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Jennyfer Jazmin Osorio Monsalve

Impacto químico y sensorial de compuestos potencialmente migrantes provenientes de materiales emergentes destinados al contacto alimentario

Director/es
Nerín de la Puerta, María Consolación Cristina
Aznar Ramos, Margarita Pilar

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Tesis Doctoral

IMPACTO QUÍMICO Y SENSORIAL DE COMPUESTOS POTENCIALMENTE MIGRANTES PROVENIENTES DE MATERIALES EMERGENTES DESTINADOS AL CONTACTO ALIMENTARIO

Autor

Jennyfer Jazmin Osorio Monsalve

Director/es

Nerín de la Puerta, María Consolación Cristina
Aznar Ramos, Margarita Pilar

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Impacto químico y sensorial de compuestos potencialmente migrantes en materiales emergentes destinados al contacto alimentario

Memoria presentada por

JAZMIN OSORIO MONSALVE

Para optar al título de Doctor en Ciencia Analítica en Química

Dirigida por

Dra. Dª. CRISTINA NERÍN DE LA PUERTA

Dra. Dª. MARGARITA AZNAR RAMOS

La **Dra. Cristina Nerín de la Puerta**, Catedrática de la Universidad de Zaragoza en el Departamento de Química Analítica y la **Dra. Margarita Aznar Ramos**, Profesora Contratada Doctora en el Departamento de Química Analítica de la Universidad de Zaragoza,

CERTIFICAN:

Que la presente Memoria, titulada: "***Impacto químico y sensorial de compuestos potencialmente migrantes en materiales emergentes destinados al contacto alimentario***" presentada por **Doña Jazmin Osorio Monsalve** para optar al grado de Doctor en Ciencia Analítica, ha sido realizada bajo nuestra codirección en la Escuela de Ingeniería y Arquitectura (EINA) de la Universidad de Zaragoza, de acuerdo con los objetivos presentados en el Proyecto de Tesis aprobado por el Departamento de Química Analítica. Por tanto, autorizamos su presentación para proseguir con los trámites oportunos y proceder a su calificación por el tribunal correspondiente.

En Zaragoza, a 18 de septiembre de 2020.

Dra. Cristina Nerín de la Puerta

Dra. Margarita Aznar Ramos

Departamento de Química Analítica
Universidad Zaragoza

Instituto Universitario de Investigación de Ingeniería de Aragón
Universidad Zaragoza

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A Rafał

“El escritor escribe su libro para explicarse a sí mismo lo que no se puede explicar”

Vivir para contarla - Gabriel García Márquez

ÍNDICE

GLOSARIO DE TÉRMINOS

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AA: Adipic Acid

AG: Aroma Groups

AMH: After Microwave Heating

AMI: Ambient Ionization Techniques

AMS: Ambient Mass Spectrometry

AOH: After Oven Heating

APCI: Atmospheric Pressure Chemical Ionization

APGC: Atmospheric Pressure Gas Chromatography

API: Atmospheric Pressure Ionization

ASAP: Atmospheric Pressure Solid Analysis Probe

BD: Butane-1,4-Diol

BH: Before Heating

BP: Starch-based Biopolymers

CE: Collision Energy

DAPCI: Desorption Atmospheric Pressure Chemical Ionization

DART: Direct Analysis in Real Time

DBDI: Dielectric Barrier Discharge Ionization

DBPG: 2,2-dibutyl-1,3-propanediol

DESI: Desorption ElectroSpray Ionization

DPG: Dipropylene glycol

DVD/CAR/PDMS: Divinylbenzene/Carboxen/Polydimethylsiloxane

EESI: Extractive Electrospray Ionization

EF: Enrichment Factor

EFSA: European Food Safety Authority

EI: Electron Ionization

ESI: Electrospray Ionization

F (%): Percentage of Frequency

FCM: Food Contact Materials

FDA: Food and Drug Administration

FG: Functional Group

GC: Gas Chromatography

GC-O: Gas Chromatography-Olfactometry

HD: 1-6-Hexanediol

HDPE: High Density Polythylene

HF-LPME: Hollow Fiber - Liquid Phase Microextraction

HPLC: High-Performance Liquid Chromatography

HRMS: High Resolution Mass Spectrometry

HS: Headspace

HS-SPME: Headspace Solid-Phase Microextraction

I (%): Average Percentage of Intensity

IAS: Intentionally Added Substances

i-BuOH: Isobutanol

IT: Ion Trap

KI: Kovats Index

LA: Lactic Acid

LDPE: Low Density Polyethylene

LLE: Liquid-Liquid Extraction

LLME: Liquid-Liquid Microextraction

LOD: Limit of Detection

LOQ: Limit of Quantification

LTP: Low-Temperature Plasma

MF: Modified Frequency

MS: Mass Spectrometry

MS^E: High and low energy acquisition mode in Mass Spectrometry

NIAS: Non-Intentionally Added Substances

NIST: National Institute of Standards and Technology

NOAEL: No Observed Adversed Ejject Level

OAV: Odor Activity Value

OML: Overall Migration Limit

PA: Polyamide

PCL: Polycaprolactone

PE: Polyethylene

PET: Polyethylene Terephthalate

PFBHA: o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride

PG: Propylene glycol

PHA: Polyhydroxy Alkanoates

PHB: Polyhydroxybutyrate

PLA: Polylactic Acid

PP: Polypropylene

PPG: polypropylene Glycol

PS: Polystyrene

PU: Polyurethane

PVA: Polyvinyl Alcohol

PVC: Polyvinyl Chloride

Q: Quadrupole

QDA: Quantitative Descriptive Analysis

R^2: Correlation coefficient

RI: Retention Index

RSD (%): Relative Standard Devation

SML: Specific Migration Limit

SPE: Solid-Phase Extraction

SPME: Solid-Phase Microextraction

SVP: Standardized Voltage and Pressur

TIC: Total Ion Chromatogram

ToF: Time of Flight

TPA: Terephthalic Acid

TPS: Thermoplastic-Like Starch

TTC: Threshold of Toxicological Concern

UPLC: Ultra-Performance Liquid Chromatography

PRESENTACIÓN

PRESENTACIÓN

La presente tesis se titula ***"Impacto químico y sensorial de compuestos potencialmente migrantes en materiales emergentes destinados al contacto alimentario"*** y se enmarca en el campo de la investigación de la seguridad del envase alimentario que está llevando a cabo el Grupo Universitario de Investigación Analítica (GUIA) del Departamento de Química Analitica de la Universidad de Zaragoza. El grupo GUIA está liderado por la Dra. Crisitna Nerín de la Puerta y está integrado en el Instituto de Investigación en Ingenieria en Aragón (I3A).

