitless), respectivamente. Como gas portador se utilizó Helio con un flujo constante de 1,5 mL/min. La temperatura de la rampa utilizada fue: isoterma a 55 ºC durante 1 min, aumento a un ratio de 25 ºC/min hasta 320 ºC durante 3 min, tiempo total 14,60 minutos.

3.2.5.3 Espectrometría de Masas (MS)

La identificación de BaP y BaP-d12 fue realizada mediante el detector espectrómetro de masas (MS) Agilent Technologies 5975 equipado con unas lentes de apertura ultra larga de 6 mm, de referencia G2589-20045. El retraso del disolvente fue de 4 minutos y el voltaje EM (utilizado a voltaje autotune) fue de 1294 voltios. Se utilizó el método SIM con 3 iones específicos (252, 264 and 126), 50 msec dwell/ion. La temperatura del cuadrupolo, de la fuente y de la línea de transferencia fueron 180, 300 y 280°C, respectivamente. Los datos fueron adquiridos utilizando el MS en el modo de monitorización selectiva de los iones (SIM) durante la cuantificación de las muestras y en SCAN (barrido completo) en una primera instancia para identificar y determinar el tiempo de retención de los patrones. Los espectros de los picos fueron comparados con los espectros de masas de los patrones de BaP y BaP-d12 y también con la librería proporcionada por el instrumento. Cada vial fue analizado por triplicado. La determinación de la recuperación obtenida en las medidas se calculó comparando la diferencia entre muestras a las que se añadió una cantidad conocida de patrón BaP (spiked), 1 ppb, y muestras a las que no se añadió patrón (unspiked). Los límites de detección y cuantificación (LOD and LOQ, respectivamente) se

definieron como la concentración de analito que produce una relación señal/ruido (S/N) de 3 y 10 respectivamente. Estos valores fueron también estimados mediante el análisis de muestras blanco y 6 diluciones de patrones de BaP y BaP-d12 en ciclohexano, por triplicado. Se obtuvo la pendiente (m) y la desviación estándar en el origen (sb0) de la respuesta analítica (y) frente a la concentración añadida (x) y LOD y LOQ fueron también estimados mediante las siguientes ecuaciones: LOD=3.3*sb0/m, LOQ=10*sb0/m.

La siguiente tabla resume las condiciones generales de GC/MS

Tabla 3.1 Condiciones generales de cromatografía de gases/espectrometría de masas (GC/MS).

Columna: Agilent Technologies HP-5MS 19091S-433			
Longitud	30.0 m		
Diámetro	250 µm		
Espesor	0,25 µm		
Modo	Flujo constante = 1,5 mL/min		
Espectrómetro de masas MSD: Agilent Technologies 5975			
BaP	Iones: 252 (target),126, 264 (qualifiers) Tiempo de retención: 12.22 min	**Modo**	SIM
BaP d12	Iones: 264 (target),126, 252 (qualifiers) Tiempo de retención: 12.22 min		
Horno : Rampa			
Min	**°C/min**	**Siguiente °C**	**Duración**
Inicial	0	55	1,00
Rampa 1	25	320	3,00
Tiempo total	14,60 min		
Cromatógrafo de gases: Agilent Technologies 6890			
Entrada	Splitless, 1 ml	**Flujo total**	34.6 mL/min
Temperatura	300 °C	**Presión**	13.00 psi

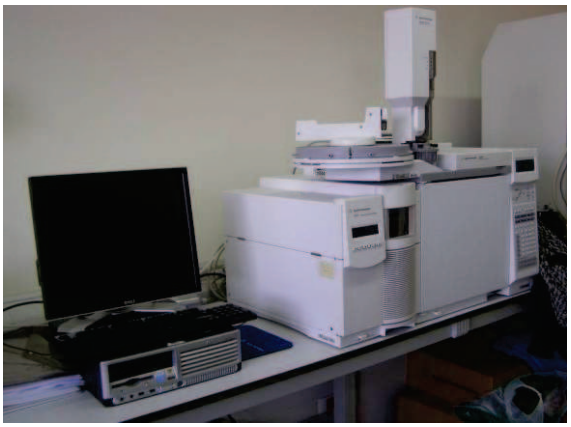


Figura 3.18. Cromatógrafo de gases (GC) 6890/ espectrómetro de masas (MS) 5975, Agilent Technologies.

3.3 CARACTERIZACIÓN FÍSICA DEL EFECTO DE LAS TRIPAS EN EL CHORIZO

3.3.1 CARACTERIZACIÓN DE LAS TRIPAS

3.3.1.1 Determinación de la porosidad

3.3.1.1.1 Pretratamiento

Las tripas sintéticas son proporcionadas por el proveedor en fase seca, mientras que las naturales presentan un elevado contenido de humedad. Mediante porosimetría no es posible el análisis de muestras húmedas. El método de liofilización fue descartado para evitar la posible degradación y/o modificación de las características físicas de las muestras. Por esta razón, se diseñó y preparó un sistema especial para el secado lento y cuidadoso de las tripas, del mismo modo que ocurre en los productos cárnicos, e impidiendo la contaminación de las tripas con los ingredientes.

Para ello se tomaron 60 cm de cada tipo de tripa, natural (de intestino de cerdo) y sintética (de colágeno) y se remojaron en agua con sal. Mientras tanto, se recortó la parte central

de un tubo hueco de cartón de 53 cm, para crear un espacio interior sin material. La superficie del la parte del tubo que permanecía de cartón se recubrió con parafilm para permitir que las tripas deslizasen bien. Entonces se embutieron los palos con las tripas y los sistemas creados se colgaron en barras independientes, las cuales fueron emplazadas en una estantería de metal colocada en una cámara de atmósfera controlada, donde fueron secadas lentamente (del mismo modo que los chorizos) durante 3 días, con unas condiciones de 12ºC de temperatura y 65% de humedad. La figura 3.19 esquematiza el sistema de secado. Una vez secas, las muestras fueron transformadas cuidadosamente, colgadas en barras colocadas en una caja (diseñada también específicamente para el estudio), previniendo el contacto de las mismas con cualquier objeto (ver figura 3.19 b).

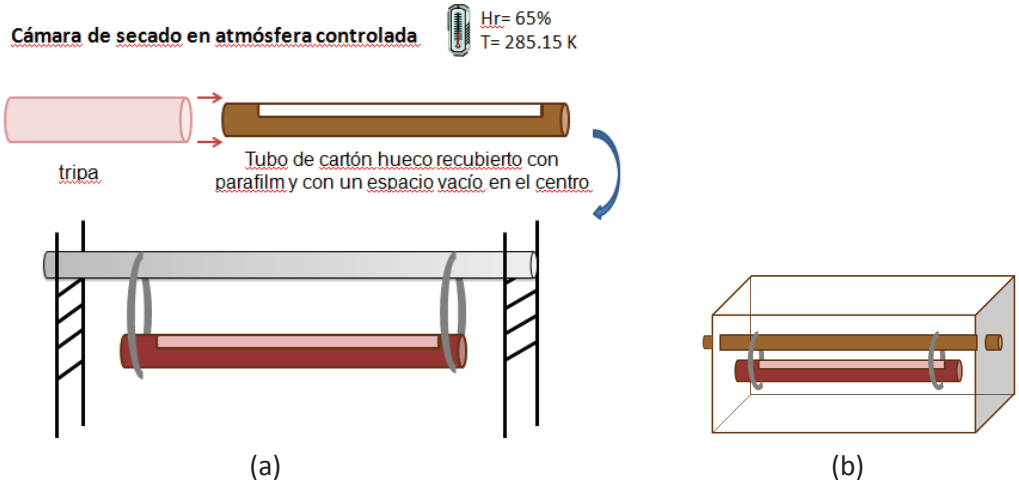


Figura 3.19. Procedimiento de secado (a) y transporte (b) de membranas (tripas).

3.3.1.1.2 Determinación de la porosidad

Una vez en estado seco, se tomaron 0,51 g y 0,42 g de las tripas sintética y natural, respectivamente, y se determinó su porosidad mediante porosimetría de intrusión de mercurio utilizando el dispositivo Micromeritics Autopore IV (Norcross, GA, EEUU), de la Unidad de Tecnología Alimentaria de los Servicios Científico Técnicos (SCTs) de la Universidad de Oviedo. Las muestras previamente evacuadas, fueron sometidas a valores de presión en el rango (0,10 - 60.000,00) psia, para obtener las siguientes características de los materiales: datos de intrusión, estructura de los poros y valores de compresibilidad.

Figura 3.20. Porosímetro Micromeritics Autopore IV.

3.3.1.2 Microscopía electrónica de barrido (SEM)

Se utilizó el microscopio electrónico de barrido (scanning electron microscope, SEM) MEB JEOL-6610LV (Tokyo, Japón) con microanálisis en la Unidad de Microscopia Electrónica de los (SCTs) de la Universidad de Oviedo, para estudiar ambas caras de las tripas natural y sintética, pretratadas como describe el apartado 3.3.1.1.

Figura 3.21. Microscopio electrónico de barrido MEB JEOL-6610LV SCTs UO.

3.3.1.3 Estereomicroscopía de fluorescencia óptica

Se utilizó el Microscopio de Fluorescencia Óptica Leica M205 FA (Wetzlar, Alemania) ubicado en la Unidad de Microscopía Fotónica y Proceso de Imágenes de los SCTs de la Universidad de Oviedo, para estudiar la morfología de las siguientes muestras: ambas caras de las tripas natural y sintética, pretratadas como describe el apartado 3.3.1.1 y sin pretratar

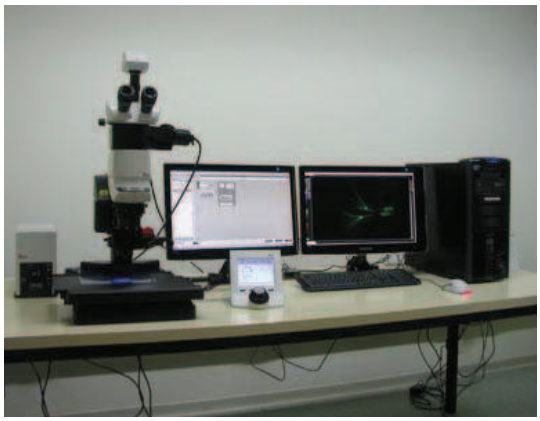


Figura 3.22. Microscopio de Fluorescencia Óptica Leica M205 FA. SCTs UO.

3.3.2 CHORIZOS EMBUTIDOS EN TRIPAS

3.3.2.1 Determinación del color

Se determinó el color de la superficie exterior de 36 chorizos (18 embutidos en cada tipo de tripa, natural y sintética), a los 0, 1, 4, 5, 7 y 11 días de ahumado fabricados como especifica el apartado 3.1.1.1. Cada día se tomaron 3 muestras de chorizo embutido en cada tripa. El color de las diferentes muestras fue determinado en el espacio de color CIELAB (L*, a*, b*) mediante el espectrofotometro UltraScan VIS (HunterLab) de la Unidad de Tecnología Alimentaria de los Servicios Científico Técnicos de la Universidad de Oviedo. El equipo fue calibrado utilizando como patrones una teja blanca y una trampa de luz y se comprobó su correcta calibración utilizando como referencia una teja verde. En la medición se empleo una cubeta de cuarzo de 10 mm de paso de luz (USVIS1034). Los análisis fueron realizados en reflectancia con el método de reflexión especular excluida, este método elimina los errores generados por la turbidez y la bruma de la

muestra, de manera que la evaluación del color es mas similar a la percepción del ojo humano (HunterLab, 2015). Los valores L*, a* y b* corresponden a claridad, (-L) negro o (+L) blanco, verde (-a) o rojo (+a) y azul (-b) o amarillo (+b), respectivamente. Las medidas de color se realizaron a temperatura ambiente (20 ± 2 ºC). Los parámetros de color se midieron 30 veces para cada chorizo estudiado. El cambio total de color (ΔE; Eq. (1)), ángulo de tonalidad cromática (Eq. (2)), chroma (índice de saturación; Eq. (3)) e índice de pardeamiento ó enmarronecimiento (browning index) (BI; Eq. (4)) se calcularon utilizando los valores Hunter L, a, y b (Homco-Ryan et al., 2004; Laca, Sáenz, Paredes, & Díaz, 2010; Maskan, 2001), de la siguiente manera:

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad \text{Eq.(2)}$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b}{a}\right) \quad \text{Ec. (3)}$$

$$\text{Chroma} = \sqrt{a^2 + b^2} \quad \text{Ec. (4)}$$

$$BI = \frac{[100 \times (x - 0.31)]}{0.17} \quad \text{Ec. (5)}$$

donde:

$$x = \frac{a + (1.75 \times L)}{(5.645 \times L) + a - (3.012 \times b)} \quad \text{Ec. (6)}$$

Fig 3.23. Colorímetro.

3.3.2.2 Determinación de la textura

Se determinó la textura de la superficie exterior de 36 chorizos (18 embutidos en cada tipo de tripa, natural y sintética), a los 0, 1, 4, 5, 7 y 11 días de ahumado fabricados como especifica el apartado 3.1.1.1. Cada día se tomaron 3 muestras de chorizo embutido en cada tripa. Para ello se utilizó el analizador de Textura TA.XTPlus. Los chorizos se colocan enteros (sin trocear) en una plataforma metálica. Se utilizó una sonda esférica de 1/2 pulgada de diámetro, de acero inoxidable para mediar la fuerza de penetración de la sonda en el producto cárnico. En los ensayos se determinaron tanto la textura como la pegajosidad.

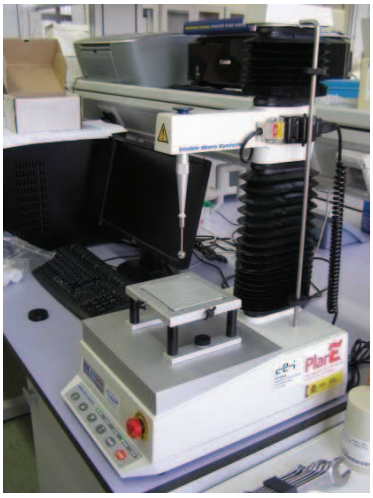


Figura 3.24. Texturómetro.

3.3.2.3 Determinación del contenido de humedad

El extracto seco total de las muestras se determinó por gravimetría, por triplicado, durante 0, 1, 4, 5, 7 y 11 días de ahumado de ChN y ChS. Para ello, se pesaron aproximadamente 20 gramos de arena de mar gruesa, de referencia 211161 (Panreac, Spain), en morteros de acero inoxidable con su varilla, y se mantuvieron en un desecador durante 30 minutos, hasta alcanzar un valor de masa constante. A continuación, se pesaron aproximadamente 6 gramos de muestra en el mortero, y se homogeneizaron con la arena con la ayuda de la varilla.

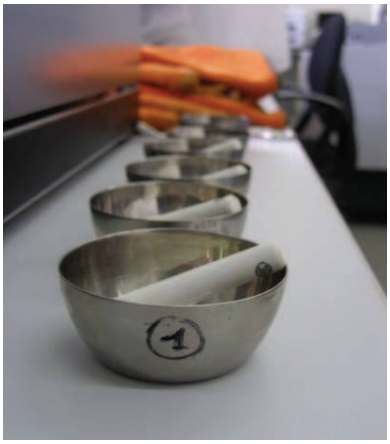


Figura 3.25. Pocillos.

El sistema (constituido por el mortero, la arena, la muestra y la varilla) se introdujo en la estufa (Memmert, Germany) a 105±2ºC durante 5 horas. Entonces, el sistema se dejó enfriar en un desecador de vacio a temperatura ambiente, durante 30 minutos aproximadamente, y finalmente se pesó.

Figura 3.26. Dispositivo para determinación de humedad: desecador, arena, pocillos y horno.

3.3.2.4 Determinación de la estructura

3.3.2.4.1 Microscopía electrónica de barrido (SEM)

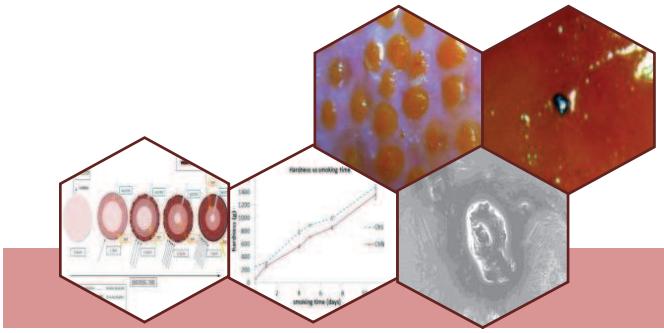
Se utilizó el microscopio electrónico de barrido (scanning electron microscope, SEM) MEB JEOL-6610LV con microanálisis (ver figura en apartado 3.3.1.2) de la Unidad de Microscopia Electrónica de los Servicios Científico Técnicos (SCTs) de la Universidad de Oviedo (UO), para estudiar las tripas natural y sintética de 8 chorizos después de 3 y 5 días de ahumado, fabricados como describe el apartado 3.1.1.1 y pretratadas mediante congelación a -80ºC durante 4 horas y secadas en un liofilizador (Telstar-Cryodos, España) durante un día.

3.3.2.4.2 Estereomicroscopía de fluorescencia óptica

Se utilizó el Microscopio de Fluorescencia Óptica Leica M205 FA (ver figura en apartado 3.3.1.3) de la Unidad de Microscopía Fotónica y Proceso de Imágenes de los Servicios Científico Técnicos de la Universidad de Oviedo, para estudiar la morfología de las tripas (superficie exterior) natural y sintética de 8 chorizos sin ahumar (4 embutidos en cada tipo de tripa) y de 40 chorizos (fabricados como especifica el apartado 3.1.1.1), ahumados entre 0 y 10 días (20 embutidos en cada tipo de tripa) y sus diferentes profundidades, externa (una vez que la tripa fue retirada), e interna.

3.4 ANÁLISIS ESTADÍSTICOS

Los datos obtenidos en al presente tesis doctoral, fueron tratados con el paquete estadístico Statgraphics Plus 3.1, para Windows 3.0® (Statpoint Technologies, Inc. Warrenton, Virginia, EEUU), tal y como se detalla en cada trabajo. Se realizaron pruebas “t-Student” para comparar entre las medias de las muestras, y determinar si presentaban diferencias estadísticamente significativas, bajo un criterio de $p\text{-valor}<0.05$. Para comparar conjuntos de datos obtenidos entre diferentes muestras, se aplicaron análisis “multirango”. Previamente se estudió si la distribución estadística correspondía a una distribución normal, estudiando las medidas de asimetría, el coeficiente de asimetría de Fisher, junto con las medidas de apuntamiento o curtosis. Se realizó la prueba F de Fisher (F-test), para comparar las desviaciones estándar de las muestras.



4

Resultados

4. RESULTADOS

4.1 DETERMINACIÓN DE B(a)P EN CHORIZOS AHUMADOS DEL PRINCIPADO DE ASTURIAS

Como se ha indicado en la presente memoria (apartado 1.1) ante las perspectivas de crecimiento de consumo mundial de carne y productos cárnicos ahumados, diversas entidades (Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO), Organización Mundial de la Salud (OMS), etc.) alertan sobre la necesidad de evaluar y controlar la presencia de HAP cancerígenos, representados por el BaP, en productos cárnicos ahumados de todo el mundo. Por todo ello, recientemente la legislación europea ha reducido la cantidad máxima permitida de HAP y BaP en estos alimentos.

La elevada tasa de atomización del sector cárnico español obliga a las pequeñas (y micro) empresas cárnicas a seguir utilizando el método de ahumado directo, como método para secar sus productos, prolongando su vida útil, y conferir buenas propiedades organolépticas. Sin embargo, las referencias científicas han demostrado que este método es susceptible de conferir estas sustancias cancerígenas a los productos.

En el siguiente artículo de investigación se presentan los resultados sobre la puesta a punto de un método analítico para la determinación de BaP en chorizo asturiano ahumado. Así mismo, se evalúa el contenido de BaP de chorizos asturianos, elaborados por 16 empresas diferentes del Principado de Asturias, recogidos en establecimientos de venta al público. Se llevó por tanto a cabo un estudio que nunca había sido realizado en esta provincia española por la comunidad científica. En el este artículo se estudió la relación entre el contenido de BaP y la humedad de los chorizos asturianos, evaluando la calidad del ahumado directo como método de secado, así como la adecuación de los chorizos ahumados del Principado de Asturias a la nueva normativa europea.

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Original research article

Spanish smoked meat products: Benzo(a)pyrene (BaP) contamination and moisture

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ABSTRACT

Traditional direct smoking is used for drying and flavouring foodstuffs, although carcinogenic compounds are added during this process, namely polycyclic aromatic hydrocarbons (PAH). The maximum permissible content of benzo(a)pyrene (BaP) (a current marker for the occurrence and effect of PAH in foods) in smoked meat products was reduced from 5 to 2 μg/kg on 1/09/2014, in compliance with European Regulation No. 835/2011. In this study, an analytical method has been developed to determine BaP content consisting of PAH extraction assisted by sonication followed by solid-phase extraction sample clean-up and analytical determination using gas chromatography/mass spectrometry. Sixteen commercial chorizo samples from 16 different Spanish producers from the Principality of Asturias were studied. Five of the samples exceeded the 2 ppb BaP limit. The relationship between moisture and BaP content in chorizo was examined, in order to verify the quality of the manufacturing process. Moisture content did not correlate with BaP content in chorizo.

1. Introduction

Food smoking is an old and traditional technological process widely applied to many foodstuffs such as meat, fish and cheese, not only for the special organoleptic profiles that it confers, but also due to the inactivating effect of smoke and heat on enzymes and microorganisms (Šimko, 2002; Codex Alimentarius CAC/RCP 68/2009). However, unwanted compounds are produced during traditional uncontrolled smoking processes because of incomplete combustion or thermal decomposition of the organic material employed (wood) (Djinovic et al., 2008; Danyi et al., 2009). These compounds are polycyclic aromatic hydrocarbons (PAH), whose carcinogenic, mutagenic and bioaccumulative capacities have been reported by the World Health Organisation (WHO), the International Agency for Research on Cancer (IARC), the European Food Safety Authority (EFSA) and the US Environmental Protection Agency (EPA).

Humans are exposed to PAH in three ways: through food, the environment and a combination of both, i.e. food contaminated due to exposure to the environment. The main source of PAH is diet (Lodovici et al., 1995; Phillips, 1999; Falcó et al., 2003; Ibáñez et al., 2005), contributing to more than 70% of total exposure in non-smokers (Gilbert, 1994; McGrath et al., 2007). PAH enter the food chain by means of a large variety of unprocessed and processed foods, due to their processing, packaging and thermal processes such as smoking, drying, roasting, baking, barbecuing and frying (Codex Alimentarius CAC/RCP 68/2009). The major contributors to PAH intake are cereals and cereal products (owing to high consumption in diets) and vegetable fats and oils (due to higher concentrations of PAH in this food group) (Codex Alimentarius CAC/RCP 68/2009).

Particular attention has been paid to smoked meat products, because the highest levels of total PAH have been detected in these foods (Larsson et al., 1988; Gomaa et al., 1993; Karl and Leinemann, 1996; Martorell et al., 2010). The Commission of the European Communities (EC) has recently amended Regulation (EC) No. 1881/2006 by means of Regulation (EU) No. 835/2011 of 19 August 2011, setting new maximum levels for PAH in foodstuffs. New maximum

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0889-1575/

levels for the sum of four substances (PAH4) (benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) were introduced, maintaining a separate maximum level for BaP, to ensure comparability between previous and future data. The maximum permissible BaP content in smoked meat and smoked meat products was 5.0 μg/kg in wet weight until 31/8/2014 and has been 2.0 μg/kg in wet weight since 1/9/2014. The permissible sum of PAH4 in these foods was 30.0 μg/kg in wet weight from 1/9/2012 to 31/8/2014 and 12.0 μg/kg in wet weight since 1/9/2014.

Likewise, the " Code of practice for the reduction of contamination of food with (PAH) from smoking and direct drying processes" (CAC/RCP 68/2009) stipulates that PAH contamination in foodstuffs via food processing should be controlled, taking into consideration the food processing technology. The food producer should be aware of the conditions under which higher levels of PAH are generated and, wherever possible, should control those conditions to minimise their formation. To accomplish this, an analysis of important points to consider in processes used or intended to be used in food production with smoking or direct drying should be carried out.

Both bodies, EU regulators and the Codex Alimentarius, conclude that more scientific data are needed to elucidate the precise influence of the smoking method employed and other processing parameters on PAH contamination of food. A variety of both old and modern pre-treatment and analytical methods has been used to determine PAH in foods. Old pre-treatment methods, like saponification, liquid–liquid extraction and Soxhlet in combination with gel permeation chromatography (GPC), are time consuming and involve high solvent consumption. Modern methods, such as microwave-assisted extraction (MAE), accelerated solvent extraction (ASE) solid-phase microextraction (SPME) and direct extraction devices (DED) require the use of expensive technologies. The combination of sonication with solid-phase extraction has been tested in some foodstuffs, affording results that are both fast and inexpensive. Typical analytical methods for PAH identification are: high pressure liquid chromatography (HPLC) combined with fluorescence (FLD) or ultraviolet (UVD) detectors and gas chromatography (GC) combined with flame ionisation (FID) or mass spectrometry (MS). GC/MS is widely used nowadays to determine PAH in foodstuffs (Moret and Conte, 2002; Šimko, 2002; Martin and Ruiz, 2007; Purcaro et al., 2009).

The main aims of this study were to determine the BaP content of a traditional Spanish meat product called chorizo in an as-yet unstudied region, the Principality of Asturias, and to test whether this content will comply with the recently modified maximum BaP content permitted by EU regulation No. 835/2011. An analytical method based on a combination of sonication, SPE and GC/MS was developed and employed for this purpose. Furthermore, the manufacturing method used for each analysed product was studied to evaluate the quality of drying versus BaP content in the final meat product. Finally, the traditional meat product manufacturing method was evaluated to test whether any changes are required to achieve high quality, safe and healthy foods.

2. Materials and methods

2.1. Samples

Chorizo is a typical Spanish smoked meat product made with the following ingredients: pork meat, pork fat, salt, garlic, sweet or spicy paprika and herbs minced, mixed and sheathed in a natural casing (or skin) made from animal intestine. Once prepared, chorizo is smoked for 7–15 days, usually employing a mixture of oak and chestnut woods for this purpose. Nowadays, antioxidants such as sodium citrate and sodium ascorbate, colourings such as carmine, emulsifiers such as sodium triphosphate, food preservatives such as sodium nitrite and other food additives are used to ensure the microbiological stability and quality of the foodstuff.

In this study, 16 samples of chorizo were analysed. All the samples were bought from a market. Each sample came from a different manufacturer in Asturias (a region in the north of Spain). The samples were purchased and analysed between 15th March and 25th November 2012.

Samples A, C, F, I, J, M, O and P are labelled as "extra" quality and samples G, H, K and N as "top" quality products. According to Spanish regulations, the maximum amount of fat, hydroxyproline and total carbohydrates (expressed in terms of glucose content) in "extra" quality chorizos is 57%, 0.8% and 8%, respectively, while the minimum amount of meat proteins allowed in these samples is 30%, expressed in terms of dry matter (DM) content. The maximum amount of fat, hydroxyproline and total carbohydrates (expressed in terms of glucose content) in "top" quality chorizos is 60%, 0.7% and 9%, respectively, and the minimum amount of meat proteins allowed in these samples is 26%, expressed in DM content. The maximum permissible moisture content for all samples is 45%. The standard amount of fat, meat proteins and carbohydrates, in "extra" and "top" quality Spanish chorizos is 57–60%, 26–30% and 8–9%, respectively. Once collected, the samples were stored in the dark at −18 °C.

2.2. Reagents and standards

Benzo[a]pyrene (BaP) analytical standard solution was supplied by Sigma–Aldrich (Kempton Park, South Africa) at a concentration of 100 μg/mL in cyclohexane and a total volume of 2 mL. Benzo[a]pyrene internal standard (d_{12}, 98%) (BaP d-12) solution was obtained from Cambridge Isotope Laboratories, (Andover, MA) at a concentration of 200 μg/mL in isooctane and a total volume of 1.2 mL.

All solvents for sample preparation, hexane, dichloromethane, cyclohexane and isooctane (2,2,4-trimethylpentane), were purchased from Sigma–Aldrich with ACS reagent purities ≥99% (GC) grade.

Solid phase extraction (SPE) cartridges were supplied by Varian Iberica (Madrid, Spain) and Agilent Technologies (Santa Clara, CA). The Mega Bond Elut cartridges employed contain a silica sorbent phase, with a particle size of 40 nm, a weight of 5 g and a volume of 20 mL.

Amber-coloured flasks and vials were used to protect the PAH from light decomposition. Specifically, 15 mL (21 mm × 70 mm) amber glass vials were purchased from Sigma–Aldrich for PAH collection during the SPE step.

2.3. Apparatus

An ultrasonic bath (J.P. Selecta, S.A., Barcelona, Spain) and a Supelco Visiprep™ SPE vacuum manifold with 12 ports (Supelco, Bellefonte, PA) were used for each meat product sample pre-treatment.

An Agilent Technologies 5975 mass spectrometer (MS) coupled to an Agilent Technologies 6890 gas chromatograph (GC) was used for BaP analytical identification. An Agilent Technologies HP-5MS (30 m × 0.25 mm × 0.25 μm) GC column was chosen for this study.

2.4. Sample pre-treatment

2.4.1. Grinding step

First, the skin of the chorizo was removed according to the procedures specified in Fretheim (1976) and Wretling et al. (2010). Then, 200 g of chorizo were finely homogenised in a meat grinder (Minirobot D81 meat grinder; Moulinex, France) following

the protocol devised by Purcaro et al. (2009). Pre-treatment of all samples was carried out in triplicate.

2.4.2. Lyophilisation step

Three aliquots of 20 g chorizo were placed on individual lyophilisation plates, carefully sealed with aluminium foil, and weighed before freeze-drying. Each sample was then frozen to –80 °C for 4 hours and freeze-dried (Telstar-Cryodos, Spain) for 1 day. Finally, the plates (containing the sample) were weighed after freeze-drying.

2.4.3. Sonication step

Two grams of freeze-dried chorizo (one from each lyophilisation plate) were placed in flasks (the remaining lyophilised chorizo was stored in a freezer). Subsequently, 20 mL of *n*-hexane, 200 μL of benzo[a]pyrene-d12 internal standard solution at a concentration of 100 μg/L and 100 μL of benzo[a]pyrene standard solution at a concentration of 100 μg/L were added (dilutions from the stock solution were made previously). This solution was carefully stored under refrigeration for 3 days to help the deuterated compound adapt to the sample matrix. This is the appropriate point to add the internal standard compound as it is the first time both compounds are in the same matrix (Stumpe et al., 2008; Purcaro et al., 2009). Finally, the samples were sonicated for 1 hour at ambient temperature.

2.4.4. Filtration

After sonication, the samples were filtered through filter paper to remove the solid meat waste, which was washed with additional *n*-hexane (2× 5 mL). The solvent was subsequently collected in a flask and the volume was made up to 10 mL with the help of a rotatory evaporator (Heidolph Laborota 4000; Schwabach, Germany) and the addition of the necessary amount of *n*-hexane.

2.4.5. Solid-phase extraction (SPE)

The extract obtained after sonication contained an appreciable amount of fat (Purcaro et al., 2009). A solid-phase extraction (SPE) procedure was used to isolate benzo(a)pyrene and benzo[a]pyrene-d_{12} (BaP-d12) (the sought after PAH in the sample), similar to the previous setup for rapid PAH determination in vegetable oils by Moret and Conte (2002).

First, a 5-g silica SPE cartridge (Mega Bond Elut, 20 mL, Varian, Palo Alto, CA) was slowly washed with 20 mL of dichloromethane, dried completely under vacuum and conditioned with 20 mL of *n*-hexane (to prevent the cartridge from drying). Next, 1 mL of the sonicated sample was diluted in 3 mL of *n*-hexane and slowly loaded onto the cartridge, avoiding complete drying of the cartridge. The aliphatic hydrocarbons were then discharged via the addition of 8 mL of a mixture of hexane and dichloromethane 70:30 (v/v) to the cartridge. Finally, after loading the cartridge with another 8 mL of the hexane and dichloromethane 70:30 (v/v) solution, the PAH, BaP and BaP-d12 contained in the sample were slowly discharged into 15-mL amber glass vials, allowing the cartridge to dry under vacuum.

2.4.6. Concentration step

The samples were concentrated after the SPE step, the volume being reduced to less than 1 mL using a nitrogen stream at the top of the 15-mL vials. Finally, the samples were loaded into 2 mL GC/MS amber glass vials and the final volume was up to 1 mL with the addition of the necessary volume of solvent calculated by weighing the vials and the application of the density of the solution. It was found that during the final concentration step only hexane remains in the sample. For this reason, in this step the density of hexane (ρ_{Hexane} = 0.66 g/mL) must be applied instead of the density of the mixture of hexane and dichloromethane 70:30 (v/v) solution ($\rho_{Mixture}$ = 0.859 g/mL), that is calculated with the theoretical following equation:

$$\frac{1}{\rho_M} = \frac{\hat{x}_{Hexane}\cdot}{\rho_{Hexane}} + \frac{\hat{x}_{Dichlorometane}}{\rho_{Dichloromethane}}.$$

where ρ_M is the density of the mixture; ρ_{Hexane} is the density of hexane at 20 °C: 0.660 g/mL; $\rho_{dichlorometane}$ is the density of dichloromethane at 20 °C: 1.32 g/mL; $\hat{x}_{Hexane}$ is the weight fraction of hexane; $\hat{\boldsymbol{X}}_{Dichloromethane}$ is the weight fraction of dichloromethane.

2.5. Analysis

2.5.1. GC/MS calibration

BaP quantification was performed via prior calibration of the GC/MS system with benzo(a)pyrene and benzo[a]pyrene-d_{12} standard solutions. For calibration purposes, benzo[a]pyrene-d_{12} standard solution was added to check the extraction recovery during pre-treatment methods.

Calibration curves were generated with the Chemstation software of the GC/MS by injecting 6 diluted standard solutions of BaP and BaP-d12 in cyclohexane, in triplicate. Calibration curves were defined as amount ratios (x) (concentration of BaP standard divided by the concentration of the BaP-d12 standard) versus response ratios (y), (ratios of BaP response divided by BaP-d12 standard response). BaP standard concentrations for each calibration vial were 0.05 ng/mL, 0.10 ng/mL, 0.50 ng/mL, 1.00 ng/mL, 2.00 ng/mL and 5.00 ng/mL. The concentration of BaP-d12 for all calibration vials was 2.00 ng/mL. BaP-d12 and BaP concentrations of the calibration method were fitted to the range of BaP concentrations found in the samples. The MS Assistant programme (Agilent Technologies) compares the relationship between both BaP standard signals (BaP and BaP-d12) to determine the final BaP concentration. A blank (i.e. a vial containing only the solvent cyclohexane, with no BaP and BaP-d12 standard compounds) was also included among all the standard vials containing BaP compounds.

2.5.2. Gas chromatography (GC)

Separation of BaP and BaP-d12 was performed on an HP-5MS column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies). The injection temperature and volume were 300 °C and 1 μL (splitless), respectively. Helium was applied as carrier gas at a constant flow rate of 1.5 mL/min. The temperature ramp used was the following: isothermal at 55 °C for 1 min, increasing at a rate of 25 °C/min to 320 °C for 3 min; total run time was 14.60 min.

2.5.3. Mass spectrometry (MS)

Identification of BaP and BaP-d12 was performed using an Agilent Technologies 5975 mass spectrometer with a G2589-20045 6-mm ultra-large aperture drawout lens. The solvent delay was 4 min and the electron multiplier (EM) voltage was 1294 V. The quadrupole temperature was 180 °C, the source temperature 300 °C and the transfer line temperature 280 °C. Full scan spectrum analysis (scan) mode method was used to obtain the full mass spectra of benzo(a)pyrene and benzo[a]pyrene-d_{12} standards, using a concentrated solution of 10 μg/mL. Scan mode was used to identify, and select target and main qualifier ions and the retention time (RT): BaP: RT 12.22 min, target ion: 252, qualifier ions: 264, 126; BaP-d12: RT 12.20 min, target ion: 264, qualifier ions: 252, 126. These data were used to create the selected ion monitoring (SIM) method applied for the analysis of all calibration vials and samples. 50 ms dwell/ion was applied in SIM mode. Peaks spectra were compared to the mass spectra of BaP and BaP-d12 standards. Qualifier-to-target ion ratio criteria were ±10% compared to the standard's ratio for compounds confirmation and assessment of spectral interferences.

Matrix-effect assessment criteria were defined as a ±20% ratio of target ions of BaP and BaP-d12 in samples compared to standards. Detection and quantification limits (*LOD*s and *LOQ*s, respectively) were evaluated on the basis of noise obtained with the analysis of the blank samples. *LOD* and *LOQ* were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively. Recoveries were calculated by comparing the difference between spiked and unspiked samples with the known amount of added BaP standard (1 ppb).

2.6. Statistical analyses

Experimental data were evaluated by running analysis of variance (ANOVA), which determined whether there were any significant differences between means at a 95% confidence level. Multiple range test was used to distinguish which means were significantly different. Standardised skewness and standardised kurtosis were used to assess if the samples came from normal distributions. These analyses were performed using STATGRAPHICS PLUS for Windows 3.0 (StatPoint, Inc., Herndon, VA, USA).

3. Results and discussion

3.1. Procedure performance

The basic analytical method of Purcaro et al. (2009) was applied. Some modifications were implemented to adapt the method for its application to the analysis of chorizo samples and GC/MS determination. Table 1 shows benzo(a)pyrene identification ions (target compound and qualifier ions), retention time, limits of detection (*LOD*) and quantification (*LOQ*) in ng/mL, instrument linear dynamic ranges, determination coefficient (r^2) and recovery ± repeatability for a spiked sample. Target ions ratios and qualifier-to-target ion ratios found in all samples were within the defined criteria (±20%).

One of the key features of the procedure performance is the definition of the appropriate moment to add the standards. All analytes, both those deliberately added (BaP and BaP-d12 standards) and native (original BaP content in the sample), must be firmly and equally bound to the sample under the same conditions. If not, recoveries will be incorrect. An appropriate reference material must be used for the method to be accurate and to achieve precision testing, i.e. one with a similar sample matrix and whose content in the target analytes has been certified. There is no suitable certified material for chorizo. In these cases, a spiking procedure must be used to define recoveries (Purcaro et al., 2009).

The pre-treatment method was accordingly applied to a 2.00-g aliquot of chorizo smoked by means of traditional combustion. During the analytical steps, the best moment to add the BaP-d12 standard is just before sonication, as this is when all the analytes (the natural analyte in the sample and the added BaP) will be in the same matrix, i.e. a liquid matrix. For this reason, hexane, BaP-d12 and BaP standards were added just before sonication and left to stand for 3 days, allowing all the analytes to adapt to the liquid matrix. Flasks were stored under refrigeration in the dark to prevent PAH decomposition due to light exposure (Šimko, 2002).

Figs. 1 and 2 show the ion chromatograms of the spiked and the unspiked sample, respectively. In the lower line, the peak of the target ion 264 of BaP-d12 can be detected at an RT of 12.20 min, and in the upper line the peak of the target ion of BaP 252 can be detected at an RT of 12.22 min. The arrow indicates the BaP peak (added compound), which increases in the spiked sample in comparison with the unspiked one, and its relationship with the BaP-d12 standard peak.

The combination of the sonication and SPE pre-treatment methods to determine the BaP content in smoked meat products by GC/MS analysis was found to be a good method, as it entails a low level of solvent consumption and waste generation, short operating times and is easy to set up.

3.2. BaP content

All the samples were successfully analysed by the proposed method. Fig. 3 shows, as an example, the ion chromatogram of a directly smoked chorizo (sample A). Fig. 4 shows the moisture and BaP content (μg/kg) found in the 16 analysed direct smoked meat products. The samples were alphabetically identified according to their order of analysis. In this figure, horizontal bold lines have been used to indicate the BaP content limit specified by the EU regulation before 31/8/2014, i.e. 5 μg/kg (upper line), and the current regulation, applied from 1/9/2014, i.e. 2 μg/kg (lower line).

3.2.1. Moisture and BaP content in smoked meat products

One of the most important goals of the smoking process during chorizo manufacturing is drying. The water content of meat products decreases during this process. This reduction in moisture content is important, as it has an inactivating effect on microbial growth, hence extending the shelf life. However, as the Codex Alimentarius CAC/RCP 68/2009 specifies and some scientific studies confirm, the PAH content in meat products generally increases during the smoking process (Djinovic et al., 2008). As the Codex Alimentarius advises, the smoking process should be optimised to obtain smoked products with a low PAH content, but a good microbiological status, organoleptic properties in the final product. The ideal method should have no adverse effects on the product's appearance, flavour, taste or nutritional properties.

As Fig. 4 and Table 2 show, there is little relationship between moisture and BaP content in the analysed smoked meat products. ANOVA indicated that there is a statistically significant difference between the means of the 16 variables at the 95.0% confidence level. In order to determine which means are significantly different from others in the whole analysis of the samples range, multiple range test was applied to the results. This test showed 10 homogeneous groups with significant differences between

Table 1
Benzo(a)pyrene (BaP) confirmation ions, retention time, limits of detection (*LOD*) and quantification (*LOQ*) in ng/mL, instrument linear dynamic ranges, determination coefficient (r^2) and recovery ± repeatability.

Determined compound	Ions	Retention time (min)	LOD (ng/mL)	LOQ (ng/mL)	Instrument linearity		Recovery assays	
					Standards concentration range (ng/mL)	r^2	Added (ng/mL)	Recovery ± RSD (%) range
Benzo(a)pyrene	Target: 252 Qualifiers: 264, 126	12.22	0.05	0.24	0.05–5	0.999	1.00	98 ± 1.04 to 102 ± 3.70

(LOQ) in μg/L (n = 8).

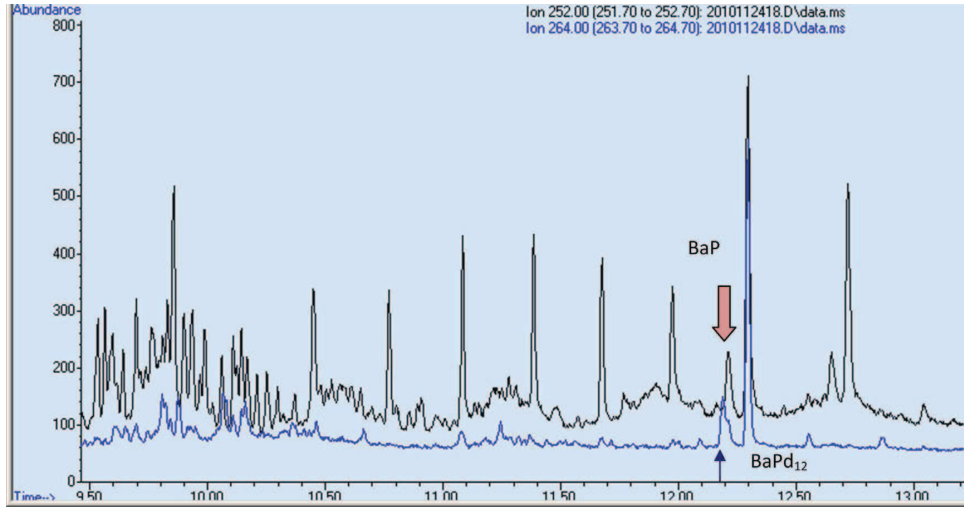

Fig. 1. 1.0 ng/mL Benzo(a)pyrene (BaP) spiked sample of chorizo.

each other ($p < 0.05$). As can be seen in Fig. 4, the sample with the highest BaP content in this study has the lowest moisture content (sample O). This could suggest that when the moisture content decreases, the BaP content increases, which could occur when increasing smoking time, in accordance with other studies (Djinovic et al., 2008). However, the other results obtained in the present study indicate that chorizos with a similar BaP content can have very different moisture contents.