Esta tesis está enmarcada en el proyecto de investigación SENEMs (AGL2015-67362-P) del Programa Estatal de Fomento de la Investigación Científica y Técnica de Excelencia. Parte de la tesis fue realizada en el Institute for Global Food Security de Queen´s University Belfast, durante una estancia de tres meses financiada parcialmente por el programa ERASMUS +

Esta tesis doctoral se centra en el estudio de los compuestos migrantes que se transfieren desde envases emergentes hacia simulantes alimentarios. Concretamente en los compuestos odorantes que pueden afectar la calidad del alimento y los potenciales migrantes provenientes de un material para cocinado en envase y biopolímeros.

Además, se ha realizado un estudio exhaustivo de la composición de estos materiales, se han determinado algunos de los parámetros de migración más influyentes y se han establecido librerías de los potenciales migrantes. Todo ello se ha logrado por medio de la optimización de una gran variedad de técnicas de tratamiento de muestras, y de la implementación de potentes técnicas analíticas para la detección y cuantificación de los compuestos.

La memoria se ha estructurado en seis secciones:

La sección I ofrece una introducción general del marco en el que se ha desarrollado la tesis.

La sección II plantea los objetivos que se pretenden conseguir durante el desarrollo del trabajo.

La sección III presenta el desarrollo experimental que se divide en seis capítulos repartidos en tres subsecciones. Todos los capítulos son autocontenidos, es decir, inicialmente incorporan un pequeño resumen y los objetivos más relevantes del capítulo en español, y posteriormente un esquema del trabajo, una introducción, los materiales utilizados, los métodos desarrollados, así como los resultados, discusiones y principales conclusiones en inglés.

Métodos de pre-concentración en muestras de migración

- **Capítulo 1:** Analysis of isophthalaldehyde in migration samples from polyethylene terephthalate packaging.

Análisis de materiales destinados al cocinado en envase

- **Capítulo 2:** Release of volatile compounds from cooking plastic bags under different heating sources.

Análisis de biopolímeros

- **Capítulo 3:** Determination of volatile non intentionally added substances coming from a starch-based biopolymer intended for food contact by different gas chromatography-mass spectrometry approaches.

- **Capítulo 4:** Identification of key odorant compounds in starch-based polymers intended for food contact materials.

- **Capítulo 5:** Rapid and simultaneous determination of polyester oligomer as migrants from biopolymers by Direct Analysis in Real Time mass spectrometry.

- **Capítulo 6:** Ambient mass spectrometry as a tool for a rapid and simultaneous determination of migrants coming from a bamboo-based biopolymer packaging.

La sección IV se recogen las conclusiones más relevantes del trabajo

La memoria finaliza con las secciones V y VI, en las cuales se muestran, respectivamente, las publicaciones que derivan de este trabajo y las referencias bibliográficas consultadas.

A continuación, se muestra un esquema con la estructura de la tesis.

Sección I: Introducción General

Sección II: Objetivos

Sección III: Desarrollo Experimental

Métodos de pre-concentración en muestras de migración

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Capítulo 6: Ambient mass spectrometry as a tool for a rapid and simultaneous determination of migrants coming from a bamboo-based biopolymer packaging.

Sección IV: Conclusiones Generales

Sección V: Publicaciones

Sección VI: Bibliografía

SECCIÓN I: Introducción General

INTRODUCCIÓN

1. MATERIALES PARA ENVASE ALIMENTARIO

En la actualidad existen una gran cantidad de materiales destinados al envase alimentario. En general todos cumplen dos funciones básicas: contener y proteger el alimento del medio exterior. Los envases evitan que agentes como la luz, los microorganismos, el oxígeno y el agua, entre otros, alteren la calidad y las propiedades organolépticas del alimento. Se busca a su vez que estos materiales sean de fácil implementación, económicos, respetuosos con el medio ambiente, reutilizables o reciclables, y que cumplan con las reglamentaciones establecidas (Rodríguez et al., 2019).

Entre los materiales más comunes para la fabricación de envases alimentarios se encuentran los plásticos, el cartón, el papel, el vidrio, el metal y los materiales multicapa (Muncke, 2016). Una de las tendencias más actuales en el campo del envase alimentario es el desarrollo y la utilización de nuevos materiales bioplásticos. Esta necesidad se ha generado debido a los daños ambientales causados por los residuos provenientes de materiales plásticos convencionales de origen fósil.

1.1. PLÁSTICOS

Los plásticos son los materiales más utilizados en la fabricación de envases alimentarios. Se pueden encontrar en una gran diversidad de formatos, como botellas, láminas, bandejas y bolsas, entre otros. Consisten en polímeros orgánicos obtenidos a partir de moléculas de bajo peso molecular, denominadas monómeros, que reaccionan entre ellas a través de reacciones de polimerización, policondensación, o poliadición, entre otras. Normalmente, los monómeros de los plásticos convencionales se obtienen a partir de productos petroquímicos. Entre los plásticos más usados para la fabricación de envases alimentarios están el tereftalato de polietileno (PET), el polipropileno (PP), el polietileno de alta densidad (HDPE) y de baja densidad (LDPE), el poliestireno (PS), la

poliamida (PA) y el cloruro de polivinilo (PVC). También es muy común la fabricación de plásticos multicapa que son combinaciones de varios tipos de polímeros e incluso papel, cartón y aluminio, unidos por capas de adhesivos, generalmente de poliuretano (PU) (Geueke et al., 2018).