In conclusion, the smoking process can be adapted to obtain smoked meat products with a low moisture content which present a good microbiological status and a low BaP content. The traditional direct smoking process is affected by a large number of parameters, making it difficult to avoid PAH contamination of foods (Djinovic et al., 2008; Stumpe et al., 2008; Lorenzo et al., 2011; Santos et al., 2011; Roseiro et al., 2012). However, producers should study and control their own traditional processes to make healthier foods, as explained in the following sections.

3.2.2. BaP content in smoked meat products with regard to the regulation limit

As can be seen in Fig. 4, the chorizo samples analysed in this study present a BaP content ranging between less than 0.38 and 3.21 μg/kg in wet weight. This means that all the studied samples comfortably met the previous legal limit set at 5 μg/kg in wet weight. However, 5 of them (31% of the samples) do not meet the new limit of 2 μg/kg applicable from 1/9/2014. Fig. 5 summarises the average, maximum and minimum BaP contents (μg/kg) found in direct smoked samples of the tested products. The results obtained in this study indicate that smoked meat product manufacturers should make some modifications in the smoking process to offer healthy smoked meat products in compliance with EU regulation No. 835/2011.

In its chapter "Code of practice for the reduction of contamination of food with (PAH) from smoking and direct drying processes" (CAC/RCP 68/2009), Codex Alimentarius points out that adaptations

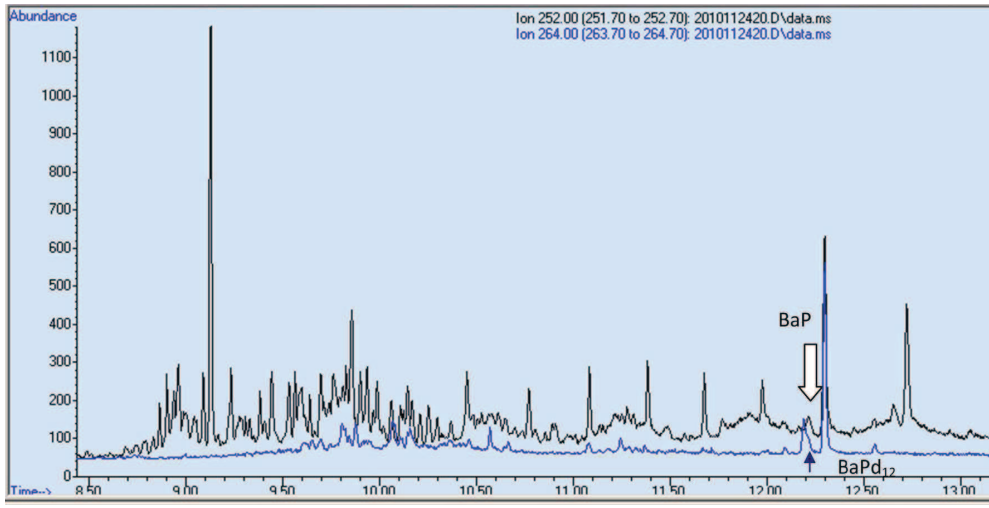

Fig. 2. Unspiked sample of chorizo.

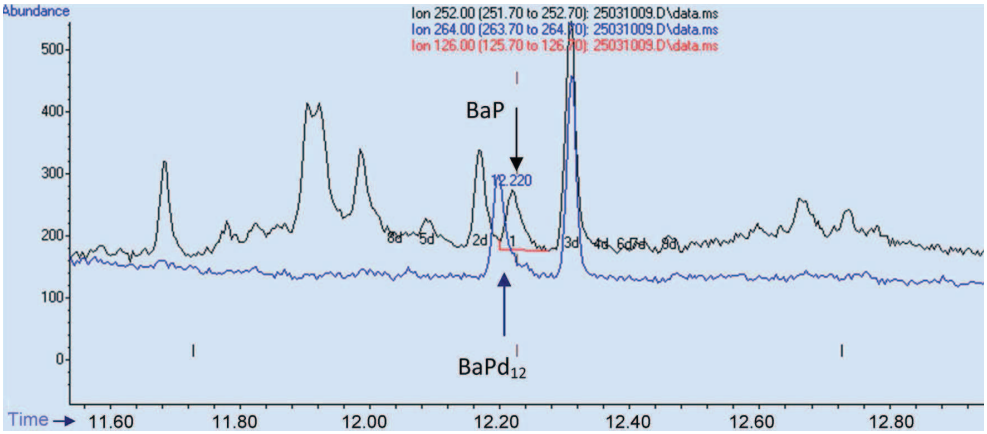

Fig. 3. 252 Benzo(a)pyrene (BaP) and 264 Benzo(a)pyrene (BaP D12) ions chromatograms of a 1.0 ng/mL BaP spiked sample of direct smoked chorizo (sample A).

of current smoking technology may be necessary in some cases. It stipulates that PAH contamination in foodstuffs via food processing should be controlled taking into account the chosen food processing technology. In order to regulate PAH formation during smoking and direct drying, the code recommends controlling a number of variables. These include the kind of fuel, smoking or drying method (direct or indirect), the process of generating smoke in relation to the temperature of pyrolysis and to airflow in the case of a smoke generator (friction, smouldering, thermostated plates) or in relation to other methods, such as direct smoking or regenerated smoke by atomising smoke condensate (liquid smoke), the distance between the food and the heat source, the position of the food in relation to the heat source, the fat content of the food and what happens during processing, the duration of smoking and direct drying, the temperature during smoking and direct drying, the cleanliness and maintenance of equipment and the design of the smoking chamber and the equipment used for the smoke/air mixture. It especially recommends replacing direct smoking processes (traditional smoking methods by means of wood combustion) by indirect ones, such as the use of a friction smoke generator. Only a few of these factors have been studied. For instance, it has been demonstrated that the kind of wood used for smoking meat

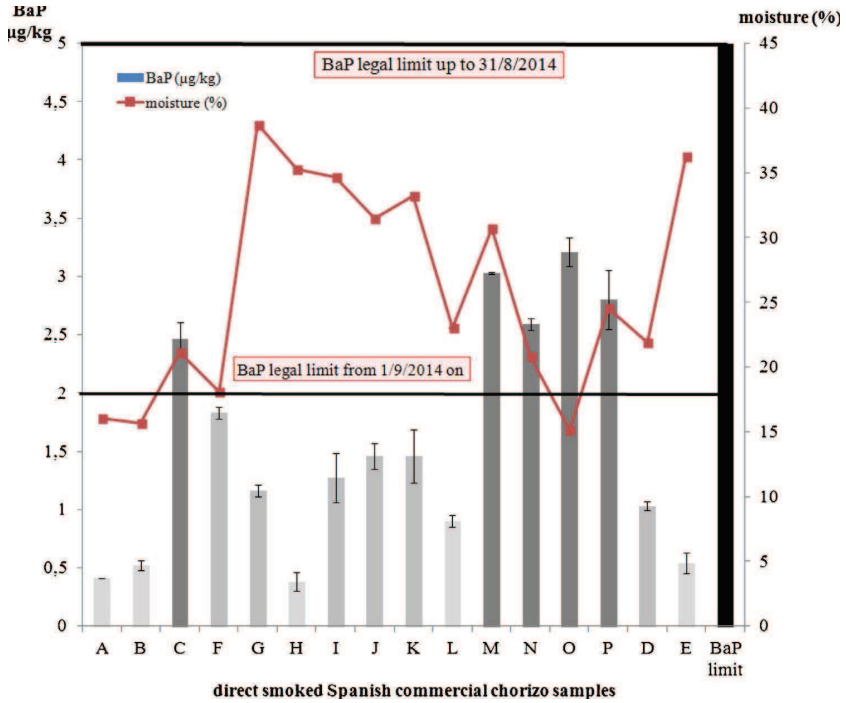

Fig. 4. Benzo(a)pyrene (BaP) content (µg/kg) and moisture (%) of direct smoked chorizo and comparison with the EU Regulation limit ($n = 3$).

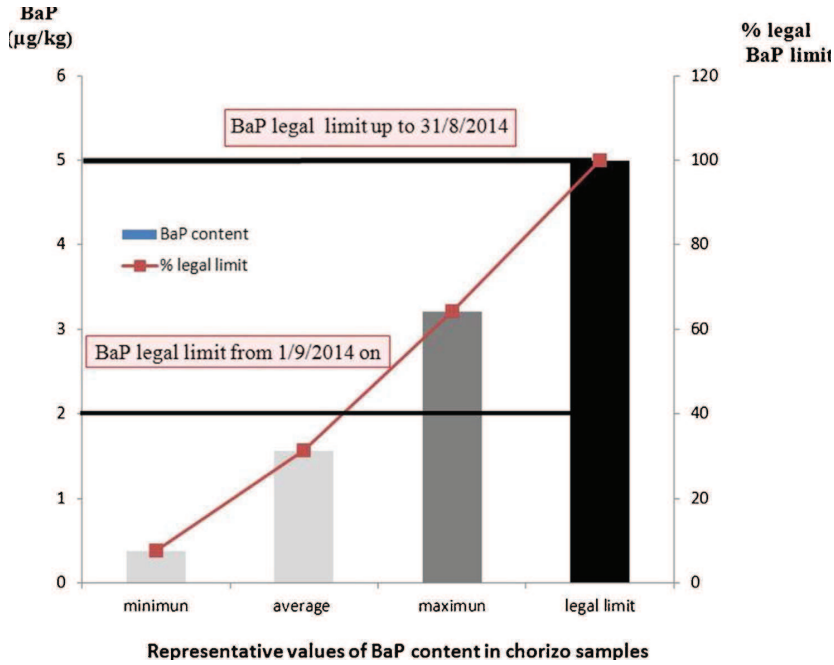

Fig. 5. Benzo(a)pyrene (BaP) content (µg/kg) in direct smoked chorizos from the north of Spain and comparison with the EC Regulation limit.

products has a significant influence on the amount of PAH in the final product (Stumpe et al., 2008). Changes in the traditional method for smoking meat products will be needed to ensure healthy products. More scientific background and data are needed to elucidate the precise influence of the use of different types of smoking methods and other process parameters in food contamination with PAH carcinogenic compounds.

Table 2
Benzo(a)pyrene (BaP) content (µg/kg) and multi-range statistical analysis of commercial chorizo samples ($n = 3$).

Sample	BaP (µg/kg) mean ± uncertainty	Moisture (%)	Multi-range statistical analysis: heterogeneous groups of BaP content samples ($p < 0.05$)
M	3.03 ± 0.01	30.73	Group 1
O	3.21 ± 0.12	15.13	
P	2.8 ± 0.25	24.54	Group 2
C	2.46 ± 0.15	21.17	Group 3
N	2.59 ± 0.05	20.84	
F	1.83 ± 0.05	18.11	Group 4
J	1.46 ± 0.11	31.45	Group 5
K	1.46 ± 0.23	33.22	
I	1.27 ± 0.21	34.65	Group 6
J	1.46 ± 0.11	31.45	
G	1.16 ± 0.05	38.74	Group 7
I	1.27 ± 0.21	34.65	
D	1.03 ± 0.04	21.93	Group 8
G	1.16 ± 0.05	38.74	
D	1.03 ± 0.04	21.93	Group 9
L	0.9 ± 0.05	23.04	
H	0.38 ± 0.08	35.31	Group 10
A	0.41 ± 0.00	16.04	
B	0.52 ± 0.04	15.68	
E	0.54 ± 0.09	36.25	

3.2.3. Improving the traditional smoked meat product smoking process

Traditional meat product smoking is a non-optimised food processing technology. The temperature and moisture parameters of traditional smoking chambers depend on the unpredictable, variable values of weather conditions. The usual smoking time for traditionally manufactured Spanish chorizo ranges between 10 and 15 days of smoking, the whole process requiring about 3 weeks. During this process, wood combustion and fire are produced, which require a temperature above 700 °C (Demirbas, 2009). The amount of PAH in smoke, formed during pyrolysis, increases linearly with smoking temperature within the interval 400–1000 °C (Tóth and Blaas, 1972). Likewise, the PAH content in smoked meat products generally increases during smoking (Djinovic et al., 2008). This process is not environmentally friendly, as an unnecessary amount of excess wood is used for combustion in some cases. In fact, the amount of raw material used to produce smoke is not monitored.

Furthermore, safety in the workplace is currently one of the most important factors of industrial quality. Safety in the meat industry is threatened by traditional uncontrolled smoking processes. For instance, over the last 5 years more than 9 smoked meat product companies located within the geographical scope of this study have burnt down because of uncontrolled combustion of wood. Moreover, product weight losses are not always the same and product standardisation and homogenisation, nowadays demanded by customers as well as wholesalers, are yet to be achieved in this northern region of Spain. All these facts indicate that the traditional smoking process needs improving.

4. Conclusions

The combination of sonication, SPE and GC/MS was optimised to reveal new data on the BaP content of 16 smoked meat products (chorizos) from different producers of an as-yet unstudied region,

the Principality of Asturias, linking it with their moisture content to verify the quality of the manufacturing process.

The range in BaP content found in direct smoked meat products was 0.38–3.21 μg/kg. Five of the 16 direct smoked samples do not meet the 2 ppb BaP limit stated by EU regulation No. 835/2011, which was introduced on 1/09/2014. The average BaP content found in the samples (1.57 μg/kg) falls below the new permissible BaP limit. The range in moisture content found in the samples was 15.1–38.7%. There was no correlation between BaP concentration and moisture content.

Uncontrolled traditional direct smoking methods do not constitute an environmentally friendly, safe or optimised food processing technology, as they do not allow product standardisation. However, the direct smoking process can be adapted to obtain healthy, good quality products with low moisture content which present a good microbiological status and low BaP content.

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4.2 MECANISMO DE PENETRACIÓN DE BENZO(A)PIRENO EN CHORIZO: INFLUENCIA DEL TIEMPO DE AHUMADO Y LA PROFUNDIDAD

El Código (CAC/RCP 68/2009) del Códex Alimentarius establece las 10 variables que han de ser controladas para reducir la contaminación por hidrocarburos aromáticos policíclicos (HAP) en los alimentos producidos por procedimientos de ahumado y secado directo. Entre estas variables se encuentran el tipo de combustible, el método de ahumado o secado (directo o indirecto), el procedimiento de generación de humo, el uso de humo líquido, la distancia entre el alimento y la fuente de calor, la posición del alimento con respecto a la fuente de calor, el contenido de grasa del alimento y lo que le sucede durante el procedimiento, la duración del procedimiento de ahumado o secado directo, la temperatura y la limpieza y el mantenimiento de los utensilios. Cada una de estas variables ha sido estudiada por varios autores, concluyendo que en muchos casos, en especial los incontrolados sistemas tradicionales de ahumado a modo directo, es difícil controlar todas las variables, por lo que deben estudiarse por separado. Por otro lado, como tratamiento posterior al ahumado el código aconseja medidas para eliminar el hollín y las partículas que contienen HAP en la superficie del alimento.

En el presente trabajo se presentan los resultados del estudio de una de estas variables, el tiempo de ahumado, en el proceso de elaboración de chorizo asturiano a modo directo. Así mismo, por primera vez se realiza un estudio pormenorizado de la penetración del marcador benzo(a)pireno y evacuación de la humedad en distintas profundidades de chorizo ahumado. Se realiza especial hincapié en evaluar el proceso de concentración del producto durante el secado. Con los resultados obtenidos se propone por primera vez un mecanismo que define el proceso de contaminación del chorizo por HAP durante el ahumado, ofreciendo datos relevantes para la comunidad científica.

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Benzo(a)pyrene penetration on a smoked meat product during smoking time

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The Codex Alimentarius gives recommendations to prevent carcinogenic polycyclic aromatic hydrocarbons (PAH) (represented by benzo(a)pyrene (BaP)) contamination during processing of meat products, including the control of smoking time. The influence of direct smoking time (0, 1, 3, 5 and 7 days) on the relationship between the BaP and moisture content of a typical Spanish smoked meat product called chorizo and the mechanism of BaP penetration and water release from four different depths in the product was studied. Chorizo was studied from the Principality of Asturias, a location never before tested. An analytical method was developed for this purpose consisting of PAH extraction assisted by sonication followed by solid-phase extraction (SPE) sample clean-up and analytical determination using GC-MS. Results show that an increase in smoking time produced contradictory and independent effects on the moisture and BaP content (μg kg^{-1}) of chorizos. The moisture content decreased from 49.9% to 31.3%. On the other hand, the BaP content increased from less than 0.24 μg kg^{-1} to 0.75 μg kg^{-1}, finally stabilising after 5 days of smoking. After this time, the natural pores of the casing could be blocked by the large size tar particles from smoke, preventing the continued penetration of PAHs. The BaP content decreased and the moisture content increased progressively from the casing to the centre of the meat product. BaP mainly accumulated in the smoked casing, being four times in excess of the legal limit. This paper analyses the mechanism for preventing PAHs contamination during the process of smoking meat products.

Keywords: benzo(a)pyrene (BaP); moisture content; smoking time; depth; polycyclic aromatic hydrocarbons (PAHs); smoked meat product

Introduction

Meat and meat products are high-value foods. Meat production is foreseen to double by 2050 due to its nutritional composition, essential for the growing world population. Meat products have high-quality protein, containing all the essential amino acids, and highly bioavailable minerals and vitamins (Food and Agriculture Organization of United Nations 2013).

Meat products have been smoked since the beginning of time, principally for the purpose of food preservation via drying (Möhler 1978; Varlet et al. 2007). Smoking technology continues to be used nowadays because of the sensory active components contained in smoke (phenol derivates, carbonyls, organic acids and their esters, lactones, pyrazines, pyrols and furan derivatives) in the aromatisation and colouring of meat products, widely demanded characteristics by the consumer (Šimko 2002) and due to the antioxidant action of certain components, especially phenol derivatives (Möhler 1978).

Food can be contaminated during smoking by undesired by-products formed via biomass gasification and pyrolysis, namely tar. Tar is a thick, black and highly viscous liquid that can create the following problems: direct condensation and deposition on foods, formation of tar aerosols and polymerisation into more complex structures. Above 750ºC, tertiary tar products begin to appear with increasing temperature. Condensed tertiary aromatics make up a series of polynuclear aromatic hydrocarbons (PAHs) without substituents (atoms or a group of atoms substituted by hydrogen in the parent hydrocarbon chain) (Basu 2010).

PAHs have been found to be carcinogenic, mutagenic, lipophilic and bioaccumulative. The most significant endpoint of PAH toxicity is cancer (ATSDR 1995, 2009). Benzo(a) pyrene (BaP) is the current marker for the occurrence and effect of PAHs in meat products according to European Union Commission (EC) Regulations No 1881/2006 and (EU) No 835/2011. This new regulation specifies that two dates must be considered as regards PAH contamination in foodstuffs. New maximum levels for the sum of four substances (PAH4) (BaP, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) were introduced, maintaining a separate maximum level for BaP, to ensure comparability between previous and future data. Moreover, a recent study by Lorenzo et al. (2011) reports it to be a good marker for the sum of 15 PAHs as well as for seven PAHs considered probable human carcinogenic by the USEPA in 'chorizo gallego'.

Humans are exposed to PAHs due to environmental contamination, though mainly because of diet (Lodovici et al. 1995; Phillips 1999; Falcó et al. 2003; Ibáñez et al.

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2005). Food can be PAHs contaminated because of environment contamination and manufacturing processes such as smoking, drying, roasting, baking, barbecuing and frying (Rey-Salgueiro et al. 2008, 2009; Rey-Salgueiro, García-Falcón, et al. 2008; Rey-Salgueiro, Martínez-Carballo, et al. 2008 Codex Alimentarius Commission, CAC/RCP 68-2009). In non-occupational settings, up to 70% of PAH exposure for a non-smoker can be associated with the diet (Skupinska et al. 2004). Although several foodstuffs are contaminated with PAHs, high levels have been found in particular in smoked meat products, thus making their study a matter of importance (Larsson et al. 1988; Gomaa et al. 1993; Karl & Leinemann 1996; Martorell et al. 2010).

To protect consumers from these contaminants, the European Commission has set maximum levels for PAHs in foodstuffs. The current maximum permissible BaP content in smoked meat and smoked meat products is 5.0 μg kg^{-1} (wet weight) until 31 August 2014 and will be 2.0 μg kg^{-1} (wet weight) from 1 September 2014 on (European Union Commission (EU) Regulation No. 835/2011). The permissible sum of PAH4 in these foods will be 30.0 μg kg^{-1} in wet weight from 1 September 2012 to 31 August 2014 and 12.0 μg kg^{-1} in wet weight from 1 September 2014 on. The USFDA regulates contaminant levels in foodstuffs, but has not still established standards governing the PAH content of foodstuffs. The maximum contaminant level goal for BaP in drinking water is 0.2 parts per billion (ppb). In 1980, the USEPA established ambient water quality criteria to protect human health from the carcinogenic effects of PAH exposure. The recommendation was a goal of zero (non-detectable level for carcinogenic PAHs in ambient water). As a regulatory agency, the USEPA establishes a maximum contaminant level (MCL) for BaP, the most carcinogenic PAHs, at 0.2 ppb (ATSDR 2009; EPA 2013).

The 'Code of Practice for the Reduction of Contamination of Food with PAHs from Smoking and Direct Drying Processes' (CAC/RCP 68/2009) and recent studies (Djinovic et al. 2008; Stumpe-Vīksna et al. 2008; Lorenzo et al. 2011; Santos et al. 2011; Roseiro et al. 2012) state that PAH contamination in foodstuffs via food processing should be controlled by taking into consideration several variables of food processing technology. One of these parameters is the duration of the smoking process. They especially specify that smoking time should be as short as possible to minimise the exposure of food surfaces to PAH-bearing smoke, but long enough to allow the product to be thoroughly cooked and to ensure food safety, shelf life, and good sensory and nutritional properties.

A variety of both classical and modern pre-treatment and analytical methods have been used to determine PAHs in foods. Classical pre-treatment methods include saponification, liquid–liquid extraction and Soxhlet in combination with gel permeation chromatography (GPC) (Grimmer & Böhnke 1975; Fretheim 1976; Chiu et al. 1997; Šimko 2002). Modern methods include microwave-assisted extraction (MAE), sonication (Purcaro et al. 2009), accelerated solvent extraction (ASE) (Djinovic et al. 2008; Martorell et al. 2010; Sun et al. 2012), ultrasound-assisted solvent extraction (USAE), ultrasound-assisted emulsification–microextraction (USAEME) (Yebra-Pimentel et al. 2013) or pressurised liquid extraction (PLE) (Pöhlmann et al. 2013), followed by SPE (García-Falcón et al. 2004, 2005; Purcaro et al. 2009; Hitzel et al. 2013), stir-bar sorptive extraction (SBSE) (García-Falcón et al. 2004) or solid-phase microextraction (SPME). Direct extraction devices (DED) have also been proposed (Martin & Ruiz 2007). The combination of sonication with SPE methods has been tested in some foodstuffs, giving results that are both fast and inexpensive (Purcaro et al. 2009). Typical analytical methods for PAH identification are: HPLC combined with fluorescence (FLD) or ultraviolet (UV) detectors (Moret & Conte 2002; Purcaro et al. 2009; Lorenzo et al. 2011; Santos et al. 2011; Roseiro et al. 2012) and GC combined with flame ionisation (FID), ion trap (ITD) and mass spectrometry (MSD) detectors (Hitzel et al. 2013; Olatunji et al. 2014). GC-MS and GC/HRMS are widely used nowadays to determine PAHs in foodstuffs (Šimko 2002; Martin & Ruiz 2007; Purcaro et al. 2007; Djinovic et al. 2008; Stumpe-Vīksna et al. 2008; Martorell et al. 2010; Wretling et al. 2010; Hitzel et al. 2013).

The main aim of the present study was to determine the influence and relationship of direct smoking time on the BaP and moisture content of a traditional Spanish meat product called chorizo in an as-yet unstudied region, the Principality of Asturias, to assess the quality of drying with regard to BaP content, according to European Union Commission (EU) Regulation No. 835/2011. Moreover, the BaP and moisture content at different depths in chorizo were assessed in order to define the mechanism of penetration of this compound into the food product during drying. An analytical method based on a combination of sonication, SPE and GC-MS was developed and employed for this purpose.

Materials and methods

Sample preparation

All samples comprise the same kind of typical Spanish smoked meat product called chorizo, made of the following ingredients: pork loin (46.8%), pork jowl (46.8%), salt (1.8%), garlic (1%), sweet or spicy paprika (2%) and herbs (1.6%), minced, mixed and encased in a natural casing made from animal intestine. Antioxidants such as sodium citrate or sodium ascorbate, colourings such as carmine, emulsifiers such as sodium triphosphate, food preservatives such as sodium nitrite and other food additives were used to ensure the microbiological stability and quality of the product.

All samples were manufactured at the El Hórreo Healthy Food S.L. meat company facilities, where 50 kg of chorizo raw materials were minced, mixed, marinated and stuffed to obtain 200 chorizo strings of four chorizos. Then chorizos were exposed to traditional direct smoking. A mixture of oak (90%) and chestnut (10%) woods was used for smoking. All the selected samples were placed in the same position in the smoking chamber, at the same distance from the smoke source, namely 10 m.

The nutritional details of a 5-day smoked chorizo manufactured by the company with the same ingredients and manufacturing process like the one studied here, determined by the Principality of Asturias Meat Industry Association Technological Centre for Supporting Innovation (Spanish acronym, ASINCAR), are: 31.41% moisture content, 22.98% protein, 37.94% fat, 2.85% carbohydrates, 4.82% ash and pH 5.2.

The samples were classified according to the two kinds of studies carried out: those made to study the influence of smoking time on the BaP and moisture content of chorizo; and those made to study the moisture content and penetration of BaP at different depths of smoked chorizos.

Study on the BaP content in chorizo during smoking time

One string of four chorizos was selected before the smoking process. This sample was denominated the 't0 sample'. Four strings of four chorizos were hung 40 cm apart on a bar, placed in the same position and location in the smoking chamber and collected after the following smoking times: 1, 3, 5 and 7 days. These samples were respectively denominated samples t1, t3, t5 and t7. Once collected, the samples were labelled and stored in the dark at 18ºC.

Study on the BaP content at different depths of chorizo

One string of four chorizos was hung on a bar, placed in the smoking chamber and collected after 5 days of smoking. Once collected, the samples were labelled and stored in the dark at 18ºC. Two chorizos from the string were chosen for the experiments. The chorizos were denominated samples A and B, respectively. The overall length and width of the samples were 12.5 and 4 cm for sample A and 15.0 and 3.5 cm for sample B, respectively. The overall chorizo weights were 116.9 and 113.4 g, respectively, for samples A and B. During the sampling process, four depths were defined for each chorizo: D1, D2, D3 and D4. The casing of the sample was denominated depth 1 (D1). Once the casings were removed, samples A and B were cut in slices to obtain the amount of product defining the following depths: the amount of chorizo situated at a distance between 0.25 cm from the casing and the casing of the chorizo was denominated depth 2 (D2). The amount of chorizo situated at a distance of 0.25 cm from the centre and the centre of the chorizo was denominated depth 4 (D4). The amount of chorizo located between D4 and D2 was denominated depth 3 (D3). Figure 1 and Table 1 show the average dimensions and weights of depths of the chorizos defined in this study.

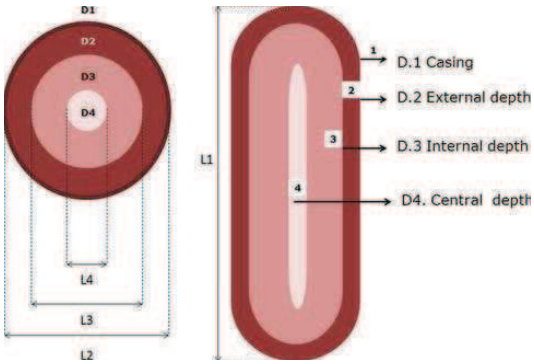

Figure 1. (colour online) Sample dimensions employed in the study on BaP content at different chorizo depths.

Table 1. Chorizo dimensions and mass for depth study.

Dimension	Length (cm)	Depth/sample	Mass (g)	Mass (%)
L1	13.7	D1	1.15	1.00
L2	3.75	D2	23.6	20.5
L3	3.25	D3	65.4	56.8
L4	0.50	D4	25.0	21.7
Total			115.1	100.0

Reagents and standards

Benzo(a)pyrene (BaP) analytical standard solution was supplied by Sigma-Aldrich (Seelze, Germany) at a concentration of 100 µg ml^{-1} in cyclohexane and a total volume of 2 ml.

BaP internal standard (D12, 98%) solution was obtained from Cambridge Isotope Laboratories (Andover, MA, USA) at a concentration of 200 µg ml^{-1} in isooctane and a total volume of 1.2 ml.

All solvents for sample preparation, hexane, dichloromethane, cyclohexane and isooctane (2,2,4-trimethylpentane) were purchased from Sigma-Aldrich, puriss grade, ACS reagent, ≥ 99% (GC).

SPE cartridges were supplied by Varian (Palo Alto, CA, USA) and Agilent Technologies (Santa Clara, CA, USA). Mega Bond Elut cartridges contain a silica sorbent phase, with a particle size of 40 nm, a weight of 5 g and a volume of 20 ml.

Amber-coloured flasks and vials were used to protect the PAHs from light decomposition. In particular, 15 ml (21 × 70 mm) amber glass vials were purchased from Sigma-Aldrich for PAHs collection during the SPE step.

Sample pre-treatment

Grinding step

First, the casing of the chorizo was removed and stored. Then, 200 g of chorizo for the A experiments and the total amount of each depth for the B experiments were finely homogenised in a meat grinder (Minirobot D81 meat grinder, Moulinex, France) following the protocol devised by Purcaro et al. (2009). Pre-treatment of all samples was carried out in triplicate.

Lyophilisation step

Three aliquots of 20 g chorizo, or the total amount in the case of the D1 samples, were taken, placed in individual lyophilisation plates, carefully sealed with aluminium foil and weighed before freeze-drying. Each sample was then frozen to –80ºC for 4 h and freeze-dried in a lyophiliser (Telstar-Cryodos, Spain) for 1 day. Finally, the plates (containing the sample) were weighed after freeze-drying.

Sonication step

A total of 2 g of freeze-dried chorizo (one from each lyophilisation plate), or the total amount in the case of the D1 samples after pulverisation, were placed in flasks (the remaining amount of lyophilised chorizo was stored in a freezer). Then, 20 ml of *n*-hexane and 200 µl of benzo(a)pyrene-d12 (BaP-d12) internal standard solution at a concentration of 100 µg l^{-1} were added (dilutions from the stock solution must be made previously). To help the deuterated compound adapt to the sample matrix, this solution was carefully stored under refrigeration for 3 days. This is the appropriate point to add the internal standard compound as it is the first time both compounds are in the same matrix (Stumpe-Vīksna et al. 2008; Purcaro et al. 2009). Finally, the samples were sonicated for 1 h in an ultrasound bath (Cod. 3000513, J.P. Selecta, S.A, Barcelona, Spain) at ambient temperature.

Filtration

After sonication, the samples were filtered on a paper filter (reference number 1242, 11 cm, Albet LabScience; Albet-Hahnemuehle S.L, Barcelona, Spain)) to remove the solid meat waste, which was washed with additional *n*-hexane (5 + 5 ml). The solvent was subsequently collected in a flask, making up the volume to 10 ml with the help of a rotary evaporator (Heidolph Laborota 4000 efficient, Heidolph-instruments, Schwabach, Germany) and the addition of the necessary amount of *n*-hexane.

SPE step

The extract obtained after sonication contained an appreciable amount of fat (Purcaro et al. 2009). An SPE procedure in a Supelco Visiprep TM SPE vacuum manifold with 12 ports (Sigma-Aldrich), was used to isolate BaP and BaP-d12 (the sought after PAHs in the sample) similar to the previously designed set-up for rapid PAHs determination in vegetable oils by Moret & Conte (2002).

First, a 5 g silica SPE cartridge (Mega Bond Elut, 20 ml, Varian) was slowly washed with 20 ml of dichloromethane, dried completely under vacuum and conditioned with 20 ml of *n*-hexane (to prevent the cartridge from drying). Next, 1 ml of the sonicated sample was diluted in 3 ml of *n*-hexane and slowly loaded into the cartridge, preventing the cartridge from drying completely. The aliphatic hydrocarbons were then discharged via the addition of 8 ml of a mixture of hexane and dichloromethane 70:30 (v/v) to the cartridge. Finally, after loading the cartridge with another 8 ml of the hexane and dichloromethane 70:30 (v/v) solution, the PAHs, BaP and BaP-d12 contained in the sample were slowly discharged into 15 ml amber glass vials, allowing the cartridge to be dried under vacuum.

Concentration step

The samples were concentrated after the SPE step. The volume being made up to 1 ml by N_2 blowing using a nitrogen stream at the top of the 15 ml vials placed in a fume hood.

Finally, the samples were loaded into 2 ml GC-MS amber glass vials and the final volume of 1 ml was checked by weighing the vials.

Analysis

GC-MS calibration

BaP quantification was performed via prior calibration of the GC-MS system with BaP and BaP-d12 standard solutions. For calibration purposes, BaP-d12 standard solution was added to check the extraction recovery during pre-treatment methods.

Calibration curves were generated by injecting six diluted standard solutions of BaP and BaP-d12 in cyclohexane, in triplicate. BaP standard concentrations for each calibration vial were: 0.05, 0.1, 0.5, 1.0, 5 and 10 ppb. The concentration of BaP-d12 for all calibration vials was 2 ppb. The MS assistant program (Agilent Technologies) compares the relationship between both BaP standard signals (BaP and BaP-d12) to determine the final BaP concentration. Identification of compound was assessed by comparing the retention time and confirm the ion ratios (qualifier ion/target ions) with a criterion of ±20% of standards compounds ratio. The criterion of BaP/BaP-d12 standards target ion ratios was ±20%. A blank (i.e. a vial containing only the solvent cyclohexane, with no BaP

or BaP-d12 standard compounds) was also included among all the standard vials containing BaP compounds.

GC

Separation of BaP and BaP-d12 was performed on an HP-5MS 19091S-433 part number column (30 m × 250 μm × 0.25 μm) in a 6890 gas chromatograph (GC) (all Agilent Technologies). The injection temperature and volume were 300ºC and 1 μl (splitless), respectively. Helium was applied as carrier gas at a constant flow rate of 1.5 ml min^{-1}. The temperature ramp used was the following: isothermal at 55ºC for 1 min, increasing at a rate of 25 °C min^{-1} to 320ºC for 3 min, total run time 14.60 min.

MS

Identification of BaP and BaP-d12 was performed using an Agilent Technologies 5975 mass spectrometer detector (MS) equipped with a G2589-20045 part number 6-mm ultra-large aperture draw-out lens. The solvent delay was 4.00 min and the EM voltage (run at auto-tune voltage) was 1294 V. The low and high masses scanned were 252 and 264 amu, respectively. SIM mode was selected for one group, three ions per group (252, 264 and 126), 50 ms dwell/ion. The quad temperature was 180°C, the source temperature 300°C and the transfer line temperature 280° C. Data were acquired by operating the MS in ion-monitoring mode. Peak spectra were compared with the mass spectra of BaP and BaP-d12 standards and the library supplied with the instrument. Nine different spiked samples (as well as unspiked controls) were prepared and each sample and vial were analysed in triplicate according to the analytical procedure. Recoveries were calculated by comparing the difference between spiked and unspiked samples with the known amount of added BaP standard (1 ppb). LODs and LOQs were evaluated on the basis of noise obtained with the analysis of the blank samples. LODs and LOQs were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively. Moreover these results were confirmed by the analysis of blank samples and six diluted standard solutions of BaP and BaP-d12 in cyclohexane, in triplicate. The slope (m) and standard deviation of intercept ($sb0$) of analytical response (y) versus concentration level added (x) were calculated, then LOD and LOQ were also estimated with following equations: LOD = 3.3*$sb0$/m and LOQ = 10*$sb0$/m.

Statistical analysis

Data from each study were analysed by running Fisher's least significant difference (LSD) procedure for multi-sample statistical comparison. This method was used to discriminate among the means. Besides t-tests between-consecutive samples were applied to compare means at a 5% probability level. Previously F-tests at a 5% probability level were carried out to compare SDs. Standardised skewness and standardised kurtosis were used to assess if the samples came from normal distributions. The software used was STATGRAPHICS Plus 3.1.

Results and discussion

All samples were successfully analysed by the proposed method. Previous studies consisting of the combination of Soxhlet and gel permeation chromatography (GPC) were carried out in order to evaluate analytical methods for BaP determination in smoked meat products. The combination of the sonication and SPE pre-treatment methods to determine the BaP content in smoked meat products by GC-MS analysis was found to be a good method, as it entails a low level of solvent consumption and waste generation, short operating times and is easy to set up. Figure 2 shows the BaP and BaP-d12 target ions chromatograms of a casing chorizo sample obtained by SIM analysis mode of

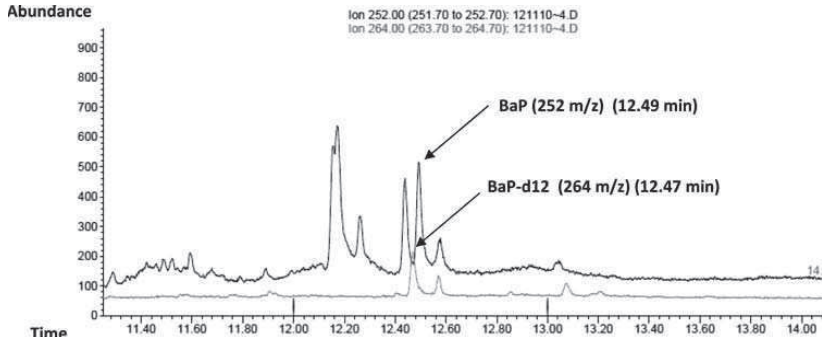

Figure 2. BaP (mass 252 m/z, black upper chromatogram) and BaP-d12 internal standard (mass 264 m/z, grey lower chromatogram) target ions chromatograms of casing sample.

GC/MS. Similar retention time was found for BaP and BaP-d12 standards, 12.47 and 12.49 s respectively. Ion ratios in all samples were within the required criterion (±20) and compounds were confirmed. This method offers high-quality quantitative results with high extraction efficiency. The LOD found was 0.05 μg kg^{-1} and the LOQ found was 0.24 μg kg^{-1}. Recoveries ranged from 99% to 102% and RSDs were lower than 4%.

Influence of direct smoking process time on the BaP and moisture content of chorizo

All the samples were analysed following the proposed method. Figure 3 shows the moisture and BaP content (μg kg^{-1}) found in the five analysed chorizos processed with different smoking times (0, 1, 3, 5 and 7 days). As Figure 3 shows, an increase in smoking time produces opposite effects on the moisture (%) and the BaP content (μg kg^{-1}) of chorizo. While the moisture content decreases (49.9% ± 3.2%, 42.9% ± 2.5%, 36.9% ± 0.4%, 33.3% ± 1.0% and 31.3% ± 1.2%), the BaP content increases and finally becoming stabilised after 5 days of smoking (from less than 0.24 μg kg^{-1} LOQ for 0 and 1 days of smoking to 0.37 ± 0.05, 0.75 ± 0.05 and 0.75 ± 0.05 for 3, 5 and 7 days of smoking respectively). Moreover, the intensity of the effects is also different. In the same smoking time (7 days), while the moisture content decreased 18.7% (from 49.9% ± 3.2% to 31.3% ± 1.2%), the BaP content increased more than 300% (from less than 0.24 μg kg^{-1} LOQ to 0.75 ± 0.05 μg kg^{-1}). The BaP content of chorizo was doubled from 3 days of smoking (0.37 ± 0.05 μg kg^{-1}) to 5 days of smoking (0.75 ± 0.05 μg kg^{-1}).

The BaP content between the samples smoked during 3 and 5 days shows a statistically significant difference ($p < 0.05$). However, no significant differences ($p > 0.05$) were observed between the BaP content of samples smoked during 5 and 7 days. Fisher's least significant difference (LSD) procedure found two groups, one for the meat products smoked for 3 days and another group for the products smoked for 5 and 7 days. Finally, it can be stated that BaP becomes stabilised after 5 days of smoking.

The differences between moisture contents between the samples was similar to the BaP content. Significant differences ($p < 0.05$) were observed between samples smoked for 1 and 3 days and for 3 and 5 days, respectively. No significant differences were observed between the samples smoked for 5 and 7 days ($p > 0.05$).

The BaP content (μg kg^{-1}) results in the freeze-dried samples compared with fresh samples shown in Figure 4 which show that the increase in BaP content is not only caused by the decrease in moisture content in the direct smoked product, as the BaP content of the samples expressed in this way also increases with smoking time. Visual inspection of the samples revealed that the stabilisation of the BaP content after 5 days of smoking may be produced by obstruction of the casing pores.

These results are in agreement with those obtained by Djinovic et al. (2008) who found that the BaP and total PAHs content in the products they studied generally increased during smoking. However, these authors found only a similar BaP content in smoked meat products, always less than 0.48 μg kg^{-1} after 9 days of smoking, whereas we found 0.75 μg kg^{-1} after 5 days of smoking. Santos et al. (2011) studied the BaP content of a chorizo with similar characteristics to those studied in this paper,

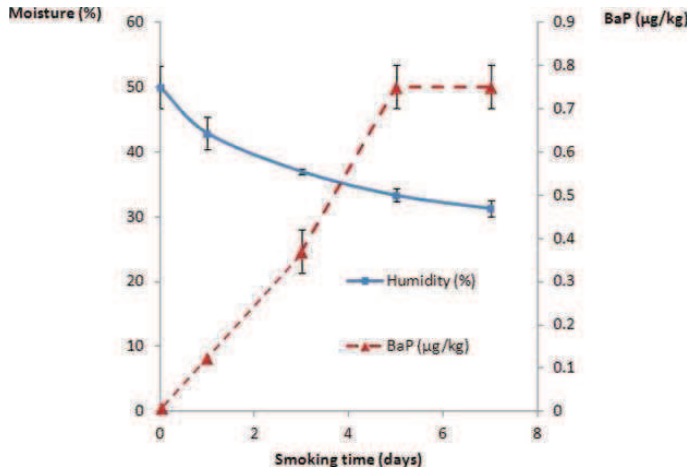

Figure 3. (colour online) Influence of direct smoking time (0, 1, 3, 5 and 7 days; $n = 3$) on the moisture (%) and BaP (μg kg^{-1}) content of a direct smoked meat product (chorizo) analysed without casing (D1).