1.2. BIOPLÁSTICOS

Debido a los problemas ambientales y económicos producidos por la contaminación de plásticos convencionales, el desarrollo de bioplásticos ha aumentado considerablemente en los últimos años. Actualmente representan un poco más del 1% del total de los plásticos del mercado, pero se prevé que en el futuro su porcentaje de uso aumente (Asgher et al., 2020). De acuerdo a la definición aportada por el *Food Packaging Forum*, los bioplásticos se pueden clasificar en dos grupos: los plásticos biodegradables y/o compostables, y los plásticos que provienen de fuentes renovables (figura 1) (Geueke, 2014).

Los biopolímeros pueden provenir de diferentes tratamientos de biomasa vegetales. Pueden ser extraídos directamente de fuentes naturales, tales como los polisacáridos (almidón, celulosa); ser producidos por fermentación bacteriana o síntesis química de azúcares, tales como los polihidroxialcanoatos (PHA) o por fermentación bacteriana a partir de monómeros biológicos, tales como el ácido poliláctico (PLA); o ser producidos por síntesis química a partir de momeros biológicos basados en polietileno y polipropileno (PE y PP) (Asgher et al., 2020)).

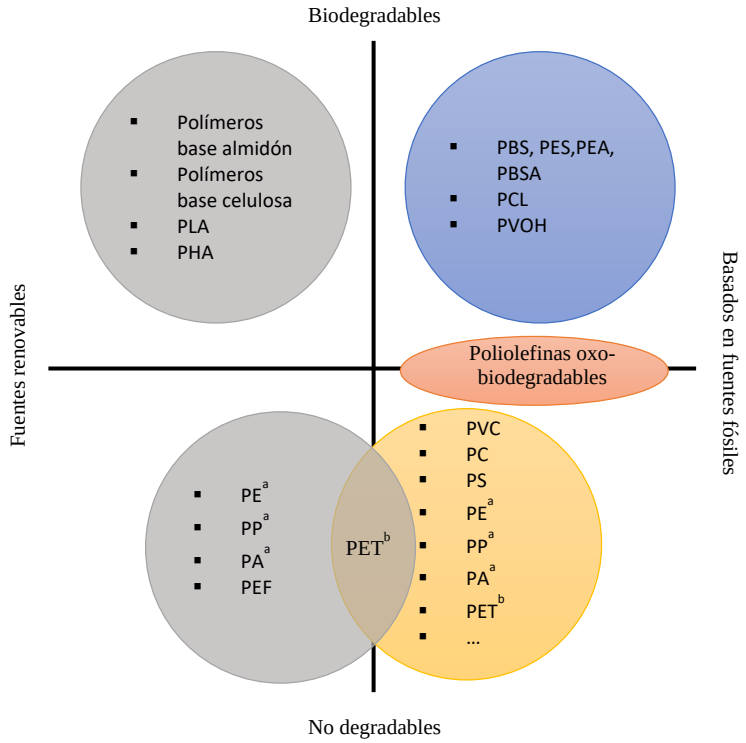


Figura 1. Clasificación de bioplásticos según su origen y su biodegradabilidad. [a]PE, PP, PA provienen de fuentes renovables o fuentes fósiles. [b]PET obtención por síntesis química (Geueke, 2014).

Normalmente los bioplásticos se combinan con poliésteres biodegradables, por medio de técnicas de co-polimerización, con el objetivo de mejorar sus propiedades físicas, como la fragilidad y la permeabilidad al oxígeno. Además, también es necesario la adición de aditivos similares a los usados en los plásticos convencionales, como plastificantes, antioxidantes o estabilizantes(Geueke, 2014).

1.2.1. Bioplásticos de PLA

El ácido poliláctico (PLA) es un poliéster alifático, compostable bajo condiciones industriales controladas y renovable, que se encuentra clasificado como bioplástico. Es uno de los biopolímeros de mayor producción a nivel mundial porque sus propiedades fisicoquímicas se asemejan mucho a las del tereftalato de polietileno (PET), uno de los plásticos más usados en la fabricación de envases (Asgher et al., 2020). Este biopolímero es termoplástico, moldeable y con una alta resistencia, características convenientes para la fabricación de films, fibras y piezas moldeadas. Debido a estas características, se usa

con frecuencia en la fabricación de envases alimentarios (Aznar et al., 2019) y en aplicaciones médicas (Farah et al., 2016).

La cadena polimérica de PLA está constituida por la unión de monómeros de ácido láctico (ácido 2-hidroxipropiónico, LA). El LA se obtiene por fermentación de la glucosa del almidón presente en alimentos como el maíz, la caña de azúcar, la yuca o la remolacha (Aznar et al., 2019). En 1932, se sintetizó por primera vez PLA de bajo peso molecular por medio de una reacción de policondensación del LA (Farah et al., 2016). Hoy en día, el PLA es obtenido principalmente mediante procesos de polimerización por apertura del anillo de la lactida (diester cíclico del ácido láctico), ya que permite la obtención de PLA de alto peso molecular (Figura 2). Todos estos procesos de síntesis requieren un control riguroso de la temperatura, presión y pH, además de largos tiempos de reacción y el uso de catalizadores (Farah et al., 2016).

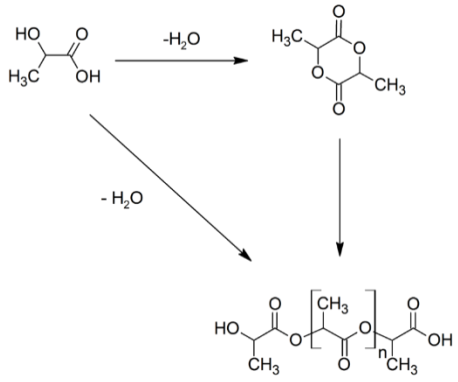


Figura 2. Proceso de deshidratación y apertura de anillo para la obtención de PLA de alto peso molecular.

La producción de PLA tiene algunas ventajas, como el respeto ambiental, la bio-compatibilidad con otros biopolímeros, compatibilidad con tejidos humanos para aplicaciones médicas, la alta eficiencia en el procesamiento térmico, y el menor coste de producción, ya que su consumo energético es un 25 – 55% menor que el de otros plásticos convencionales. Sin embargo, los materiales de PLA son relativamente frágiles, son permeables al oxígeno y se degradan fácilmente por la hidrólisis de los

grupos éster de la cadena principal (Farah et al., 2016). En la industria del envase alimentario, estas desventajas se superan por medio de la formación de co-polímeros mediante la adición de poliésteres en el proceso de fabricación. Algunos de los poliésteres usados son polímeros biodegradables (base almidón o celulosa) o poliésteres producidos por exceso de carbohidratos durante crecimiento bacteriano, polihidroxialcanoatos (PHAs), polihidroxibutiratos (PHBs), entre otros. También se agregan en el proceso algunos aditivos poliméricos como plastificantes (Aznar et al., 2019).