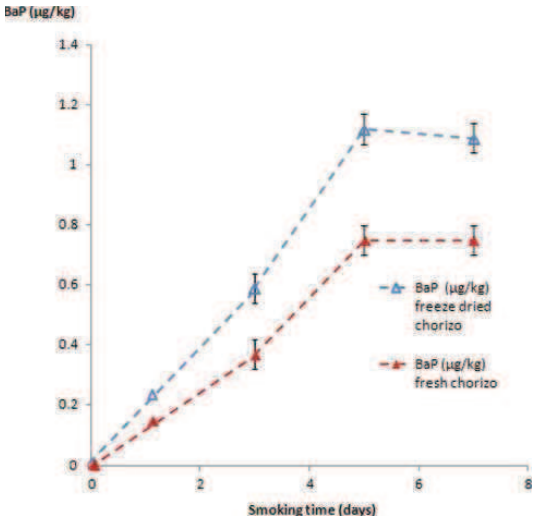

Figure 4. (colour online) BaP content in freeze dried and fresh chorizo during smoking time (0, 1, 3, 5 and 7 days; $n = 3$).

with 25.1% fat content, 5 days of smoking and 2 cm in diameter. These authors reported a BaP content of 0.38 µg kg^{-1} in this chorizo, nearly the same BaP content we found after 3 days of smoking. After 5 days of smoking, we found twice the content of BaP, 0.75 µg kg^{-1}. These results may be considered similar bearing in mind the great number of parameters that influence the traditional direct smoking method, such as the kind of wood used for combustion, the position in the smoking chamber or smoking time (Djinovic et al. 2008; Stumpe-Vīksna et al. 2008, Codex Alimentarius, CAC/RCP 68-2009, Lorenzo et al. 2011; Santos et al. 2011; Roseiro et al. 2012; Hitzel et al. 2013).

BaP penetration at different depths in a meat product during the direct smoking process

In this study the BaP (µg kg^{-1}) and moisture (%) content at four different depths of two meat products (chorizos A and B) smoked in the same conditions (smoking time, smoking room, distance to the smoking source, ingredients, etc.) were analysed in triplicate. The results obtained were similar for the two studied meat products, so an average of both has been used in the discussion of the results. Figure 5 shows the moisture (%) and BaP content (µg kg^{-1}) found at the four studied depths. As can be appreciated in Figure 5, several differences were found in the BaP content at the studied depths. There are important differences in the moisture and BaP content found at depth D1 (casing) compared with the rest of the studied depths. The BaP content found in the samples decreases from the casing towards the inside of the meat product. The BaP content found in the casing (D1, 20.0 ± 1.1 µg kg^{-1}) is very high, while the BaP contents found inside the chorizo are similar and low (1.63 ± 0.11 µg kg^{-1} D2, 1.12 ± 0.10 µg kg^{-1} D3 and 0.88 ± 0.10 µg kg^{-1} D4), with concentrations decreasing with depth. It is important to note that significant differences between the samples ($p < 0.05$) were obtained by running t-tests (D2–D3, D3–D4). Three different groups were found by running Fisher's LSD procedure of D2, D3

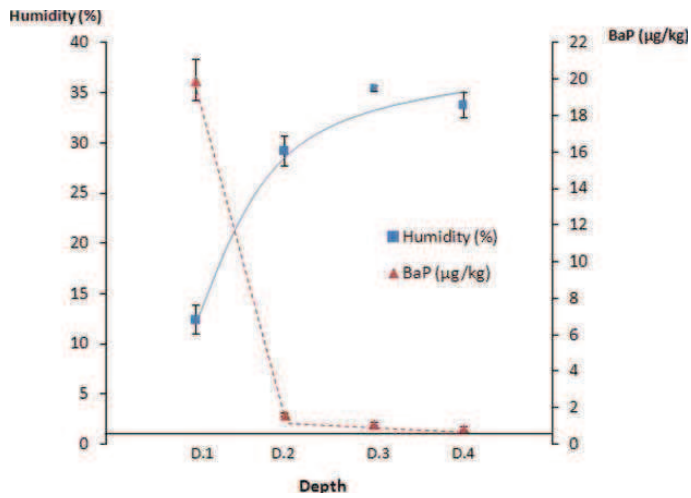

Figure 5. (colour online) BaP (µg kg^{-1}) and moisture (%) content at different depths (D1, D2, D3 and D4) in a 5 days' direct smoked meat product (chorizo).

and D4 samples. Figure 2 shows the BaP and BaP-d12 ions chromatogram found in the casing. As Figure 2 shows, the BaP peak size is higher than the added internal standard BaP-d12 one.

The results obtained in this study indicate that, during direct smoking process, the greatest amount of BaP, PAH content indicator, was deposited in the casing of the meat product, not inside the product. This is an important finding, as the inhabitants of the region where this study was carried out do not remove the casing of the meat product before eating it. Chorizo is commonly used as ingredient in traditional Spanish stews such as white beans, lentils or chick peas, as well as in rice dishes such as paella. Chorizo casing is not usually removed before preparing these dishes.

However, the amount of BaP found inside the chorizo (at depths D2, D3 and D4) is similar to that found in other studies. These results are in agreement with those reported by other authors in similar smoked meat products. Garcia-Falcón & Simal-Gándara (2005), Djinovic et al. (2008), Andrée et al. (2010) and Santos et al. (2011) report that PAHs accumulate on the surface of the smoked meat product during smoking and then migrate into the products being smoked. Santos et al. (2011) specifically found a substantially higher PAHs content in the casing of chorizo than inside the product.

As is well known, a great number of variables alter the PAH content of smoked foods, such as the smoking or drying method (direct or indirect) or the wood used for combustion (Codex Alimentarius CAC/RCP 68-2009; Stumpe-Vīksna et al. 2008) and should be specified in scientific papers. However, the present study shows that it is also important to specify whether the samples have been analysed with or without the casing.

The results obtained in this study could explain the findings of Djinovic et al. (2008). They explain that a decrease in BaP content during smoking time could be caused by differences in the content of the smoke during this process. We would further add that the depth of the sample taken for analysis and the obstruction of the pores caused by the components of the smoke could also have an influence on the results. Moreover, an excessive drying time sometimes produces deterioration or tearing of the casing, allowing BaP to pass inside the product. This effect could explain an increase in BaP in meat products smoked over 7 days. Furthermore, we assume that the stabilisation of the BaP content in chorizo after 5 days of smoking may be produced by a coating created by the smoke components on the surface of the product, impeding the penetration of BaP.

On the other hand, as Figure 5 shows, there are also important differences in the moisture content found at depth D1 (casing) compared with the rest of the studied depths. In this case, the moisture content found inside the meat product increases from the casing inwards. The moisture content found in the casing (D1, 12.4% ± 1.4%) is very low, while the values found inside the chorizo are similar and higher than that at D1 (D2: 29.2% ± 1.5%, D3: 35.5% ± 0.3% and D4: 33.8% ± 1.3%). Significant differences ($p < 0.05$) between D1–D2 and D2–D3 were found. No significant differences ($p > 0.05$) between D3 and D4 were found.

One of the most important goals of the smoking process during chorizo manufacturing is that of drying. The water content of meat products decreases during drying, thus leading to a drop in moisture content. This reduction in moisture content is important because it has an inactivating effect of the growth of microorganisms. During the traditional direct smoking process using natural casings, the product is dried from the outside of the product inwards. This process has an effect on the firmness, colour, taste and other organoleptic properties of the product. The outer layer is harder and more colourful and tasty.

Bearing all this in mind, a scheme of the mechanism of BaP penetration and water exiting the different depths of a smoked meat product during smoking time is proposed in Figure 6. As summed up, before smoking chorizo is a raw mix of pink ingredients stuffed in a casing without any BaP content. During smoking, chorizo is immersed in smoke in direct contact with smoke components. After 1 day of smoking, smoke deposits start to accumulate on the surface of the casing; BaP is mainly deposited in the casing and the first depths of the product start to be BaP contaminated and dried. From 1 to 5 days of smoking, smoke components (including BaP) continue to accumulate mainly on the casing and BaP penetrates deeper inside the product, while water exits the product from the external to the internal depths of chorizo. After about 6 days of smoking, smoke components have built a barrier impeding BaP penetration, although in some cases the casing becomes degraded or torn, thus allowing BaP to penetrate. During this process, water exits the meat product resulting in different depths, being harder and darker on the outside than inside the product.

To sum up, it may be proposed that the sides of the product, which are in direct contact with the smoke, become more dried and PAHs contaminated than those that are protected inside the product. The external sides can become obstructed by smoke components, thereby constituting a barrier to PAHs penetration into the internal sides.

BaP content in chorizo with regard to regulations

According to European Union Commission (EU) Regulation No. 835/2011, in addition to the new PAH4 sum, the separate maximum level for BaP is maintained to ensure comparability of previous and future data. The recent study by Lorenzo et al. (2011) concluded that BaP is a good marker for the sum of 15 PAHs in chorizo

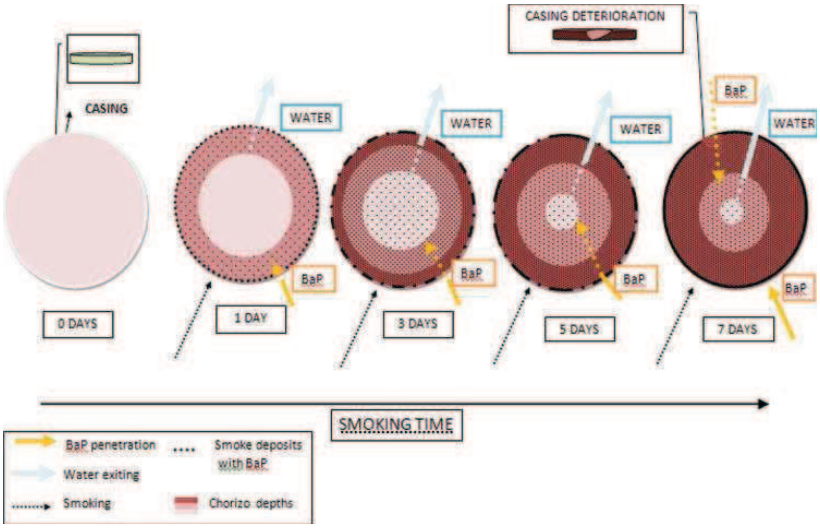

Figure 6. (colour online) Mechanism of BaP penetration and drying at different depths in a smoked meat product (chorizo) during smoking time.

gallego as well as for seven PAHs considered probable human carcinogens by the USEPA in 'chorizo gallego' and 'chorizo de cebolla', similar smoked meat products to the one studied here ('chorizo asturiano').

As Figure 3 shows, the BaP content of a typical chorizo manufactured in 7 days of direct smoking (0.75 μg kg^{-1}) meets the current (5 μg kg^{-1}) and future (2 μg kg^{-1}) legal limits for BaP in smoked meat products. The BaP content increases twofold in 2 days of smoking (from 3 to 5). As already stated, these samples were analysed without casing.

Figure 5 shows that the chorizo depth samples analysed in this study present different BaP contents. With a content of 20.0 ± 1.1 μg kg^{-1}, the casing of the smoked product (D1) presents a value four times in excess of the current legal limit set at 5 μg kg^{-1} (wet weight). However, the internal depths (D2: 1.63 ± 0.11 μg kg^{-1}, D3: 1.12 ± 0.10 μg kg^{-1} and D4: 0.88 ± 0.10 μg kg^{-1}) comfortably meet the current (5 μg kg^{-1}) and future (2 μg kg^{-1}) legal limits for BaP in smoked meat products.

The total average mass of the studied meat products is 115.1**4** g and their total average BaP content is 0.16 μg. This means that the total average BaP content (μg kg^{-1}) in the meat products (CT) is 1.36 μg kg^{-1}. If all the studied parts of the meat product were mixed together, a concentration of 1.36 μg kg^{-1} of BaP would be obtained. On the other hand, if the average BaP content of the internal depths (D2, D3 and D4) is studied, a concentration of 1.21 μg kg^{-1} is obtained. Both results will meet the future legal limit (2 μg kg^{-1}) for BaP in smoked meat products.

Bearing in mind the large number of variables that influence the smoking process, such as the unpredictable and variable values of temperature and humidity depending on weather conditions, the distance between the heat source and the product, the time of smoking, the amount of fat in the product, the kind of wood, etc., corroborated by CAC (2009) and reported by several authors (Djinovic et al. 2008; Stumpe-Vīksna et al. 2008; Lorenzo et al. 2011; Santos et al. 2011; Roseiro et al. 2012), it is quite difficult to optimise and have full control of traditional direct smoking processes. However, a BaP content below 1 μg kg^{-1} in similar smoked meat products has been reported by other authors (Jira et al. 2006; Purcaro et al. 2009; Santos et al. 2011; Roseiro et al. 2012) and also below the maximum value allowed by European Union regulations (5 μg kg^{-1}) (Fontcuberta et al. 2006; Wretling et al. 2010; Lorenzo et al. 2011). In contrast, a BaP content higher than 2 μg kg^{-1} has been reported in some kinds of direct smoked chorizo (Roseiro et al. 2012).

Finally, we can state that the traditional direct smoking process is affected by a large number of parameters. A low BaP content has been found in recent studies of traditional direct smoked meat products, indicating that it can be achieved. However, information on innovative smoking technologies or the introduction of simple modifications in the smoking process will be necessary in some cases to meet the forthcoming maximum level of BaP allowed in smoked meat products by the European Union regulation, i.e. 2 μg kg^{-1}. For instance, we propose replacing direct smoking methods by indirect techniques, such as friction smoke generation, particularly controlling direct smoking time, the kind of wood employed and all the raw materials used.

Conclusions

An increase in smoking time from 0 to 7 days produces opposite effects on the moisture and the BaP content of chorizo. While the moisture content decreases (from 49.9% ± 3.2% to 31.3% ± 1.2%), the BaP content increases (from less than 0.24 μg kg^{-1} to 0.75 ± 0.05 μg kg^{-1}), finally becoming stabilised after 5 days of smoking. After this time, BaP is mainly deposited in the casing of the product. The BaP content decreases and the moisture content increases progressively from the casing (20.0 ± 1.1 μg kg^{-1}, 12.4% ± 1.4%) towards the inside of the meat product (depths D2: 1.63 ± 0.11 μg kg^{-1}, 29.2% ± 1.5%; D3: 1.12 ± 0.10 μg kg^{-1}, 35.5% ± 0.3%; and D4: 0.88 ± 0.10 μg kg^{-1}, 33.8% ± 1.3%).

Obstruction of the pores of the casing could be caused by smoke components, making this casing a barrier preventing further PAHs contamination of the foodstuff. However, degradation of the casing during a long smoking time may produce the opposite effect.

All the chorizo samples (without casing) studied in this paper will meet the current (5 μg kg^{-1}) and future (2 μg kg^{-1}) BaP limits stipulated in European Union Commission (EU) Regulation No. 835/2011, to be applied from 1 September 2014. With a content of 20.0 ± 1.1 μg kg^{-1}, the casing of the smoked product (D1) is four times in excess of the current legal limit.

PAH contamination of traditional smoked meat products is difficult to control because the direct smoking process is affected by a great number of parameters such as the time of smoking, the distance between the heat source and the smoked product, the amount of fat in the product and the kind of wood. However, a low BaP content has been found in recent studies of traditional direct smoked meat products, indicating that it can be achieved (Jira et al. 2006; Purcaro et al. 2009; Santos et al. 2011; Roseiro et al. 2012). Information on innovate smoking technologies or the introduction of simple modifications in the smoking process will be necessary in some cases to meet the forthcoming maximum amount of BaP allowed in smoked meat products by the European Union regulation, i.e. 2 μg kg^{-1}, such as replacing direct smoking methods by indirect techniques, e.g. friction smoke generation.

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4.3 INFLUENCIA DEL TIPO DE TRIPA EN LA PENETRACIÓN DE BENZO(A)PIRENO EN CHORIZO AHUMADO

El trabajo anteriormente presentado indica que la mayor cantidad de BaP se queda depositado en la tripa natural sin penetrar en el interior del chorizo asturiano ahumado a modo directo. Algunos estudios realizados hace algunos años proponían que el uso de distintos tipos de tripa podría influenciar la contaminación de los productos. Por ello, el estado del arte actual está enfocado en estimar la influencia del uso de distintos tipos de tripa, en el contenido de BaP y HAP en productos cárnicos embutidos.

En el trabajo expuesto a continuación se publicaron por primera vez las causas que producen las diferencias en la contaminación por BaP de chorizos ahumados embutidos con distinto tipo de tripa, natural (ChN) y sintética (ChS). Para ello se realizaron varios estudios novedosos. En primer lugar se diseñó un sistema basado en aceite embutido para eliminar las interferencias que causan las distintas profundidades, de acuerdo a los resultados encontrados en el trabajo anterior. Así mismo, se realizaron los estudios de contenido de BaP en ChN y ChS, controlando el resto de variables influyentes. Por otro lado, se diseñó un dispositivo que permite secar las tripas de manera controlada y semejante al proceso de ahumado industrial, con el objetivo de determinar la porosidad de distintos tipos de tripa mediante porosimetría. Así mismo se estudió la evolución de las tripas y ChN y ChS durante el ahumado directo, mediante el uso de microscopía electrónica de barrido (SEM) y estereomicroscopía de fluorescencia óptica. En el trabajo se encontraron y publicaron por primera vez las diferencias físicas que ocurren entre ChN y ChS durante el procedimiento de ahumado, causantes de la contaminación por HAP. Con estos resultados se detalló el mecanismo de contaminación por HAP del chorizo asturiano ahumado.

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Characterization of natural and synthetic casings and mechanism of BaP penetration in smoked meat products

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ABSTRACT

Meat product casings are able to prevent the penetration of chemical and potentially carcinogenic compounds. Some components play an important role in the food safety of smoked meat products. In this study, the penetration of benzo(a)pyrene (BaP) (a polycyclic aromatic hydrocarbon [PAH] marker) in smoked meat products stuffed in different casings (natural and synthetic) has been tested. It was found that, in contrast to natural casing, collagen casing prevents BaP contamination of smoked meat products. The reasons of this fact have never been explained. Porosimetry and physical morphology determination by means of scanning electron microscope (SEM) and Fluorescence Stereo Microscope images of both types of unsmoked and smoked casings and different depths of 48 chorizos stuffed with both casings subjected to 0–10 days of smoking were studied to explain this effect. Based on the results, a mechanism can be inferred to explain BaP penetration inside the products. During smoking, meat products are heated and dried. The wrinkled morphology and high porosity (66.8%) of natural casing makes the fat flows outward through the casing making its surface sticky. Soot particles and tar aerosols containing polycyclic aromatic hydrocarbons (PAH) stick to this surface and then start to migrate inwards. In contrast, the smooth morphology and low porosity (16.6%) of synthetic casing make it a barrier, impeding the fat from exiting the inside of the casing. In this case, the product shows a hard, dry external surface with less affinity for soot particles, whose possibility of penetrating the product is hindered by the small pore size of the casing.

1. Introduction

The growing demand for meat, meat products and other protein-rich food sources in many parts of the world is of increasing concern in the light of the growing population figures, environmental sustainability issues and land-use and food safety concerns. Questions related to optimal production and processing methods, location, health effects, environmental impact and legal issues remain unanswered (EC, 2013; FAO, 2013). One of the oldest processes invented by humans to extend the storage period of the aforementioned products, maintaining their quality and providing flavour and the consistency required by consumers is smoking. During uncontrolled smoking, however, many chemical contaminants are formed, including polycyclic aromatic hydrocarbons (PAH), dioxins, formaldehyde, nitrogen and sulphur oxides (relevant for the formation of nitrosamines, for instance) and even heavy metals (CAC, 2009). The World Health Organization (WHO), International Agency for Research on Cancer (IARC), European Food Safety Authority (EFSA) and US Environmental Protection Agency (EPA) have warned of the carcinogenic activity of PAH. The most significant endpoint of PAH toxicity is cancer (ATSDR, 1995; ATSDR, 2009). The Commission of the European Communities (EC) has recently amended Regulation (EC) No. 1881/2006 (EC, 2006) by means of Regulation (EU) No. 835/2011 of 19 August 2011 (EC, 2011), setting new maximum levels for PAH in foodstuffs. This new regulation specifies that two dates must be considered as regards PAH contamination in foodstuffs. New maximum levels for the sum of four substances (PAH4) (benzo(a)pyrene, benzo(a) anthracene, benzo(b)fluoranthene and chrysene) were introduced,

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maintaining a separate maximum level for BaP to ensure comparability between previous and future data. The maximum permissible BaP content in smoked meat and smoked meat products was 5.0 μg/kg in wet weight until 31/8/2014 and is 2.0 μg/kg in wet weight from 1/9/2014 on. The maximum permissible sum of PAH4 in these foods was 30.0 μg/kg in wet weight from 1/9/2012 to 31/8/2014 and is 12.0 μg/kg in wet weight from 1/9/2014 on. Although humans may be exposed to PAH because of environmental conditions, the main source of contamination is diet (Falcó et al., 2003; Ibáñez et al., 2005; Lodovici, Dolara, Casalini, Ciappellano, & Testolin, 1995; Martorell et al., 2010; Phillips, 1999; Skupinska, Misiewicz, & Kasprzycka-Guttman, 2004). Due to their widespread consumption, the major contributors to PAH intakes in diet are cereals, cereal products, vegetable fats and oils.

There has been a great deal of concern about the PAH content in meat products, although low values of below 1 μg/kg have recently been reported (Jira, Ziegenhals, & Speer, 2006; Purcaro, Moret, & Conte, 2009; Roseiro, Gomes, Patarata, & Santos, 2012; Santos, Gomes, & Roseiro, 2011). Lorenzo et al. (2011) also found low BaP levels and concluded that BaP is a good marker for the Σ 15 US EPA PAH in "Chorizo gallego" samples as well as for 7 US EPA probably carcinogenic PAH in "Chorizo gallego" and "Chorizo de cebolla" samples. PAH are mainly accumulated in the fat content of foods. Therefore, oils have been found to be one of the highest PAH-contaminated foods. This fact is mainly due to atmospheric pollution contaminating the raw ingredients (Guillén, Sopelana, & Palencia, 2004; Rodríguez-Acuña, Pérez-Camino, Cert, & Moreda, 2008). Meat product mixtures after processing are also not exposed to smoking processes. However, olives and meat product ingredients can become PAH contaminated because of exposure of their skin or surface to polluted air or, in the case of olives, during milling as well (Fromberg, Højgård, & Duedahl-Olesen, 2007; Rodríguez-Acuña et al., 2008), resulting in these carcinogenic compounds appearing in the final products (CAC, 2009; Rodríguez-Acuña et al., 2008).

The "Code of practice for the reduction of contamination of food with (PAH) from smoking and direct drying processes" (CAC/RCP 68/2009) stipulates that PAH contamination in foodstuffs via food processing should be minimized, providing producers with the variables that need to be controlled. These variables have been studied by several authors, confirming that the PAH content of meat products can be reduced by means of its control. They include the kind of fuel (Hitzel, Pöhlmann, Schwägele, Speer, & Jira, 2013; Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2012; Stumpe-Vīksna, Bartkevics, Kukare, & Morozovs, 2008), smoking method (Pöhlmann et al., 2012, Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013a; Škaljac et al., 2014; Varlet et al., 2007), smoking duration (Djinovic, Popovic, & Jira, 2008; Essumang, Dodoo, & Adjei, 2013; Ledesma, Rendueles, & Díaz, 2014), fat content (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013b), distance between the food and the heat source, position of the food in relation to the heat source (Pöhlmann et al., 2013b), temperature during smoking and direct drying (Pöhlmann et al., 2012), cleanliness and maintenance of equipment and the design of the smoking chamber, and the equipment used for the smoke/air mixture (Pöhlmann et al., 2013a). The use of woods from apple (Stumpe-Vīksna et al., 2008), oak and hickory (Hitzel et al., 2013), indirect smoking methods, like friction smoke generation (Pöhlmann et al., 2013a), reducing smoking time (Djinovic et al., 2008; Ledesma et al., 2014), reducing fat content (Pöhlmann et al., 2013b), reducing smoke generation temperatures (below 600 °C) (Pöhlmann et al., 2012), or put the products in front, centre-front position of smoking chamber (Pöhlmann et al., 2013b) helps to reduce final BaP and PAH contents of meat products. Moreover, as a post-smoking treatment, CAC (2009) advises cleaning the product by means of rinsing or immersion in water, as these processes may remove PAH-containing soot and particles on the surface of the food. It has been found that the greatest amount of BaP (and probably other PAH) is deposited in the meat product casing and then migrates into the product (Andrée, Jira, Schwind, Wagner, & Schwagele, 2010; Djinovic et al., 2008; García-Falcón & Simal-Gándara, 2005; Ledesma et al., 2014; Santos et al., 2011). Some authors report that the use of different types of casing (natural and synthetic) has an influence on the PAH content of meat products (Filipovic & Toth, 1971; Toth, 1973). Some studies have recently continued this line of research, finding different results (Gomes, Santos, Almeida, Elias, & Roseiro, 2013; Pöhlmann et al., 2013b; Škaljac et al., 2014), although the reasons underlying this effect have yet to be studied.

The aim of this study was to test the influence of the type of casing (natural or synthetic) on BaP penetration in a typical Spanish smoked meat product called "chorizo", determining the physical differences between the two casings and proposing a mechanism to explain PAH contamination of stuffed meat products during the smoking process.

2. Materials and methods

2.1. BaP determination in smoked oil, casing and meat samples

The BaP content of meat products decreases progressively from the casing to the centre of the meat product. For this reason, samples from different products must be taken from the same product depth and location to compare results. Analysis of samples with or without casing must be specified (Ledesma et al., 2014). In this work 2 methods have been applied to test penetration of BaP in different natural and synthetic casings. With the aim of create an ideal system of BaP dilution without product depths interferences, a system of oil stuffed in casings was developed. On the other hand, real meat products were analyzed to confirm results.

2.1.1. Oil samples preparation

400 mL of extra virgin olive oil (EVOO) (Manufactured by Carbonell, Spain) were poured into 40 cm of each one of 2 types of casings, natural (from pig intestine) and synthetic (collagen) previously wetted in salty water. 40 mL of unprocessed oil were kept in the original bottle, in the dark, for analysis. The samples were hung 40 cm apart on a bar, placed in the same position and location in the smoking chamber, at the same distance from the smoke source, namely 10 m, and smoked for 7 days employing a mixture of oak (90%) and chestnut (10%). Once smoked, samples were hung on a bar placed into a box and sent for laboratory analysis. The oil content of the samples and the casings were subsequently kept separately in individual glass flasks in a fridge, at 5 °C in the dark. Fifteen samples were finally analyzed, three for each one of the following products: original oil, smoked oil stuffed in natural casing, smoked oil stuffed in synthetic casing, smoked natural casing, and smoked synthetic casing.

2.1.2. Meat products preparation

Chorizo is a typical Spanish smoked meat product made from the following ingredients: pork meat, pork fat, salt, garlic, sweet or spicy paprika and herbs. Antioxidants such as sodium citrate or sodium ascorbate, colourings such as carmine, emulsifiers such as sodium triphosphate, food preservatives such as sodium nitrite and other food additives were used to ensure the microbiological stability and quality of the foodstuff. Two kinds of chorizo were prepared for this study: chorizo encased in natural casing, and chorizo encased in synthetic casing. Fifty kg of each chorizo were manufactured at the El Hórreo Healthy Food S.L. meat company facilities in the following steps: reception of raw ingredients, mincing,

mixing, marinating, stuffing, direct smoking, packaging and labelling. The chorizo samples were stuffed in strings of 4 chorizos. A mixture of oak (90%) and chestnut (10%) wood was used to smoke directly the chorizo for 7 days. All the selected samples were placed in the same position in the smoking chamber, at the same distance from the smoke source, namely 10 m. Once smoked, the chorizos were hung in a cardboard tube and placed in a box to prevent the materials from coming into contact with any other object. The nutritional details of a chorizo manufactured by the company with the same ingredients and manufacturing process as the one studied here, determined by the Principality of Asturias Meat Industry Association Technological Centre for Supporting Innovation (Spanish acronym, ASINCAR), are 31.41% moisture content, 22.98% protein, 37.94% fat, 2.85% carbohydrates, 4.82% ash and pH 5.2. Six samples were finally analyzed, three for each of the following products: smoked chorizo stuffed in natural casing, and smoked chorizo stuffed in synthetic casing.

2.1.3. Reagents and standards

Benzo[a]pyrene (BaP) analytical standard solution was supplied by Sigma–Aldrich at a concentration of 100 μg/mL in cyclohexane and a total volume of 2 mL. Benzo[a]pyrene internal standard (d12, 98%) solution was obtained from Cambridge Isotope Laboratories at a concentration of 200 μg/mL in isooctane and a total volume of 1.2 mL. All solvents for sample preparation, hexane, dichloromethane, cyclohexane and isooctane (2, 2, 4-Trimethylpentane), were purchased from Sigma–Aldrich with ACS reagent purities ≥ 99% (GC) grade. Solid phase extraction (SPE) cartridges were supplied by Varian and Agilent Technologies. The Mega Bond Elut cartridges employed contain a silica sorbent phase, with a particle size of 40 nm, a weight of 5 g and a volume of 20 mL. Amber-coloured flasks and vials were used to protect the PAH from light decomposition. Specifically, 15 mL (21 × 70 mm) amber glass vials were purchased from Sigma–Aldrich for PAH collection during the solid phase extraction (SPE) step.

2.1.4. Sample pre-treatment of chorizo samples

2.1.4.1. Grinding step. First, the chorizo casing was removed and stored for its analysis, following the procedures specified by Fretheim (1976) and Wretling, Eriksson, Eskhult, and Larsson (2010). Then, samples were cut in slices and the amount of chorizo situated at a distance between 0.25 cm from the casing and the casing of the chorizo, denominated depth 2 (D2) according to Ledesma et al. (2014) was taken. 20 g of D2 were finely homogenised in a meat grinder (Minirobot D81 meat grinder, Moulinex, France) following the protocol developed by Purcaro et al. (2009). Pre-treatment of all samples was carried out in triplicate.

2.1.4.2. Lyophilisation step. Three aliquots of 20 g chorizo were taken, placed in individual lyophilisation plates carefully sealed with aluminium foil and weighed before freeze-drying. Each sample was then frozen to 193.15 K for 4 h and freeze-dried in a lyophilizer (Telstar-Cryodos, Spain) for 1 day. Finally, the plates (containing the sample) were weighed after freeze-drying.

2.1.4.3. Sonication step. Two grams of freeze-dried chorizo (one from each lyophilisation plate) were placed in flasks (the remaining amount of lyophilised chorizo being stored in a freezer). Subsequently, 20 mL of n-hexane and 200 μL of benzo[a]pyrene-d_{12} internal standard solution at a concentration of 100 μg/L were added (dilutions from the stock solution must be made previously). This solution was carefully stored under refrigeration for 3 days to help the deuterated compound adapt to the sample matrix. This is the appropriate moment to add the internal standard compound as it is the first time both compounds are in the same matrix (Purcaro et al., 2009; Stumpe-Viksna et al., 2008). Finally, the samples were sonicated for 1 h in an ultrasound bath (Cod. 3000513, J.P. Selecta, S.A, Barcelona, Spain) at ambient temperature.

2.1.4.4. Filtration. After sonication, the samples were filtered on a paper filter (reference number 1242, 11 cm, Albet) to remove the solid meat waste, which was washed with additional n-hexane (5 + 5 mL). The solvent was subsequently collected in a flask and the volume was made up to 10 mL with the help of a rotatory evaporator (Heidolph Laborota 4000 efficient, Germany) and the addition of the necessary amount of n-hexane.

2.1.4.5. Solid phase extraction (SPE) step. The extract obtained after sonication contained an appreciable amount of fat (Purcaro et al., 2009). A solid phase extraction (SPE) procedure in a Supelco Visiprep TM SPE vacuum manifold with 12 ports (Sigma–Aldrich, USA), was used to isolate benzo(a)pyrene and benzo[a]pyrene-d12 (BaP-d12) (the sought-after PAH in the sample), similar to the previously established setup for rapid PAH determination in vegetable oils by Moret and Conte (2002). First, a 5 g silica SPE cartridge (Mega Bond Elut, 20 mL, Varian) was slowly washed with 20 mL of dichloromethane, dried completely under vacuum and conditioned with 20 mL of n-hexane (to prevent the cartridge from drying). Next, 1 mL of the sonicated sample was diluted in 3 mL of n-hexane and slowly loaded into the cartridge, avoiding complete drying of the cartridge. The aliphatic hydrocarbons were then discharged via the addition of 8 mL of a mixture of hexane and dichloromethane 70:30 (v/v) to the cartridge. Finally, after loading the cartridge with another 8 mL of the hexane and dichloromethane 70:30 (v/v) solution, the PAH, BaP and BaP-d12 contained in the sample were slowly discharged into 15 mL amber glass vials, allowing the cartridge to dry under vacuum.

2.1.4.6. Concentration step. The samples were concentrated after the SPE step, the volume being made up to 1 mL by N_2 blowing using a nitrogen stream at the top of 15 mL volume vials placed in a fume hood. Finally, the samples were loaded into 2 mL GC/MS amber glass vials and the final volume of 1 mL was checked by weighing the vials.

2.1.4.7. Sample pre-treatment of oil samples. A solid phase extraction (SPE) procedure was used to isolate benzo(a)pyrene and benzo[a]pyrene-d_{12} (BaP-d12), similar to that previously described for meat product pre-treatment. 2.5 g ± 0.001 g of oil were weighed into a 10 mL volumetric flask, 200 μL of benzo[a]pyrene-d_{12} internal standard solution at a concentration of 100 μg/L were then added and the volume was made up to 10 mL with n-hexane. This solution was carefully stored under refrigeration for 3 days to help the deuterated compound adapt to the sample matrix. Then, 1 mL of the sonicated sample was slowly loaded into the cartridge and the same SPE and concentration procedures, as described for meat product pre-treatment, were applied.

2.1.4.8. Sample pre-treatment of casing samples. Casings were placed in flasks, adding 20 mL of n-hexane and 200 μL of benzo[a]pyrene-d_{12} internal standard solution at a concentration of 100 μg/L. This solution was carefully stored under refrigeration for 3 days. The samples were then treated following the same procedure as chorizo samples from the sonication step on.

2.1.5. BaP determination by GS/MS

2.1.5.1. GC/MS calibration. BaP quantification was performed via prior calibration of the GC/MS system with benzo(a)pyrene and benzo[a]pyrene-d_{12} standard solutions. For calibration purposes, benzo[a]pyrene-d_{12} standard solution was added to check

extraction recovery during the pre-treatment methods. Calibration curves were generated by injecting 6 diluted standard solutions of BaP and BaP-d12 in cyclohexane, in triplicate. BaP standard concentrations for each calibration vial were 0.05 ppb, 0.1 ppb, 0.5 ppb, 1.0 ppb, 5 ppb and 10 ppb. The concentration of BaP-d12 for all calibration vials was 2 ppb. The MS assistant program (Agilent Technologies) compares the relationship between both BaP standard signals (BaP and BaP-d12) to determine the final BaP concentration. Identification of compound was assessed comparing the retention time and confirm the ion ratios (qualifier ion/target ions) with a criterion of ±20% of standards compounds ratio. Criterion of BaP/BaP-d12 standards target ion ratios was ±20%.A blank (i.e. a vial containing only the solvent cyclohexane, with no BaP or BaP-d12 standard compounds) was also included among all the standard vials containing BaP compounds.

2.1.5.2. Gas chromatography (GC). Separation of BaP and BaP-d12 was performed on an HP-5MS 19091S-433 part number column (30 m × 250 μm × 0.25 μm) (Agilent Technologies) in an Agilent Technologies 6890 gas chromatograph (GC). The injection temperature and volume were 300 °C and 1 μL (splitless), respectively. Helium was applied as carrier gas at a constant flow rate of 1.5 mL/min. The temperature ramp used was the following: isothermal at 55 °C for 1 min, increasing at a rate of 25 °C/min to 320 °C for 3 min, total run time 14.60 min.

2.1.5.3. Mass spectrometry (MS). Identification of BaP and BaP-d12 was performed using an Agilent Technologies 5975 mass spectrometer detector (MS) equipped with a G2589-20045 part number 6-mm ultra-large aperture drawout lens. The solvent delay was 4 min and the EM voltage (run at autotune voltage) was 1294 V. The low mass was 252 amu and the high mass was 264 amu. SIM mode was selected for 1 group, 3 ions per group (252, 264 and 126), 50 msec dwell/ion. The quad temperature was 180 °C, the source temperature, 300 °C and the transfer line temperature, 280 °C. Data were acquired by operating the MS in ion monitoring mode. Peak spectra were compared to the mass spectra of BaP and BaP-d12 standards and the library supplied with the instrument. Ten different spiked samples (as well as unspiked controls), were prepared and each of these samples and vials was analyzed in triplicate according to the analytical procedure. Recoveries were calculated by comparing the difference between spiked and unspiked samples with the known amount of added BaP standard (1 ppb). Detection and quantification limits (LODs and LOQs, respectively) were evaluated on the basis of the noise obtained through analysis of the blank samples. LOD and LOQ were defined as the concentration of the analyte that produced a signal-to-noise ratio of 3 and 10, respectively. Moreover, these results were confirmed by the analysis of blank samples and 6 diluted standard solutions of BaP and BaP-d12 in cyclohexane, in triplicate. The slope (m) and standard deviation of intercept (sb_0) of the analytical response (y) vs concentration level added (x) were calculated, then the LOD and LOQ were also estimated via the following equations: $LOD = 3.3^{*}sb_0/m$, $LOQ = 10^{*}sb_0/m$.

2.2. Porosimetry determination of natural and synthetic casings

2.2.1. Casing samples preparation

Synthetic casings are marketed in the dry state, while natural casings have a high moisture content. Porosimetry equipment does not allow wet samples analysis. The lyophilisation method was dismissed because of possible degradation and transformation of the samples. For this reason, a special system was prepared to dry casings in the same way as meat products, avoiding contamination of the casings by ingredients. Sixty centimetres of each type of casing, natural (from pig intestine) and synthetic (collagen), were wetted in the same salty water. Meanwhile, the central section of a 53 cm hollow cardboard tube was removed to create a space with no material where air could pass through after making a hole in the tube. Then the surfaces of the cardboard tube were covered with a plastic parafilm to ensure the casings did not slip, thus preventing their degradation. Then, systems of casings were hung on independent bars and placed on a metal shelve and slowly and carefully dried for 3 days in a controlled-atmosphere drying shed, with temperature and humidity conditions of 285.15 K and 65%, respectively. Fig. 1 shows the system. Once dried, the samples were carefully transported hung on bars placed inside a box, preventing their contact with any other object.

2.2.2. Determination of casing porosimetry

0.51 g and 0.42 g of dried synthetic and natural casing, respectively, were analyzed by mercury intrusion porosimetry using a Micromeritics Autopore IV device. Analyses were performed under pressure values ranging from 0.10 to 60,000.00 psia on previously evacuated samples to obtain the following material characteristics: all the intrusion data, pore structure data and material compressibility data.

2.3. Microscopic morphology of casings and meat products

A Jeol 6100 scanning electron microscope operating at 30 kV was used to determine the microscopic morphology of the following samples: both sides of the casings pre-treated as stated in Section 2.2; and the natural and synthetic casings of eight chorizos smoked after 3 and 5 days, manufactured as stated in Section 2.1.2 and pre-treated by freezing to 193.15 K for 4 h and freeze-drying in a lyophilizer (Telstar-Cryodos, Spain) for 1 day.

A Leica M205 FA Fluorescence Stereo Microscope was used to determine the microscopic morphology of the following samples: the natural and synthetic casings pre-treated as stated in Section 2.2; the natural and synthetic casings of 8 unsmoked chorizos (4 for each type of casing); and 40 chorizos smoked between 0 and 10 days (20 for each type of casing) and their external and internal surfaces (once the casing was removed), manufactured as stated in Section 2.1.2.

2.4. Statistical analysis

Data from each study were analysed by running Fisher's least significant difference (LSD) procedure for multisample statistical

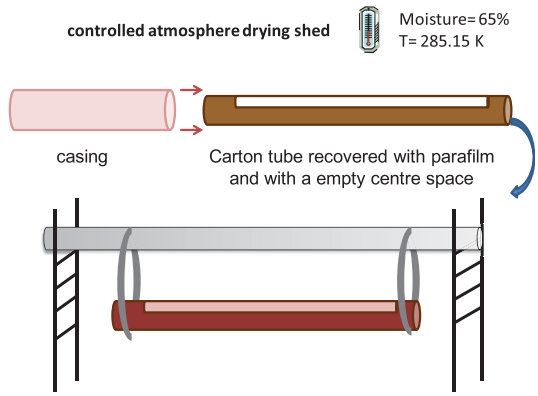

Fig. 1. Casings slow drying system.

comparison. This method was used to discriminate between the means. Furthermore, *t*-tests between consecutive samples were applied to compare means, at significant level ($p < 0.05$). F-tests at significant level ($p < 0.05$) were previously carried out to compare standard deviations. Standardized skewness and standardized kurtosis were used to assess whether the samples came from normal distributions. The software used was STATGRAPHICS Plus 3.1.

3. Results and discussion

3.1. BaP content of oil and chorizo stuffed in natural and synthetic casings

All the samples were successfully analysed by the proposed methods. Previous studies consisting of the combination of soxhlet and Gel Permeation Chromatography (GPC) were performed in order to evaluate analytical methods for BaP determination in smoked meat products. The combination of sonication and SPE pretreatment methods to determine the BaP content in smoked meat products by GC/MS analysis was found to be a good method, as it entails a low level of solvent consumption and waste generation, short operating times and is easy to set up. A similar retention time was found for the BaP and BaP-d12 standards, respectively 12.47 s and 12.49 s. Ion ratios in all samples were within the required criterion (±20) and compounds were confirmed. This method offers high quality quantitative results with high extraction efficiency. The limit of detection (LOD) for both compounds was 0.05 μg/kg and the limit of quantification (LOQ) was 0.24 μg/kg. Recoveries ranged from 98% to 101% and relative standard deviations (RSDs) were lower than 4%.

The BaP content found in the unsmoked oil system and meat products, smoked meat products and oils stuffed into natural and synthetic casings are shown in Table 1 The oil system was prepared to test the penetration of BaP across natural and collagen casings. As Table 1 shows, BaP was not found in the unsmoked oil or in the oil stuffed in the collagen-casing system, while it did appear in the system prepared with natural casing. The same effect was also found in meat products. The absence of BaP in all unsmoked samples is a coherent result, as EVOO is not exposed to PAH during its production processes. However, PAH can be found in unsmoked oil and also in raw sausage mixtures due to environmental contamination of ingredients (CAC, 2009; Djinovic et al., 2008; Rodríguez-Acuña et al., 2008). According to reported results, a range level between less than 0.2 μg/kg and 0.4 μg/kg of BaP was found by Fromberg et al. (2007) in 46 samples of merchant EVOO for human consumption from Italy, Spain, Greece, France and Holland. A content of between 0.07 and 0.28 μg/kg of BaP was found by Teixeira, Casal, and Oliveira (2007) in 2 virgin olive oils from the Portuguese market. A content of 0.08, 0.18 and 0.04 μg/kg of BaP was found by Djinovic et al. (2008) in unsmoked Sremska and Cajna sausages and pork ham, respectively. After 5 days of exposure to smoking, different BaP contents were found in oils and meat products stuffed in natural and synthetic casings. While an amount of 2.16 ± 0.12 μg/kg was found in the oil stuffed in the natural casing, BaP was not found in the smoked oil stuffed in the synthetic casing. However, a great amount of BaP was found in the external part of both the natural (19.9 ± 2.9 μg/kg) and synthetic (**14**.0 ± 0.2 μg/kg) casing. Significant differences ($p < 0.05$) between BaP content in both casings were found.

Likewise, BaP was not found in smoked meat products stuffed in collagen casings, although 1.71 ± 0.15 μg/kg was found in the D2 depth of products stuffed in natural casings.