1.2.2. Bioplásticos base almidón

El interés por el desarrollo de materiales base almidón se debe principalmente a que el almidón proviene de fuentes renovables y es biodegradable. La producción y degradación del almidón es un proceso cíclico. Las plantas lo sintetizan por medio de la fotosíntesis, usando dióxido de carbono y agua; posteriormente, enzimas y/o microorganismos se encargan de la degradación, metabolizándolo en dióxido de carbono y agua. Además, los bioplásticos base almidón no presentan tanta permeabilidad al oxígeno, al contrario que los materiales de PLA (Averous et al., 2014). Aunque en un principio el almidón se empleó como relleno para aumentar la biodegradación de los materiales, la tendencia actual es la producción de bioplásticos con un porcentaje de almidón superior al 80%. La mayoría de sus aplicaciones están relacionas con materiales destinados al contacto alimentario (González et al., 2011).

El almidón es un carbohidrato natural que se encuentra en gran variedad de alimentos, tales como la patata, el maíz, el trigo, la yuca o el arroz. También se encuentra en forma de gránulos en las raíces, semillas y tallos de las mismas plantas. La estructura consiste en un polisacárido formado a partir de amilosa, un polímero lineal, y amilopectina, un polímero ramificado, que se unen por medio de moléculas de glucosa. La cantidad de amilosa en los gránulos de almidón suele estar cerca del 30%, aunque puede variar levemente en función de la fuente de almidón. Los gránulos de almidón también contienen pequeñas cantidades de lípidos y proteínas (González et al., 2011).

Los bioplásticos base almidón pueden ser procesados usando las técnicas empleadas para la producción de los plásticos convencionales. Sin embargo, el procesamiento de almidón puro es complicado debido a su alta viscosidad, la gran cantidad de agua, las formulaciones de los materiales, y principalmente la baja calidad del material obtenido. Para solucionar estos problemas, se recurre a la adición de plastificantes como el glicerol que ayuda a disminuir el grado de fragmentación molecular. También es común la adición de otros copolímeros procedentes de fuentes naturales como PLA, PHAs y PHBs, o derivados de combustibles fósiles (Dilkes-Hoffman et al., 2018). La extrusión por mezclado es un método eficiente, porque permite controlar la interacción entre el plastificante, el almidón y los aditivos. Sin embargo, en la industria también se utilizan metodologías como extrusión de película, moldeado por inyección y termoformado (Zhang et al., 2014) .

1.2.3. Bioplásticos base bambú

En los últimos años el interés por los materiales procedentes de bambú ha incrementado, principalmente porque el tiempo de crecimiento de las plantas de bambú es corto y el proceso de producción es sostenible. Además, el bambú tiene buenas propiedades mecánicas porque sus fibras están alineadas longitudinalmente, favoreciendo la resistencia de la planta y por tanto de los materiales (Wang et al., 2016). Debido a sus características, los materiales de bambú se utilizan en diversas aplicaciones, entre ellas en la producción de envases destinados al contacto con alimentos (Zuo et al., 2018).

Las fibras de bambú pueden ser obtenidas por varias metodologías de extracción. Sin embargo, los tres métodos más usados son: la extracción mecánica (exposición de vapor, trituración, molienda, laminación), la extracción química (extracción alcalina o ácida) y una combinación de ambas. En estos procesos se cuida que las fibras obtenidas sean largas, finas y rectas, para que puedan mantener las características originales de la planta de bambú (Wang et al., 2016).

Los materiales que consisten en la mezcla de las fibras de bambú con diversos polímeros han resultado tener algunas ventajas ambientales sobre los productos provenientes casi en exclusiva de bambú. Por ejemplo, la producción de estos no genera tantos problemas de contaminación por el alto consumo de energía (Zuo et al., 2018), su producción es de alto rendimiento, y además tienen un gran valor agregado (Wang et al., 2016). Algunos de los materiales de bambú se producen por la mezcla entre la fibra de bambú y polietileno, polipropileno u otros polímeros no degradables (Zuo et al., 2018); o por adición de resinas poliméricas que ayudan a aumentar la resistencia térmica, como la melamina (Geueke, 2013). Sin embargo, una de las desventajas de estos materiales es que no se degradan fácilmente lo que puede causar serios problemas de contaminación. En la actualidad, se está trabajando en la producción de biomateriales base bambú por medio de la adición de co-polímeros biodegradables, como PLA, celulosa, base almidón, entre otros (Jena, 2018; Nakanishi et al., 2019).

2. FENOMENOS DE INTERACCIÓN

Cuando un alimento esta envasado se pueden dar varios fenómenos de interacción, beneficiosos o perjudiciales, entre el envase, el alimento y el entorno (Rodríguez et al., 2019). Las principales interacciones se describen en tres fenómenos.

- Permeación que permite el paso de agua, compuestos volátiles y energía en forma de luz, desde el entorno hacia el alimento, y viceversa.
- Absorción/Adsorción donde diversidad de sustancias del entorno y del alimento quedan retenidas en el material polimérico del envase.
- Migración donde se transfieren compuestos propios del envase al alimento.

3. MIGRACIÓN

El fenómeno de migración es de gran importancia, ya que supone la incorporación de sustancias no deseadas procedentes del material de envase a los alimentos. Estas sustancias pueden producir la degradación sensorial y nutricional del alimento alterando la calidad del producto; o ser compuestos tóxicos que comprometen la seguridad del alimento.

La migración es un proceso de trasferencia de masa complejo, que depende de factores físico-químicos, tanto del envase como de las sustancias migrantes y del alimento. Los procesos de migración se rigen por la difusión de los migrantes a través del envase y la partición, distribución, de los migrantes entre el alimento y el envase. Los dos parámetros que rigen el proceso de migración son (Aznar et al., 2015):

- Coeficiente de partición que expresa la relación de las concentraciones de los migrantes cuando se encuentran en equilibrio, entre el envase y el alimento.