Significant differences ($p < 0.05$) were found in the BaP content of oils and chorizos stuffed in natural casings.

Similar results of BaP content in natural casing (20.0 ± 1.14 μg/kg) and D2 depth of chorizo (1.63 ± 0.11 μg/kg) where found in previous works (Ledesma et al., 2014).

These results are in line with recent studies, the main amount of PAH remains in the casing and does not penetrate inside the meat product (Andrée et al., 2010; Djinovic et al., 2008; García-Falcón & Simal-Gándara, 2005; Gomes et al., 2013; Pöhlmann et al., 2013b; Santos et al., 2011), and then higher level of PAH was found inside the meat products stuffed in natural than in collagen casings smoked under the same conditions (Gomes et al., 2013; Lorenzo et al., 2011; Roseiro et al., 2012; Santos et al., 2011; Škaljac et al., 2014). However, a recent study by Pöhlmann et al. (2013b) reports that smoked Frankfurter sausages with natural (sheep intestine) casing had a similar BaP and PAH4 content to those with a collagen casing while lower levels of these contaminants were found in Frankfurter sausageswith peelable cellulose casings. The amount of BaP found in the tested chorizos falls well below the

Table 1
BaP content (μg/kg) of the studied samples: unsmoked extra virgin olive oil, smoked extra virgin olive oil, and meat products stuffed into natural (from pig intestine) and synthetic (collagen) casings ($n = 3$).

Stuffed sample	BaP content (μg/kg) of Sample stuffed into this casing	
	Natural casing	Collagen casing
Unsmoked oil	n.d	n.d
Unsmoked meat	n.d	n.d
smoked oil	2.16 ± 0.12	n.d
Meat product	1.71 ± 0.15	n.d
Casing only	19.9 ± 2.9*	14.0 ± 0.2*

$N = 3$.
n.d: non detectable.
*: statistical significance: (<0.05).

Table 2
Intrusion data, pore structure and Mayer Stowe summaries of natural (from pig intestine) and synthetic (collagen) casings.

	Casing	
	Natural	Collagen
Intrusion data summary		
Total intrusion volume (mL g^{-1})	1.37	0.14
Total pore area (m^2 g^{-1})	9.11	11.5
Median pore diameter (Volume) (nm)	66018.7	44815.1
Median pore diameter (Area) (nm)	4.90	5.10
Average pore diameter (4 V/A) (nm)	599.8	48.2
Bulk density at 1.49 psia (g mL^{-1})	0.49	1.20
Apparent (skeletal) density (g mL^{-1})	1.47	1.44
Porosity (%)	66.8	16.6
Stem volume used (%)	52.0	6.00
Pore structure summary		
Threshold pressure (psia)	1.98	2.65
Characteristic length (nm)	91261.6	68141.3
Conductivity formation factor	0.36	0.07
Permeability constant	0.00442	0.00442
Permeability (mdarcy)	13459.7	1380.2
BET surface area (m^2 g^{-1})	133.8	133.8
Pore shape exponent	1	1
Tortuosity factor	1.47	2.04
Tortuosity	3.65	2.86
Percolation fractal dimension	3.00	2.99
Backbone fractal dimension	2.98	2.99
Mayer stowe summary		
Interstitial porosity (%)	47.6	25.9
Breakthrough pressure ratio	3.35	8.32
Material compressibility		
Linear coefficient ($psia^{-1}$)	−6.21E-01	N/A
Quadratic coefficient ($psia^{-2}$)	4.40E-02	N/A

current European legal limit (2 ppb). These results are in line with the low BaP content found in smoked meat products reported by other authors (Jira et al., 2006; Purcaro et al., 2009; Roseiro et al., 2012; Santos et al., 2011).

3.2. Physical characterization of casings and meat products

3.2.1. Mercury intrusion porosimetry

Table 2 shows the intrusion data, pore structure and Mayer-Stowe data of natural (from pig intestine) and synthetic (collagen) casings.

As can be seen in Table 2, higher values of intrusion data parameters, i.e. total intrusion volume, median pore diameter (expressed in volume and area), average pore diameter, apparent density and finally porosity, were found in the natural casing than in the collagen casing.

In fact, with a porosity of 66.8%, the natural casing is four times more porous than the collagen casing (16.6%). The average pore diameter of the natural casing (599.8 nm) is considerably higher than that of the collagen casing (48.2 nm). Moreover, the interstitial porosity of the natural casing (47.6%) is higher than that of the collagen casing (25.9%). However, the total pore area of both casings is similar.

As Table 2 shows, the total intrusion volume of the natural casing (1.37 mL/g) is higher than that of the collagen casing (0.14 mL/g). To sum up, with a higher pore diameter and total intrusion volume, the natural casing is four times more porous than the collagen casing. However, the tortuosity of the natural casing (3.65) is higher than that of the collagen casing (2.86). The collagen casing is made from a synthetic material, more homogeneous than the natural casing. Most likely, a greater variation in pore diameter can be found in natural casing, making its tortuosity higher than that of the collagen material.

3.2.2. Scanning electron microscopy (SEM)

Fig. 2 shows SEM images of both the studied sides of natural and synthetic (collagen) casings. Fig. 2a–d shows SEM images of the natural casing. Fig. 2a and b were taken on the "A" external side of the natural casing. Fig. 2c and d were taken of the "B" internal side of the natural casing, that contacts with stuffed chorizo mass. Fig. 2e and f shows SEM images of the synthetic casing. No differences were found in the SEM images of both sides of the synthetic

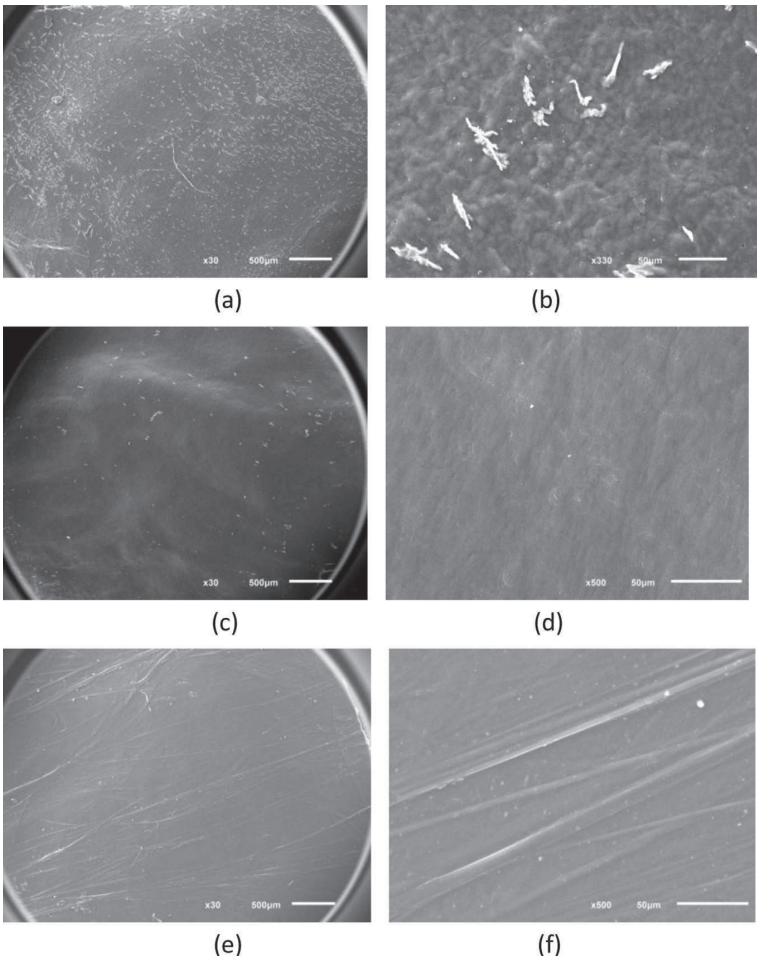

Fig. 2. SEM images of faces "A" (a,b) and "B" (c,d) of natural casing (from pig intestine) and synthetic (collagen) casing (e,f). Scale of figures a, c and e is 500 µm. Scale of figures b, d and f is 50 µm.

casing. As Fig. 2a and e shows, a higher number of small, elongated, white objects were found in the natural casings than in the collagen casings. These objects were identified as salt crystals by elemental analysis by SEM (42.7% Na and 57.2% Cl). A different morphology was found on the different sides of the natural casing. Side A (Fig. 2a and b) shows more salt crystals than side B (Fig. 2c and d). As Fig. 2b and d shows, side A of the natural casing is coarser and more wrinkled than side B. The same smooth surface was found on both sides of the collagen casing, where the collagen fibres can be clearly identified (Fig. 2e and f).

To sum up, the natural casing has 2 sides with different surfaces, one of them (external side, called side A) has a wrinkled surface with the capacity to capture small objects. The other (internal side, called side B) has a smooth surface that does not capture small objects. The morphology of the surface of both sides of the collagen casing is similar, being flat and smooth, the artificial material of which is identified by collagen fibres.

Fig. 3 shows SEM images of the natural and synthetic collagen casings of chorizos after 5 days of smoking. Fig. 3a and c (×23 magnification) show the general morphology and appearance of natural and synthetic smoked casings of a chorizo. Comparison of these images indicates that the natural casing is covered with more bodies or materials than the synthetic casing. The natural casing is dirtied by these materials, whereas the synthetic casing seems to be cleaner. Fig. 3b and d (×430 magnification) show the detailed morphology and appearance of natural and synthetic smoked casings of a chorizo. The body shown on the left side of the images is a soot particle. Comparison of these images indicates that the soot particle is embedded in the wrinkled surface of the natural casing, while the collagen casing presents a smooth surface.

Fig. 3e and f (×120 magnification) show the detailed morphology and appearance of a soot particle from smoke found in a natural and synthetic casing of smoked chorizos, respectively. Comparison of these images indicates that the soot particle harms the natural casing, managing to penetrate into the product. In contrast, the soot particle is deposited on the synthetic casing and do not harm or penetrate it.

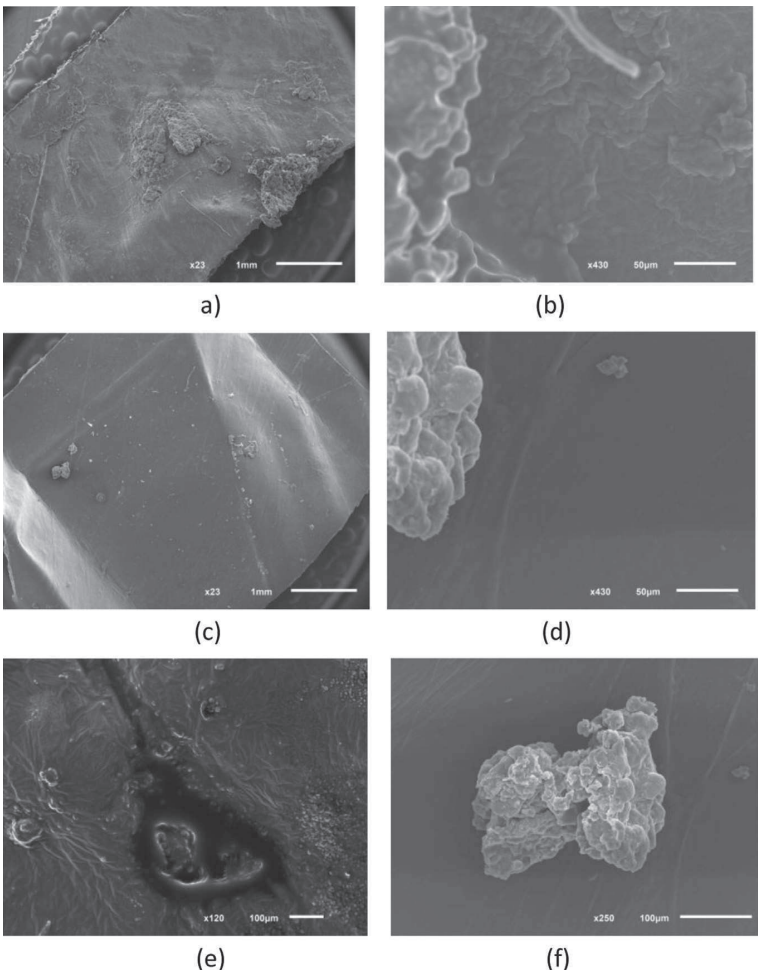

Fig. 3. SEM images of natural (from pig intestine) (a,b,e) and synthetic (collagen) (c,d,f) casings of smoked chorizos. Scale of figures "a" and "c" is 1 mm, "b" and "d" 50 μm, "e" and "f" 100 μm.

3.2.3. Fluorescence Stereo Microscope

Fig. 4 shows Fluorescence Stereo Microscope images of natural (4a) and synthetic (4b) casings, uncured chorizo mass stuffed in natural (4c) and synthetic (4d) casings, chorizo smoked for 10 days stuffed in natural (4e, 4f) and synthetic (4g, 4h) casings and the external (4i, 4k) and inner (4j, 4l) parts of chorizo smoked for 10 days stuffed in natural (4i, 4j) and synthetic (4k, 4l) casings, respectively. All 48 chorizos studied in this work showed a similar behaviour. A surface covered with a gelatinous material is found on the natural casings (Fig. 4a), while synthetic fibres are clearly identified in the collagen casings (Fig. 4b). According to Feiner (2006) natural casing is composed by several layers, organized in 5 sections: serosa (tunica serosa), 2 muscular layers, (tunica muscularis: stratum longitudinale and stratum circulare), submucosa (tela submucosa) and mucosa (mucous coat). Once the raw ingredients of the meat product are stuffed in the casings, the collagen and natural casings are seen to behave differently. As Fig. 4c shows, the morphologies and surfaces of the natural casings were modified by their content. Once stuffed, the meat product mixture seems to cross the natural casings, describing small spheres on the surfaces. As Fig. 4d shows, the raw material remains inside the collagen casing, the morphology of which does not present any modification caused by the process. The collagen casing is a barrier between the external and internal part of its content. Fig. 4e–h shows that, after 10 days of smoking, the surface of chorizos manufactured with natural and synthetic casings are very different. Fig. 4e shows that the surface of chorizos stuffed in natural casing is full of fat that has come from inside the product, making it sticky. Hands are soiled with fat when touching the product. As Fig. 4f shows, numerous soot particles are found embedded in the fat, which flows from inside the chorizo and covers its external surface. Numerous soot particles were found in the external and even the internal parts of chorizo when the casing was removed. The products seem to swell up. However, as can be seen in Fig. 4g, the surface of synthetic chorizos is dry, does not soil the hands when touched and is clean and practically free of soot particles, as in the internal parts of the products once the casings were removed. Fig. 4h shows that all the products seem to have shrunk, presenting a great deal of wrinkles on their surface. The fat from the products remains inside. When the casings are removed,

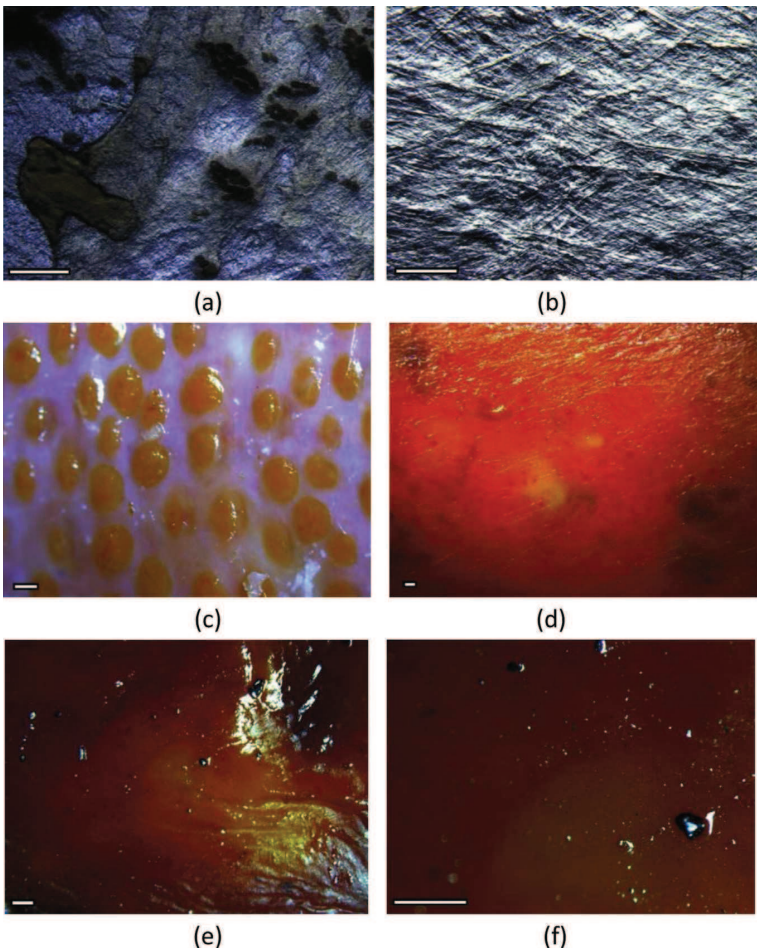

Fig. 4. Fluorescence Stereo Microscope images of natural (a) and synthetic (b) casings, uncured chorizo mass stuffed in natural(c) and synthetic (d) casings, 10 days smoked chorizo stuffed in natural (e, f) and synthetic (g, h) casings and external (i, k) and deepest (j, l) sides of 10 days smoked chorizo stuffed in natural (i, j) and synthetic (k, l) casings respectively. The scale of all images is 500 μm, except image i, whose scale is 200 μm.

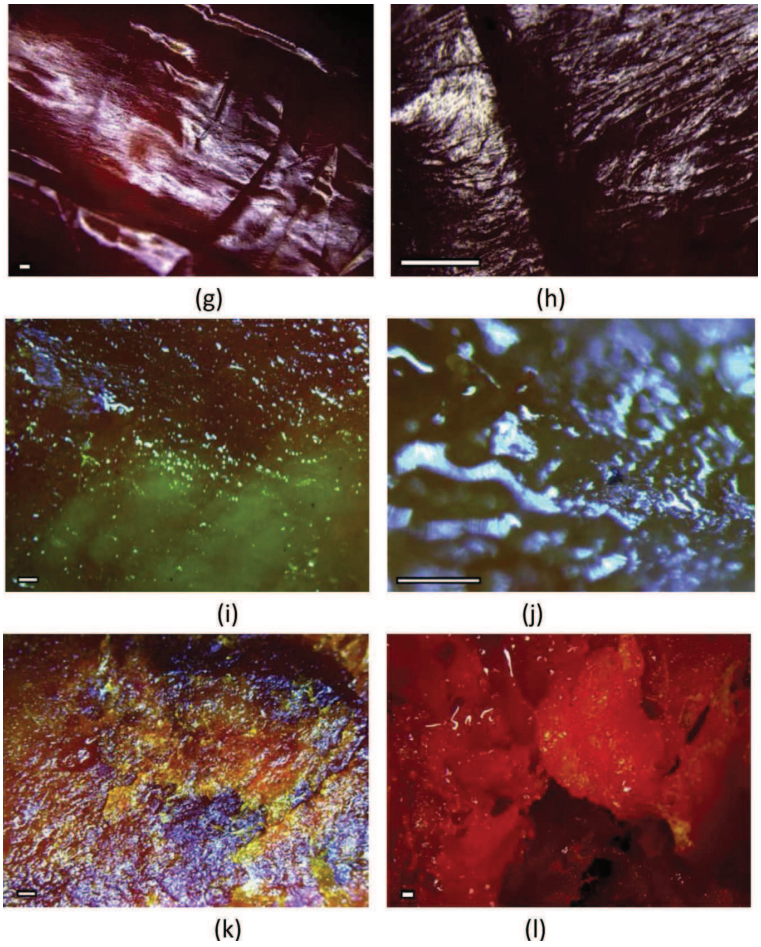

Fig. 4. (*continued*).

the morphology and surface of chorizos stuffed in natural and synthetic casings are also different. As Fig. 4i shows, the surface of natural casing of stuffed chorizos is moist, full of juicy fat, whereas Fig. 4k shows that the fat of chorizos whose synthetic casing had been removed has become dried, making it hard to the touch. Fig. 4j and l shows that more soot particles were found in the chorizos whose natural casing had been removed than in those stripped of their synthetic casing, where not many of these particles were found. Fig. 4j and l shows that, while only a few, some soot particles were found in the centre of the chorizos (the deepest part of a section) stuffed in natural (Fig. 4j) casings, while none was found in the innermost part of chorizos stuffed in synthetic casings (Fig. 4l).

3.3. Overall comments

All the results presented in this study help us to understand the process of BaP contamination of smoked meat products stuffed in different casings. During traditional smoking, biomass pyrolysis and incomplete combustion processes occur forming undesired tar aerosols. When the temperature rises above 1023 K, tertiary tar products begin to appear with increasing temperature and PAH are formed (Basu, 2010, chap. 4). PAH are transported in these smoke aerosols, finally being deposited on the meat products. Meanwhile, different processes occur in meat products stuffed in natural or synthetic casings. Both products are heated and dried during smoking (Möhler, 1978; Varlet et al., 2007). However, the porosity of natural casings (66.8%) is higher than that of synthetic casings (16.6%). Accordingly, different effects occur in chorizos stuffed in natural and synthetic casings. First, when stuffing, both products swell and the raw mix of meat products passes through the natural casing, but not through the synthetic casing. These meat products then enter the smoking chamber, where they are heated and placed in contact with smoke. Heat makes chorizos stuffed in natural casing sweat fat. The fat content of these chorizos flows through the casing and covers its external surface, making it sticky and moist. Then, when PAH aerosols reach the sticky, wrinkled surface of the natural casing of chorizos, soot particles are easily captured and adhere to it, damage the casing and start to migrate inside the product. Once captured, the soot particles slowly penetrate inside the meat products, accessing the inner part through the lumps of fat. Although the main amount of BaP stays in the casing, some cross the membrane and penetrate inside the product due to the high porosity of the natural casing. It can be also seen that, during the first 5 days of smoking, the membranes of the casing are more

moist and sticky, the product being more swollen. Hence, a greater amount of BaP may be captured by the casing. In previous works it was found that BaP increases during smoking time, finally becoming stabilized after 5 days of smoking (Ledesma et al., 2014). In contrast, the fat content of chorizos stuffed in synthetic casings remains inside the product, its surface remaining dry, making it non-sticky and smooth without any affinity for soot particles. Accordingly, a lesser amount of soot particles containing PAH (aerosols) stays on the outside of the casing and does not damage its surface. Moreover, the smaller size of the synthetic casing pores hinders the small amount of coal tars from penetrating the product. For all these reasons, high BaP levels were found on the external surface of the smoked meat products stuffed in natural casing (19.9 ± 2.9 μg/kg) and were then also found inside the meat products (D2) stuffed in natural casing (1.71 ± 0.15 μg/kg). However, no BaP content was found inside (D2) the meat products stuffed in synthetic casings. These results are in keeping with the findings of Gomes et al. (2013) and Škaljac et al. (2014), in which the use of synthetic instead of natural casings contributed to reducing PAH levels in smoked meat products.They likewise explain the results reported by Pöhlmann et al. (2013b), who found only a small increase in BaP content in the smoked meat products stuffed in natural casings (0.57 ± 0.21 μg/kg) compared to collagen casings (0.40 ± 0.12 μg/kg), which may be caused by prior environmental PAH contamination of the raw ingredients.

4. Conclusions

Natural and synthetic casings have physical differences that lead to different BaP content into smoked meat products. The natural casings have a wrinkled morphology and high porosity (66.8%). This makes the fat content flows outward through the casing, making its surface sticky and product swollen. A high number of soot particles and tar aerosols containing BaP adhere in the casing, mainly during the first 5 days of smoking. Only a small amount of soot particles migrates into the product, through the high size pores and the viscous fat. In contrast, synthetic casings have a smooth morphology and low porosity (16.6%). The fat remains inside the product They present a dry, hard external surface with less affinity for soot particles, whose possibility of penetrating into the product is hindered by the small pore size of the casing and dry surfaces. For all these reasons, a high BaP content was found in the casings, but it was only detected inside the meat products stuffed in natural casing (1.71 ± 0.15 μg/kg). No BaP was found inside the meat products stuffed in synthetic casings. Finally synthetic casings contribute to reducing BaP levels in smoked meat products.

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4.4 INFLUENCIA DEL TIPO DE TRIPA EN LAS PROPIEDADES DEL CHORIZO DURANTE EL AHUMADO: TEXTURA, COLOR Y CARACTERÍSTICAS ÓPTICAS

Los resultados obtenidos en el capítulo 4.3 indican que, a diferencia de las tripas naturales, y debido principalmente a su menor tamaño de poro y porosidad, las tripas de colágeno minimizan el flujo del contenido graso del chorizo hacia la superficie exterior, previniendo la captura y penetración en el producto de partículas procedentes del ahumado que contiene BaP y HAP cancerígenos.

Las tripas naturales han sido utilizadas desde hace más de 2000 años y numerosas entidades, como la Asociación Europea de Tripas Naturales para Embutidos (ENSCA), o la Asociación Europea de Tripas Naturales para Embutidos (INSCA), defienden sus propiedades para el desarrollo de productos cárnicos, destacando entre otros factores su arraigo a lo tradicional, su menor grosor, su resistencia a romper, o su dificultad para despegarse del producto (ENSCA, 2015; FEDECARNE, 2013; INSCA, 2015). Por otro lado, la creación de nuevas tripas artificiales ha sido necesaria para el desarrollo de la industria cárnica. Las tripas de colágeno fueron creadas por primera vez en Alemania, en el año 1925. Desde entonces han sido optimizadas, y su fabricación se consolida sobre los años 60 (Devro, 2015). Los productores de tripas artificiales defienden sus propiedades, su capacidad para homogeneizar la producción, el ahorro de tiempo productivo y en definitiva de costes industriales para el fabricante (Devro, 2015; Viscofan, 2015). La competencia entre los 2 sectores es muy elevada. Según datos del informe anual de Viscofan (líder mundial en la producción de tripas sintéticas), el tamaño mundial del mercado de venta de tripas para embutidos es mayor de 4,2 billones de euros (Viscofan, 2012). El mercado de tripas sintéticas está creciendo, pues el reparto global era de 70%-30% y 63%-37% para tripa natural y sintética, en 2011 y 2012, respectivamente, según Devro (Devro, 2013; Ryan, 2013) y al 58-42% y 50-50%, según Viscofan (Viscofan, 2012).

En el siguiente trabajo se determinaron y compararon científicamente la textura, el color, la humedad y la evolución morfológica de chorizos asturianos embutidos en tripa natural y de colágeno, mediante el estudio de 48 chorizos durante 11 días de ahumado. Mediante este trabajo se obtuvieron nuevas evidencias de la contaminación de los chorizos embutidos en tripa natural por las partículas de hollín que transportan HAP.

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TEXTURE, COLOUR AND OPTICAL CHARACTERISTICS OF A MEAT PRODUCT DEPENDING ON SMOKING TIME AND CASING TYPE

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ABSTRACT

Synthetic casings have recently been found to prevent the penetration of carcinogenic compounds in meat products during smoking, in contrast to natural casings. In this paper, physical characteristics of 48 chorizos encased in natural (ChN) and synthetic (ChS) casings, during 11 days of direct smoking, were compared by means of texturometry, colorimetry, moisture, Scanning Electron Microscopy and Fluorescence Stereo Microscopy analyses. The lightness (L*) of ChS is lower ($p=0$) than ChN. During 5 days, ChS presents higher hardness and browning index, and less redness (a*), yellowness (b*) and chroma than ChN ($p<0.05$). At 7 days, no differences ($p>0.05$) were found in a*, b*, hue angle or chroma. Except at 1 day, no differences ($p>0.05$) were found in their moisture. Physical characteristics were suitable in both cases, but synthetic casing reduces production time, prevents the penetration and accumulation of soot particles in mass cavities, and enables the standardization of chorizo.

Keywords: chorizo, smoked meat products, casing, colour, texture, microstructure.

1. Introduction

The consumption of meat and meat products, which contain important levels of protein, vitamins, minerals and essential micronutrients, is growing in developing countries. Meat

processing provides the opportunity to add value, reduce prices, improve food safety and extend shelf life. However, unsuitable processing can lead to undesirable health effects and environmental impact (EC, 2013; FAO, 2013). Chorizo is a typical Spanish meat product. In northern Spain and other European countries, chorizo is exposed to direct smoking with the main aim of prolonging its shelf life and conferring sensorial characteristics. During non-controlled smoking, however, chemical contaminants are also formed, such as polycyclic aromatic hydrocarbons (PAH), dioxins, formaldehyde, nitrogen and sulphur oxides (relevant in the formation of nitrosamines, for instance) and even heavy metals (CAC, 2009). PAH have been found to be carcinogenic for humans (ATSDR, 2009).

In order to protect consumers from PAH contamination, laws and codes of best practices in food processing have been proposed. Regulation (EU) No. 835/2011 of 19 August 2011 (EC, 2011) has recently reduced the maximum permissible content in BaP (a marker of the presence of PAH) in smoked meat and smoked meat products from 5.0 to 2.0 µg/kg in wet weight from 1/9/2014 on. The permissible sum of PAH4 (benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) in these foods has been reduced from 30.0 to 12.0 µg/kg in wet weight from 1/9/2014 on. The "Code of practice for the reduction of contamination of food with (PAH) from smoking and direct drying processes" (CAC/RCP 68/2009) advocates the control of certain variables to prevent PAH contamination. A number of studies have been carried out to test these variables, including the kind of fuel (Hitzel, Pöhlmann, Schwägele, Speer, & Jira, 2013; Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2012; Stumpe-Vīksna, Bartkevics, Kukare, & Morozovs, 2008), smoking method (Pöhlmann et al., 2012; Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013a; Škaljac et al., 2014; Varlet et al., 2007), duration of smoking (Djinovic, Popovic, & Jira, 2008; Essumang, Dodoo, & Adjei, 2013; Ledesma, Rendueles, & Díaz, 2014), fat content (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013b), distance between the food and the heat source, position of the food in relation to the heat source (Pöhlmann et al., 2013b), temperature during smoking and direct drying (Pöhlmann et al., 2012), cleanliness and maintenance of equipment, and design of the smoking chamber and the equipment used for producing the smoke/air mixture (Pöhlmann et al., 2013a). Moreover as a post-smoking treatment, CAC (2009) advocates cleaning the product by rinsing or immersing it in water to remove soot and particles containing PAH from the surface of the food. It has been found that the greatest amount of BaP (and probably other PAH) is deposited in the casing of the meat product, subsequently migrating into the product (Andrée, Jira, Schwind, Wagner, & Schwägele, 2010; Djinovic et al., 2008; García-

Falcón, & Simal-Gándara, 2005; Ledesma et al., 2014; Santos, Gomes, & Roseiro, 2011). Some authors have reported that the use of different types of casing influences the PAH content of meat products (Filipovic & Toth, 1971; Toth, 1973). Recent studies (Gomes, Santos, Almeida, Elias, & Roseiro, 2013; Ledesma et al., 2015; Škaljac et al., 2014) have concluded that the use of synthetic instead of natural casings contributes to reducing PAH levels in smoked meat products. The causes of this effect have been attributed to differences in the morphology and porosity of natural (16.63%) and synthetic (66.84%) casings (Ledesma et al., 2015).

The aim of the present study was to compare the physical characteristics (texture, colour, moisture and morphology) of chorizos encased in natural and synthetic casings during direct smoking time in order to determine which casing is better for producing high quality chorizos safe for human consumption.

2. Materials and methods

2.1 Preparation of meat products

Two kinds of meat products were prepared for this study: chorizo encased in natural casing (ChN), and chorizo encased in synthetic casing (ChS). Twenty-four chorizos of each kind were manufactured at the El Hórreo Healthy Food S.L. meat company using pork loin (46.8%), pork jowl (46.8%), salt (1.8%), garlic (1%), sweet or spicy paprika (2%) and herbs (1.6%), all of which were minced, mixed and encased. Antioxidants such as sodium citrate or sodium ascorbate, colourings such as carmine, emulsifiers such as sodium triphosphate, food preservatives such as sodium nitrite and other food additives were used to ensure the microbiological stability and quality of the foodstuff. Nutritional analysis of the product was carried out by the Principality of Asturias Meat Industry Association Technological Centre for Supporting Innovation (Spanish acronym, ASINCAR), providing the following results: 31.41% moisture content, 22.98% protein, 37.94% fat, 2.85% carbohydrates, 4.82% ash and pH 5.2.

A batch of chorizo was processed with the aim of reproducing industrial manufacturing conditions. The minimum amount of chorizo considered as an industrial production volume is 50 kg. If an insufficient amount of chorizo is introduced in the smoking chamber, it could be exposed to an excess of smoke. Thus, 50 kg of raw chorizo ingredients were minced, mixed, marinated

and stuffed to obtain 200 strings of chorizo, of which 12 strings (containing 48 chorizos) were analyzed. All the selected samples were placed in the same position in the direct smoking chamber at the same distance from the smoke source, namely 10 m. Only oak wood was used for direct smoking of the chorizos. Temperature and humidity parameters in the direct smoking chamber ranged between 7-17 ºC and 49-100 % respectively, depending on weather conditions and intermittent smoking. One string of each kind of chorizo was sampled at the following smoking times: 0, 1, 4, 5, 7 and 11 days, between 7th and 18th November. Once smoked, the chorizos were hung from a cardboard tube inside a box, avoiding any contact of the materials.

2.2 Apparatus

2.2.1 Fluorescence Stereo Microscope

The external and internal surfaces (once the casing was removed) of the chorizos were studied using a Fluorescence Stereo Microscope **(**Leica M205 FA, Wetzlar, Germany) to determine the microscopic morphology of the samples.

2.2.2 Texture measurement

In this study, the hardness of ChN and ChS during smoking time (0, 1, 4, 5, 7, and 11 smoking days) was analyzed in triplicate. Whole chorizo samples were placed on the heavy duty platform of the texture analyser (TA.XTPlus, Hamilton, MA, USA) and the arm of the texture analyser moved down to penetrate the product before returning to its initial position. A TA-18 rounded end probe with a 1/2" diameter stainless steel ball was used for this purpose.

2.2.3 Colour measurement

Chorizo colour measurements (L*, a*, b* CIELAB values) were carried out using an UltraScan VIS spectrophotometer (HunterLab, Reston, VA, USA). The instrument was standardized using a white title and calibration was tested using a green ceramic plate. The Hunter L*, a*, and b* values correspond to lightness, black (-l) or white (+l), greenness (-a) or redness (+a), and blueness (-b) or yellowness (+b), respectively. The colour measurements were performed on chorizos at room temperature (20 ± 2 ºC). Three different samples of each kind of chorizo (ChN and ChS) were selected after different days of smoking (0, 1, 4, 5, 7 or 11) and colour parameters were measured 30 times for each chorizo per smoking day.

Total colour change (ΔE; Eq. (1)), hue angle (Eq. (2)), chroma (saturation index; Eq. (3)), and browning index (BI; Eq. (4)) were calculated using Hunter L, a, and b values (Bozkurt & Bayram, 2006; Homco-Ryan et al., 2004; Laca, Sáez, Paredes, & Díaz, 2010; Maskan, 2001) as:

$$\Delta E = \sqrt{\Delta L^{*\,2} + \Delta a^{*\,2} + \Delta b^{*\,2}} = \sqrt{L^* - L_0^{*\,2} + a^* - a_0^{*\,2} + b^* - b_0^{*\,2}} \quad \text{Eq.(1)}$$

$$\text{Hue angle} = \tan^{-1}\left(\frac{b}{a}\right) \quad \text{Eq. (2)}$$

$$\text{Chroma} = \sqrt{a^2 + b^2} \quad \text{Eq. (3)}$$

$$BI = \frac{[100 \times x - 0.31\ ]}{0.17} \quad \text{Eq. (4)}$$

where:

$$x = \frac{a + (1.75 \times L)}{5.645 \times L\ + a - (3.012 \times b)}$$

2.2.4 Scanning electron microscopy (SEM)

A Jeol 6100 scanning electron microscope operating at 30 kV was used to determine the microscopic morphology of the samples. Chorizos were divided in two parts for SEM analysis: the casings, and the chorizos without casings. Samples were pretreated by means of freezing to 193.15 K for 4 hours followed by freeze-drying in a lyophilizer (Telstar-Cryodos, Spain) for 1 day.

2.2.5 Determination of moisture content

Moisture content was determined by gravimetry, in triplicate, during 0, 1, 4, 5, 7 and 11 days of smoking of ChN and ChS. Twenty g of thick grain sea sand (ref. 211161, Panreac, Spain) were weighed in a stainless steel mortar with its pestle The mortar was then kept in a vacuum desiccator for 30 minutes until its weight value was constant. Subsequently, approximately 6 g of sample were weighed into the mortar and the sea sand and chorizo sample were mixed with the pestle. The mortar was placed in an oven (Memmert, Germany) at 105±2ºC for 5 hours. The mortars were then allowed to cool in a vacuum desiccator at room temperature for 30 minutes before finally being weighed.

2.2.6 Statistical analyses

Data were analysed by running t-tests to compare means, at a significance level of $p<0.05$. Previously, F-tests were carried out at a significance level of $p<0.05$ to compare standard deviations. Standardized skewness and standardized kurtosis were used to assess whether the samples came from normal distributions. Multiple range tests were applied between chorizo samples stuffed in the same kind of casing during smoking time in order to determine which means were significantly different from which others. The software used was Statgraphics Plus 3.1.

3. Results and discussion

3.1 Texture measurement

Figure 1 shows the hardness results for ChN and ChS during smoking time (0, 1, 4, 5, 7, and 11 smoking days). As can be seen in this figure, the hardness of both types of chorizos presents a similar trend: hardness increases during drying. However, the hardness of ChN and ChS was found to be different ($p<0.05$) from the moment when the products were stuffed (before smoking) to 7 days of smoking. The hardness of ChS (from 255.24g ± 9.60 to 999.27 ± 24.13) was always higher than that of ChN (from 66.57g ± 1.14 to 851.62g ± 22.93). However, no statistical significant differences ($p>0.05$) were found between the two groups at 11 days of smoking.

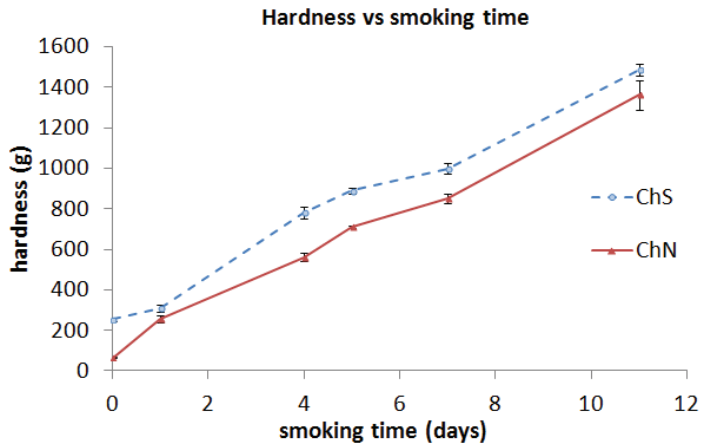

Figure 1. Hardness (g) texture profile analysis of chorizos stuffed in natural (ChN) and synthetic (ChS) casings during smoking time.

According to our results, higher values of hardness were found in more dried Spanish "chorizo de Pamplona" after about 30 days of fermentation, smoking and drying processes (Gimeno, Ansorena, Astiasarán, & Bello, 2000). Softness can be a positive attribute in some types of chorizo (Melendo, Beltran, Jaime, Sancho, & Roncales, 1996), while in others (like ''chorizo de Pamplona") it can be considered a defect (Gimeno, Astiasarán, & Bello, 1999). In fact, the Spanish chorizo studied in this paper (chorizo from northern Spain) is used as an ingredient in typical Spanish stews with a long cooking time, such as "fabada" (about 3 hours). For this reason, this kind of chorizo should not be too hard before cooking so as to allow its ingredients to provide a good taste to the final dish. Synthetic casing could hence reduce processing time to obtain the same hardness as that of ChN.

3.2 Colour analysis

Figure 2 shows the lightness (L*) (2A), redness (a*) (2B), yellowness (b*), (2C) browning index (BI) (2D), hue angle (2E), chroma (2F) and total colour change (ΔE) (2G) of the analyzed Asturian ChN and ChS during smoking time (0, 1, 4, 5, 7 and 11 days).

There were no differences in the ingredients or preparation of the ChN and ChS, except for the use of different types of casing (natural and synthetic). However, as Figure 2 shows, the two types of casings produce differences in chorizo colour from the moment the products are stuffed. Before smoking, synthetic casings produced significantly ($p<0.05$) less lightness (L*: 44.01±1.29), redness (a*: 33.09±0.96), yellowness (b*: 32.50±0.81) and chroma (46.38±1.21) and a higher browning index (170.46±2.53) in unsmoked chorizo than natural casings (L*: 47.14±0.90; a*: 33.92±0.57; b*: 33.16±0.45; BI: 159.90±4.69; CH: 47.44±0.22). In contrast, no significant differences ($p>0.05$) were found in the hue angle of either type of chorizo before smoking.

During 1 to 11 days of smoking, ChN always continues to show more lightness ($p=0$ from 0 to 7, $p= 0.000012$ at 11 smoking days, from 39.18±0.32 to 29.73±2.55) than ChS (from 35.39±0.56 to 26.41±0.68). Redness showed more variable behaviour in chorizos stuffed in the two types of casing during smoking. At the beginning of smoking (1 day), no differences in redness ($p>0.05$) were found in the two types of chorizos. After 4 and 5 days of smoking, ChN showed higher redness ($p<0.05$) than ChS, a finding which was also observed ($p>0.05$) at 7 days of smoking. Finally, the opposite effect appeared after 11 days of smoking, when a higher a* was found in ChS (19.99±0.17) than in ChN (18.84±0.75). Likewise, from 1 to 5 days of smoking, the yellowness

of ChN was higher than ChS, with no differences ($p>0.05$) finally being found between the two groups from 7 days of smoking on, when b* dropped to 12 after 11 days. From 4 to 11 days of smoking, the BI of ChS was higher ($p=0$) (from 148.24±11.80 to 111.52±5.06) than that of ChN (from 133.36±15.32 to 94.70±4.21). From 1 to 5 days of smoking, the HA and CH of ChN was found to be higher ($p<0.05$) than that of ChS. Like yellowness, no differences were found ($p>0.05$) in HA from 7 days of smoking on in the two kinds of chorizos. Like redness, no differences were found ($p>0.05$) in the CH of the two types of chorizos at 7 days of smoking. The opposite effect was found at 11 days of smoking, when the CH of ChS was significantly ($p<0.05$) higher (24.03±1.16) than that of ChN (22.13±0.97). Finally, both kinds of casings produced differences in the colour of smoked chorizo. Synthetic casing gives a darker colour at the same smoking time, maintaining redness and affording the product a "more cooked" appearance. Differences in the colour of meat products stuffed in different casings were also found by Pöhlmann et al. (2013b).

As Figure 2G shows, the highest total colour change (ΔE) in ChN and ChS was produced between before (0 days) and after 4 days of smoking, probably because the product is modified from "unprocessed" to "processed" and constitutes the most intense change. The total colour change (ΔE) was less pronounced from 1 to 4 days of smoking, probably because of the occurrence of a weekend between these days, when stoking of smoke is lower. From 4 to 7 days of smoking, the product is already cooked and intense changes are not produced, obtaining a very low increase of ΔE. In fact, manufacturers used to define 5-6 days of smoking as the end of processing. Finally, ΔE increased from 7 to 11 days, when the product is "over cooked".