- Coeficiente de difusión que determina la transferencia de los migrantes debida a movimientos aleatorios de una región de alta concentración a otra de menor concentración.

Estos mecanismos de migración se ven afectados por las características físico-químicas de los migrantes, como peso molecular y polaridad. En su gran mayoría los compuestos potencialmente migrantes tienen bajo peso molecular, inferior a 1000 Da, debido a que tienen coeficientes de difusión más altos. En la migración también influyen factores como la estructura, naturaleza y espesor del envase; el contenido de agua, grasa y cristalinidad del alimento; y las condiciones de almacenamiento y transporte del alimento (Aznar et al., 2015).

El análisis de la migración puede clasificarse en dos tipos: global y especifica. La migración global hace referencia la inercia química del material y se mide a través de la cantidad total de componentes que se transfieren desde el envase hacia el alimento, pero sin llevar a cabo la identificación de los componentes. Por otro lado, la migración

específica se refiere a la cantidad de cada sustancia concreta que se transfiere al alimento, lo que requiere la identificación de todas y cada una de las sustancias transferidas y su posterior cuantificación. En estas migraciones se evalúan las condiciones menos favorables de almacenaje y preparación de los alimentos (Muncke, 2016).

Los ensayos de migración del material se realizan de acuerdo al Reglamento Europeo EU/2011/10 (European Commission, 2011a), que establece las posibles condiciones de tiempo, temperatura y los simulantes alimentarios que debe usarse según el tipo de material del envase y el uso para el que esté previsto. Normalmente se estudian más en profundidad los NIAS con peso molecular menor a 1000 Da. (Wagner et al, 2018).

4. SUSTANCIAS MIGRANTES

Las sustancias que migran del envase al alimento pueden provenir de los materiales y aditivos usados para fabricar el envase o pueden ser formados durante el proceso de producción del mismo. Los migrantes se pueden clasificar en dos grupos dependiendo de si han sido añadidos de forma intencionada, los IAS, o no lo han sido, los NIAS (García Ibarra et al., 2019; Nerín et al., 2018)

4.1. IAS

Las sustancias intencionalmente adicionadas (IAS) son aquellas que han sido añadidas durante el proceso de fabricación del envase alimentario. Los monómeros y los solventes son las principales sustancias para la fabricación, pero es necesario el uso de aditivos para mejorar las propiedades fisicoquímicas del material. Entre los aditivos más comunes se encuentran los monómeros, plastificantes, antioxidantes, colorantes, estabilizadores térmicos, espesantes, tensoactivos, emulsionantes, ceras, agentes deslizantes, estabilizadores de luz, biocidas, entre otros. Los IAS también pueden encontrarse, no solo en el polímero, sino en los adhesivos y las tintas (Canellas et al.,

2015; Peters et al., 2019). La mayoría de estos compuestos pueden migrar a los alimentos si no se encuentran unidos de forma covalente al material (García et al., 2018).

4.2. NIAS

Las sustancias no añadidas intencionalmente (NIAS) pueden provenir de diferentes orígenes: reacciones durante el proceso de producción o productos de descomposición, pero también pueden tratarse de impurezas que se adicionan de forma involuntaria a partir de IAS o contaminantes provenientes de las etapas de producción, almacenamiento y/o transporte del envase alimentario. Estas sustancias también pueden llegar a interaccionar con el alimento bajo diversas condiciones de temperatura y almacenamiento (Nerin et al., 2013). Entre los diversos tipos de NIAS que son potencialmente migrantes de los envases plásticos se encuentran las sustancias odorantes, que comprometen la calidad, y los oligómeros, que comprometen la seguridad.

4.2.1. Sustancias odorantes

Las sustancias odorantes son compuestos volátiles responsables del aroma y de la percepción sensorial de todo lo que nos rodea. En la industria alimentaria, estos compuestos son de gran importancia, porque al ser asociados a sensaciones agradables o desagradables pueden influir positiva o negativamente en la calidad sensorial de un producto (Piergiovannyet al., 2019)

Entre las familias de compuestos odorantes que pueden migran del envase a los alimentos, se encuentran:

- Aldehídos, cetonas, alcoholes, éteres, alcanos, entre otros, como productos de degradación térmica formados por la oxidación de los compuestos orgánicos presentes en el material plástico. Entre los odorantes reportados se encuentran por ejemplo: el acetaldehído con olor frutal proveniente del PET (Piergiovanny et al., 2019); el nonanal y decanal con olores a verde y/o pepino en bolsas de PET (Aznar et al., 2020), el 2-etil-1-hexanol con olor a verde, el benzaldehído con olor a

almendra y/o azúcar, la acetofenona con olor a mosto, y naftaleno con olor a alquitrán detectados en muestras de PP, PE, papel y cartón (Vera et al., 2020)

- Ácidos carboxílicos, alcoholes y aldehídos, que pueden migrar de los adhesivos utilizados para fabricar algunos materiales multicapa. Por ejemplo: el ácido butírico, el ácido acético, el butirato de metilo, el 1-butanol y el nonanal han sido identificados en cinco tipos diferentes de adhesivos y en muestras de PE y PP; y produciendo olores desagradables como mantequilla, rancios, ácidos, medicinales y verdes (Vera et al., 2014).

- Solventes orgánicos, como el tolueno, el xileno y las cetonas, que son utilizados en la aplicación de tintas de impresión, adhesivos, barnices, recubrimientos y lacas. Estos solventes son reportados como responsables de olores muy desagradables en los alimentos (Piergiovannyet al., 2019).

- Los aldehídos insaturados y saturados, los fenoles y las lactonas, que fueron reportados como los compuestos responsables de los malos olores generados en los materiales plásticos reciclados postconsumo (Strangl et al., 2017).

- Los haloanisoles, compuestos halogenados con olor a moho y/o humedad generados por la acción de microorganismos en materiales naturales tratados con desinfectantes halo-fenólicos. Con frecuencia son encuentran haloanisoles como 2,4,6-tricloroanisol, 2,3,4-tricloroanisol, 2,3,6-tricloroanisol, tetracloroanisol, pentacloroanisol y 2,4,6-tribromoanisol en vino (Jeleń et al., 2013).