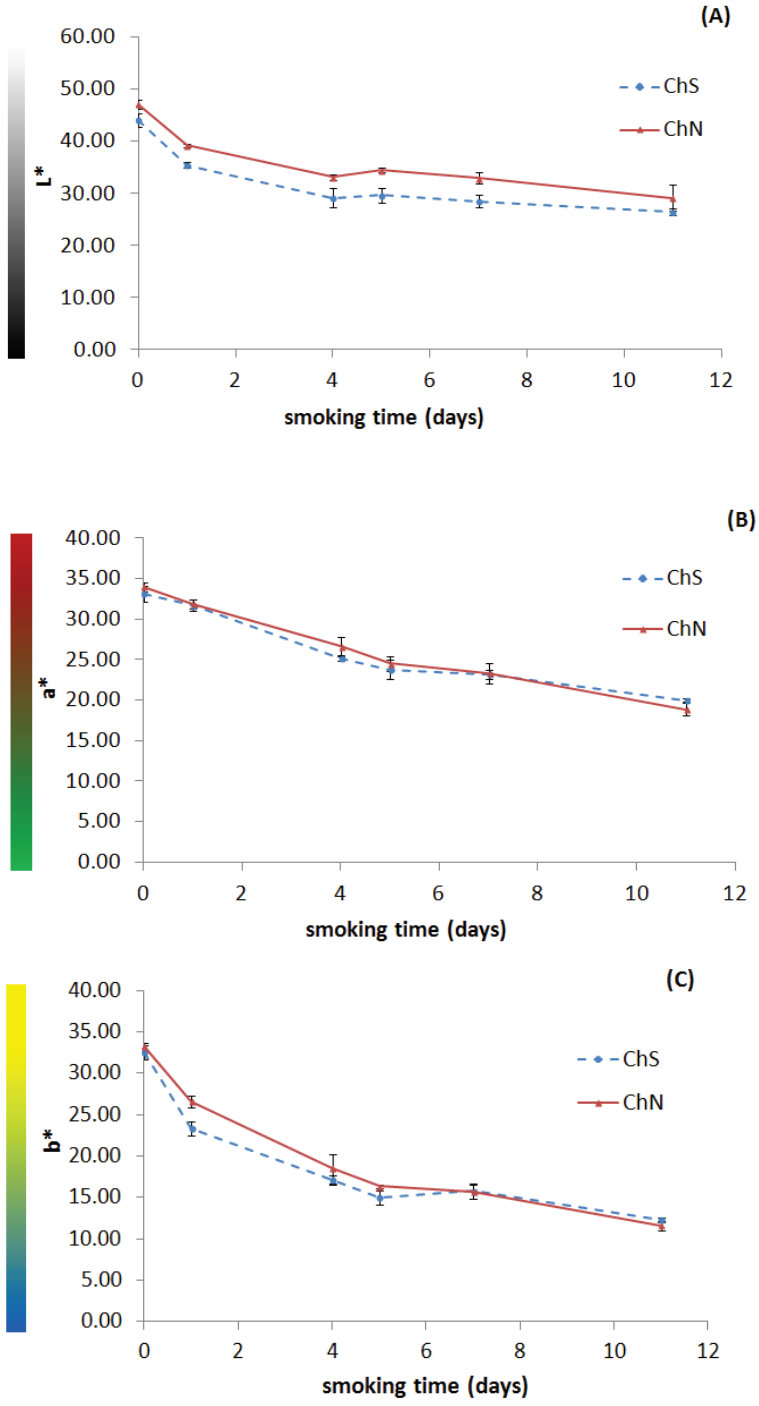

Figure 2. Lightness (L*) (2.a), redness (a*) (2.b), yellowness (b*) (2.c), browning index (BI) (2.d), hue angle (2.e), chroma (2.f) and total colour change (ΔE) (2.g) of Asturian chorizo stuffed in natural (ChN) and synthetic (ChS) casings during smoking time.

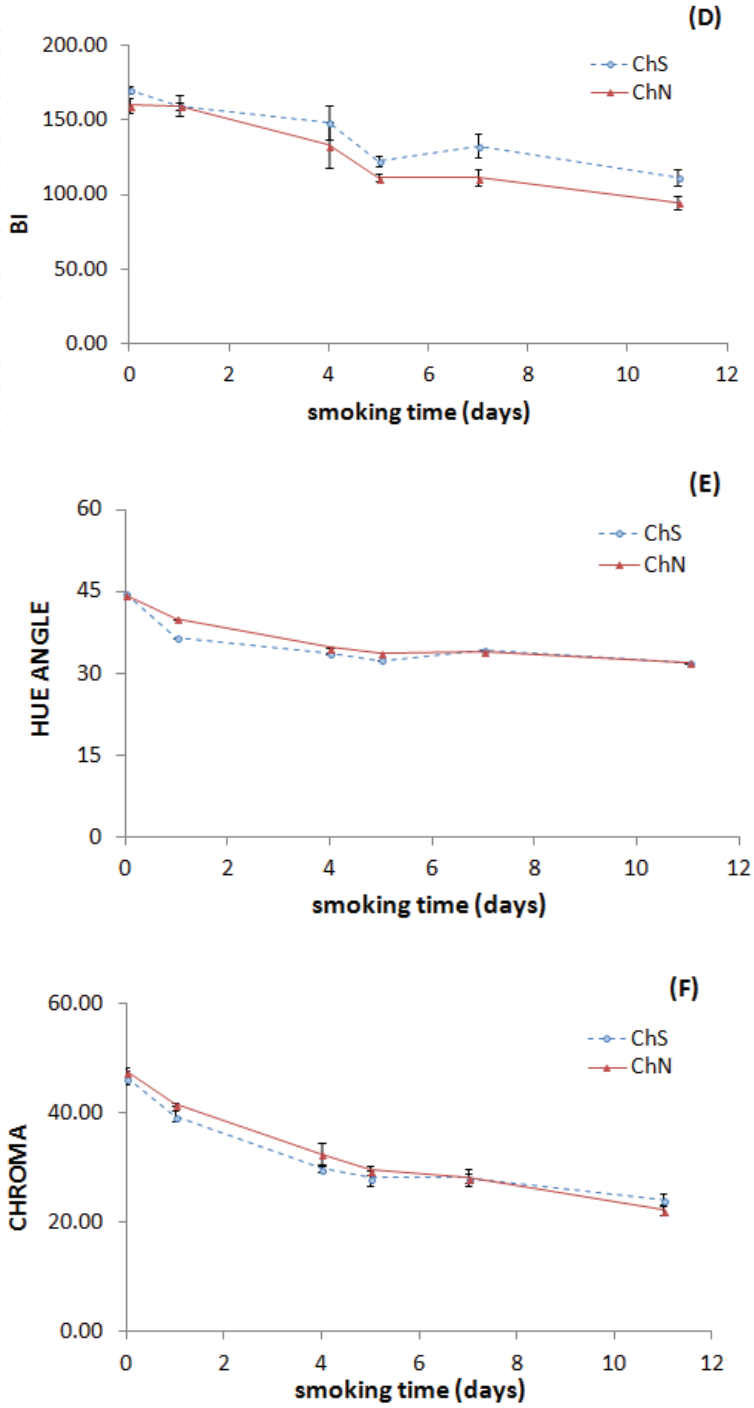

Figure 2. (continued).

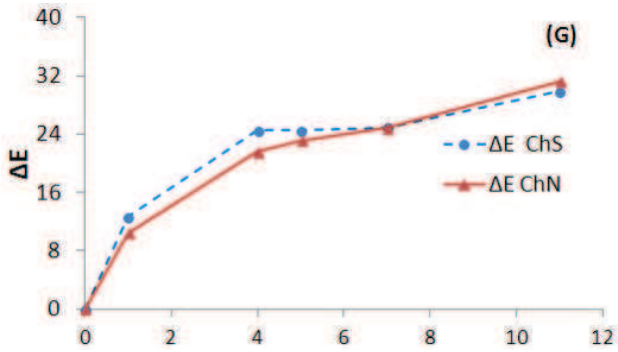

Figure 2. (continued).

3.3 Moisture content study

Table 1 shows the moisture content results for ChN and ChS during smoking time (0, 1, 4, 5, 7 and 11 days). As can be seen in this table, the moisture content of both kinds of chorizos shows a similar trend. Before smoking, both kinds of chorizos have the same moisture content (47.22% ±3.36), as they have the same ingredients. From 4 days of smoking on, ChN always shows a higher moisture content than ChS, although no significant differences ($p>0.05$) were found between ChN and ChS. Furthermore, the moisture content stabilized ($p>0.05$) after 5 days of smoking in each type of chorizo.

Table 1. Moisture profile analysis of chorizo stuffed in natural (ChN) and synthetic (ChS) casing during smoking time (n=3).

Smoking time (days)	**0**			**1**			**4**		
Chorizo and "p"	**ChS**	**ChN**	**p**	**ChS**	**ChN**	**p**	**ChS**	**ChN**	**p**
Average moisture content (%)	47.22	47.22	1	44.29	37.80	0.04	33.02	35.51	0.48
S.D.	3.36	3.36		3.04	2.14		4.26	3.49	
Smoking time (days)	**5**			**7**			**11**		
Chorizo and "p"	**ChS**	**ChN**	**p**	**ChS**	**ChN**	**p**	**ChS**	**ChN**	**p**
Average moisture content (%)	28.21	32.38	0.14	27.36	31.29	0.09	25.67	26.45	0.40
S.D.	2.97	2.62		2.49	1.26		0.88	1.12	

ChS: Chorizo stuffed in Synthetic casing
ChN: Chorizo stuffed in Natural casing
p: probability of significant differences between 2 samples
($p<0.05$: there is a significant difference between the two means).
S.D.: Standard deviation
n: number of analyzed samples

Figure 3 shows the SEM images for ChN and ChS during smoking time. Figure 3.a and 3.b show the physical structure of raw chorizo mass before it is introduced in a casing. As these figures show, the unsmoked raw mass of chorizo is a non-homogeneous or non-compact material, full of large cavities. Figure 3.c shows the external surface of ChN after 3 days of smoking, where soot particles and small holes can be seen on the casing surface. The holes could be cavities on a fat or soot layer. Figures 3.d to 3.g show the physical structure of external (3.d, 3.f) and internal (3.e, 3.g) depths of slices of 5-day-smoked chorizo stuffed in natural (3.d, 3.e) and synthetic (3.f, 3.g) casings. As these figures show, after 5 days of smoking the smoked chorizo mass has become a more homogeneous and compact material, with no cavities, the opposite to what is observed in Figures 3.a and 3.b. The internal depth of ChN (3.e) and ChS (3.g) is smoother and less irregular than the unsmoked raw mass (3.a and 3.b). During smoking, the water content in chorizo probably starts to flow up from the internal depth of the product across the large cavities present in the raw material. The components of the inner mass of both types of chorizo start to blend together and cavities get smaller until disappearing. This fact could explain the finding that the water exiting both kinds of chorizos and their moisture content is similar. As result of drying, the roughness of the chorizo surface probably decreases from the external to the internal depth of the product, where more protuberances were found.

Figures 3.h and 3.i show the structure of natural and synthetic unsmoked casings (before processing), respectively. As can be seen in Figure 3.h and 3.i, a higher number of small, elongated white objects were found in natural (3.h) than in collagen (3.i) casings. The casings were found to present a different morphology. According to previous results **(**Ledesma et al., 2015**),** these objects were identified by elemental analysis using SEM as crystals of salt (42.75% Na and 57.25% Cl). Natural casings were found to be wrinkled, while synthetic casings were found to be smooth and formed by clear collagen fibres. Figures 3.j and 3.k show the SEM images of ChN and ChS casings after 5 days of smoking, respectively. Soot particles from the smoke damage and penetrate the natural casing (3.j), while they seem to be deposited on the synthetic casing (3.k), without penetrating it.

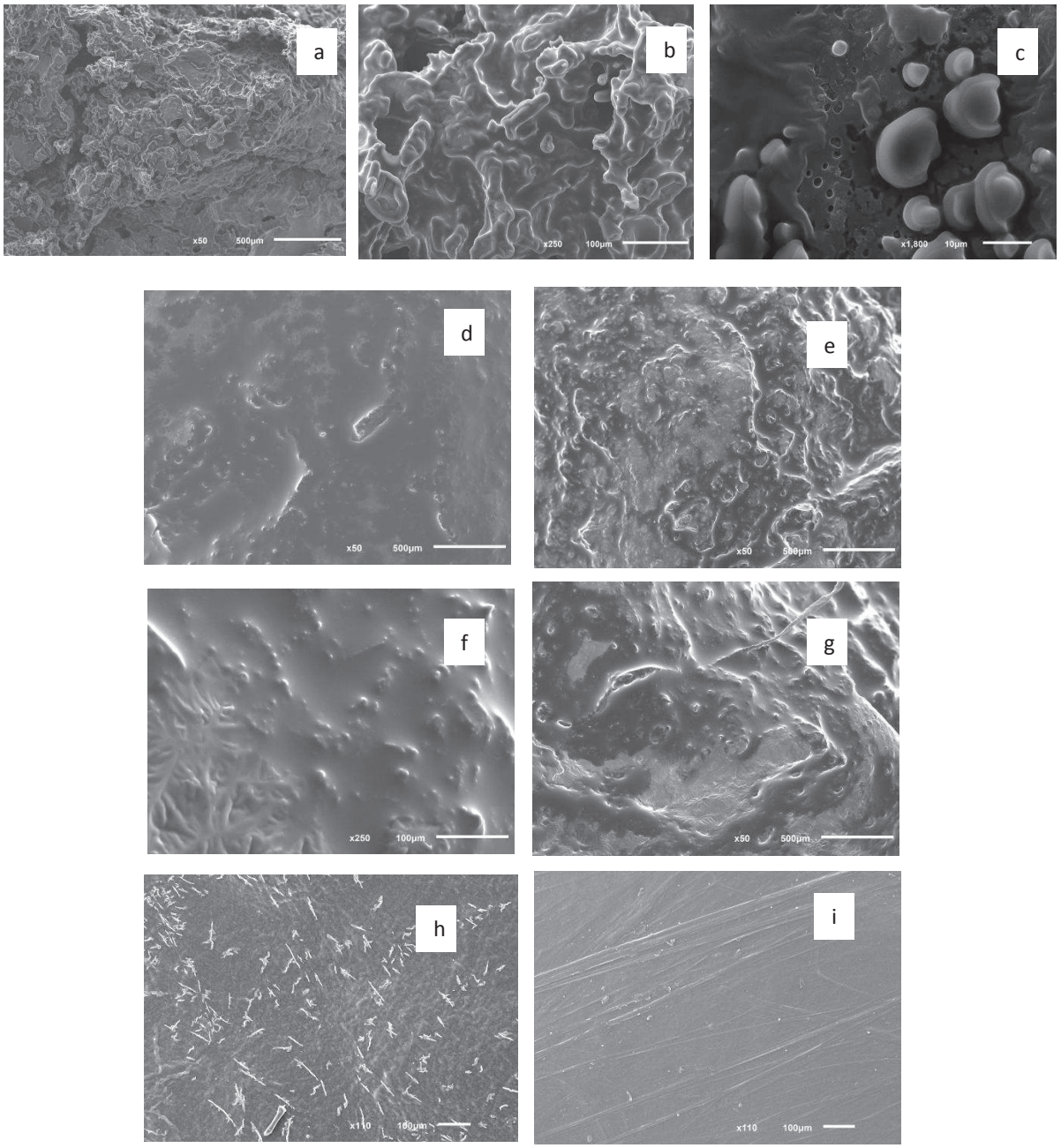

Figure 3. SEM images of chorizo before processing (a,b), external surface of 3-day-smoked chorizo stuffed in natural casing (ChN) (c), external and internal depths of 5-day-smoked chorizo stuffed in natural (d,e) and synthetic (ChS) (f,g) casings. Natural and synthetic casings before (h,i) and after (j,k) 5 days of smoking. The scale of Figures "a", "d", "e", "g" and "k" is 500 µm; the scale of Figures "b", "f", "h" and "i" is 100 µm. The scale of Figures "c" and "j" is 10 µm and 200 µm, respectively.

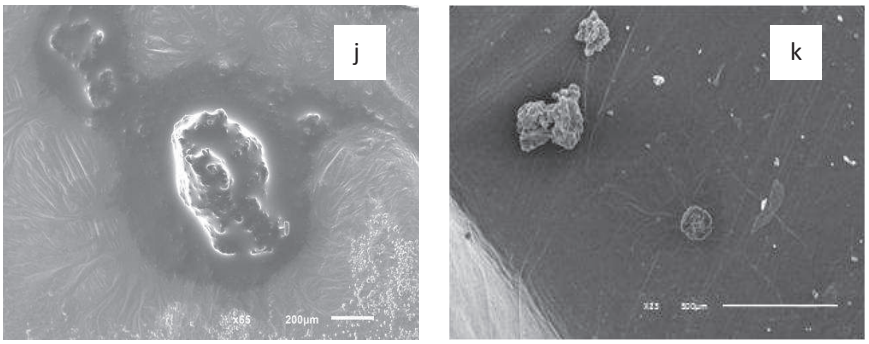

Figure 3. (continued).

In previous studies (Ledesma et al., 2015), a higher degree of porosity was found in natural casings (66.84%) than in synthetic casings (16.63%). The wrinkled morphology of natural casing is more able to capture particles than synthetic casing. The fat content in chorizos flows through the natural casings, covering their external surface and making it sticky and wet. Soot particles slowly penetrate the internal parts of these meat products and migrate to the interior, preferentially through the lumps of fat. Finally, it can be stated that raw chorizo mass is full of cavities before smoking. During smoking, soot particles pass through the pores in natural casing. They can then pass through cavities in the raw ingredients and reach the internal parts of the product. At the same time, water exits the chorizo through these cavities, raw ingredients blend together and possible soot particles can be captured in the final compact mass.

3.4 Fluorescence Stereo Microscope analysis

Figures 4 and 5 show the general (without zoom) and enlarged (with zoom) external surface of ChN (left column, images a, c, e, g and i) and ChS (right column, images b, d, f, h and j) from 0 to 10 days of smoking, with the exception of Figures 5.a and 5.b, which respectively show unprocessed natural and synthetic casings (without raw ingredients stuffed inside them). Natural and synthetic casings produce different behaviour in chorizos throughout the manufacturing process, before and during each smoking day. As Figures 4.a and 4.b show, the chorizo mass seems to exit natural casings (4.a), while it remains inside synthetic casing (4.b). As can be seen in Figures 4.c, 4.e, 4.g and 4.i, the external surface of ChN gets progressively stickier and covered in

fat during 1, 4, 5 to 10 days of smoking time, respectively. The chorizo seems to swell up when flat flows across the casings. In contrast, the external surface of ChS (4.d, 4.f, 4.h and 4.j) is always dry and the casing is clearly identified by the shiny plastic material. During smoking time, ChS seems to be wrinkled and clear lines are found in these chorizos (4.f, 4.i).

Figure 5.a shows that unprocessed natural casing is irregular. In contrast, Figure 5.b shows that synthetic casing is full of aligned, elongated collagen fibres. After only 1 day of smoking, small soot particles can be found on the sticky surface of ChN (Figure 5.c). The production of these soot particles and tar aerosols during meat product smoking and pyrolysis of biomass at high temperatures (above 750ºC) produces contamination of meat products by carcinogenic PAH (Basu, 2010, Ledesma et al., 2015). With increasing smoking time, the number of soot particles was seen to increase and they also appeared to increase in size (Figures 5.e, 5.g, 5.i). In contrast, no fat was found on the dry surface of ChS and almost no soot particles were found on them either, even after 10 days of smoking (5.j). The elongated, lined fibres of collagen are found in the surface of ChS and wrinkles seem to get deeper with increasing smoking time. Areas where soot particles were found on the external surface of chorizos with casing were selected for analysis as shown in Figures 5.k and 5.l. Figures 5.k and 5.l show the external surface of ChN and ChS, respectively, when casings were removed. As can be appreciated in these figures, soot particles seem to cross the natural casing and are found when the casing was removed (5.k). In contrast, no soot particles were found when the synthetic casing was removed (5.l). The findings of this study are in agreement with previous research by the authors (Ledesma et al., 2015). Synthetic casing prevents the penetration of soot particles and carcinogenic compounds into meat products.

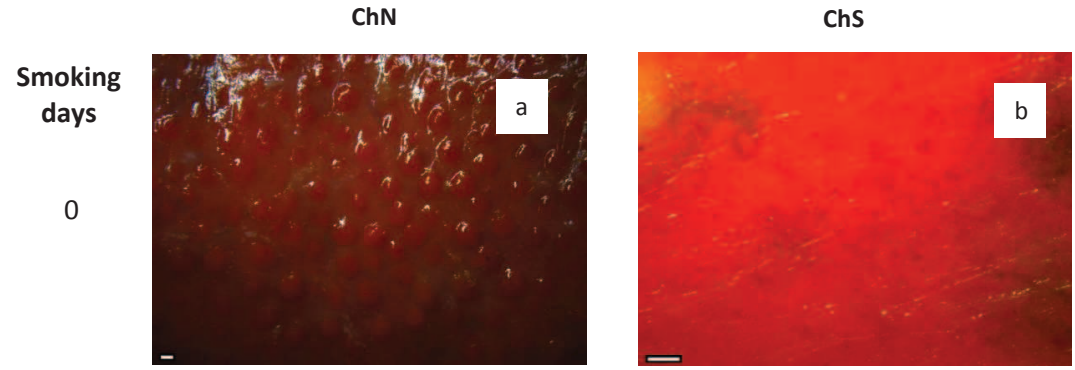

Figure 4. Fluorescence Stereo Microscope general images (no zoom) of chorizo before (a, b) and after the following smoking days: 1 (c,d), 4 (e,f), 5 (g,h), 10 (i,j). Pairs of images are chorizo stuffed in natural (ChN) and synthetic (ChS) casings, respectively. The scale of all images is 500 µm.

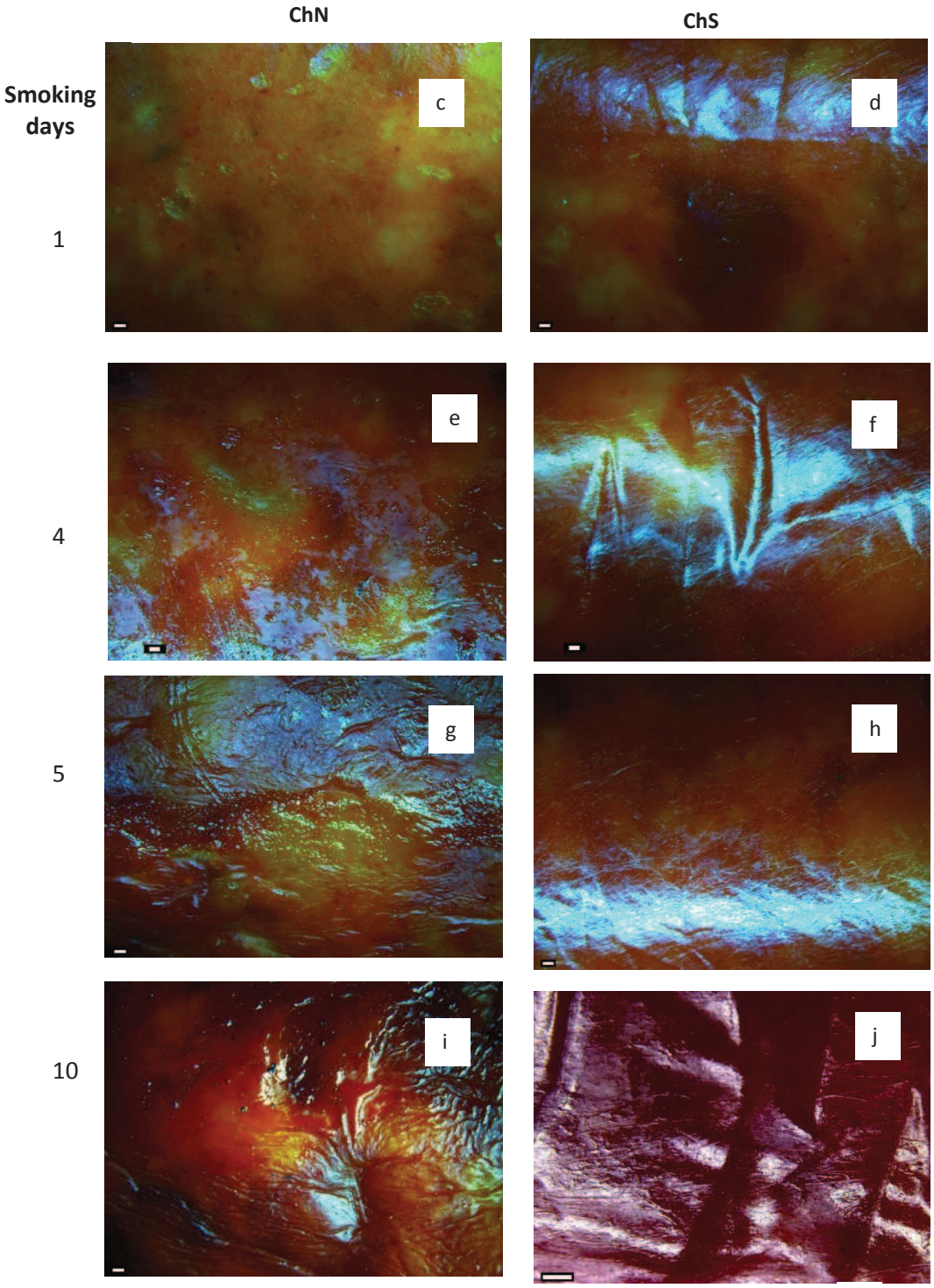

Figure 4. (continued).

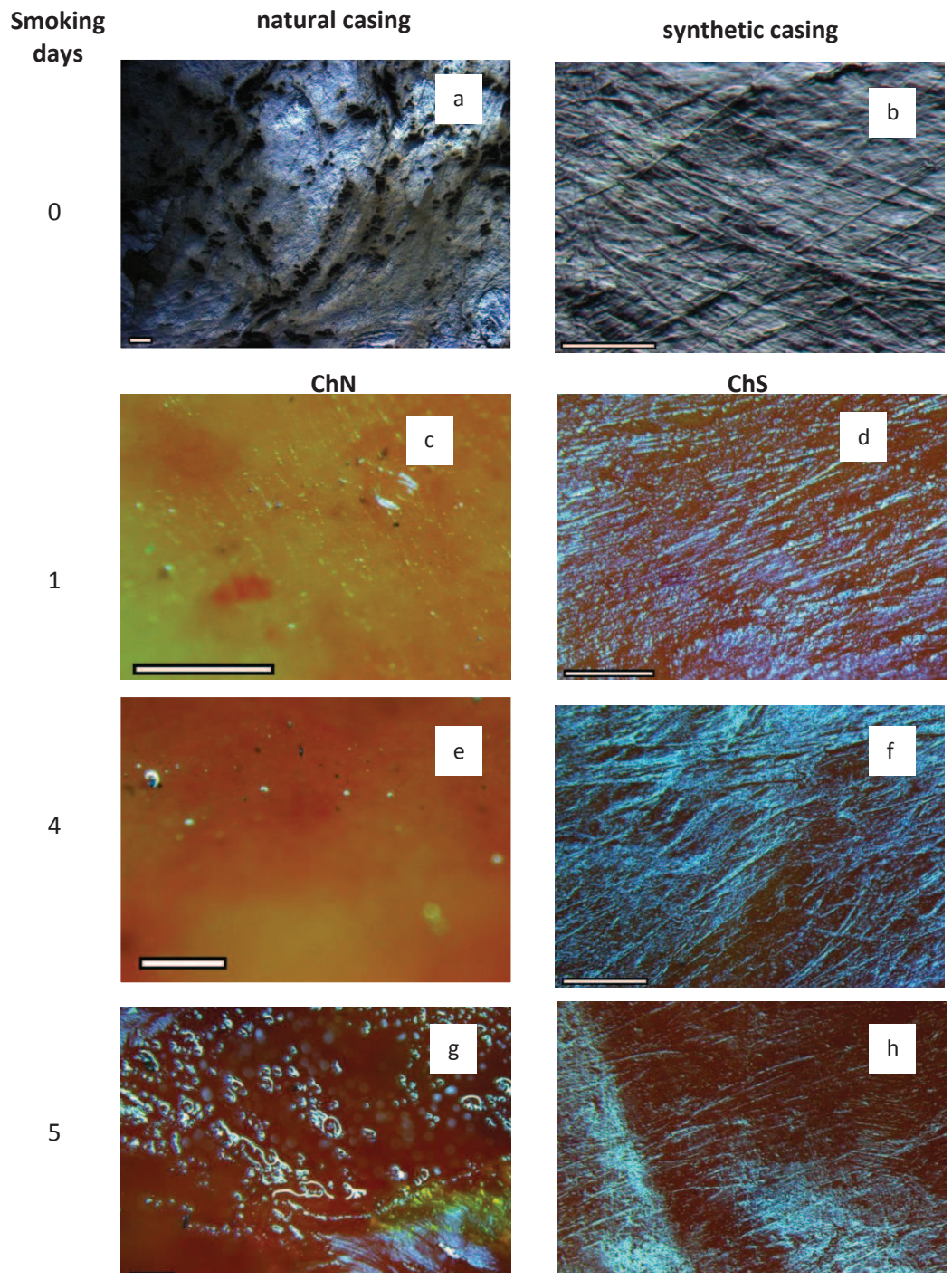

Figure 5. Fluorescence Stereo Microscope images of natural and synthetic casing before smoking (a, b) (without zoom) and chorizo after the following days of smoking: 1 day (c,d), 4 days (e,f), 5 days (g,h) and 10 days (i,j and k,l).Pairs of images are chorizo stuffed in natural (ChN) and synthetic (ChS) casings, respectively. K and l images were taken from the external surface of ChN and ChS, once the casings were removed. The zoom factor of all images is 59, except for e (X43) and g (X21). The scale in all images is 500 µm.

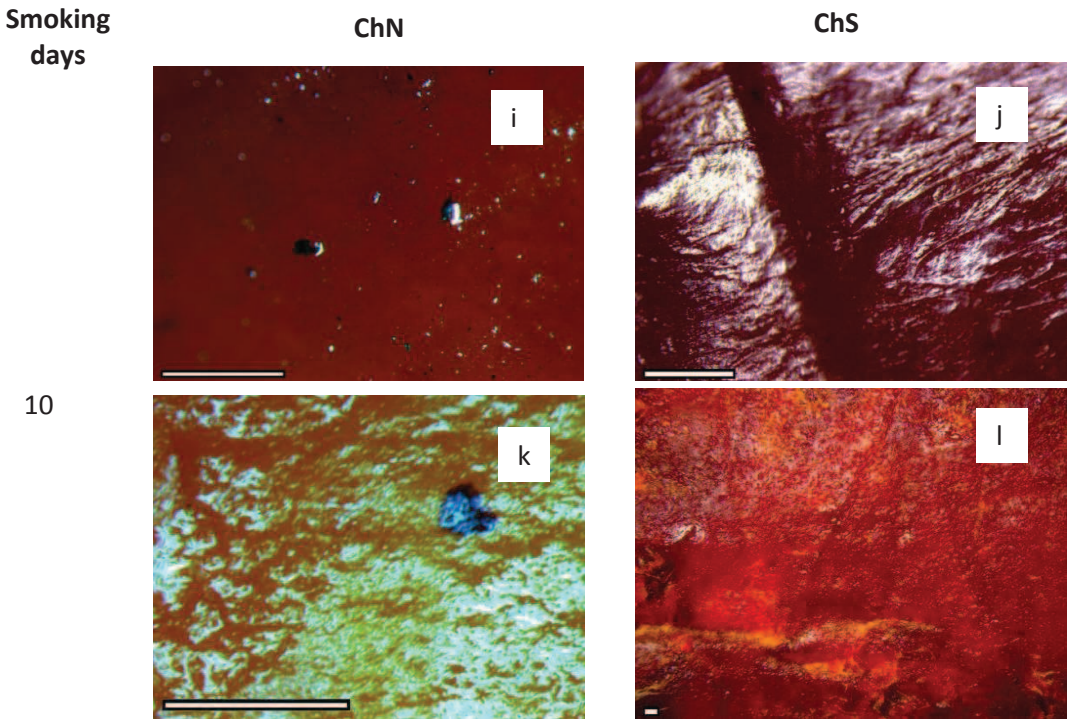

Figure 5. (continued)

4. Conclusions

The use of different types of casing (natural and synthetic) produces differences in the physical properties of chorizo during smoking. The texture, colour and moisture content values of chorizos stuffed in natural and synthetic casings were suitable in both cases. Over a period of 11 days of smoking, chorizos stuffed in synthetic casings showed darker colour, harder texture and drier mass than chorizos stuffed in natural casing. However, no significant differences ($p>0.05$) were found in the moisture content between the two groups. According to previous results, the structure of synthetic casing was found to prevent the accumulation and penetration of soot particles into the chorizo, while new evidence is linked to the capture of soot particles in the cavities of raw chorizo mass during drying. Finally, the use of synthetic casings in chorizo production could help to reduce processing time, prevent contamination by unhealthy products from smoking and achieve product standardization and homogenization, food properties demanded by customers and wholesalers.

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4.5 CONTAMINACIÓN DE PRODUCTOS CÁRNICOS POR HAP DURANTE EL AHUMADO: PROCESOS Y PREVENCIÓN.

Este apartado de la memoria realiza una revisión sobre el proceso tecnológico de ahumado de productos cárnicos y el control de la contaminación por HAP durante el mismo. En esta revisión se narra la historia del ahumado, buscando las primeras referencias históricas de aplicación de este proceso tecnológico alimentario y el avance de las técnicas hasta la actualidad. Así mismo, se describen los distintos tipos de ahumado, directo e indirecto, utilizados actualmente en la industria cárnica. Por otro lado se define la composición del humo y su objetivo en el ahumado de productos cárnicos. Se describe la formación química de HAP durante el ahumado y los distintos mecanismos mediante los cuales estos compuestos llegan a los productos en la industria cárnica, contaminándolos con sustancias cancerígenas. Se realiza una revisión de las distintas regulaciones internacionales publicadas y aplicadas durante los últimos 10 años para proteger a los consumidores sobre la ingesta de HAP en la dieta, en concreto, mediante el consumo de productos cárnicos. Estas regulaciones determinan la concentración máxima permitida de estos compuestos en los alimentos. Se describen y comparan los distintos métodos, clásticos y modernos, de pretratamiento de muestras y determinación analítica utilizados por la comunidad científica para identificar y cuantificar la presencia de estos compuestos en los productos cárnicos.

La parte central está enfocada en 2 apartados. Por un lado, en la revisión de la presencia en la actualidad de HAP en productos cárnicos ahumados a lo largo del mundo, y por otro lado, en las 10 variables del proceso tecnológico de ahumado que, de acuerdo al Codex Alimentarius Code CAC/RCP68/2009, se deben controlar para minimizar y prevenir la contaminación de HAP en alimentos ahumados y que por lo tanto han sido estudiadas en profundidad por la comunidad científica. Finalmente la revisión concluye con la comparación de todas las variables, seleccionando las que tienen más relevancia de acuerdo a la comunidad científica y evaluando la coherencia de los límites de HAP en productos cárnicos establecidos por la reglamentación.

Publicación: Contamination of meat products during smoking by polycyclic aromatic hydrocarbons: Processes and Prevention.

Situación: Enviado a la revista Food Control (Elsevier).

CONTAMINATION OF MEAT PRODUCTS DURING SMOKING BY POLYCYCLIC AROMATIC HYDROCARBONS: PROCESSES AND PREVENTION.

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ABSTRACT

Meat products may be contaminated by carcinogenic polycyclic aromatic hydrocarbons (PAH) during smoking. The maximum PAH content allowed in meat products has recently been reduced by EU Regulation No 835/2011. Codex Alimentarius Commission code of practice CAC/RCP 68/2009 specifies the 10 variables that must be controlled in order to minimize and prevent PAH contamination of meat products during smoking. Each variable has been separately studied by several researchers. The aims of this paper are to describe PAH contamination of meat products during smoking, review recent data on PAH in meat products, and compare the influence of the different variables involved in smoking. PAH limits are met in the majority of meat products reported worldwide, but are still greatly exceeded in others. Three variables seem to have a greater effect in reducing PAH content: smoke generation temperature, type of casing, and smoking method (direct or indirect). A smoke generation temperature below 600°C must be applied to prevent the formation of PAH. The use of synthetic instead of natural casings prevents PAH from penetrating inside the products. Indirect smoking systems (i.e., friction or liquid smokes) can highly reduce the PAH content of meat products. Finally, meat product smoking can be adapted to prevent PAH contamination and comply with the new European limits.

Keywords: Polycyclic aromatic hydrocarbons (PAH), meat products, smoking process, CAC/RCP68/2009, contamination, control.

1. Introduction

Meat and meat products are high economic interest foodstuffs, constituting the most valuable livestock products. World meat production is forecast to double by 2050. In 2015, the average meat consumption per capita worldwide is expected to be 41.3 kg/year. The projected consumption in developing and industrial countries will be 31.6 kg/year and 95.7 kg/year, respectively (FAO, 2013, 2014). Meat has played a crucial role in human evolution and is an important component of a healthy, well-balanced diet due to its nutritional richness (De Castro Cardoso Pereira & Dos Reis Baltazar Vicente, 2013). From the nutritional point of view, meat's importance derives from its high biological value protein, containing all the essential amino acids, as well as highly bioavailable minerals, vitamins and micronutrients like iron, selenium, zinc and vitamin B12 (De Castro Cardoso Pereira & Dos Reis Baltazar Vicente, 2013; FAO, 2014; USDA/HHS, 2010; WHO/FAO, 2003). Offal meats like liver are also crucial sources of vitamin A and folic acid (Biesalski, 2005). However, the consumption of meat and meat products has been associated with an increased risk of certain chronic diseases such as colorectal cancer (CRC) (Alexander, Miller, Cushing, & Lowe, 2010; Alexander, Weed, Cushing, & Lowe, 2011; Aune et al., 2013; Biesalski, 2005; Corpet, 2011; Demeyer, Honikel, & De Smet, 2008; Ferguson, 2010; McNeill & Van Elswyk, 2012; WCRF/AICR, 2007; WHO/IARC, 2008; WHO/FAO, 2003; Wyness et al., 2011), the third most common cancer in men (746,000 cases, 10.0% of the total) and the second in women (614,000 cases, 9.2% of the total) worldwide (IARC, 2012). This association has been rejected by recent studies (McAfee et al., 2010), as there is a certain degree of inconsistency between observational and experimental data on red meat and cancer (Dragsted et al., 2014). More studies and reviews are needed (Kim, Coelho, & Blachier, 2013), while the prevention of the carcinogenic activity of meat products should be researched, especially in terms of controlling the processing of meat products. Processing increases the value of meat products, prices can be reduced and shelf life can be extended (FAO, 2014). However, the composition of meat can be damaged by non-desirable substances such as nitrosamines, polycyclic aromatic hydrocarbons (PAH), heterocyclic amines, biogenic amines and lipid oxidation products (Olmedilla-Alonso, Jiménez-Colmenero, & Sánchez-Muniz, 2013). Special attention has been paid to the smoking process because of the contamination of meat products by PAH. The carcinogenic, mutagenic and bioaccumulative capacities of PAH have been reported by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), (WHO, 2006), the International Agency for Research on Cancer (IARC), the European Scientific

Committee on Food (SCF), (SCF, 2002), the European Food Safety Authority (EFSA) and the US Environmental Protection Agency (EPA). The maximum PAH levels in meat products have recently been reduced by European Union (EU) Regulation No 835/2011. PAH contamination in smoked meat products can be controlled, maintaining the beneficial effects of smoking and preventing its undesirable effects. In particular, Codex Alimentarius Commission code of practice CAC/RCP 68/2009 specifies the 10 variables that need to be controlled to minimize and prevent carcinogenic PAH contamination of meat products during smoking (CAC, 2009). Each of these variables has accordingly been separately studied by several researchers.

The aims of this paper are to define the smoking process, describe the contamination of meat products with carcinogenic PAH during smoking, review recent data on PAH in meat products worldwide, compare the incidence and effect of the different smoking variables and, finally, evaluate the regulations.

1.1 History

Food smoking is one of the oldest food technologies used by humankind since the beginning of time (Tóth, 1982). Humans probably first hung meat over the fire as a means of protecting it from canines. Subsequently, the preservative action of smoke in prolonging the meat's "shelf life" was probably discovered (Šimko, 2002, 2009). The first evidence of smoking as a technological process dates back 90,000 years to Poland. The oldest smoking house was discovered by archaeologists in a Stone Age colony located in Zwierzymec, near Krakow (Möhler, 1978). The greatest amount of information on meat smoking dates from Roman cultures, especially in the book by Marcus Cato *De Agri Cultura*, dating from 160 BC (Leroy, Geyzen, Janssens, De Vuyst, & Scholliers, 2013; Mateo, Caro, Figueira, Ramos, & Zumalacarregui, 2009; Möhler, 1978; Zeuthen, 2007), and from the Middle Ages, in the book by Marx Rumpolt's "New Cookbook", published in 1581 (Möhler, 1978). Some researchers claim that the Romans may have learned the technique of smoking meat from the Gauls and Celts. Europeans emigrants exported the method worldwide, including the Americas, South Africa and Australia (Leroy et al., 2013). Nowadays, with the exception of some African and Asian countries, where a cold chain has not been widely established (Ogbadu, 2014; FAO-Thiaroye, 2015), smoking is only used to give foodstuffs an enhanced, specific organoleptic profile, improving their flavor, color and smell, as these are properties widely demanded by consumers (Lorenzo, Purriños, García Fontán, &

Franco, 2010; Šimko, 2002; Vaz-Velho, 2003). It has been proved that the profile of meat products (color, odor, and flavor) is enhanced by increasing the time the smoke is in contact with meat products (Pöhlmann, Hitzel, Schwägele, Speer, & Jira, 2013a). The alternatives to smoking as a meat preservation method include modern techniques such as controlled drying chambers, liquid smoke (Kostyra & Baryłko-Pikielna, 2006; Lingbeck et al., 2014; Theobald et al., 2012) and other methods for preserving fresh meat by means of refrigeration (such as chilling, freezing and superchilling), ionizing radiation, chemical preservatives and biopreservation, high hydrostatic pressure (HHP) and packaging methods like vacuum packaging, modified atmosphere packaging (MAP), active packaging (AP), antimicrobial packaging and hurdle technology (HT) (Zhou, Xu, & Liu, 2010).

2. Smoking methods

Since the beginning of traditional, uncontrolled burning of biomass, the technique of smoking food has been improved until became a food technology process. There are a number of ways of classifying smoking methods based on the temperature of the smoke, the location of smoke generation with respect to the position of the foodstuff, and the device used for generating smoke. In this paper, the different smoking methods are classified in two main groups: direct and indirect smoking.

2.1 Direct smoking methods

During direct smoking, smoke is produced in the same chamber where the meat is processed (CAC, 2009). Direct smoking methods mainly comprise traditional techniques. The traditional smoking method consists of direct thermal degradation of wood to produce smoke (Ahmad, 2003). This definition can be extended, as others kinds of fuels were erroneously used in the past all over the world. In fact, humans used to burn any kind of waste in bonfires to produce smoke, such as food waste (coconut shells, ears of corn, fruit stones, etc.) and even newspapers or furniture (wood treated with paint) (CAC, 2009). Various unhealthy compounds are formed if the correct fuel is not used, especially PAH. Direct smoking methods can be classified according to the temperature of the smoke.