4.2.2. Oligómeros

Los oligómeros son moléculas que consisten en la unión de un número reducido de monómeros, normalmente entre 2 y 40 unidades repetidas. Los oligómeros provenientes de materiales para envase alimentario son considerados NIAS por ser subproductos de reacción entre las materias primas. Además, son migrantes potenciales, especialmente cuando tienen pesos moleculares por debajo de los 1000 Da, debido a que las moléculas pequeñas tienen velocidades de difusión más altas y, por lo tanto, un

mayor potencial de migración. Además, aquellos compuestos con peso molecular inferior a 1000 Da pueden ser absorbidos en el tracto digestivo, dependiendo de factores como la estructura molecular y la vía de absorción, por lo que debe evaluarse su toxicidad (Groh et al., 2017; Hoppe et al., 2016)

Se han encontrado oligómeros procedentes de diferentes tipos de polímeros utilizados en la fabricación de envases alimentarios, tales como poliésteres, poliolefinas, poliamida, o PLA (Aznar et al., 2019; Hoppe et al., 2016; Ubeda et al., 2019; Ubeda et al., 2018).

- En los poliésteres, los oligómeros más comunes se forman a partir de la unión de di-ácidos y di-alcoholes, mediante una reacción de trans-esterificación. Los oligómeros pueden ser cíclicos o lineales debido a reacciones de hidrólisis de los enlaces éster. Por ejemplo, en PET se han detectado oligómeros formados por la unión entre el ácido tereftálico (TPA) y los alcoholes dietilenglicol (DEG) y/o etilenglicol (EG), con estructuras como: $[TPA\text{-}DEG]_n$ *cíclico,* $[TPA\text{-}EG]_n+H_2O$ *lineal,* $[TPA\text{-}EG]_n$ *cíclico* o $[TPA_{n+1}\text{-}EG_n\text{-}DEG]+H_2O$ *lineal*.

- En Poliolefinas, tales como el PE y el PP,los oligómeros los conformarían cadenas de alcanos con la estructura $[C_2H_4]_n$ y $[C_3H_6]_n$, respectivamente. En el caso del PP es posible que se formen oligómeros isoméricos debido a estéreo centros formados durante el proceso de polimerización.

- En las poliamidas que se producen por reacciones de polimerización con apertura de anillo se han detectado oligómeros cíclicos, como en el nylon 6 $[C_6H_{11}NO]_n$, proveniente de la caprolactama, reportandose oligómeros cíclicos desde n:2 hasta n:9. En el nylon 6,6 $[C_{12}H_{22}N_2O_2]_n$, formado por la unión de hexametilendiamina (HDMA) y ácido adípico, se han detectado desde el monómero hasta oligómeros cíclicos de n:4.

- En el PLA, los oligómeros formados por la unión de monómeros de ácido láctico pueden ser cíclicos o lineales, por la hidrólisis de los grupos esteres del polímero en medios acuosos o en solventes con grupo hidroxilos.

La producción de los oligómeros puede tener lugar durante el proceso de fabricación del polímero o posteriormente durante el almacenaje o contacto con el alimento. La cantidad total de oligómeros producida puede llegar a ser una cantidad significativa (Hoppe et al., 2016) y por tanto su evaluación es necesaria. Además, la gran variedad de monómeros usados en la actualidad para la producción de polímeros destinados al contacto alimentario, puede dar lugar a un gran número y diversidad de oligómeros presentes en los envases alimentarios. El aumento de la variedad y cantidad de oligómeros hace que la investigación de su migración potencial y efectos toxicológicos sea más importante (Hoppe et al., 2016).

5. LEGISLACIÓN

Los materiales destinados a contacto alimentario están sujetos a diferentes reglamentos. Éstos tienen por finalidad garantizar la seguridad para la salud humana. El Reglamento Marco (CE) Nº 1935/2004 del Parlamento Europeo, tiene como objetivo garantizar la seguridad del consumidor de todos los materiales y objetos destinados a entrar en contacto directo o indirecto con alimentos. Este reglamento establece que "el envase no debe transferir sus componentes a los alimentos en cantidades que puedan: representar un peligro para la salud humana, provocar una modificación inaceptable de la composición de los alimentos o provocar una alteración de las características organolépticas de éstos". Además del Reglamento Marco existen otros reglamentos específicos para diferentes materiales de envase y sustancias específicas (Bajpai, 2019).

Los materiales plásticos, que constituyen un gran porcentaje de los materiales destinados al envase de alimentos, están legislados por el Reglamento Europeo EU/2011/10 (European Commission, 2011a). El reglamento especifica las sustancias que pueden utilizarse en la elaboración de los plásticos destinados al contacto con alimentos y sus límites máximos de migración. Además, hace referencia a los compuestos que no han sido adicionados de forma intencionada (NIAS) pero que se

forman por diversos procesos, ya que representan un riesgo potencial de migración a los alimentos.

En el Reglamento EU/2011/10, y sus sucesivas enmiendas también se encuentran las condiciones de los ensayos de migración, (simulantes, temperatura y tiempos) necesarios para garantizar la inocuidad de los materiales. Este reglamento especifica por una parte el límite de migración global (OML), que es la cantidad total de los componentes que puede ser transferida del envase al alimento, OML < 10 mg/dm^2 o 60 $mg\ Kg^{-1}$ simulante. Además, establece el límite de migración específica para cada sustancia de la lista positiva (SML), que se define como la cantidad máxima de una sustancia que puede ser transferida al alimento. Para una sustancia que no se encuentra en la lista positiva del anexo 1, la legislación dispone que no deben ser detectables, estableciendo el límite de detección en 10 $\mu g\ Kg^{-1}$ simulante, siempre y cuando no sean mutágenicas, carcinógenas o reprotóxicas (European Commission, 2011a).

En Europa, los bioplásticos están regulados por el Reglamento Europeo EU/2011/10, el mismo reglamento que para los plásticos convencionales. Todos los monómeros y aditivos permitidos en la fabricación de bioplásticos están listados en el anexo 1 del reglamento. Estos materiales deben cumplir normas sobre compostabilidad, biodegradabilidad y determinación del contenido de carbono de base biológica. Los encargados de estas certificaciones en el etiquetado son las organizaciones como DIN CERTCO, Bioderadable Products Institute, Vincotte, and Japan BioPlastics Association (Geueke, 2014).