2.1.1 Traditional cold smoking

During cold smoking, wood is burned and smoke is produced. Meat products are hung from shelves placed above the hearth, located in a grilled floor through which the smoke passes. Smoking chambers are usually large. When burning finishes, the fire is not poked and the smoke cools. According to different authors, the required temperature in a smoking chamber to achieve cold smoking conditions should be below 20°C (Möhler, 1978), between 15 -25°C (Šimko, 2009; Woods, 2003), or below 30°C (Ahmad, 2003).

Raw hams (Möhler, 1978; Šimko, 2009), heat fermented untreated products like salami (Šimko, 2009) and other stuffed meat products, like chorizo, are usually produced by cold smoking.

2.1.2 Traditional hot smoking

During traditional hot smoking, the chamber is heated by the burning of wood in a similar process to a typical old baking oven. Once placed inside the chamber, the meat is heated and dried by embers of burnt wood. Sawdust is then introduced into the chamber and the fire is stoked with the aim of producing a large amount of smoke (Möhler, 1978). Temperatures of 130°C in the smoke and 80°C in the meat are needed in hot smoking (Ahmad, 2003; Möhler, 1978), although some authors specify lower temperatures, between 55 and 80°C (Woods, 2003).

2.2 Indirect smoking methods

Indirect smoking comprises a number of new methods that help reduce PAH contamination of meat products. These are summarized below.

2.2.1 Smoke produced by a friction generator

Primitive tribes produced smoke during the discovery of fire by means of the uninterrupted friction of wood on wood. This may well have provided the inspiration for friction smoke generation methods. The first development of this technique for producing smoke was first developed as a modern technology by Rasmussen and Rasmussen (1961) and was protected by US patent 3.001, 879. Since then, new models have been introduced. Nowadays, smoking chambers are designed to control all the processing steps, including preheating and reddening,

friction smoke generation, smoke evacuation and drying. These steps are repeated in cycles, the number and duration of which depend on the type of meat product. Typical smoke generation values are 20-second intervals of continuous friction of a gearwheel with wood followed by a pause of between 70 and 175 seconds. During this process, a temperature range between 180 and 380°C is reported by Varlet et al. (2007). The process, adapted for the production of a typical Spanish meat product called chorizo, includes 5 cycles of 30 minutes of preheating (150 minutes), reducing the temperature from 18 to 2°C and the humidity from 95 to 90%, 6 hours of drying at 25°C and 90% humidity, 41 cycles, consisting of 10 minutes of smoking, 3 minutes of smoke evacuation, and 20 minutes of drying at 25°C and 90% humidity, and a final step of 3 cycles of 8 hours of drying (24 hours) at 80% humidity, decreasing the temperature from 23 to 18°C. According to Pöhlmann et al. (2013a), Frankfurter-type sausages are exposed to reddening for 10 min at 52°C, drying for 12 min at 56°C and friction smoking for 26 to 40 hours, followed by a final step of scalding at 75°C for 25 min. With friction smoke generation, operation time and wood requirements are reduced and production is controlled and optimized. Furthermore, meat industry safety is increased, as PAH production is very low (Pöhlmann et al., 2013a), workers' health is improved by preventing fire hazards, product weight losses are reduced, product flavor is enhanced by avoiding the concealing of the taste of the ingredients, product shelf life and quality are suitable and product standardization and homogenization is made possible.

2.2.2 Liquid smoke

Liquid smoke is a modern way of producing commercial smoked meat products more rapidly. It is more environmentally friendly and easier to apply than traditional smoking and allows good reproducibility of desired characteristics in the end product. Liquid smoke is produced by condensing wood smoke formed by the controlled, minimal oxygen pyrolysis of sawdust or wood chips. The wood is placed in large retorts where intense heat is applied, causing the wood to smolder (not burn), releasing the gases seen in ordinary smoke. These gases are quickly chilled in condensers, thus liquefying the smoke. The liquid smoke is then forced through refining vats and subsequently filtered to remove toxic and carcinogenic impurities containing PAH. Finally, the liquid is aged for mellowness (Lingbeck et al., 2014). In addition, liquid smoke exhibits antimicrobial activity against *Listeria* (Gedela, Gamble, Macwana, Escoubas, & Mariana, 2007; Martin et al., 2010; Messina, Ahmad, Marchello, Gerba, & Paquette, 1988), *Escherichia coli* (Van Loo, Babu, Crandall, & Ricke, 2012), *Staphylococcus aureus* and staphylococcal enterotoxins

(Lingbeck et al., 2014; Taormina & Bartholomew, 2005). During its production, liquid smoke is filtered and subjected to fractionation and purification processes to remove toxic and carcinogenic particles and compounds. Therefore, its use is generally considered to be of less health concern than the traditional smoking process. However, the possibility of broader applications of smoke flavorings compared to conventional smoking has to be taken into account in safety assessments (EC No 2065/2003; Lingbeck et al., 2014).

2.2.3 Electrostatic smoking

In this type of smoking, the product is positioned in a continuous tunnel between live electrical wires that are charged to between 20 and 60 kV. Smoke passing through this system is charged according to its phase (smoke is a two-phase system, particulate and vapor), and smoke components can precipitate on the oppositely charged food surface (Vaz-Velho, 2003; Woods, 2003). The movement of gas and liquid in chambers has barely been studied (Pinilla, Díaz, & Coca, 1984). In order to ensure sedimentation of the smoke components on the surface of the product, the smoking step is usually followed by infrared irradiation (Möhler, 1978). This process helps to avoid PAH contamination of foodstuffs.

2.2.4 Other smoke generation technologies

Other well-known smoke generation technologies include steam, fluidization, touch and smoldering smoke generators. Steam smoke is produced by passing superheated steam through chopped wood, inducing pyrolysis. The resulting smoke passes through the smoking chamber, being cooled to 80°C (Prändl, Fisher, Schmidhofer, & Sinell, 1994; Tóth & Potthast, 1984). According to Müller (1982), the steam smoke generation temperature varies between 450 and 650°C. A fluidization smoke generator allows pyrolysis of wood shavings suspended in air that has been previously heated to 300-400°C. Pyrolysis is carried out within a reaction chamber and smoke and solid particles are separated on passing through a cyclone refiner (Nicol, 1960; Klettner, 1979; Tóth & Potthast, 1984; Prändl et al., 1994). The schematic representations of the different smoking generators are reported by Prändl et al. (1994). Smoldering, steam and touch smoke generation systems for the production of Frankfurter-type sausages are described in detail by Pöhlmann et al. (2012, 2013a). In these technologies, smoking conditions are highly controlled and optimized, including different smoke densities (light, medium, and intensive), temperatures of smoke generation (ranging between 300 and 520°C), ventilator speeds (750, 1500, and 3000

rpm) and exposure times to smoke (from 3 to 40 minutes). The control of smoking conditions in these smoke generation technologies allows the prevention of final contamination of meat products with PAH (Pöhlmann et al., 2013a).

3. The smoking process

3.1 Aim and composition of smoke

Smoke composition is defined by the kind of fuel (wood), smoking conditions (temperature, time, humidity, air flow rate) and subsequent treatments of smoke. Up to 1100 chemical compounds have been identified in wood smoke (Wilms, 2000). Smoke composition has been described in detail (Ahmad, 2003; Hitzel et al., 2013; Stumpe-Vīksna, Bartkevics, Kukare, & Morozovs, 2008). Wood smoke is composed by over 400 volatile components comprising 48 acids, 22 alcohols, 131 carbonyls, 22 esters, 46 furans, 16 lactones, 75 phenols, and some 50 miscellaneous compounds (Woods, 2003). The main components of smoke condensates are water (82.42%), tar (4.81%), residue (4.21%), extracts from activated charcoal (4.08%), acetic acid and acids of higher molecular weight (1.71%), methanol (0.96%), ketones (0.67%), aldehydes of highe molecular weight (0.57%), formic acid (0.38%), formaldehyde (0.12%) and phenols (0.07%).

Table 1 (Ahmad, 2003; Guillén & Manzanos, 2002; Kostyra & Baryłko-Pikielna, 2006; Möhler, 1978; Ojeda, Barcenas, Pérez-Elortondo, Albisu, & Guillén, 2002; Woods, 2003) summarizes the effects and function of the different components of smoke during meat processing, as described by Möhler (1978). The chemical compounds of smoke are organized in 20 groups. However, these can be divided in 2 main groups depending on their desirable or non-desirable effects in the end product. For instance, Thymol, formaldehyde, formic, acetic and benzoic acids, orthocresol, meta-cresol, para-cresol, guaiacol, methylguaiacol, cresol and xinelone have desirable bactericidal, antimicrobial, biocidal, fungicidal and preservative effects. Guaiacol, vinilguaiacol and butyric, valerianic, caproic, enanthic, caprylic, and pelargonic acids confer a good smell on products. During the first days of ripening, the coloring of meat products is mainly caused by the nitrogenous compounds present in meat in combination with myoglobin, resulting in the desired color pigment (Bozkurt & Bayram, 2006). Coloring of meat products is mainly caused by the well-known, non-enzymatic Maillard browning.

Table 1. Chemical composition of smoke and technological effect.

MAIN GROUPS OF COMPOUNDS IN SMOKE		EFFECT ON MEAT PRODUCTS
Group 1 **CH series compounds**	Saturated and unsaturated aliphatic hydrocarbons (paraffin and olefins).	Low effect due to their low reactivity
Group 2 **CH series compounds**	Aromatic hydrocarbons (benzol, polyphenol).	Negative effects: Bad taste
Group 3 **COH_2 series compounds:** **Aliphatic compounds with one hydroxyl, alcohol and ethers:**	Methyl alcohol: precursor of formaldehyde and formic acid. Ethyl alcohol, propyl alcohol and iso propyl alcohol: oxidation to produce carbonyls and acids. Butyl alcohol and amyl alcohol. Fenyl alcohols (benzyl alcohols, phenethyl alcohol)	Non-desirable, toxic Desirable Desirable (oil aroma) Desirable (smell of roses)
Group 4 **COH_2 series compounds:** **Aromatic compounds with one hydroxyl, phenol**	Guaiacol, methylguaiacol, cresol and xinelone. Phenol Phenol derivates: Orthocresol, meta cresol, para-cresol Xylenols Thymol	Desirable, preservatives and flavoring Non desirable, bad taste Desirable in low quantities Good effects in preservation, smoke smell and color Strong antimicrobial attributes, biocide Fungicide effect Preservative Thyme aroma
Group 5 **COH compounds (carbonyls): Aliphatic aldehydes**	Formaldehyde	Desirable Hardening of natural casing Connective tissue Bactericide Food preservative
Group 6 **COH compounds (carbonyls): Aromatic aldehydes**	Benzaldehyde	Desirable, Bitter almond aroma
Group 7 **CO compounds: Aliphatic ketones**	Acetone and unsaturated long-chain compounds.	Undesirable aroma
Group 8 **CO compounds:** **Aromatic ketones**	Acetophenone Hydrindenes	Hay smell Soft aroma
Group 9 **COO Compounds: Alyphatic carboxylic acids**	Formic acid and acetic acid. Butyric acid, valerianic acid, caproic acid, enanthic acid, caprylic acid, pelargonic acid. Unsaturated carboxylic acid (crotonic and tiglic)	Desirable for: Color setting, Good smell.Bactericidal effect Smell Desirable for color No desirable for taste

Table 1. (continued).

MAIN GROUPS OF COMPOUNDS IN SMOKE		EFFECT ON MEAT PRODUCTS
Group 10 **COO compounds: Aromatic carboxylic acids**	Benzoic acid Salycilic acid, gallic acid, toluic acid, phthalic acid, isophthalic acid, terephthalic acid	Specific action against the aerobic sporadic germ of the genus bacillus which contaminates meat products
Group 11: **Aromatic polyvalent hydroxy compounds**	Dihydroxybenzole Guayacol	
Group 12: **Hydroxy-oxo- compounds:** **Aliphatic hydroxyaldehydes and hydroxy ketones.**	Acetol	Desirable Reaction with meat proteins to develop the characteristic color of smoke
Group 13: **Hydroxy-oxo- compounds:** **Aromatic compounds, phenol aldehydes and phenol ketones**	Salicylaldehyde (2-hydroxybenzaldehyde), 4-Anisaldehyde, Vanillin and coniferaldehyde	Desirable: intense aroma and flavor of vanilla and conifers
Group 14: **Compounds with various oxo-groups:** **Di aldehydes and Di ketones**	Glyoxal Diacetyl	Casing hardening Margarine and bread color and smell
Group 15 **Compounds with 2 or more carboxyl groups:** **Saturated and unsaturated dicarboxylic acids**	Maleic acid	Desirable for color: pigment
Group 16 **Oxo- carboxy compounds : Keto acids**	Pyruvic acid, levulinic acid	
Group 17 **Nitrogenous organic compounds**	Pyrrole, pyrazine, indole, carbazole	Darkening of color
Group 18 **Non-aromatic cyclic compounds of C**	Cyclotene	Food aroma
Group 19 **Heterocyclic compounds**	Furan Lactone (Maltol)	Polymerization to obtain dark pigments. Good aroma. Flavor enhancer
Group 20 **Polycyclic aromatic hydrocarbons (PAH)**	Benzo(a)pyreno, PAH 4 (ΣPAH: Benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene.	Non-desirable in food science: Toxic, carcinogenic

On the other hand, some compounds have non-desirable effects on products, such as phenol (bad taste), acetone and unsaturated long-chain compounds (undesirable aroma). Among non-desirable compounds, polycyclic aromatic hydrocarbons (PAH) are the most important. The most significant endpoint of PAH toxicity is cancer (ATSDR, 1995; ATSDR, 2009). The major contributors to PAH intake are cereals and cereal products (owing to their high levels of consumption in diets) and vegetable fats and oils (due to higher concentrations of PAH in this food group) (CAC, 2009). PAH are found in many kinds of foodstuffs in the human diet, such as smoked fish, mussels, coffee, bread and smoked cheese (Martorell et al., 2010). However, particular attention has been paid to smoked meat products because the highest levels of total PAH have been detected in these products, especially in infrequent cases of a high consumption of these products in the diet (CAC, 2009; Gomaa, Gray, Rabie, Lopez-Bote, & Booren, 1993; Karl & Leinemann, 1996; Larsson, Pyysalo, & Sauri, 1988; Martorell et al., 2010).

3.2 PAH formation and transport mechanisms

The chemical formation of PAH during meat product smoking is defined by several food technology researchers as the incomplete combustion or thermal decomposition (pyrolysis) of wood (Conde, Ayala, Afonso, & González, 2005; Djinovic, Popovic, & Jira, 2008; Gomes, Santos, Almeida, Elias, & Roseiro, 2013; Hitzel, Pöhlmann, Schwägele, Speer, & Jira, 2013; Pöhlmann et al., 2013b; Lorenzo et al., 2010; Rey-Salgueiro, García-Falcón, Martínez-Carballo, González-Barreiro, & Simal-Gándara, 2008; Škaljac et al., 2014; Wretling, Eriksson, Eskhult, & Larsson, 2010). However, for an in-depth understanding of this process, scientific research papers on PAH formation during biomass combustion should be taken into account. PAH can be found as Tertiary Tar Products formed during biomass pyrolysis (Basu, 2010). Pyrolysis products can be classified as solids (mostly char or carbon), liquids (tars, heavier hydrocarbons and water) and gases (CO_2, H_2O, CO, C_2H_2, C_2H_4, C_2H_6, C_6H_6, etc.). Tar, also known as bio-oil or biocrude, is a black, tarry fluid formed by a mixture of complex hydrocarbons containing large amounts of oxygen and water (Basu, 2010). Tar is a complex microemulsion mixture of condensable hydrocarbons, including oxygen-containing, 1- to 5-ring aromatic and complex polyaromatic hydrocarbons, among others (Devi, Ptasinski, & Janssen, 2003). The International Energy Agency (IEA) Bioenergy Agreement, the U.S. Department of Energy (DOE) and the DGXVII of the European Commission agreed to classify all components of product gas with a molecular weight higher than benzene as

tar (Knoef, 2005). Figure 1 shows the formation of tertiary tar products as a function of the temperature of biomass conversion (Evans & Milne, 1997). Tertiary tar products are produced after the destruction of primary tar products, at about 750°C, with increasing temperature. Condensed tertiary aromatics thus make up a series of polynuclear aromatic hydrocarbons (PAH) without substituents (atoms or a group of atoms substituted by hydrogen in the parent hydrocarbon chain) (Basu, 2010; Evans & Milne, 1997). The process of producing aerosols containing PAH from the decomposition of primary tar products of biomass has been studied by several authors (Li et al., 2009; Liu et al., 2013; McGrath, Sharma, & Hajaligol, 2001; Simoneit, 2002), who found that PAH formation increases with temperature and residence time. It was also been found to depend on the cell wall components of the biomass (hemicellulose, cellulose and lignin) and may even contain heavy metals (Wobst, Wichmann, & Bahadir, 2003).

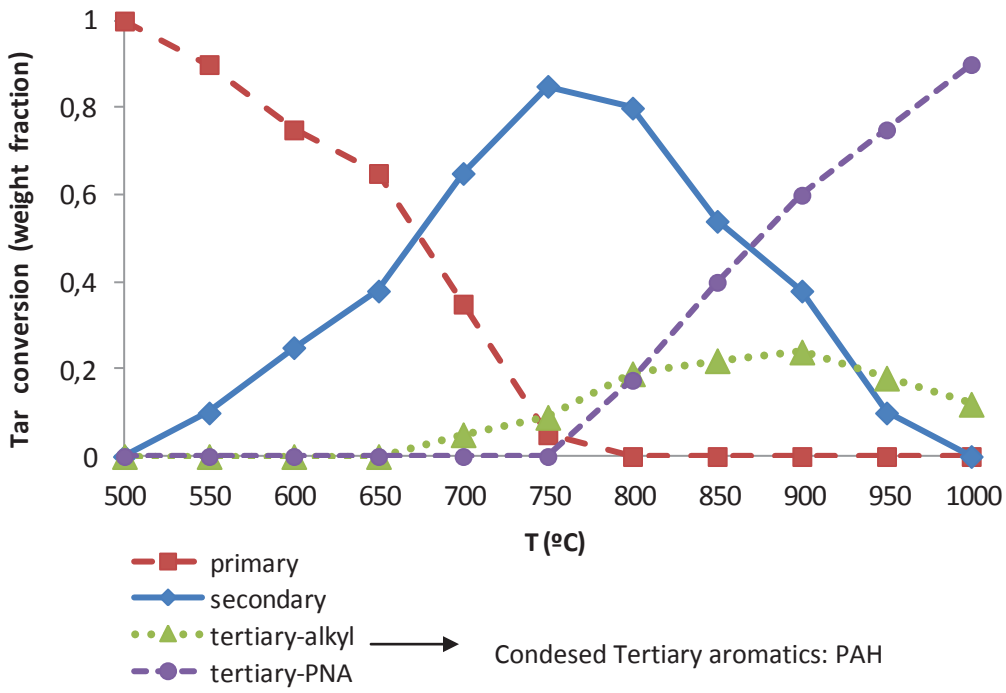


Figure 1. Temperature of polycyclic aromatic hydrocarbons (PAH) formation: tertiary tar products production during biomass conversion. (Adapted from Evans & Milne, (1997), page 804 and Basu, (2010), page 102).

During meat processing, tar is transported through the smoking chamber by aerosols, conveying PAH to the meat products being smoked, which eventually become contaminated. Several processes occur at the same time in the traditional conversion of biomass during meat smoking. The wood starts to dry, pyrolysis commences in some parts of the fire, while complete combustion occurs in the parts nearest the flames. However, PAH contamination of meat products can have different origins. First, raw ingredients can be PAH "pre-contaminated" due to atmospheric pollution. Animals (pigs, cows, etc.) and vegetables (onions, garlic, etc.) can be contaminated by PAH present in the air (by deposition), soil (by transfer) or water (deposition and transfer). There are numerous natural and mostly anthropogenic sources of PAH in the environment, including exhaust gases from motive sources (motor vehicles and aircrafts), industrial plants, forest fires, volcanic eruptions, domestic heating with open fireplaces, etc. (CX/FAC 05/37/1, 2004; CAC, 2009). Second, food technology processes other than smoking, such as packaging, drying, grilling, roasting, (Chung et al., 2011), baking, barbecuing (Kazerouni, Sinha, Hsu, Greenberg, & Rothman, 2001) and frying, can also contribute to the final PAH content of meat products (CAC, 2009). Third, some of the products in a smoking chamber are hung above others on different bars on the same shelving (see a graphical representation of the smoking room in Figure 2 or in Gomes et al. (2013)). Products are heated inside the chamber, causing their fat content to flow outward through their natural casing (Ledesma, Rendueles, & Díaz, 2015b). On the one hand, some fat falls down onto or comes into contact with the fire, thereby increasing the PAH content of the smoke (Chung et al., 2011; Janoszka, Warzecha, Blaszczyk, & Bodzek, 2004). On the other hand, some fat already contaminated with tar falls onto other products, increasing the PAH content of the latter (CAC, 2009; Viegas, Novo, Pinto, Pinho, & Ferreira, 2012). Finally, meat products are contaminated with PAH during direct smoking due to the deposition of tar aerosols from the smoke (Ledesma et al., 2015b).

4. Products and regulations

4.1 Analytical methods for PAH determination

The determination of PAH in meat products involves two critical steps: sample preparation and analytical determination. The determination of PAH in meat products depends on their fat content, which could make their pre-treatment more difficult and lengthier than that of other environmental or non-fatty food samples, such as water or beverages. Classical pre-

treatment and analytical methods have thus been enhanced over time to determine PAH in meat. Several articles focusing on the comparison of classical and modern methods for PAH determination in foods have been published (Moreda, Pérez-Camino, & Cert, 2001; Moret & Conte, 2000; Plaza-Bolaños, Garrido Frenich, & Martínez Vidal, 2010; Šimko, 2002), the most recent to date being the paper by Purcaro, Moret, and Conte (2013). Classical pre-treatment methods included saponification, liquid–liquid extraction and Soxhlet extraction in combination with gel permeation chromatography (GPC) (Chiu, Lin, & Chen, 1997; Grimmer & Böhnke, 1975; Fretheim, 1976; Šimko, 2002). Modern methods include microwave-assisted extraction (MAE), sonication (Ledesma et al., 2014; Purcaro, Moret, & Conte, 2009), accelerated solvent extraction (ASE) (Djinovic et al., 2008; Martorell et al., 2010; Sun, Ge, Lv, & Wang, 2012), ultrasound-assisted solvent extraction (USAE), ultrasound-assisted emulsification–microextraction (USAEME) (Yebra-Pimentel, Martínez-Carballo, Regueiro, & Simal-Gándara, 2013) and pressurized liquid extraction (PLE) (Pöhlmann et al., 2013a, 2013b), followed by SPE (García-Falcón & Simal-Gándara, 2005; Hitzel et al., 2013; Purcaro et al., 2009), stir-bar sorptive extraction (SBSE) or solid phase microextraction (SPME). SPME coupled to a direct extraction device (DED) has also been proposed (Martin & Ruiz, 2007). The combination of sonication with the SPE pre-treatment method has been found to be a good method. It entails a low level of solvent consumption and waste generation, short operating times and is easy to set up and has been found to be better than the classical combination of Soxhlet extraction and gel permeation chromatography (GPC) (Ledesma et al., 2014; Purcaro et al., 2009).

Analytical methods for identifying PAH include HPLC combined with fluorescence (FLD) or ultraviolet (UV) detectors (Lorenzo et al., 2011; Moret & Conte, 2002; Purcaro et al., 2009; Roseiro, Gomes, Patarata, & Santos, 2012; Santos, Gomes, & Roseiro, 2011) and GC combined with flame ionization (FID), ion trap (ITD) and mass spectrometry (MSD) detectors (Hitzel et al., 2013; Olatunji, Fatoki, Opeolu, & Ximba, 2014). GC-MS and GC/HRMS are widely used today to determine PAH in meat products (Djinovic et al., 2008; Hitzel et al., 2013; Martin & Ruiz, 2007; Martorell et al., 2010; Šimko, 2002; Stumpe-Vīksna, Bartkevics, Kukare, & Morozovs, 2008; Wretling et al., 2010).

4.2 Regulation of PAH in meat products

To protect consumers against PAH intake from diet, the European Commission (EC) has adopted several regulations over the last 10 years. In accordance with the European Scientific Committee on Food (SCF) (SCF, 2002), PAH levels in foods have been regulated via EC Regulation No 208/2005 (EC, No 208/2005), and European Union (EU) Commission Regulation No 1881/2006 (EU, No 835/2011). Benzo(a)pyrene (BaP) was set as the marker for the occurrence and effect of carcinogenic PAH in food, also including benz(a)anthracene, benzo(b)fluoranthene, benzo(j)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, chrysene, cyclopenta(c,d)pyrene, dibenz(a,h)anthracene, dibenzo(a,e)pyrene, dibenzo(a,h)-pyrene, dibenzo(a,i)pyrene, dibenzo(a,l)pyrene, indeno(1,2,3-cd)pyrene and 5-methylchrysene. In addition, the JECFA also recommended including benzo(c)fluorene (WHO, 2006). Table 2 shows the names, abbreviations, relative molecular weights and chemical structures of the 16 PAH regulated in meat products by the European Union. A maximum level of 5 µg/kg BaP was set for smoked meats and smoked meat products in this EC regulation. States were asked to make further analyses of the relative proportions of these PAH in foods to inform a future review of the suitability of maintaining BaP as a marker. EC Directives 2005/10/EC (EC, No 2005/10) and No 333/2007 (EC, No 333/2007) established the sampling methods and the methods of analysis for the official control of the levels of BaP in foodstuffs. In accordance with a new study of data and opinion of the EFSA Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) (EFSA, 2008), European Union (EU) Regulation No 835/2011 (EU, No 835/2011) (Table 3) specified that two dates must be considered as regards PAH contamination in foodstuffs. New maximum levels for the sum of four substances (PAH4) (BaP, benzo(a)anthracene, benzo(b)fluoranthene and chrysene) were introduced, maintaining a separate maximum level for BaP to ensure comparability between previous and future data. The maximum permissible BaP content in smoked meat and smoked meat products was reduced to 2.00 µg/kg wet weight as of 1/9/2014. The permissible sum of PAH4 in these foods was set at 30.0 µg/kg wet weight from 1/9/2012 to 31/8/2014 and was reduced to 12.0 µg/kg wet weight as of 1/9/2014. This regulation specified that the separate maximum level for BaP should be re-assessed based on new data that will be generated in the future. A recent study by Lorenzo et al. (2011) reports that BaP is a good marker for the sum of 15 PAH as well as for 7 PAH classified as probable human carcinogenics by the USEPA in 'chorizo gallego', a typical Spanish smoked meat product. A new revision of the legislation has not yet been carried out.

Table 2. Name, abbreviations, relative molecular weights and chemical structures of the 16 polycyclic aromatic hydrocarbons (PAH) regulated in meat products by the European Union.

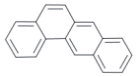
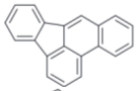
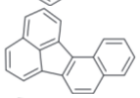
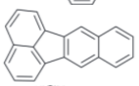
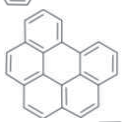
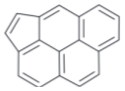
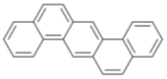
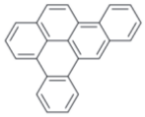
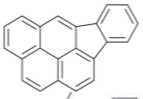
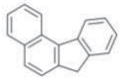
PAH compound	Abbreviation	Molecular weight	Chemical structure
Benzo(a)pyrene	BaP	252	
Benz(a)anthracene	BaA	228	
Benzo(b)fluoranthene	BbF	252	
Benzo(j)fluoranthene	BjF	252	
Benzo(k)fluoranthene	BkF	252	
Benzo(g,h,i)perylene	BghiP	276	
Chrysene	Ch	228	
Cyclopenta(c,d)pyrene	CPP	226	
Dibenz(a,h)anthracene	DBahA	278	
Dibenzo(a,e)pyrene	DBaeP	302	
Dibenzo(a,h)pyrene	DBahP	302	
Dibenzo(a,i)pyrene	DBaiP	302	
Dibenzo(a,l)pyrene	DBalP	302	
Indeno(1,2,3cd)pyrene	IP	276	
5-methylchrysene	5MeCh	242	
Benzo(c)fluorene	BcF	216	

Table 3. New European limit for polycyclic aromatic hydrocarbons (PAH) in meat products set by EU Regulation No. 835/2011, applied since 1/9/2014.

Meat Product	PAH limit (µg kg^{-1})	
	BaP	PAH4*
Smoked meat and smoked meat products	2.0	12.0

(*)PAH4: ΣPAH: BaP, BaA, BbF and CHR.

The USFDA regulates contaminant levels in foodstuffs, but has still not established standards governing the PAH content of foodstuffs. The maximum contaminant level goal for BaP in drinking water is 0.2 parts per billion (ppb). In 1980, the US EPA established ambient water quality criteria to protect human health from the carcinogenic effects of PAH exposure. The recommendation was a goal of zero (non-detectable level for carcinogenic PAH in ambient water). As a regulatory agency, the USEPA establishes a maximum contaminant level (MCL) for BaP, the most carcinogenic PAH, at 0.2 ppb (ATSDR, 2009; EPA, 2013).

4.3 PAH in smoked meat products around the world

Table 4 summarizes the recent PAH levels found in a number of different smoked meat products around the world studied by different researchers. The maximum BaP content found in various studies falls below the maximum value currently allowed by European Union regulations (2 µg/kg), e.g. in commercial samples from various local supermarkets in the Republic of Korea (Chung et al., 2011), some Spanish commercial meat products and chorizos (Ledesma et al., 2015a; Martorell et al., 2010), smoked meat products from Italian markets (Purcaro et al., 2009) and Portuguese traditional smoked meat products (Santos et al., 2011).

However, a high BaP content is still found in smoked meat products around the world: 36.9 µg/kg in Swedish ham produced by direct “sauna” smoking with birch logs (Wretling et al., 2010), 10.02 µg/kg in smoked pork from Cape Town, South Africa (Olatunji et al., 2014), 6.98±2.01 µg/kg in Beef satay from a Malaysian market (Jahurul et al., 2013), 31.20 µg/kg in commercial smoked meat from Estonia (Reinik et al., 2007), 17.63 µg/kg in smoked belly of pork from Germany (Jira, Ziegenhals, & Speer, 2008), and 3.21 ± 0.12 µg/kg in commercial smoked chorizos from Spain (Ledesma et al., 2015a). It is thus still important to control and study manufacturing conditions in order to minimize PAH contamination of smoked meat products.

Table 4. Polycyclic aromatic hydrocarbons (PAH) in different smoked meat products worldwide.

References	Studied variable	Sample	Studied smoking conditions	PAH results interval (µg/kg)			
				BaP (µg/kg)		PAH (µg/kg)	
				Maximun	Minimun	Maximun	Minimun
Chung et al., (2011)	Commercial samples from various local supermarkets in the Republic of Korea.	Ham, bacon, processed products and sausages (smoked products).	Commercial samples from various local supermarkets in the Republic of Korea.	0.08 Sausages	0.01 Processed products	PAH[2] 1.09 Bacon	PAH[2] 0.15 Processed products
Jahurul et al., (2013)	Different kinds of chicken, beef, mutton, sausage and nuggets from a Malaysian market.	Different kinds of chicken, beef, mutton, sausage and nuggets from a Malaysian market.	Grilling, boiling, frying, stir frying,	6.98±2.01 Beef satay (grilled)	n.d in any products except for: - Chicken satay (grilled) -Sausage (fried) -Beef satay (grilled)		
Jira et al., (2008)	22 smoked meat products, mainly very smoked hams	Smoked raw meat products (mainly raw smoked hams) from different producers: Ham, belly pork ham and smoked wild boar ham	Commercial hams from different producers.	17.63 Smoked belly of pork with a strongly fading black surface.	0.02 Smoked ham	BaP can be used as the leading substance in smoked meat products. This has to be confirmed by the investigation of representative samples.	
Ledesma et al., (2015a)	16 smoked meat products (chorizos) from different producers in Spain.	16 chorizos asturianos without casing.	Commercial chorizos from different producers.	3.21 ± 0.12	0.38±0.08		
Lorenzo et al., (2011)	Spanish traditional smoked sausage varieties: "Chorizo gallego" and "Chorizo de cebolla".	16 Chorizos gallegos without casing. 16 Chorizos de cebolla without casing. (16 different manufacturers)	Chorizos from 16 different industrial pork manufacturers following traditional methods.			PAH[1] 6.76 Chorizo de cebolla	PAH[1] 5.01 Chorizo gallego
Martorell et al., (2010)	Food and intake by the population of Catalonia, Spain.	Veal steak, hamburger, loin of pork, pork sausage, chicken breast, lamb, boiled ham, Frankfurt sausage, salami, cured ham.		1.10 Salami	<0.06 Veal steak, loin of pork	Total PAH[3] 364.91 Salami	Total PAH[3] 1.18 Loin of pork

Table 4. (continued).

Olatunji et al., (2014)	Processed meat products from Cape Town, South Africa.	Beef stripe fillet. Pork fillet. Chicken fillet.	Smoking Grilling Boiling	10.02 smoked pork	n.d Unprocessed pork and chicken		
Purcaro et al., (2009)	Smoked meat products from an Italian market	Smoked bacon, smoked speck, smoked pork meat, smoked beef meat, pitina.	"Pitina": Smoking in a traditional way, placing the meat near the fireplace for several days.	0.8 Pitina	<0.05 smoked bacon, smoked pork and beef meats		
Reinik et al., (2007)	Commercial meat products in Estonia 2001–2005.	Smoked sausage, ham, meat and chicken.	Commercial meat products	31.20 Smoked meat	<0.3 in all products	PAH[4] 8.0 Smoked ham	PAH[4] 5.7 smoked chicken
Roseiro et al., (2012)	Portuguese traditional meat and blood sausages	Samples, (%fat): A) "Alentejo'' products: <u>Smoked meat (M):</u> a) Chouriço de carne (25.1%), b) Paínho (24.2%) and c) Paio tradicional (40%). <u>Blood sausages (BP):</u> d) Chouriço mouro (53%), e) Cacholeira (41.8%) and f) Morcela (46.4%). B) "Trás-os-Montes" products: <u>Smoked meat (M):</u> g) Alheira (16%), h) Chouriço de carne (20.6%) and i) Salpicão (14.4%) <u>Blood sausages (BP):</u> j) Chouriço doce (11.7%), k) Morcela (39.5%) and l) Moura (23.9%). Natural casings.	Combustion of wood from: "Alentejo" *Quercus ilex* and/or *Quercus suber* wood. Smoking times (days): a) 5, b) 15, c) 30, d) 8, e) 6, f) 8. ''Trás-os-Montes'': *Quercus faginea* and/or *Castanea sativa Miller* wood. Smoking times (days): g),h),i),j),k),l): unknown	M: 4.75 i) Salpicão from "Trás-os-Montes" BP: 5.65 l) Moura from "Trás-os-Montes"	M:0.36 c) Paio tradicion. from "Alentejo" B.P:0.32 f) Morcela from "Alentejo"	PAH4 M: 294.50 i) Salpicão from "Trás-os-Montes" B.P: 271.83 l) Moura of 'Trás-os-Montes''	PAH4 M: 3.47 c) Paio tradicion. from "Alentejo" B.P: 1.84 f) Morcela from "Alentejo"

Table 4. (continued).

Santos et al., (2011)	Portuguese traditional smoked meat products	Samples, (%fat): MEAT SAUSAGES (M): a) Chouriço de carne (25.1%), b) Paínho (24.2%) and c) Paio tradicional (40.0%) BLOOD SAUSAGES (BS): d) Chouriço mouro (53.0%), e) Cacholeira (41.8) and f) Morcela (46.4). All samples stuffed in natural casing.	Direct traditional smoking Wood: *Quercus ilex*. Smoking time (days): a) 5: b, 15; c) 30; d) 8; e)6 ; f) 8.	Mean contents			
				M: 0.63 b) Paínho BP: 0.39 e) Cacholeira	M:0.36 c) Paio tradicion. B.P:0.32 f) Morcela	PAH4 M: 4.80 b) Paínho B.P: 6.94 d) Chouriço mouro	PAH4 M: 3.47 c) Paio tradicional B.P: 1.84 Morcela
				BaP max and min: 1.00 (painho), 0.21 (morcella)			
Wretling et al., (2010)	Swedish smoked meat	Ham, bacon, pork tenderloin, pork shoulder, elk, heart, elk and reindeer meat combined, lamb, leg, reindeer meat, lean, chicken, turkey, sausages, elk sausage.	Different producers with the following smoking systems: a) Direct, b)Indirect, c)"Direct sauna". Fuels: Sallow, alder or birch logs. Sallow or alder chips.	36.9 Ham smoked by direct sauna with birch logs	n.d Ham, ham indirect alder chips. Pork shoulder, lean reindeer meat, chicken, turkey, sausages.	PAH4:209 Ham smoked by direct sauna with Birch logs	PAH4: n.d Ham, pork tenderloin and shoulder, lean reindeer meat, chicken, turkey, sausage, elk sausage.

PAH4: ΣPAH: Sum of BaP, BaA, BbF and CHR.
1: ΣPAH: US EPA PAH7: BaP, BaA, CHR, BbF, BkF, DhA, IcP.
2: ΣPAH: PAH: CHR, BbF, BkF, BaP, DBahA, BghiP, IP
3: ΣPAH: Na, Ap, Ac, F, Pa, A, Fl, P, BaA, Ch, BbF, BkF, BaP, IP, DBahA, BghiP.
4: ΣPAH: BaA, BbF, BjF, BkF, BghiP, BaP, Ch, DBahA, DBaeP, DBalP, IP, 5MeCh.
n.d: non-detected.

5. Prevention of PAH contamination of smoked meat products

The Codex Alimentarius Commission (CAC) established by FAO and WHO recognizes the benefits of smoking, including the availability of food products produced by traditional smoking and the prevention of deterioration, microbiological contamination and growth. The "Code of practice for the reduction of contamination of food with (PAH) from smoking and direct drying processes" (CAC/RCP 68/2009) stipulates that PAH contamination in foodstuffs via food processing should be minimized, providing producers with the 10 variables that need to be controlled. Figure 2 shows a scheme of these variables in a chamber during direct smoking of meat products. These include the kind of fuel (different woods and other plant materials, diesel, gases, liquid/solid waste and other fuels), smoking or drying method (direct or indirect), the process of generating smoke in relation to the temperature of pyrolysis and airflow in the case of a smoke generator (friction, smoldering, thermostated plates) or in relation to other methods, such as direct smoking or regenerated smoke by atomizing smoke condensate (liquid smoke), the distance between the food and the heat source, the position of the food in relation to the heat source, the fat content of the food and its evolution during processing, the duration of smoking and direct drying, the temperature during smoking and direct drying, the cleanliness and maintenance of equipment, and the design of the smoking chamber and the equipment used for the smoke/air mixture.

All these variables should be controlled in each smoking process. For this reason, the difficulty in controlling the PAH content of smoked meat products has been highlighted by several researchers (Djinovic et al., 2008, Lorenzo et al., 2011; Roseiro et al., 2012; Santos et al., 2011; Stumpe-Vīksna et al., 2008). Table 5 summarizes the latest published studies on the parameters affecting the smoking process with the aim of understanding PAH contamination of smoked meat products. An overview of these studies is presented next.

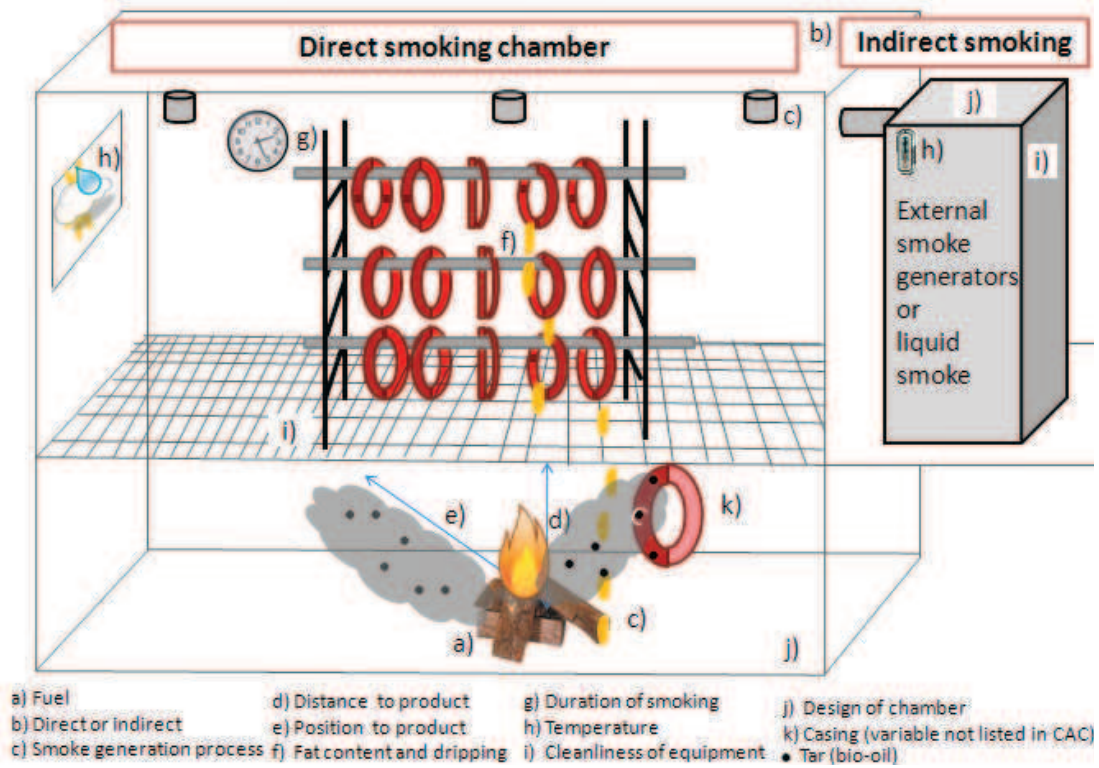


Figure 2. Smoking chamber: Representation of CAC/RCP 68/2009 variables to control polycyclic aromatic hydrocarbons (PAH) contamination of meat products in direct and indirect smoking processes.

Table 5. Codex alimentarius (CAC/RCP 68/2009) parameters studied by researchers with the aim of controlling PAH contamination (µg/kg) of smoked meat products.