6. MÉTODOLOGIA ANALÍTICA PARA IDENTIFICACIÓN DE MIGRANTES

La determinación de posibles compuestos migrantes que provienen de los envases es un desafío continuo. Los NIAS representan un mayor desafío para la química analítica porque el proceso de identificación es bastante complejo y lento. El proceso se

complica debido a la falta de estándares comerciales para la confirmación de estructuras, y a la diversidad de interacciones químicas que se pueden dar entre monómeros y aditivos, y entre posibles contaminantes y el alimento. Además, es muy común no encontrar suficiente información acerca de las materias primas y los aditivos usados en la fabricación del polímero, porque los fabricantes consideran que la composición es estrictamente confidencial.

Antes de realizar un estudio de migración, se puede realizar un análisis de detección de los aditivos y las materias primas del envase, para realizar una identificación previa de las sustancias que tienen mayor probabilidad de migrar al alimento. Este procedimiento generalmente se lleva a cabo por medio de una extracción del material con un disolvente orgánico o bien a partir de la disolución total del mismo. Posteriormente se puede usar una técnica de tratamiento de la muestra para concentrar y limpiar el extracto, facilitando la identificación de compuestos no volátiles, volátiles (odorantes y no odorantes) y semi-volátiles. La identificación de los compuestos normalmente se realiza por medio de técnicas cromatográficas como UPLC-Q/ToF, GC-MS y/o GC-MS-O.

Constantemente se desarrollan nuevos envases de alimentos que deben ser evaluados antes de su salida al mercado. Además de los procedimientos convencionales para la identificación de los NIAS, las nuevas investigaciones apuntan también a desarrollar metodologías de análisis que permitan la detección de migrantes de forma sencilla, rápida y apta para aplicar de forma rutinaria. El análisis a presión atmosférica por medio de técnicas como sonda de análisis de sólidos a presión atmosférica (ASAP), análisis directo en tiempo real (DART) o desorción ionización por electrospray (DESI) acopladas con un espectrómetro de masas de alta resolución (HRMS) han demostrado ser muy útiles, principalmente porque no requieren un paso previo de tratamiento de muestra. Estas técnicas se utilizan principalmente para verificar la presencia de compuestos, porque al no estar acopladas a una técnica previa de separación, la complejidad del espectro de masas obtenido hace imposible el proceso de identificación. (Nerin et al., 2013).

7. TECNICAS INSTRUMENTALES DE ANALISIS

7.1. Tratamiento de muestras

La identificación de diversos compuestos utilizando las técnicas convencionales requiere un paso previo de limpieza y/o concentración. Este procedimiento en muchos casos puede llegar a ser complejo. Por esta razón, durante muchos años la química analítica se ha dedicado al desarrollo y mejora de las técnicas para tratar las muestras. En la figura 3 se muestra un esquema general del protocolo de tratamientos de muestras que pueden usarse para la determinación de sustancias migrantes, tanto en el material de envase como en los ensayos de migración (Nerin et al., 2013).

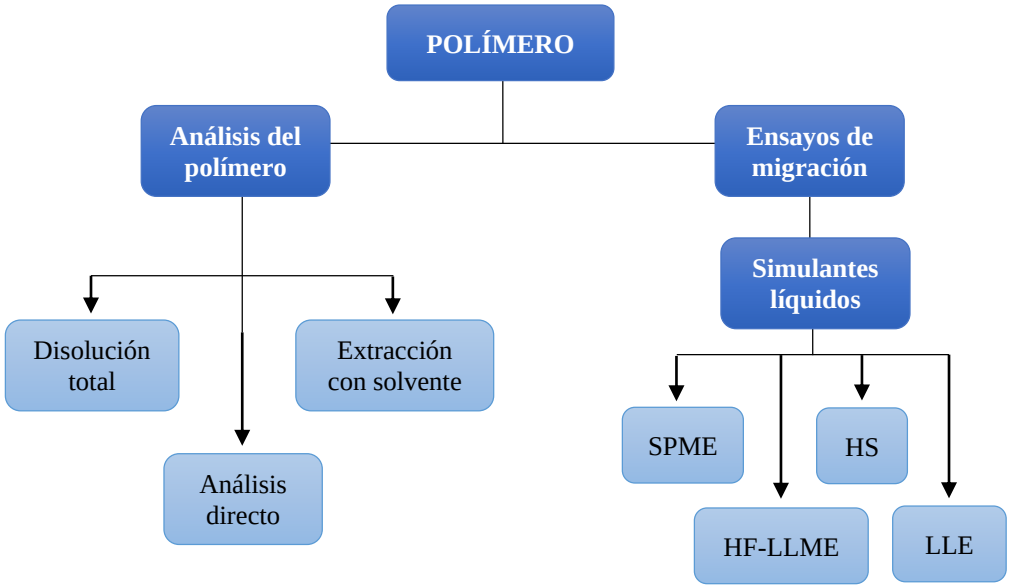


Figura 3. Esquema del procedimiento general para realizar el tratamiento de muestras de extracción y migración. SPME: Solid-Phase Microextraction, HF-LPME: Hollow Fiber - Liquid Phase Microextraction, HS: Headspace, LLE: Liquid-Liquid Extraction (Nerín, 2016).

7.2. Técnicas convencionales

La técnica analítica utilizada para la de identificación de los compuestos migrantes, debe seleccionarse de acuerdo con las propiedades fisicoquímicas de los

mismos. La figura 4 muestra un esquema a seguir para la identificación, confirmación y cuantificación de los compuestos migrantes, usando las técnicas analíticas convencionales. Del esquema se puede resaltar el uso de las técnicas cromatográficas. Estas técnicas se basan en la distribución de los componentes entre dos fases miscibles, una fase estacionaria y una fase móvil. Según la constante de distribución, los compuestos tendrán tendencia a estar en una fase u otra.