CODEX ALIMENTARIUS PARAMETER	References	Studied variable	Sample	Studied smoking conditions	PAH results interval (µg/kg)			
					BaP (µg/kg)		PAH (µg/kg)	
					Maximun	Minimun	Maximun	Minimun
a) Fuel: woods and other plant materials, diesel, gases, liquid/solid waste and other fuels.	Hitzel et al., (2013)	Wood: Beech (*Fagus sylvatica*), oak (*Quercus robur*), spruce (*Picea abies*), poplar (*Populus spp.*) and fir (*Abies alba*) from Rettenmaier & Söhne (Rosenberg, Germany) and alder (*Alnus spp.*) and hickory (*Carya spp.*) from Thomsen GmbH & Co. KG (Handewitt, Germany). Spiced beech wood chips with apple smoking spice mix (BA), spiced beech-wood chips with cherry smoking spice mix (BC), spiced beech wood chips with juniper berries and bay leaves, fir.	Samples: (predried). Sheep casings, sub-mucosa of small intestine. -Frankfurter-type sausages (F) 29.6% fresh pork, 19.8% fresh beef, 25.9% back fat, 22.6% ice, 1.4% salt (containing sodium nitrite 0.4%), 0.04% ascorbic acid, 0.17% dipotassium hydrogen phosphate, and 0.42% "Goldwürstchen" spice mix. -Mini-salamis (M) 76.9% frozen pork, 19.6% frozen back fat, 2.4% salt (containing 0.4% sodium nitrite), 0.4% glucose, 0.4% spice mix "Mild French-Style Salami" from Raps and at least 1011 "Optistart Sprint" active microorganisms from Raps.	Equipment: T1900 smoking chamber, smoke generator (RZ 325) (Fessmann). Pretreatment of samples: -F: reddening for 10 min at 52°C and drying for 12 min at 56°C. -M: drying and curing for 2 days at 22°C in a climatic chamber. Smoking conditions -Smoking time: 12 min (F), 30 min (M). -Smoking temperature: 58°C (F), 22°C (M) -Smoke density: intensive. -Ventilator velocity: 3000 rpm Post treatment of samples: F: Scalding for 25 min at 75°C.	F: Alder 0.80±0.15 M: Alder 0.60±0.01 BC 0.60±0.29	F: BA 0.32±0.05 M: Oak 0.23±0.03	F: Alder (4.70±0.49) M: Alder 4.38±0.15 BC 5.00±1.51 (PAH4)	F: BA (2.16±0.31) M: Poplar (2.37±0.11) (PAH4)
	Stumpe-Vīksna et al., (2008)	Wood: Apple, alder, alder + juniper, spruce, maple, hazel, plum, aspen, bird-cherry, rowan tree, charcoal.	Pork meat	Smoking: home kiln Smoking temperature: 80°C Smoking time: 5 hours	Aspen 35.07	Apple 6.04	(PAH^1) Spruce 470.91	(PAH^1) Apple (47.94)

Table 5. (continued).

CODEX ALIMENTARIUS PARAMETER	References	Studied variable	Sample	Studied smoking conditions	PAH results interval (µg/kg)			
					BaP (µg/kg)		PAH (µg/kg)	
					Maximun	Minimun	Maximun	Minimun
b) Smoking or drying method (direct or indirect).	Gomes et al., (2013)	Direct (d) and indirect (i) smoking placing an obstacle (a stainless steel plate) above the smoke generator, or not.	Lean and fatty pork trimmings, salt (1%), garlic paste (3%), paprika paste (3.5%), curing salts [0.25% ($NaNO_3$ 4.9%; KNO_3 5%), antioxidants [0.15% (sugars; E301 relative composition unknown) and water(2.5%)].Fat(a)20%,(b):40%.	4hours/day, 8 days, combined with drying in a controlled environmental chamber (5–15°C; 25–55% relative humidity).Oak wood (*Quercus ilex L.*), 2 smoking exposures: Direct (d) and indirect (i), placing an obstacle (a stainless steel plate) above the smoke generator, or not.	0.32 Hog casing, a:20% fat, indirect smoking	0.09 Collagen casing, a:20% fat, indirect smoking	PAH4:10.35 Hog casing, b:40% fat, direct smoke	PAH4: 4.21 Collagen casing, a:20% fat, indirect smoking
c) Smoke generation process in relation to the temperature of pyrolysis and to airflow in the case of a smoke generator(friction, smoldering, thermostated plates) or with other methods such as direct smoking or liquid smoke.	Pöhlmann et al., (2012)	Different conditions of glow smoke	Frankfurter-type sausages: 1.8 kg fresh pork, 1.2 kg fresh beef, 1.62 kg back fat, 1.38 kg ice, 84.5 g salt (containing $NaNO_2$); 0.4%, 2.5 g ascorbic acid, 10 g dipotassium hydrogen phosphate (K2HPO4) and 26 g "Goldwürstchen" spice mix from Raps. Sheep casing from submucosa of the small intestine.	Equipment: FPC 100 smoking chamber from Fessmann. Pretreatment of samples: Reddening for 10 min at 52°C and drying for 12 min at 56°C. Smoking times (min) (t): 10,11,12, 13,14,20,21,22,28,29,30. Temperature (T): 58°C. Smoke density (SD): intensive(i), medium(m), light(l). Ventilator velocity (V) (rpm): 750, 1500, 3000.Moisture content of wood (M)(%): [9.8-29.4]	0.48±0.09 T:58°C t:11 min SD: i V: 1500rpm M:12,6%	0.11±0.04 T: 58°C t:28min SD: l V:750rpm M:12,2%	2.96±0.43 T:58°C t:11 min SD: i V: 1500rpm M:12,6% (PAH4)	1.10±0.22 T: 58°C t:28min SD: l V:750rpm M:12,2% (PAH4)
					Comparison of samples of similar moisture			
	Pöhlmann et al., (2013a)	Smoldering (S), Steam (St), Friction (F), Touch smoke (T) at different smoking times, ventilator velocities [rpm]: 750rpm (a), 1500rpm (b) and 3000rpm (c) and smoke densities: intensive (i), medium (m), light (l).	Frankfurter-type sausages: 29.5% fresh pork, 19.6% fresh beef, 26.4% back fat, 22.5% ice, 1.4% salt (containing sodium nitrite 0.4%), 0.04% ascorbic acid, 0.17% dipotassium hydrogen phosphate and 0.42% "Goldwürstchen" spice mix from Raps. Sheep casings made from the submucosa of the small intestine.	S: 10,20,28 min.(i,m,l).750rpm (a). 11,21,29 min. (i,m,l).1500 rpm (b). 12,22,30 min. (i,m,l).3000 rpm (c). St: 3, 4, 5, 6, 8 min at 750 rpm (1 study 4 min with 3000 rpm), Different steam smoke temperatures. F: ventilator velocity (1500 rpm) 26, 37 and 40 min, 1.2,2 and 2.8 bar, 30, 60 and 240sec interval of friction. T:15,20,25,30,35,40 min at 750 rpm	BaP Means S: 0.27 ± 0.14 (l):0.16 (m):0.24 (i):0.40 (a): 0.18 ± 0.07 (b): 0.29 ± 0.16 (c): 0.34 ± 0.15 St: 0.08 ± 0.07 F: 0.03 ± 0.01, T: 0.09 ± 0.02		PAH4 means S: 1.88±0.69 (l):1.32 (m):1.71 (i):2.60 (a): 1.51 ± 0.39 (b): 1.96 ± 0.83 (c): 2.17 ± 0.72 St: 0.94± 0.66 F: 0.29 ± 0.11 T: 1.57 ± 0.32	

Table 5. (continued).

CODEX ALIMENTARIUS PARAMETER	References	Studied variable	Sample	Studied smoking conditions	PAH results interval (µg/kg)			
					BaP (µg/kg)		PAH (µg/kg)	
					Maximun	Minimun	Maximun	Minimun
d) Position of the food with respect to the heat source.	Pöhlmann et al., (2013b)	Different positions in the smoking chamber	Sheep casing. Fresh pork, Fresh beef , Back fat, ice : 29.4%, 19.6%, 26.5%, 22.5%.	Positions in the smoking chamber: Front (a), center-front (b), center-back (c), back (d). T1900 smoking chamber, Fessmann. Smoking time:12 min Smoke density: intensive. Ventilator velocity: 3000 rpm	1.10 centre back	0.88 back	PAH4 4.57 centre back	PAH4 3.62 back
f) Fat content of the food and what happens to it during processing.	Pöhlmann et al., (2013b)	Fat content (F): 10%,20%,30%,39%	Sheep casing. Fat (F), Fresh pork, Fresh beef , Back fat, ice : 10%, 35.8%, 23.9%, 9.9%, 28.3% 20%, 32.0% ,21.3%, 19.6%, 25.0% 30%, 28.0%, 18.7%, 29.5%, 21.8% 39% 24.1%, 16.1%, 39.1%, 18.7 %	T1900 smoking chamber, Fessmann. Smoking time: (t):12,13,14,15 min Smoke density (SD): intensive (i) and medium (m). Ventilator velocity (V) (rpm): 750, 1500, 3000.	1.37 F:39.1% t: 15min SD: i V: 3000	0.28 F:9.9% t: 12min SD: m V: 1500	6.2 (PAH4) F:19.6% t: 15min SD: i V:3000	1.5 (PAH4) F:9.9% t: 12min SD: m V:1500
g) Duration of smoking and direct drying.	Djinovic et al., (2008)	Smoking time: a,e: 0,6,9,12,15,18 days b,c,d,f: 0,6,9,12,15 days	Beef ham (a) Pork ham (b) Bacon without skin (c) Bacon with skin (d) Cajna sausage (e) Sremska sausage (f)	Beech wood combustion. Distance from the smoking source: a,b,c,d: 2m e,f: 5 m.	BaP, days a:1.09, 18 b:0.52, 15 c:1.04, 15 d:0.55, 15 e:0.24, 18 f: 0.33, 15	BaP, days a: 0.02, 0 b: 0.04, 0 c: 0.03, 0 d: 0.03, 0 e: 0.18, 0 f: 0.08, 0	PAH^2, days a: 21.3, 18 b: 10.2, 15 c: 22.7, 15 d: 12.2, 15 e: 4.4, 18 f: 10.5, 15	PAH^2, days a: 0.4, 0 b: 0.9, 0 c: 0.6, 0 d: 0.7, 0 e: 3.5, 0 f: 2.0, 0
	Ledesma et al., (2014)	Smoking time: 0,1,3,5,7 days Product depth: D1: casing (1.15g); D.2: 0.25 cm from the casing to the casing (23.6g).D.3 Part between D2-D4 (65.4g). D.4: 0.25 cm from the center and the center (25.0g).	Chorizo: Length:13.7 cm, mass: 115.1 g. Ingredients: Pork loin (46.8%), pork jowl (46.8%), salt (1.8%), garlic (1%), sweet or spicy paprika (2%) and herbs (1.6%). Antioxidants colorings,emulsifiers and otheradditives.Natural casing.	Traditional direct smoking. oak (90%) and chestnut (10%) woods Distance from the smoke source: 10 m.	BaP, days 0.75±0.05,5 D1 20.0±1.1, 5 days	BaP, days <0.24, 0 D4 0.88±0.10 5 days		

Table 5. (continued).

CODEX ALIMENTARIUS PARAMETER	References	Studied variable	Sample	Studied smoking conditions	PAH results interval (µg/kg)			
					BaP (µg/kg)		PAH (µg/kg)	
					Maximun	Minimun	Maximun	Minimun
k) Casing	García-Falcon & Simal-Gándara, (2005)	Natural casing (N) Collagen casing (C)	Chorizo: pork meat, water, paprika, salt and oregano. Fat: a) 46% (low fat) b) 53% (high fat) Natural (N) and collagen (C) casings.	Smoking room:3.5m X 1.6m X 2.7m-Wood: Q. *robur* Distance to the smoking source: 2m. Smoking time: 8 days. Drying after smoking: 16 days.	2 Natural cased chorizo with 53% fat	1 Collagen cased chorizo with 53% fat,both chorizos with 46% fat	57 ΣPAH[3] Natural casing cased chorizos, low fat content (46%)	22.1 ΣPAH[3] Collagen casing with low fat content (46%)
	Gomes et al., (2013)	Collagen casing (C) Hog casing (H)	Lean and fatty pork trimmings, salt (1%), garlic paste (3%), paprika paste (3.5%), curing salts [0.25% ($NaNO_3$ 4.9%; KNO_3 5%), antioxidants [0.15% (sugars; E301 relative composition unknown) and water (2.5%)]. Fat content: (a) 20% , (b): 40%.	4hours/day, 8 days, combined with drying in a controlled environmental chamber (5–15°C; 25–55% relative humidity). Oak wood (*Quercus ilex L.*), 2 smoking expositions: Direct (d) and indirect (i) placing an obstacle (a stainless steel plate above the smoke generator, or not.)	0.32 Hog (natural) casing, a:20% fat, indirect smoking	0.09 Collagen casing, a:20% fat, indirect smoking	PAH4:10.35 Hog casing, b:40% fat, direct smoking	PAH4: 4.21 Collagen casing, a:20% fat, indirect smoking
	Ledesma et al., (2015b)	Natural casing (N) Collagen casing (C)	Chorizo ingredients: Pork loin (46.8%), pork jowl (46.8%), salt (1.8%), garlic (1%), sweet or spicy paprika (2%) and herbs (1.6%). Antioxidants (sodium citrate or sodium ascorbate), colorings (carmine) emulsifiers (sodium triphosphate), food preservatives (sodium nitrite) and other additives. Natural and collagen casings	Traditional direct smoking. oak (90%) and chestnut (10%) woods smoking time: 7 days. Distance from the smoke source: 10 m.	n.d unsmoked chorizo, and chorizo in synthetic casing (collagen)	1.71 ± 0.15 Chorizo in natural casing. 19.9 ± 2.9 Casing only		

Table 5. (continued).

CODEX ALIMENTARIUS PARAMETER	References	Studied variable	Sample	Studied smoking conditions	PAH results interval (µg/kg)			
					BaP (µg/kg)		PAH (µg/kg)	
					Maximun	Minimun	Maximun	Minimun
k) Casing	Pöhlmann et al., (2013b)	Collagen casing(C) Cellulose-peelable casing (Ce) Sheep casing (S)	Frankfurter-type sausages: Fresh pork, Fresh beef , Back fat, ice : 29.4%, 19.6%, 26.5%, 22.5%. Before smoking, samples were reddened for 10 min at 52°C, and then dried for 12 min at 56°C.	T1900 smoking chamber, Fessmann. Smoking time: 12 min. Smoking temperature: 58°C. Smoke density: intensive Ventilator velocity: 3000 rpm	0.81 Sheep cased sausage	0.08 Peeled Cellulose cased sausage	4.77 Sheep cased sausage	0.58 Cellulose cased sausage
	Škaljac et al., (2014)	Natural casing (N) Collagen casing (C)	-Natural (pig colon; 400-500 mm 125 long; 38-55 mm in diameter) -Collagen casings (500 mm long; 55 mm in diameter). Traditional fermented sausage from Serbia "Petrovská klobása". Lean pork meat (80 %) and pork fat (20 %) home-made spicy paprika powder (2.50 %), salt (1.80 %), crushed garlic (0.20%), caraway (0.20 %) and sugar (0.15 %). Moisture content of all samples: less than 35.0%.	1.Traditional conditions: Wood: sweet cherry. Distance from smoking source: 3m. Temp. and humidity of the smoking room: A) [8.10 to 14.6°C] (average 10.7°C), [70.1 to 91.6%] (average 78.8%), B) [8.30 to 10.7°C] (average 9.30°C) [60.7% to 90.4%] (average 76.8%). Smoking time: 10 days. Drying (after smoking):90 days. Stored conditions: 10°C, 75%, until 270 days. 2. Industrial conditions: Smoke generator (indirect smoking) Wood: beech Smoke Temp.: 28.0°C. Temp., humidity chamber: 10.3°C, 83.8% Smoking time: 6 hours (in 3 days). Drying (after smoking):45 days. Storage conditions: 10°C, 75%, until 270 days.	n.d	n.d	PAH [4] 495 ± 7.65 (end of storage) 220 ± 6.30 (end of drying) Natural casing. Traditional smoking (direct)	PAH [4] 54.1 ± 4.25 (end of storage) 31.3 ± 0.55 (end of drying) Collagen casing. Industrial smoking (indirect)

1: ΣPAH: CPP, BaA, CHR, 5MC, BbF, BjF, BkF, BaP, IcdP, DBahA, BghiP, DalP, DaeP, DaiP, DahP.
2: ΣPAH: BcL, BaA, CPP, Ch, 5MC, BbF, BjF, BkF, BaP, BgP, DhA, IcP, DeP, DhP, DiP, DlP.
3: ΣPAH: F, BaA, BbF, BkF, BaP, DahA, BghiP, IcdP.
4: ΣPAH: Σ 13 US-EPA PAH: Acy, Fln, Phe, Ant, Pyr, BaA, CHR, BbF, BkF, BaP, IcP, DhA, BgP.
n.d: non-detected.

5.1 Kind of fuel

The studies by Stumpe-Vīksna et al. (2008) and Hitzel et al. (2013) stand out for researching the influence of the kind of fuel used during meat product smoking. In both studies, the samples smoked with apple (Stumpe-Vīksna et al., 2008) and spiced beech wood chips with apple smoking spice mix (BA) and oak (Hitzel et al., 2013) were found to have the lowest BaP and PAH contents. However, the BaP contents found in the products were very different, 6.04 µg/kg (apple), 0.32 µg/kg (BA) and 0.23 (oak), respectively. This difference could be explained by the differences in smoking time, temperature and samples analyzed in each study: 5 hours, 80°C and pork meat in Stumpe-Vīksna et al. (2008) (higher BaP content); and 12 minutes, 58°C and a mix of pork and beef meats (probably less fat) in Hitzel et al. (2013) (lower BaP content). According to Hitzel et al. (2013), low BaP contents have been found in meat products smoked with oak wood; for instance, 0.75 µg/kg after 5 days smoking chorizo (Ledesma et al., 2014) and 0.32 µg/kg after 4 hours/day over 8 days of smoking Portuguese dry fermented sausages (a product similar to chorizo, but with a lower fat content) (Gomes et al., 2013), both products being stuffed in natural casings. Products smoked with aspen, (Stumpe-Vīksna et al., 2008) and alder (Hitzel et al., 2013) have the highest BaP contents (35.07 µg/kg, 0.80 µg/kg), respectively. Chung et al. (2011) studied the PAH content of grilled and roasted meat products, reporting a BaP content of 8.49 µg/kg in a pork shoulder loin exposed to charcoal grilling for only 30 min. However, they used gasoline on the charcoal to start the fire. Using this type of fuel greatly increases the PAH of meat products. CAC/RCP 68/2009 recommends discouraging the use of resinous woods or woods treated with chemicals (for preserving, waterproofing, fireproofing, etc.) and other fuels rather than natural woods (even as partial components) such as diesel oil, rubber (for example, tyres) or waste oil.

5.2 Smoking or drying method (direct or indirect), grilling and barbecuing.

CAC/RCP 68/2009 established that replacing direct smoking with indirect smoking (such as the use of a friction smoke generator) can significantly reduce PAH contamination of smoked foods. Gomes et al. (2013) studied the influence of direct (d) and indirect (i) smoking, placing an obstacle (a stainless steel plate) or not above the smoke generator, providing a detailed graphical scheme of the smoking room. They found a reduction in the PAH content of indirectly smoked meat products, especially those stuffed in natural (hog) casing. The reduction ranges between 33 and 45 %. CAC/RCP 68/2009 states that domestic food preparation such as roasting, baking,

barbecuing and frying are recognized as important sources of PAH contamination. Home barbecuing and grilling could be classified as direct methods of PAH contamination. Grilled or barbequed meat was the cause of a significant proportion (21%) of the average total daily BaP intake in USA, according to the data obtained by Kazerouni et al. (2001).

Table 6 shows recent studies on PAH in grilled meat products. Various studies have been conducted on the influence during the grilling and barbecuing of meat products of marinating (Farhadian, Jinap, Faridah, & Zaidul, 2012), preheating and wrapping (Farhadian, Jinap, Hanifah, & Zaidul, 2011), the addition of onion and garlic (Janoszka, 2011), home cooking, and commercial samples from different countries, like Estonia (Reinik et al., 2007), Denmark (Aaslyng, Duedahl-Olesen, Jensen, & Meinert, 2013), Korea (Chung et al., 2011), Malaysia (Farhadian, Jinap, Abas, & Sakar, 2010) and Kuwait (Alomirah et al., 2011).

In order to reduce the PAH content of grilled meat products, the best reported pretreatment methods are steam and microwave preheating, and aluminum wrapping (100% reduction in the two carcinogenic PAH, BaP and BbFln) (Farhadian et al., 2011), an acidic marinade containing 1.2% lemon juice (70% reduction of PAH) (Farhadian et al., 2012) and the addition of onion and garlic as meat additives (Janoszka, 2011) (decrease of 60% in the total content of 6 PAH in meat samples). According to Jägerstad and Skog (2005), marinating can often result in a charred meat surface with high PAH levels. This could be caused by the type of marinade applied. Farhadian et al. (2012) recommends the following order: basic-lemon>basic> basic-oil-lemon> basic-oil. The highest BaP contents found in commercial and home-grilled samples were 24 μgkg^{-1} in Danish beef (Aaslyng et al., 2013), 12.5 μgkg^{-1} in Malaysian beef satay (Farhadian et al., 2010), 8.49 μgkg^{-1} in pork from Korea (Chung et al., 2011) and 5.79 μgkg^{-1} in meat burgers from Kuwait (Alomirah et al., 2011). BaP was not found in grilled products in several studies (Aaslyng et al., 2013; Alomirah et al., 2011; Chung et al., 2011; Farhadian et al., 2010).

Table 6. Polycyclic aromatic hydrocarbons (PAH) in grilled meat products.

Reference	Studied variable	Sample	Studied grilling conditions	Results interval		
				PAH (µg/kg)		Conclusion
				Maximum	Minimum	
Aaslyng et al., (2013)	Meat products barbecued at home in Denmark.	Boneless pork loin (intramuscular fat content of 1.4%–2.0%, 1.9% average fat content (3mm) sliced into 14 pieces of 2 cm-thick chops/loin. Beef strip loin: (intramuscular fat content of 2.7%–5.9%, 4.6% average fat content) sliced into 10 pieces of 2-cm steaks per strip of loin. Chicken breast fillets (0.6%–1.0%, intramuscular fat content, 0.9% average fat content). 1 muscle/sample.	Normal barbecue practice by consumers in Roskilde (Denmark).	Beef: 24	0 content found in all samples Chicken shows the lowest max BaP content: 1.2.	Beef, pork and chicken were affected differently by the barbecuing process. The content of some of the PAH varied significantly between the 3 meat products (pork, beef and chicken). However, time–temperature seemed to be the most important factor compared with the type of meat.
Alomirah et al., (2011)	Grilled and smoked foods from Kuwait	28 lamb meat (4 mandi, 6 kabab, 4 tikka, 6 shawerma, 3 burger, 2 smoked meat and 3 arayes), 21 chicken (4 mandi, 4 shish tauk, 4 whole grilled chicken, 6 shawerma and 3 burger).	-Charcoal grilled (indirect heat): Mandi (meat and chicken) -Charcoal grilled (direct heat): Meat kabab, meat tikka, meat arayes, Shish tauk, whole grilled chicken. -Gas grilled (indirect heat) Shawerma (meat and chicken). -Electric grilled (indirect heat) Burger (meat and chicken). -Electric smoked (indirect heat): Smoked meat	BaP 5.79 Meat burger ΣPAH[1] 1292 Meat tikka Meat tikka contained the highest mean concentrations of BaP and ΣPAH[1] (2.48, 648), respectively	BaP 0 content in all samples except for Meat tikka, smoked meat and Chicken mandi. ΣPAH[1] 6.41 chicken burger	Meat tikka, whole grilled chicken, meat burger and grilled vegetables were the major contributors to the daily intake of BaP, PAH8 and SBaPeq for the child/adolescent and adult population in Kuwait. Major influence of the type of heat source, duration of grilling, geometry of the grill and the use of marinating sauces and fat content.

Table 6. (continued).

Reference	Studied variable	Sample	Studied grilling conditions	Results interval		
				PAH (µg/kg)		Conclusion
				Maximum	Minimum	
Chung et al., (2011)	Grilling and roasting of meat products	Samples (%fat) Beef: loin (18.4-18.7%), ribs (6.7-7.5%) and ribs with sauces (14.0-16.6%). Raw pork: shoulder loin (13.2- 14.9%), belly (17.7-20.1%) and ribs with sauces (14.06-16.6%). From local supermarkets in the Republic of Korea. Samples cut into small 0.5 cm cubes.	Gas roasting: 30 min at 200 °C. Charcoal roasting: 30 min at 200 °C. Grilling: 2 kg of charcoal were placed in the bottom of the grill and 100 mL of gasoline were poured onto charcoal to start a fire. The beef and pork samples were grilled for 30 min.	BEEF: 0.916 Charcoal grilling of Ribs PORK: 8.49 Charcoal grilling of shoulder loin pork.	nd in all samples except for charcoal-grilled ribs and ribs with sauces of beef and pork	Charcoal grilling produces the highest PAH levels
Farhadian et al., (2010)	Malaysian popular grilled meat dishes	1. Charcoal grilled (well done): a) Ayam bakar (chicken), b) beef satay, c) chicken satay. 2. Gas grilled – direct heat (well done):d) Beef kebab, e) chicken kebab, f)grilled chicken. 3. Oven grilled – indirect heat (well done): g)Tandoori (chicken), h) Grilled chicken.	1. Charcoal grilled (well done): a) 4–5 pieces of chicken, 6–7 min grilling on each side using garden-type grill fuelled by charcoal. b) 4–5 small pieces of beef, 5–6 min grilling, on each side using a satay-type grill fuelled by charcoal. c) 4–5 small pieces of chicken. 4–5 min grilling on each side using a satay type grill fuelled by charcoal. 2. Gas grilled – direct heat (well done) d) Sliced beef skewered onto a vertical rotisserie with a vertical gas heat element. e) Sliced chicken skewered onto a vertical rotisserie with a vertical gas heat element. f) 1 large piece of chicken was grilled for 8–10 min on each side on a gas stove. 3. Oven grilled – indirect heat g) 2 pieces chicken cooked in a clay oven (Tan door. The) heat source was gas under a clay surface. h) 1 whole chicken (1 kg) cooked in an electric oven.	BaP 12.5 Beef satay (b)	BaP n.d in all samples except for a) ayam bakar (chicken), b) beef satay, and c) chicken satay.	The highest concentration of PAH was detected in charcoal-grilled followed by flame-gas grilled and oven grilled dishes. In charcoal grilled dishes. Beef satay had the highest concentration of PAH. PAH concentrations of flame-gas grilled dishes was found to be low when the gas-flame source was vertical. Furthermore, there were no significant difference in PAH concentrations ($p < 0.5$) between beef & chicken grilled by a vertical source.

Table 6. (continued).

Reference	Studied variable	Sample	Studied grilling conditions	Results interval		
				PAH (µg/kg)		Conclusion
				Maximum	Minimum	
Farhadian et al., (2011)	Preheating and wrapping of charcoal grilled meat products.	Beef (filet) and chicken (breast and leg with skin) exposed to different preheating and wrapping methods.	Samples of small uniformly sized pieces (about 0.5 cm). Marinating in satay spice: a mix of onion, sugar, cooking oil, lemon grass and lemon-juice for 4 h and kept in the refrigerator (4ºC) until use. **1. Pretreatments:** **1.1)Preheating:** a) Microwave preheating: 40, 60 and 120 s for beef and 20, 40 and 60 s for chicken samples at 180°C. b) Steam preheating: 100°C for 2, 4 and 6 min for beef and 1, 2 and 3 min for chicken. **1.2) Wrapping** c) Aluminum wrapping d) Banana leaf wrapping **2.Charcoal grilling:** Samples skewered onto 12 satay sticks (4 pieces on each stick) and processed on the satay charcoal grill). Duration: Between 2 and 10 min depending on sample	BEEF: 3.16 ± 0.41 CHICKEN: 2.44 ± 0.26 Both charcoal grilled (8 and 6 min for beef and chicken respectively) samples without preheating treatments	BEEF and CHICKEN: n.d in Steam and microwave preheating and aluminum wrapping samples.	The steam and microwave preheating treatments and aluminum wrapping have the same, maximum effects (100%) in reducing the two carcinogenic PAH (BaP and BbFln) for beef and chicken meat samples. The effect of banana wrapping on these two PAH was also quite satisfactory for beef samples. For chicken samples, wrapping treatments (aluminum and banana) were shown to cause a greater reduction in PAH compared to preheating treatments (steam and microwave).
Farhadian et al., (2012)	Effects of marinating	Beef (filet) meat, commonly used for charcoal grilling of satay, from local markets in Seri Serdang, Selangor. Samples were chopped into small pieces (about 0.5 cm).	Marinade treatments: 1) Basic marinade (B): sugar, water, onion, turmeric, lemon grass, salt, garlic, coriander and cinnamon. 2) Basic-oil (B-O): (B) plus oil. 3) Commercial (Cmr): spices, onion, garlic, salt, sugar, water and cooking oil. Modified marinade: 4) Basic-oil-lemon juice (B-O-L) 5) Basic-lemon juice (B-L) 6) Basic-oil-tamarind (B-O-T) 7) commercial-tamarind (Cmr-T) Grilling on a satay-type grill fueled by charcoal for 8 min until the color turned yellowish brown (well done).	BaP 4.78 ± 0.41 Control: beef satay samples without marinating. (8 hours without marinating) BaP +BbF +F 109 B-O, 12 hours of marinating	BaP B 0.11±0.01 0 hours of marinating BaP +BbF+F 35.8 B-L , 4 hours of marinating	Cooking oil as ingredients of marinade treatment for beef satay contributed to a higher concentration of PAH. Preferred marinating order to reduce PAH: basic- lemon> basic> basic-oil-lemon> basic-oil

Table 6. (continued).

Reference	Studied variable	Sample	Studied grilling conditions	Results interval		
				PAH (µg/kg)		Conclusion
				Maximum	Minimum	
Janoszka, (2011)	Addition of onion and garlic to pork meat and its fried gravy.	3 dishes (collars, chops and minced chops) made with fried pork joint, onion (30g/100g meat), garlic (15g/100g meat), without bones and small fat pieces. Pan residues obtained from collars and chops. 3 kinds of samples: a)Without additives b)With onion c)With garlic	-Collars: 3-cm diameter.10-cm length. Frying for 20 min in a pan preheated to 200°C. Addition of 200 mL of water and kept for 1 h at 95–98°C. -Chops: Onion & garlic mixture spread on the meat and kept for 12hours. Frying for 6 min on each side without fat in a Teflon-coated frying pan preheated to 200°C. Addition of 100 mL of water and left for 10 min at 95–98°C. -Minced chops: Minced and mixed meat and vegetables. Chops form: Petri dishes (9 X 1.5 cm) Grilling in an electric oven at 270°C for 17 min on each side. The grill was 20 cm above the meat. -Pan residues obtained from collars and chops: collected and evaporated nearly to dryness.	Without additives: Collar meat & gravy: 1.09 ± 0.06, 0.08± 0.02 Chops & gravy 1.61 ± 0.14, 0.11± 0.01 Minced chop: 0.50 ± 0.10	Collar meat with garlic 0.32 ± 0.08 gravy &onion: 0.01 ± 0.002 Chops & gravy with onion 0.41 ± 0.05 0.01 ± 0.002 Minced chop & onion: 0.38 ± 0.06	Onion and garlic as meat additives caused a considerable decrease in PAH in the samples of pan fried pork. The addition of onion led to an average decrease of 60% in the total content of 6 PAH in meat samples and of over 90% in gravies. Garlic reduced the concentration by 54% in meat and from 13.5–79% in gravies. No marked effect was found for minced chops.
Reinik et al., (2007)	Commercial meat products in Estonia 2001–2005	Estonian home-grilled meat products Grilled pork, grilled sausage, grilled chicken.	2 types of home grilling: A) Traditional wood-burning grill: Distance between burning coals and meat: 20 cm Temp. at the surface of the meat: 180°C-240°C. B) Disposable charcoal grill: "Less distance than A". Shorter preparation time.	1.8 Pork grilled over disposable charcoal	0.3 Chicken grilled chicken over burning wood	

1: Σ16PAH: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3- c,d]pyrene, dibenz[a,h]anthracene and benzo[ghi]perylene.

n.d: non-detected

5.3 Operating parameters of smoke generators and liquid smoke.

The main operating parameters affecting PAH formation in friction, steam smoke, touch smoke smoldering and thermostated smoke generators are the temperature of pyrolysis and airflow. Several authors (Hitzel et al., 2013; Pöhlmann et al., 2012, 2013a, 2013b) have conducted a number of detailed studies on the PAH content of Frankfurter-type sausages as a function of smoking conditions. They found that smoking conditions have a significant influence on the PAH content of smoked meat products. PAH content increases with smoke density and ventilator velocity (Pöhlmann et al., 2012). Frankfurter-type sausages smoked by friction smoke generation were found to have the lowest BaP and PAH4 contents (0.03 ± 0.01 μgkg^{-1} and 0.29 ± 0.11 μgkg^{-1}, respectively) compared to steam, touch smoke and smoldering methods. Samples produced by smoldering with an intensive smoke density have the highest BaP and PAH4 contents (0.40 μgkg^{-1} and 2.60 μgkg^{-1}, respectively) (Pöhlmann et al., 2013a). The most important parameter influencing PAH content is the smoke generation temperature. Temperatures over 600°C must to be avoided to obtain lower PAH contents in smoked meat products (Pöhlmann et al., 2012).

Table 7 shows the PAH content found in meat products with smoke flavorings. In fact, concentrations of up to 2.86 ± 0.39 µg/kg, 3.4 µg/kg and 336.6 µg/kg BaP have been detected in commercial smoke flavorings (Gomaa, Gray, Rabie, Lopez-Bote, & Booren, 1993; Guillén, Sopelana, & Partearroyo, 2000a, 2000b, 2000d; Šimko, Petrík, & Karovičová, 1992; Yabiku, Martins, & Takahashi, 1993). The use of different kinds of wood and the material used in the storage container influence the final content of PAH in smoke flavorings. Flavoring obtained from poplar wood presents the highest number and concentrations of both total and carcinogenic PAH compared to that produced with oak, cherry tree, beech, poplar or vine shoot woods. Storing smoke flavorings in polyethylene flasks reduces the concentration of some PAH (Guillén, Sopelana, & Partearroyo, 2000c). The EC has accordingly set 10 µg/kg as the maximum permissible BaP content in smoke flavorings (EC No 2065/2003) and has recently established the EU list of authorized smoke flavoring primary products for use as such in or on foods and/or for the production of derived smoke flavorings (EU, No 1321/2013). Finally, different collaborative projects have been carried out to assess analytical methods for PAH determination in smoke flavorings (Simon, Gómez-Ruiz, & Wenzl, 2010).

Table 7. Polycyclic aromatic hydrocarbons (PAH) in meat products with smoke flavorings.

Reference	Studied variable	Sample	Studied liquid smoke conditions	Results		
				BaP (µg/kg)		Conclusion
				Maximum	Minimum	
Guillén et al., (2000a)	PAH in commercial liquid smoke flavorings with different compositions.	5 commercial liquid smoke flavorings: A, B, C, D and E.	A: phenol, guaiacol, and syringol derivatives, with a small proportion of lignin dimers and trimers and an insignificant proportion of carbonyl and carboxyl derivatives. B, C: typical smoke components in similar proportions to those found in smoke. D: typical smoke components in similar proportions to those found in smoke (lower than B and C). E: similar to A, but lower concentrations.	2.86± 0.39 Commercial liquid smoke flavouring (sample A)	n.d Samples D, E	BaP was detected in 3 samples, but its concentration did not exceed the 10 µg/kg level fixed by the FAO/WHO.
Guillén et al., (2000b)	PAH in liquid smoke flavorings.	2 commercial liquid smoke flavorings. (2 smoke flavorings obtained in the laboratory under controlled conditions by pyrolysis of sawdust from beech wood and vine shoots)	2 commercial liquid smoke flavorings. (2 smoke flavorings obtained in the laboratory under controlled conditions by pyrolysis of sawdust from beech wood and vine shoots).	2.86± 0.39 Commercial liquid smoke flavoring	n.d Smoke flavoring obtained in laboratory	"The flavoring from beech wood (hardwood) has higher concentrations of carcinogenic PAH than that from vine shoots (softwood)".
Guillen et al., (2000c)	PAH in liquid smoke flavorings obtained from Different types of wood. Effect of storage in polyethylene flasks on their concentrations	5 liquid smoke flavorings obtained in the laboratory using dry sawdust from different woods: Oak, cherry tree, beech, poplar, and vine shoots.	Round-bottom flask smoke generator made of quartz. Pyrolysis was started using a rheostat-controlled heating mantle. Temperatures reached during the process: 530°C (oak), 550°C (cherry tree), 532°C (beech), 536°C (poplar), and 559°C (vine shoots). Beech, poplar, and vine shoots flavorings were stored in both glass and polyethylene flasks. Storage time: 4 years for poplar and vine shoot samples, and 2.5 years for beech flavoring.	0.06± 0.02 Poplar stored in glass flasks	n.d in flavorings from oak, cherry tree and vine shoots stored in glass flasks and in all flavorings stored in polyethylene flasks except for poplar.	The flavoring obtained from poplar wood presents the highest number and concentrations of both total and carcinogenic PAH. Storing smoke flavorings in polyethylene flasks reduces the concentration of some PAH.

Table 7. (continued).

Reference	Studied variable	Sample	Studied liquid smoke conditions	Results		
				BaP (µg/kg)		Conclusion
				Maximum	Minimum	
Guillén et al., (2000d)	PAH in liquid smoke flavorings.	Mixtures either of pure PAH and of smoke flavoring compounds, with the aim of simulating smoke flavorings and, on the other hand, aliquots from two commercial liquid smoke flavorings, A and B.	Commercial flavorings.	3.1± 0.2	2.6± 0.0	
Gomaa et al., (1993)	PAH in commercial liquid smoke flavorings and seasonings	Eighteen commercial liquid smoke flavorings and seasonings	Commercial liquid smoke flavorings and seasonings.	3.4	0.1	In liquid smoke flavorings and seasonings, total PAH concentrations ranged from 6.3 to 43.7 µg/kg, with the carcinogenic PAH ranging from 0.3 to 10.2 µg/kg.
Yabiku et al., (1993)		11 samples of liquid smoke flavor	Liquid smoke flavor.	336.6	0.1	BaP was found in 73%, of the liquid smoke flavor samples. Three liquid smoke flavor samples showed levels of BaP above the maximum level recommended by FAO/WHO (10 µg/kg).
Šimko et al., (1992)	Benzo(a)pyrene in liquid smoke preparations	Liquid smoke preparations	Liquid smoke preparations.	0.8	0.3	
Simon et al., (2010)	15 + 1 EU priority PAH	Primary Smoke Condensate (PSC).	PSC obtained from 6 different manufacturers from the smoke flavor industry and mixed in approximately equal amounts. 2 materials evaluated by an inter-laboratory comparison (25 laboratories).	13.4	5	

n.d: non-detected

5.4 Position and distance of the product in relation to the heat source.

The distance of the food from the heat source is specified, though not selected as the main variable to study, in several studies on PAH in smoked meat products. The studied distances ranged between 2 and 10 meters (Djinovic et al., 2008; García-Falcón & Simal-Gándara., 2005; Ledesma et al., 2014; Škaljac et al. 2014).

The study by Pöhlmann et al. (2013b) stands out for researching the influence of the position in the smoking chamber on the final PAH content of meat products, maintaining the remaining variables constant. The positions studied were front, center-front, center-back, and back of the chamber. Results show that meat products placed in the center-back and back have the highest and lowest PAH4 (4.57 µg/kg, 3.62 µg/kg) and BaP (1.10 µg/kg, 0.88 µg/kg) contents, respectively.

5.5 Fat content of the product and its evolution during processing.

Similar to the variables of distance and position in the smoking chamber, the fat content is specified in the sample description in several studies on PAH content in smoked meat products (Hitzel et al., 2013; Ledesma et al., 2014, 2015a, 2015b; Pöhlmann et al., 2012, 2013a, 2013b; Škaljac et al., 2014). Several papers may be highlighted for studying this variable: Pöhlmann et al. (2013b), Gomes et al. (2013), García Falcón and Simal Gándara (2005), and Ledesma et al. (2015b). Pöhlmann et al. (2013b) found that BaP content clearly increases with fat content from 0.28 µg/kg in 9.9% fat content to 1.37 µg/kg in 39.1% fat content in smoked Frankfurter-type sausages. Different smoking conditions were adopted in the experiments (different smoke density and ventilator velocity). The combination of intensive (highest) smoke density, the highest ventilator velocity (3000 rpm) and the highest fat content leads to the reported highest BaP contents. In fact, Gomes et al. (2013) concluded that the fat content and smoking regime alone did not influence the total amount of PAH, whereas the casing type has a greater influence on the PAH content of meat products. Studying these two variables, Ledesma et al. (2015b) observed that, during smoking, the fat content flows through natural casings and covers the external surface of the product, making it sticky and moist. Subsequently, when PAH aerosols reach the sticky, wrinkled surface, soot particles are easily captured and adhere to it, damage the casing and start to migrate inside the product. Once captured, the soot particles slowly penetrate inside meat products, accessing the inner part through the lumps of fat. In contrast, the fat

content of meat products stuffed in synthetic casings remains inside the product. The surface of the casing remains dry, making it non-sticky and smooth, without any affinity for soot particles. Accordingly, a lower amount of soot particles containing PAH (aerosols) was found outside the casing and did not damage its surface. Finally, BaP was found inside meat products stuffed in natural casing (1.71 ± 0.15 mg/kg), while no BaP content was found inside meat products stuffed in synthetic casings (Ledesma et al., 2015b).

5.6 Duration of smoking and direct drying.

Similar to the previously mentioned variables, the duration of smoking is also specified in the sample description in several studies on PAH content in smoked meat products (García-Falcón & Simal-Gándara, 2005; Gomes et al., 2013; Hitzel et al., 2013; Pöhlmann et al., 2012, 2013a, 2013b; Škaljac et al., 2014; Stumpe-Vīksna et al., 2008). Two papers stand out for studying this variable, Djinovic et al. (2008) and Ledesma et al. (2014). In these studies, smoking time ranged from 0 to 18 and 0 to 7 days, respectively, whereas minutes (10-30) are applied in the studies by Pöhlmann et al. (2012, 2013a, 2013b), depending on the type of smoking method employed. Djinovic et al. (2008) and Ledesma et al. (2014) found that the BaP content of meat products clearly increases with smoking time. However, smoking time may not have been the main variable influencing PAH content of smoke meat products. The BaP content of chorizo stuffed in natural casing increases from less than 0.24 μg kg^{-1} to 0.75 ± 0.05 μg kg^{-1}, finally stabilizing after 5 days of smoking (Ledesma et al., 2014). BaP is mainly deposited in the casing of the product (Andrée, Jira, Schwind, Wagner, & Schwagele, 2010; Djinovic et al., 2008; García-Falcón & Simal-Gándara, 2005; Ledesma et al., 2014; Santos et al., 2011), which works like a barrier to BaP penetration after 5 days of smoking of chorizo (Ledesma et al., 2014). Different BaP contents have been found in similar meat products when applying a similar smoking time: for example, less than 0.48 μg kg^{-1} after 9 days of smoking of different meat products (Djinovic et al., 2008), and 0.38 μg kg^{-1} and 0.75 μg kg^{-1} in chorizo after 3 and 5 days of smoking, respectively (Ledesma et al., 2014).

5.7 Type of casing

It has been found that the greatest amount of BaP is deposited on the meat product casing, only a small amount then migrating into the product (Andrée et al., 2010; Djinovic et al., 2008; García-Falcón & Simal-Gándara, 2005; Ledesma et al., 2014; Santos et al., 2011). In a recent

study (Pöhlmann et al. (2013b)), lower levels of PAH were found in Frankfurter sausages with peelable cellulose casings compared to natural or collagen casings. CAC recommends cleaning the product by rinsing or immersion in water, as these processes may remove soot and particles containing PAH on the surface of the food (CAC/RCP 68/2009). Some time ago, some authors had already reported that the use of different types of casing (natural and synthetic) has an influence on the PAH content of meat products (Filipovic & Tóth, 1971; Tóth, 1973). A number of studies have recently continued this line of research (Gomes et al., 2013; Pöhlmann et al., 2013b; Škaljac et al., 2014). Gomes et al. (2013) studied the effect of fat content, casing type and smoking procedures on the PAH contents of Portuguese traditional dried fermented sausages. They found that fermented sausages with collagen casing had the lowest PAH content, showing that the use of synthetic instead of natural casings contributes to reducing PAH levels in smoked meat products. Likewise, Škaljac et al. (2014) studied the influence of smoking under traditional and industrial conditions on the PAH content in dry fermented sausages ("Petrovská klobása") from Serbia. These authors found that the total levels of the sum of 13 US-EPA PAH were significantly higher in sausages stuffed in natural casings (220 ± 6.30 µg/kg) than in sausages stuffed in collagen casings (31.3 ± 0.55 µg/kg), even when smoked under the same conditions. Likewise, García-Falcón and Simal-Gándara (2005) found that collagen-based casings act as a better barrier to the penetration of PAH into chorizo meat. Ledesma et al. (2015b) found that this effect is caused by differences in the physical properties of the two types of casing. To sum up, the high porosity (66.8%) of natural casings allows the fat content to flow through the casing and cover its external surface, making it sticky and moist. This effect, added to the product's wrinkled surface, allows soot particles to be easily captured and adhere to it. These particles subsequently damage the casing and start to migrate inside the product. In contrast, the low porosity (16.6%) of synthetic casings makes the fat content remain inside the product. The surface of synthetic casing is dry, non-sticky and smooth, showing no affinity for soot particles. Moreover, the smaller size of the pores prevents any small amount of coal tars from penetrating the product. More differences were also found between casings. Besides pore characteristics, the diffusivity of the compounds in the continuous phase is also an important factor (Díaz, Vega, & Coca, 1987). The average pore diameter (599.8 nm) and the interstitial porosity (47.6%) of natural casing are considerably higher than those of collagen casing (48.2 nm, 25.9%). However, the total pore area of both types of casing is similar (Ledesma et al., 2015b). The typical size of smoke particles (containing PAH) ranges between 200nm and 400 nm, while the total values range between 50 nm and 1000nm (CAC, 2009). Thus, smoke particles are larger than collagen casing pores and

smaller than those of natural casing. This fact explains the penetration of smoke particles in natural casings, as opposed to collagen casings. Casing type is a variable that has an important effect on the PAH content of smoked meat products and should be considered in meat product manufacturing in order to obtain healthier products.

5.8 Evaluation of variables

As shown, a great number of variables alter the PAH content of smoked meat products. It may thus be very difficult to control all these variables, although important conclusions can be drawn from the research studies reported here. In order to prevent PAH contamination of smoked meat products, three variables seem to have a greater influence than others. These variables are: a) the type of casing, b) the smoking method, which may be direct (traditional smoking) or indirect (friction, smoldering, thermostated plates, liquid smoke), and c) the temperature of smoke generation. A smoke generation temperature below 600°C should be applied to prevent the formation of PAH. The use of synthetic instead of natural casings prevents the penetration of PAH inside products smoked by any method. Casings should be removed by consumers before consuming meat products; peelable cellulose casings, which have already been implemented by some producers, can contribute in this respect. Indirect smoking systems (such as friction smoke generation) can highly reduce the PAH content of meat products, while the use of liquid smoke could be a good alternative if the fractionation and purification processes are really improved and optimized to guarantee the absence of toxic and carcinogenic impurities and the most appropriate wood is used. Moreover, a final step involving the storing of smoke flavorings in polyethylene flasks helps to reduce PAH content. Other variables such as the use of different kinds of wood (never using gasoline, treated woods or wasted oil) and controlling the smoking time and fat content can help reduce the final PAH content of the product. Research focused on methods for reducing the tar content could be applied to improve the design of the meat product smoking process and smoking chambers to prevent PAH contamination of meat products. Moreover, microwave preheating, wrapping in aluminum, acidic marinades and the addition of onion and garlic as meat additives can help to reduce the PAH content of grilled meat products considerably. The PAH content of smoked food could be minimized taking into account the variables recommended by CAC/RCP 68/2009 and studied by several researchers. This is especially important in those developing countries were the cold chain has not been established yet (Ogbadu, 2014; FAO-Thiaroye, 2015). Considering this, The National Training Centre for

Fisheries and Aquaculture Technicians (CNFTPA) of Senegal in collaboration with FAO have designed and developed the FTT-Thiaroye technique. This technique and its diffusion help to minimize the PAH content of the smoked food in the developing countries (FAO-Thiaroye, 2015). More research and application of the CAC/RCP 68/2009 code will improve the safety of smoked food in the world.

6. Conclusions

Meat and meat products are an important component of human diet, especially due to their high protein content. Smoking has traditionally been used to prolong the shelf life of meat products, and is it still applied as preserving method in developing countries. Smoking is still used in developed countries to improve food flavor, color and smell. However, undesirable carcinogenic substances are produced in this process such as polycyclic aromatic hydrocarbons (PAH). The formation of PAH during smoking can be explained by means of biomass conversion. PAH are tertiary tar products formed via biomass gasification and pyrolysis, starting above 750°C. Tar aerosols are produced, pass through the smoking chamber and are deposited on meat products. Meat products can be contaminated by PAH in different ways, as a result of pre-contamination of ingredients, technological processes different to smoking, and fat falling from other products in the smoking chamber. The current data on BaP and PAH4 contents in meat products around the world show that, while the new legal limits are respected in the majority of cases, they are still greatly exceeded in others. According to the literature reviewed in this paper, 3 out of the 10 variables recommended by CAC/RCP 68/2009 seem to have a greater influence on preventing PAH contamination: the temperature of smoke generation, the type of casing and the smoking method (direct or indirect). A smoke generation temperature below 600°C should be applied to prevent the formation of PAH. The use of synthetic instead of natural casings prevents PAH from penetrating inside products. Indirect smoking systems (e.g., friction or liquid smokes) can highly reduce the PAH content of meat products. On the other hand, the control of other variables such as wood (never gasoline, treated woods or wasted oil), marinating, smoking time and fat content can help to reduce the final PAH content of smoked meat products. Finally, meat product smoking can be adapted to obtain PAH contamination levels below the new European limits.

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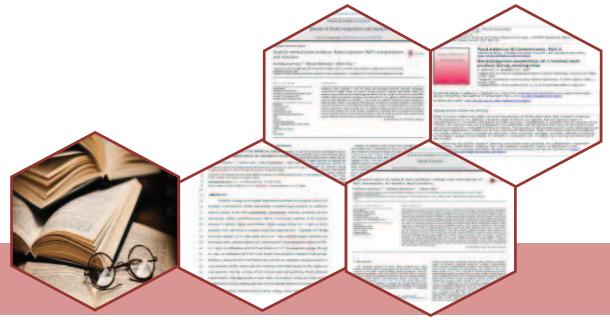


5

Discusión general

5. DISCUSIÓN GENERAL

5.1 DISCUSIÓN DE RESULTADOS

La presente tesis doctoral ofrece resultados científicos relevantes que permiten controlar y reducir la contaminación de productos cárnicos ahumados a modo directo por las sustancias cancerígenas hidrocarburos aromáticos policíclicos (HAP). La aplicación de estos resultados es especialmente importante para la creciente industria cárnica de los países desarrollados (FAO, 2014a), y las comunidades de los países en desarrollo (FAO-Thiaroye, 2015; Ogbadu, 2014; Vaz-Velho, 2003) que continúan utilizando el ahumado directo como método productivo y de conservación de los alimentos.

Haciendo una revisión (**Capítulo 4.5**) se encontró que algunas investigaciones recientes reportan valores de BaP por debajo del nuevo límite (2 µg/kg) establecido por el Reglamento (UE) No 835/2011, e introducido el 1/09/2014. Por ejemplo no superan este valor algunos productos cárnicos ahumados de la República de Korea (Chung et al., 2011), España (Martorell et al., 2010), Italia (Purcaro et al., 2009) o Portugal (Santos, Gomes, & Roseiro, 2011). Sin embargo, todavía se reportan también valores muy elevados, como 36,9 µg/kg en jamón ahumado de Suecia (Wretling, Eriksson, Eskhult, & Larsson, 2010), 10,02 µg/kg en productos de cerdo ahumado de Sudáfrica (Olatunji et al., 2014), 6,98 µg/kg en productos cárnicos bovinos en Malasia (Jahurul et al., 2013), 31,20 µg/kg en carne ahumada de Estonia (Reinik et al., 2007) y 17,63 µg/kg en productos de cerdo ahumado de Alemania (Jira, Ziegenhals, & Speer, 2008).

Estos datos indican que todavía es necesario establecer medidas para prevenir la contaminación por HAP de productos cárnicos ahumados. Para ello, la presente tesis doctoral se ha basado en las normativas y recomendaciones oficiales, como el "Código de prácticas para reducir la contaminación por hidrocarburos aromáticos policíclicos (HAP) en los alimentos producidos por procedimientos de ahumado y secado directo (CAC/RCP 68-2009), de la Comisión del Codex Alimentarius (CCA), las regulaciones europeas N^{o} 1881/2006 y N^{o} 835/2011, así como en el estudio del estado del arte a nivel científico.

Las normas y la comunidad científica indican que la contaminación por HAP de productos cárnicos ahumados está afectada por un elevado número de factores, englobados por el Codex

Alimentarius en 10 variables del proceso. Estas variables han sido estudiadas por varios investigadores actuales. Haciendo una revisión de todas ellas (**Capítulo 4.5**), se pudo concluir que 3 son las más importantes para controlar la presencia de HAP en los productos: la temperatura de generación de humo, el tipo de tripa, y el método de ahumado (directo (ahumado tradicional) o indirecto (ahumado por fricción, fuego sin llama, autocombustión o uso de humo líquido)). Se deberían aplicar temperaturas por debajo de los 600ºC para prevenir la formación de HAP (Pöhlmann et al., 2012). El uso de tripas sintéticas previene la penetración de los HAP en los productos cárnicos ahumados por cualquier método, hecho confirmado en esta tesis en ahumado directo de chorizo, por García Falcón y Simal Gándara (2005), Gomes, Santos, Almeida, Elias, y Roseiro (2013), Pöhlmann et al. (2013a) y Škaljac et al. (2014). Cualquier tipo de tripa debería ser retirada antes del consumo de un producto cárnico ahumado, por lo que el uso de tripas pelables de celulosa es muy aconsejado (Pöhlmann et al., 2013a). Los sistemas indirectos de ahumado (como el ahumado por fricción) pueden reducir en gran medida la cantidad de HAP de los productos (Pöhlmann et al., 2013b), mientras que el humo líquido es una buena alternativa si los procesos de fabricación y purificación han sido realmente optimizados para garantizar la ausencia de impurezas cancerígenas y tóxicas, y la madera utilizada ha sido correctamente seleccionada. Además la conservación de humos líquidos en contenedores de polietileno ayuda a reducir el contenido de HAP (Guillén, Sopelana, & Partearroyo, 2000). Otras variables como el uso de distintos tipos de madera (nunca utilizar gasolina, maderas pretratadas o aceite usado) (Hitzel, Pöhlmann, Schwägele, Speer, & Jira, 2013; Stumpe-Vīksna et al., 2008) o el control del tiempo de ahumado, tal y como se ha estudiado en esta tesis, y por Djinovic, Popovic, y Jira (2008), la posición del alimento respecto a la fuente de calor (Pöhlmann et al., 2013a), y la grasa del producto y su evolución, tal y como se ha estudiado en esta tesis, y por García Falcón y Simal Gándara (2005), Gomes et al. (2013) y Pöhlmann et al. (2013a), puede ayudar a reducir la cantidad de HAP del alimento final. Además el precaliento con microondas, envoltura en aluminio (Farhadian, Jinap, Hanifah, & Zaidul, 2011), marinados ácidos (Farhadian, Jinap, Faridah, & Zaidul, 2012) y la adición de cebolla y ajo como aditivos (Janoszka, 2011) podría ayudar a reducir el contenido de HAP en el caso de productos cárnicos ahumados y hechos a la parrilla.

Considerando todos estos aspectos, la presente tesis doctoral se ha enfocado en el estudio de la contaminación por HAP durante el ahumado a modo directo de chorizo en el Principado de Asturias. Para ello, en la presente tesis doctoral, además de estudiar otras, se ha seleccionado una de las 3 variables más importantes, y la única que se puede controlar utilizando

el ahumado directo tradicional del chorizo asturiano: el tipo de tripa. Así mismo, se ha centrado en el hallazgo de los motivos, que expliquen científicamente, el proceso de transporte y penetración de HAP en los productos, para proponer métodos de prevención de la contaminación de estos alimentos por los cancerígenos HAP.

En particular, mediante el primer estudio (**Capítulo 4.1**), se puso a punto un método para determinar BaP en chorizo ahumado. Este método está basado en la combinación de las técnicas de pretratamiento de muestra modernas, sonicación y SPE, que garantizan la reducción de consumo de disolventes y tiempo de análisis respecto a las técnicas clásicas, como la combinación de Soxhlet y GPC. La aplicación de este método en 16 chorizos ahumados fabricados por 16 empresas diferentes del Principado de Asturias, obtenidos en establecimientos comerciales, confirmó que 5 de los 16 productores sobrepasaron el nuevo límite máximo de BaP (2 µg/kg) en productos cárnicos ahumados establecido por la regulación europea Nº 835/2011, e introducido el 1/09/2014. El rango de BaP encontrado fue 0,38–3,21 g/kg. Se encontró que el contenido de humedad y BaP de las muestras no estaba correlacionado. En definitiva, algunos productos contenían un elevado contenido tanto de BaP como de humedad, relevando la baja calidad del ahumado como sistema de secado. Este hecho enfatiza la necesidad de facilitar recomendaciones a los productores.

En el segundo estudio (**Capítulo 4.2**), se confirmó que utilizando un sistema de ahumado controlado, bajo las variables "tipo de combustible, ingredientes del producto, distancia entre el alimento y fuente de calor" constantes, y estudiando la variable "tiempo de ahumado", la cantidad de BaP del chorizo (sin tripa) aumenta desde menos de 0,24 µg/kg, antes de ahumar, hasta 0,75 µg/kg, estabilizándose entre los 5 y 7 días de ahumado. El contenido de humedad disminuye desde el 49,9% al 31,3% en este periodo. Otros investigadores (Djinovic et al., 2008) han encontrado recientemente una tendencia similar de aumento del contenido de BaP con el tiempo de ahumado, si bien con valores menores a los hallados en esta tesis doctoral, incluso con mayor tiempo de ahumado. Además, en nuestro trabajo, se realizó un estudio del contenido de BaP en distintas profundidades de chorizo, ya que el Codex Alimentarius proponía, como tratamiento posterior al ahumado, el rasurado de la superficie del alimento para eliminar el hollín y las partículas que contienen HAP (CCA, 2009). En efecto, varias referencias científicas actuales estaban confirmando este hecho en productos de todo el mundo (Andrée, Jira, Schwind, Wagner, & Schwagele, 2010; Djinovic et al., 2008; García-Falcón & Simal-Gándara, 2005; Santos

et al., 2011). En este trabajo se realizó un estudio completo y novedoso sobre la penetración de BaP en diferentes profundidades en el chorizo asturiano ahumado. Se encontró que la cantidad de BaP disminuye y el contenido de humedad aumenta progresivamente desde la superficie (profundidad D1: 20,0 ± 1.1 µg/kg, 12,4% ± 1,4%), hasta el interior del producto (profundidades D2: 1,63 ± 0.11 µg/kg, 29.2% ± 1,5%; D3: 1,12 ± 0,10 µg/kg, 35,5% ± 0,3%; y D4: 0,88 ± 0,10 µg/kg, 33,8% ± 1,3%). Estos datos permitieron proponer un mecanismo esquemático que explica la penetración de BaP y el secado del chorizo durante el proceso de ahumado. Resultó de especial relevancia encontrar un contenido de BaP de 20 µg/kg en la superficie del producto. Por ello, los productores deben supervisar el estado de las tripas durante el procesado. Si la tripa se rompe los HAP pueden entrar más fácilmente en el interior del producto. Por otro lado, se aconseja a los consumidores que eliminen la tripa natural (también llamada "piel") del chorizo ahumado, antes de su consumo.

Dada la relevancia de los resultados obtenidos en el segundo estudio, relacionados con el elevado contenido de BaP en la superficie del producto, el tercer estudio (**Capítulo 4.3**) se enfocó en evaluar el flujo de este compuesto a través de la tripa, que funciona como una membrana. Varias referencias científicas habían dilucidado en los años 70 que el uso de distintos tipos de tripa tendría influencia en el contenido de HAP de los productos cárnicos (Filipovic & Toth, 1971; Toth, 1973), siendo por lo tanto el punto de mira de las investigaciones actuales (García-Falcón & Simal-Gándara, 2005; Gomes et al., 2013; Pöhlmann et al., 2013a; Škaljac et al., 2014), las cuales coincidieron en el tiempo con las nuestras. Por tanto, en esta tesis se estudió el contenido de BaP durante el ahumado de chorizo asturiano embutido en tripa natural (ChN) y sintética, de colágeno (ChS). Teniendo en cuenta los resultados obtenidos en el segundo estudio, se diseñó paralelamente un sistema novedoso de tripa llenada con aceite, que permitiera eliminar las barreras causadas por las distintas profundidades encontradas en el producto cárnico, siendo las grasas un medio idóneo para la dilución y concentración de los compuestos de interés, ya que los HAP son compuestos lipofílicos (Vázquez Troche et al., 2000), que se disuelven en las grasas en estado líquido de manera uniforme. En ambos sistemas (chorizo y aceite), se encontró que el BaP no atravesaba la tripa sintética. Al contrario, se encontró un contenido de 2,16 ± 0,12 µg/kg y 1,71 ± 0,15 µg/kg en el interior de los sistemas de aceite y ChN, respectivamente. Por otro lado, confirmando los resultados del estudio previo, se encontró un contenido de 19,9 ± 2,9 µg/kg en el exterior de la tripa natural. Nuestros resultados fueron corroborados por otros autores que realizaron estudios simúlatenos en el tiempo, encontrando que las tripas sintéticas previenen la

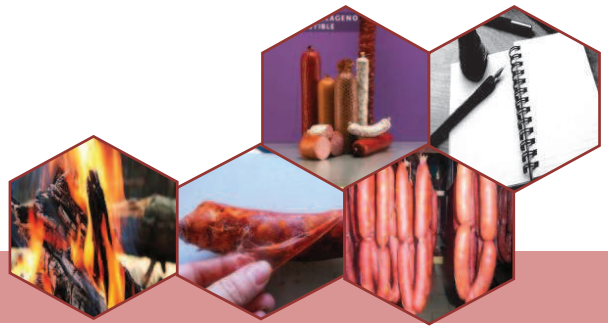
penetración de HAP en otros productos cárnicos ahumados (Gomes et al., 2013; Pöhlmann et al., 2013a; Škaljac et al., 2014). Dado que el hecho estaba confirmado, se planteó como estudio en la frontera del conocimiento encontrar una explicación científica que justificara estos hechos. Para ello se caracterizaron las diferencias físicas entre los 2 tipos de membrana (tripa natura y de colágeno), y la evolución de los productos cárnicos durante el tiempo de ahumado. Los estudios planteados fueron la determinación de la porosidad de las tripas, y caracterización morfológica de ambos tipos de tripas sin ahumar y ahumadas, y 48 productos embutidos en ellas durante el proceso de ahumado, de 0 a 10 días. Para la caracterización morfológica se utilizaron las técnicas microscopía electrónica de barrido (SEM) y microscopía de fluorescencia óptica. La mayor dificultad fue la imposibilidad de determinar la porosidad de la tripa natural en estado húmedo, pues el dispositivo experimental (porosímetro) no permite la medida de muestras húmedas. Para resolver este problema se creó un sistema novedoso de secado lento de ambos tipos de tripa, que no dañase su estructura y sometiera a ambas a las mismas condiciones. Los resultados obtenidos fueron los más novedosos a nivel científico de la presente tesis doctoral y permitieron proponer un mecanismo que explica porqué el BaP atraviesa la membrana natural y no la sintética, basado a demás en una de las variables fijadas por el Codex Alimentarius: lo que sucede al contenido graso del producto durante el ahumado. Además, el mecanismo que lo explica fue completado mediante el estudio bibliográfico del proceso tecnológico de ahumado y el estado del arte sobre contaminación de HAP en productos cárnicos, lo cual dio lugar a la redacción de un review (**Capítulo 4.5**). A modo de resumen, del estudio bibliográfico se concluye que los productos cárnicos pueden ser "precontaminados" por HAP debido a la contaminación ambiental de las materias primas (CCA, 2004, 2009), en segundo lugar, los procesos tecnológicos diferentes al ahumado pueden contribuir al contenido total de HAP de los alimentos (CCA, 2009; Chung et al., 2011; Kazerouni, Sinha, Hsu, Greenberg, & Rothman, 2001). Finalmente, durante el proceso tecnológico de ahumado, la combustión incompleta y pirólisis de biomasa a temperaturas superiores a 750 ºC da lugar a la formación de compuestos de tar terciarios, los cuales contiendo los HAP (Basu, 2010, chap.4), son transportados en forma de aerosol hasta los productos cárnicos dentro de la cámara de ahumado. Por otro lado, en los experimentos realizados en esta tesis doctoral, se encontró que las tripas naturales se caracterizan por su rugosidad y capacidad para atrapar cuerpos pequeños, mientras que las tripas de colágeno se caracterizan por formar una red-barrera de fibras sin capacidad para capturar cuerpos pequeños. Una vez dentro de la cámara de ahumado, los chorizos embutidos en distintos tipos de tripa, natural (ChN) y sintética (ChS), se calientan y comienzan a secarse. La elevada porosidad de las tripas naturales (66,8%)

hace que incluso las materias primas crucen sus poros. Durante el ahumado, estos chorizos (ChN) se calientan, se hinchan y la grasa fluye a través de la tripa natural, convirtiendo la superficie de los chorizos en un medio pegajoso donde las partículas de humo son fácilmente capturadas y adheridas, y finalmente migran a través de los poros de gran diámetro (599,8 nm) de la tripa natural hacia el interior del producto. Al contrario, la morfología lisa y la baja porosidad (16,6%) de las tripas sintéticas hace que éstas funcionen como una barrera. Los chorizos embutidos en esta tripa (ChS) no se hinchan, y la grasa permanece en el interior del producto, mostrando una superficie seca, no pegajosa y lisa, con menos afinidad a capturar las partículas de humo que contienen HAP, cuya dificultad de penetrar en el producto se incrementa debido al pequeño diámetro de los poros (48,2 nm). Estos hechos explican los resultados sobre penetración de BaP a través de la tripa natural encontrados en nuestros estudios previos, así como por García-Falcón y Simal-Gándara (2005), Gomes et al. (2013) y Škaljac et al. (2014). El código de prácticas para reducir la contaminación por HAP en los alimentos producidos por procedimientos de ahumado y secado directo, (CCA, 2009), indica que el humo consta de particulados líquidos y sólidos suspendidos en una fase gaseosa, formados por numerosos componentes, entre los que se encuentran los HAP. Según el código, se estima que el tamaño de las partículas del humo oscila entre 0,2 y 0,4 µm (o un tamaño tan pequeño como 0,05 a 1 µm), las cuales constituyen el 90% del peso total del humo. Estos datos justifican la teoría expuesta en esta tesis. Así, el BaP es un HAP transportado por las partículas del humo hasta los productos cárnicos. Estas partículas no penetran a través la tripa sintética, dado que el tamaño de las partículas más pequeñas (50 nm), es mayor que el diámetro medio de los poros de la tripa sintética (48,2 nm); sin embargo si penetran a través de la tripa natural, ya que el tamaño medio de las partículas (entre 200 y 400 nm) es menor que el tamaño medio del diámetro de los poros de la tripa natural (599,8nm).

Por último, se planteó determinar si las propiedades físicas y organolépticas de los chorizos son adecuadas en ambos casos, embutidos en tripa natural (ChN) y en sintética (ChS). Por tanto, en el último estudio (**Capítulo 4.4**), se determinaron las características físicas (textura, color y humedad) de 48 chorizos asturianos, embutidos en distintos tipos de tripa, durante el tiempo de ahumado (0 a 11 días). Además también se evaluó el proceso de adherencia y penetración de las partículas en distintas profundidades del producto. A modo de resumen, la tendencia general encontrada es que las propiedades son adecuadas en los dos casos, sin embargo los productos se oscurecen y endurecen significativamente antes ($p<0.05$) si son embutidos en la tripa sintética. Además, aunque las diferencias no fueron significativas ($p>0.05$),

la chorizos embutidos en esta tripa podrían secarse antes, pues mostraron siempre menor contenido de humedad. En este estudio se demostró que una vez que las partículas de humo llegan y penetran a través de la tripa natural, se colocan en las cavidades de la masa del producto, las cuales se cierran durante el secado, trasladándolas hasta el interior del producto (hecho que explica encontrar BaP en el interior del producto en el **capítulo 4.2**). Además, los chorizos embutidos en tripa sintética muestran una morfología homogénea que facilita la estandarización del producto, característica demanda por los distribuidores y consumidores. Estos resultados demuestran que las tripas sintéticas son capaces de reducir el tiempo de procesado, y garantizar la estandarización del producto, validando estos factores sugeridos por los fabricantes de tripas sintéticas (CCTA, 2015; DEVRO, 2015; FIBRAN, 2015; VISCOFAN, 2015), y además protegen el producto de la contaminación por HAP, con la ventaja de seguridad alimentaria que esto implica. Es importante señalar que el uso de tripas naturales es correcto y ofrece buenas propiedades físicas y tecnológicas al producto, tal y como sugieren sus fabricantes (ENSCA, 2015; FEDECARNE, 2013; INSCA, 2015), siempre y cuando no sea sometido a un proceso tecnológico de ahumado directo intenso, prolongado e incontrolado, que los contamine con HAP. El control de las variables del proceso de ahumado, de acuerdo a las recomendaciones del CCA y los resultados obtenidos por muchos investigadores, permite el uso de las tripas naturales para la producción de cárnicos ahumados.

Finalmente, los datos actuales de contenido de BaP y HAP4 en productos cárnicos y alimentos ahumados de todo el mundo son todavía muy elevados en algunos casos, especialmente donde se aplica el ahumado directo, en las pequeñas empresas cárnicas de los países desarrollados y más extensamente en los países en desarrollo. Las técnicas de ahumado pueden controlarse para alcanzar valores por debajo de la normativa europea, que garanticen la seguridad alimentaria de estos productos de importante valor económico y nutricional para la dieta humana.



6

Conclusiones

6. CONCLUSIONES

El trabajo expuesto en la presente tesis doctoral permite obtener las siguientes conclusiones:

- El ahumado directo es una de las técnicas de conservación de alimentos más antiguas, y ha tenido un rol importante en la evolución y la dieta humana. Los principales tipos de alimentos ahumados son las carnes, los pescados, los quesos, algunas especies y bebidas. Esta técnica ha sido remplazada en algunos países desarrollados por modernas técnicas de preservación de alimentos frescos y tecnologías optimizadas de ahumado. Sin embargo sigue siendo utilizado por algunas pequeñas empresas de los países desarrollados, y más extensamente en los países en desarrollo.

- Durante el ahumado directo se forman numerosos compuestos, que ejercen efectos deseados y no deseados sobre los alimentos. Entre los efectos deseados destacan la prolongación de la vida útil y la mejora de las propiedades organolépticas, color, textura y flavor, de los alimentos. Entre los efectos no deseados destaca la contaminación por sustancias tóxicas y cancerígenas, como los hidrocarburos aromáticos policíclicos (HAP), las n-nitrosaminas, las aminas aromáticas heterocíclicas y los β-carbonilos. Las investigaciones se han focalizado en los HAP debido a su elevada actividad cancerígena, y especialmente en productos cárnicos ahumados, ya que se ha encontrado que son los principales contribuyentes de HAP en la dieta, y que contienen los niveles más elevados de HAP.

- El control de las variables del proceso tecnológico de ahumado, recomendadas por el Codex Alimentarius CAC/RCP68/2009, permite minimizar la contaminación de los productos cárnicos por HAP durante el proceso, obteniendo valores de benzo(a)pireno (BaP) (un marcador de la presencia y efecto de los HAP en alimentos) por debajo del contenido máximo permitido, reducido a 2 µg/kg por la Regulación Europea No. 835/2011, e introducido el 1 de septiembre de 2014.

- La combinación de las técnicas de pretratamiento de muestras, sonicación y extracción en fase sólida (SPE), y la determinación analítica mediante cromatografía de gases/espectrometría de masas (GC/MS), es un método de análisis óptimo para la determinación del contenido de BaP en chorizo ahumado a modo directo, implicando además una reducción del consumo de disolventes, generación de residuos, tiempo de operación y complejidad del montaje, respecto a técnicas clásicas, como la combinación de soxhlet y cromatografía de permeación de gel (GPC).

- El rango de contenido de BaP encontrado en muestras de chorizo ahumado (medido sin tripa) elaborado por empresas del Principado de Asturias fue de 0,38–3,21 µg/kg. Cinco de las 16 muestras superan el contenido máximo legal de BaP. No se encontró correlación entre el contenido de BaP y la humedad, relevando la baja calidad del ahumado directo, utilizado como método de secado. Por tanto, las empresas del Principado de Asturias deben optimizar su método de ahumado directo, para obtener chorizos de elevada calidad y seguridad alimentaria.

- El incremento del tiempo de ahumado, desde 0 a 7 días, produce efectos contrarios entre el contenido de BaP y la humedad del chorizo, medido sin tripa. Mientras que el contenido de humedad decrece (desde 49,9% ± 3,2% hasta 31,3% ± 1,2%), el contenido de BaP aumenta con el tiempo de ahumado (desde menos de 0,24 µg/kg hasta 0,75 ± 0,05 µg/kg), y finalmente se estabiliza desde los 5 hasta los 7 días de ahumado, con un valor por debajo del límite legal europeo.

- La selección y descripción de la profundidad de la muestra tomada es un parámetro fundamental al definir el contenido de BaP del chorizo ahumado a modo directo, embutido en tripa natural. El contenido de BaP decrece, y la humedad aumenta, progresivamente desde la tripa (20,0 ± 1,1 µg/kg, 12,4% ± 1,4%) hasta el interior del producto (hasta el núcleo: 0,88 ± 0,10 µg/kg, 33,8% ± 1,3%). El contenido de BaP en la tripa del chorizo es 10 veces superior al límite legal y casi 20 veces mayor que en el núcleo. Por tanto, se recomienda retirar la tripa del chorizo ahumado, antes del consumo. Con el aumento del tiempo de ahumado los componentes del humo podrían obstruir los poros de la tripa, generando una barrera para la penetración de mayor

contenido de BaP. Sin embargo, la degradación de las tripas podría producir el efecto contrario.

- Durante el ahumado directo, tienen lugar la pirolisis y la combustión incompleta de la biomasa, dando lugar a la formación de tar. Cuando la temperatura alcanza los 750ºC se comienzan a formar los productos de tar terciaros, entre los cuales se encuentran los HAP. En la cámara de ahumado, los HAP son transportados hacia los productos cárnicos a través de aerosoles de tar, que forman parte del humo. Al mismo tiempo, los chorizos se calientan y comienzan a secarse. Las tripas natural (de cerdo) y sintética (de colágeno) presentan diferencias físicas que producen un comportamiento diferente de los chorizos embutidos en ellas.

- La morfología irregular, la elevada porosidad (66,84%), y el gran diámetro de los poros (599,8 nm diámetro medio) de la tripa natural, hace que la grasa del chorizo fluya a través de ella, el chorizo se hinche, y su superficie se convierta en un material pegajoso donde se adhieren fácilmente las partículas del humo, cuyo tamaño, de acuerdo al Codex Alimentarius, oscila entre los 200 y 400 nm. Por ello, las partículas penetran a través de los poros de la tripa, se colocan en las grandes cavidades de la masa cruda, y comienzan a migrar hacia el interior del producto a través de la grasa, y la compactación de la masa durante el secado, contaminado el interior del chorizo con BaP.

- Por el contrario, la tripa de colágeno tiene una morfología lisa y baja porosidad (16,6%). La grasa permanece en el interior del chorizo embutido en ella, mostrando una superficie seca y dura, con menor afinidad a capturar las partículas de humo, cuya posibilidad de penetrar en el producto está limitada por el pequeño diámetro de sus poros (48,2 nm), impidiendo la penetración y contaminación del producto con BaP.

- Durante el ahumado directo, los chorizos embutidos en tripa de colágeno se oscurecen y endurecen significativamente ($p<0,05$) antes, y se secan más rápidamente (aunque las diferencias no fueron significativas, $p>0,05$), que los embutidos en tripa natural. Los chorizos embutidos en tripa de colágeno muestran una morfología homogénea, que facilita la estandarización del producto, característica demanda por las grandes empresas distribuidores y de platos precocinados. Los chorizos embutidos en tripa natural presentan una morfología irregular, característica demandada por el consumidor de

producto tradicional. Las tripas natural y de colágeno confieren al chorizo buenas propiedades de textura, color y humedad, pero la tripa de colágeno permite reducir el tiempo de procesado y prevenir la contaminación por BaP en el interior del producto, si es ahumado a modo directo.

- El contenido de BaP en productos cárnicos ahumados mediante diferentes técnicas, reportado por la comunidad científica, todavía sobrepasa en algunos casos el límite reglamentario. De la revisión de las referencias científicas, se puede concluir que 3 de las variables recomendadas por el Codex Alimentarius tienen mayor influencia para prevenir la contaminación por HAP de productos cárnicos ahumados: la temperatura de generación del humo, el tipo de tripa y el método de ahumado (directo o indirecto). Se deben aplicar temperaturas por debajo de 600ºC para evitar la formación de HAP, el uso de sistemas de ahumado indirecto, como el ahumado por fricción o el humo líquido, minimiza el contenido de BaP generado, y el uso de tripas sintéticas previene la penetración de los HAP en los productos cárnicos ahumados por cualquier método. El control de otras variables, como la madera, el tiempo de ahumado, la posición del alimento respecto a la fuente de calor, y la grasa del producto y su evolución, puede ayudar a reducir la cantidad de HAP del producto final.

- El proceso de ahumado directo de chorizo debe, y puede, ser optimizado mediante el control de variables recomendadas por el Codex Alimentatius, como el tiempo de ahumado, la selección del tipo de tripa y su eliminación antes del consumo, para minimizar la contaminación por BaP, y respetar los nuevos límites establecidos por la regulación europea.

- La aplicación de tecnologías modernas de ahumado y ahumado directo de un modo controlado, permite mantener los efectos deseados y prevenir los no deseados del ahumado directo. Esto es importante en algunas pequeñas empresas alimentarias de los países desarrollados, y especialmente en los países en desarrollo, donde el ahumado directo es todavía el principal método de conservación de alimentos.

7

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Anexos

8. ANEXOS

8.1 DIFUSIÓN DE LA TESIS DOCTORAL

8.1.1 ARTÍCULOS CIENTÍFICOS

- Ledesma, E., Rendueles, M., & Díaz, M. (2015). Spanish smoked meat products: Benzo(a)pyrene (BaP) contamination and moisture. Journal of Food Composition and Analysis, 37, 87–94.

- Ledesma, E., Rendueles, M., & Díaz, M. (2014). Benzo(a)pyrene penetration on a smoked meat product during smoking time. Food Additives and Contaminants, 31, 1688-1698.

- Ledesma, E., Rendueles, M., & Díaz, M. (2015). Characterization of natural and synthetic casings and mechanism of BaP penetration in smoked meat products. Food Control, 51, 195-205.

- Ledesma, E., Laca, A., Rendueles, M., & Díaz, M. Texture, colour and optical characteristics of a meat product depending on smoking time and casing type. Enviada a la revista Food Chemisty (Elsevier) para su evaluación, bajo revisión.

- Ledesma, E., Rendueles, M., & Díaz, M. Contamination by polycyclic aromatic hydrocarbons in the meat products smoking: Processes and Prevention. Enviado a la revista Food Control (Elsevier) para su evaluación, bajo revisión.

- Ledesma, E., Rendueles, M., & Díaz, M. Smoked Food. Capítulo de libro de Enciclopedia “Biotechnology” de Elsevier. Pendiente de envío.

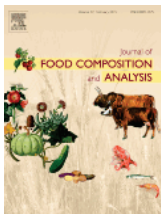

Figura 8.1. Revistas científicas de difusión de la presente tesis doctoral.

8.1.2 COMUNICACIONES A CONGRESOS

- E. Ledesma, M. Rendueles, M. Díaz. Physical characterization of natural and synthetic casings for polycyclic aromatic hydrocarbons (PAH) prevention in smoked meat products (keynote). II International Congress of Chemical Engineering (ICCE) of the Spanish's leading organization for Chemistry and Chemical Engineering professionals (ANQUE). Madrid (Spain). July 1-4, 2014.

Figura 8.2. ICCE.

- E. Ledesma, J.S.Monte, J.Monte, M.Rendueles, M.Díaz. Optimisation of analytical methods for BaP determination in smoked meat products (póster). 11th International Nutrition & Diagnostics Conference (INDC). Brno (Czech Republic).
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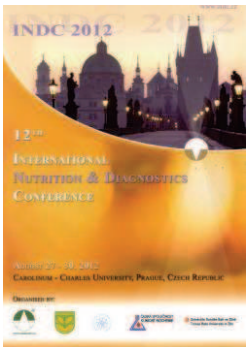

Figura 8.3. INDC.

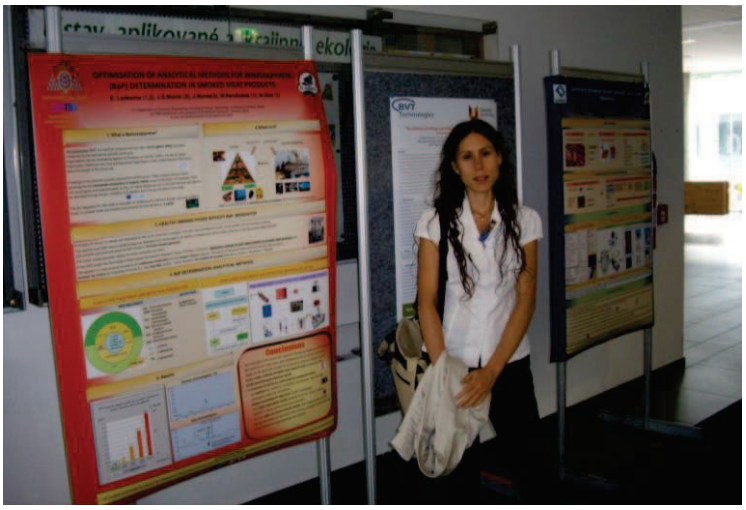

Figura 8.4. Presentación de poster INDC. Brno, República Checa, 28-31 de agosto, 2011.

8.2 INFORME DEL FACTOR DE IMPACTO DE LAS PUBLICACIONES PRESENTADAS

Los artículos que conforman la presente memoria de tesis doctoral, han sido publicados en revistas científicas incluidas en el Science Citation Index (Thomson Reuters), cuyos factores de impacto, y su posición en el área, en función del mismo, se presentan a continuación:

- ***Food Additives and Contaminants, part A*:**

  -Factor de impacto (2013): **2,341.**

  -Factor de impacto de los últimos 5 años (2013): **2,621.**

- ***Food Control*:**

  -Factor de impacto (2013): **2,819.**

  -Factor de impacto de los últimos 5 años (2013): **3,038.**

- ***Journal of Food Composition and Analysis*:**

  -Factor de impacto (2013): **2,259.**

  -Factor de impacto de los últimos 5 años, (2013): **2,799.**

8.3 NOMENCLATURA

8.3.1 ACRÓNIMOS

AETRIN	Asociación Española de Tripa Natural.
AICR	American Institute for Cancer Research.
ANICE	Asociación Nacional de Industrias de la Carne de España.
ASINCAR	Centro Tecnológico Agroalimentario "Asociación de Industrias Cárnicas del Principado de Asturias".
BOE	Boletín Oficial del Estado.
CCA	Comisión del Codex Alimentarius.
CCTA	Collagen Casings Trade Association.
CE	Comisión Europea.
EEUU	Estados Unidos de América.
ENSCA	European Natural Sausage Casings Association.
EPA	Agencia de Protección Ambiental de Estados Unidos.
FAO	Organización de las Naciones Unidas para la Alimentación y la Agricultura.
FEDECARNE	Federación Madrileña de Detallistas de la Carne.
HHS	United States Department of Health and Human Services.
IARC	Agencia Internacional para la Investigación del Cáncer.
INE	Instituto Nacional de Estadística.
INSCA	International Natural Sausage Casing Association.
JECFA	Comité Mixto FAO/OMS de Expertos en Aditivos Alimentarios.

MAGRAMA	Ministerio de Agricultura, Alimentación y Medio Ambiente.
OMS	Organización Mundial de la Salud.
SCF	Scientific Committee on Food of European Commission.
SCTs	Servicios Científico Técnicos de la Universidad de Oviedo.
UE	Unión Europea.
USA	United States of America.
USDA	United States Department of Agriculture.
WCRF	World Cancer Research Fund.

8.3.2 ABREVIATURAS

BaP	Benzo(a)pireno.
BaP d12	Patrón interno Benzo(a)pireno deuterado.
CCR	Cáncer colorectal.
ChN	Chorizo embutido en tripa natural.
ChS	Chorizo embutido en tripa sintética.
GC	Cromatógrafo de gases
HAP	Hidrocarburos aromáticos policíclicos
HAP 4	Suma de los siguientes 4 hidrocarburos aromáticos policíclicos: benzo(a)pireno, benzo(a)antraceno, benzo(b)fluoranteno y criseno.
IGP	Indicación Geográfica Protegida.
LOD	Límite de detección.
LOQ	Límite de cuantificación.

MS	Espectrómetro de masas.
O.E	Objetivo específico.
O.G	Objetivo general.
PVDC	Cloruro de polivinilideno.
SCAN	Full scan spectrum analysis.
SEM	Microscopía electrónica de barrido.
SIM	Selected Ion Monitoring (Monitorización selectiva de iones).
S/N	Relación señal/ruido.

8.3.3 SÍMBOLOS

a*	Parámetro del espacio de color CIELAB. Representa la variación entre el magenta y el verde (valores negativos indican verde mientras valores positivos indican magenta).
b*	Parámetro del espacio de color CIELAB. Representa la variación entre el amarillo y el azul (valores negativos indican azul y valores positivos indican amarillo).
BI	Indice de pardeamiento ó enmarronecimiento.
d	Distancia (m).
Hr	Humedad relativa (%).
L*	Parámetro del espacio de color CIELAB. Representa la luminosidad de color (L*= 0 indica negro y L* = 100 indica blanco).
T	Temperatura (ºC).

8.3.4 SIMBOLOS CON LETRAS GRIEGAS

ΔE	Cambio total de color.
µL	Microlitros.
ρ_M	Densidad de la mezcla.
ρ_{hexano}	Densidad del hexano a 20 ºC (0,660 g/mL).
$\rho_{diclorometano}$	Densidad del diclorometano a 20 ºC (1,325 g/mL).
$\hat{x}_{hexano}$	Fracción en peso del hexano.
$\hat{x}_{diclorometano}$	Fracción en peso del diclorometano.