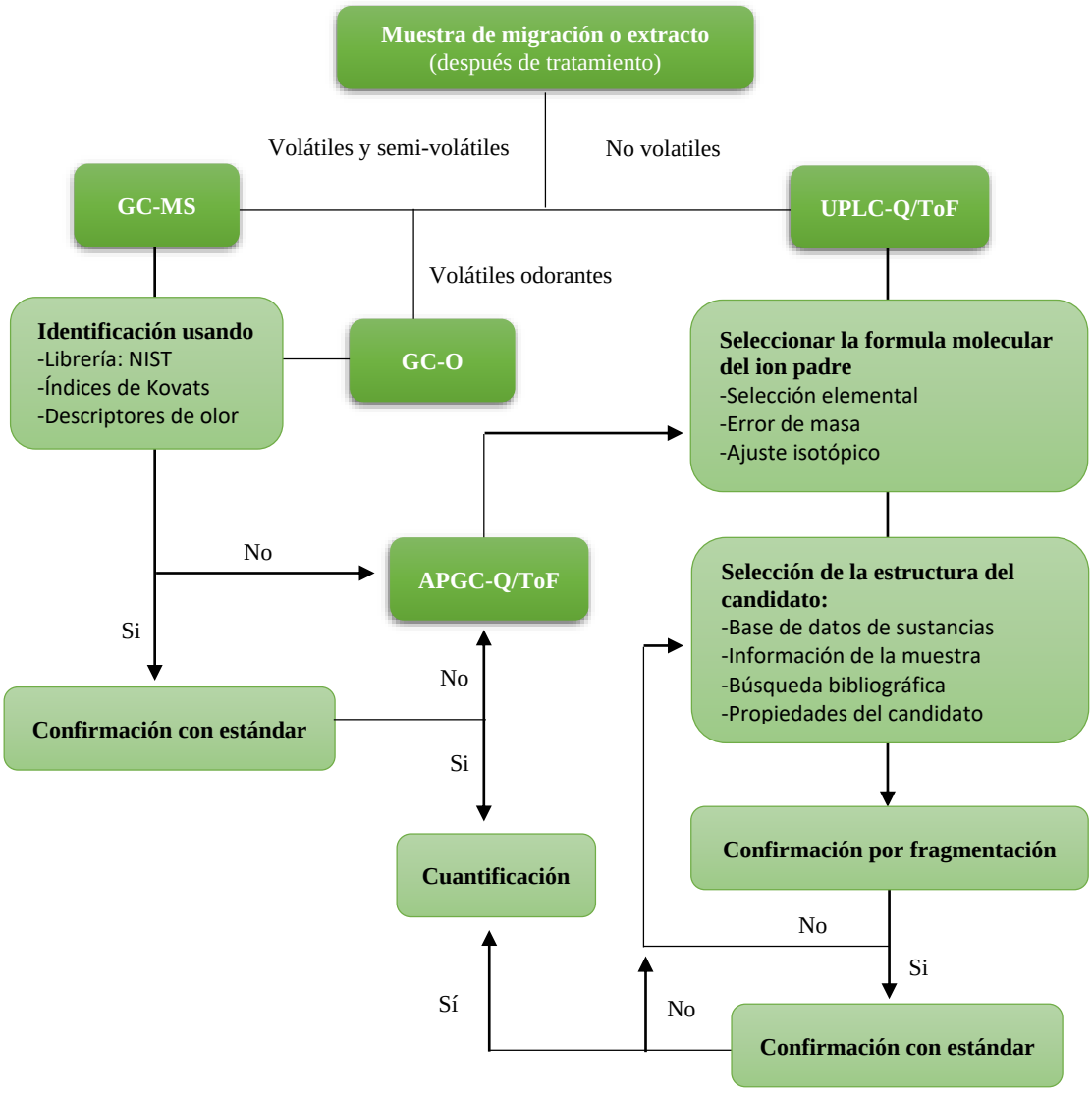


Figura 4. Esquema de la metodología utilizada para la identificaron de compuestos migrantes no volátiles, volátiles odorantes, volátiles y semi-volátiles (Nerín, 2016).

7.2.1. Identificación de migrantes volátiles

La cromatografía de gases acoplada a un detector de espectrometría de masas (GC-MS) es adecuada para la identificación de migrantes con alta volatilidad y estables térmicamente. La identificación se realiza mediante la comparación de espectros de masas experimentales con los espectros de masas reportados en las librerías, y mediante el cálculo de los índices retención (RI). Los espectros de las librerías están obtenidos por ionización por impacto electrónico (EI) con analizador Q, por tanto, la comparación suele ser bastante precisa (Paseiro-Cerrato et al., 2019).

Cuando la identificación mediante GC-MS (EI) no es posible, el acoplamiento con un HRMS, como el tiempo de vuelo (ToF), puede convertirse en una alternativa interesante a la GC-MS convencional. Los espectros de HRMS proporcionan masas más exactas y mayor información respecto a las fragmentaciones de los compuestos, aunque la identificación puede llegar a ser compleja (Nerin et al., 2013). Una vez se tiene un posible candidato, la confirmación final se lleva a cabo mediante la inyección de un estándar puro. También, la cromatografía de gases a presión atmosférica (APGC) acoplada a un HRMS puede ser una buena alternativa para identificar migrantes, porque su ionización química es relativamente suave, lo que permite obtener el ion molecular y a partir de él, encontrar la estructura química del compuesto (Domeño et al., 2012). Debe resaltarse, que incluso utilizando la misma columna cromatográfica, los tiempos de retención obtenidos en GC-MS (Q) y APGC-MS-QTOF no son los mismos (Su et al., 2019).

La GC-MS con un detector olfatométrico (GC-O) permite identificar los migrantes odorantes y conocer su impacto aromático en una muestra. De esta forma es posible detectar con la nariz humana el olor originado por un compuesto y relacionar los descriptores olfativos con los picos cromatográficos (Piergiovanny et al., 2019; Song et al., 2018). En la figura 5 se puede observar el funcionamiento de la técnica. La caracterización del aroma es posible siempre que los compuestos odorantes tengan bajo punto de ebullición, bajo peso molecular y contengan un grupo osmóforo (un grupo funcional activo) dentro de su estructura. También deberían tener una estereoquímica

adecuada y estar en concentración mayor a la del umbral de olfacción (concentración mínima determinada por estudios estadísticos que puede ser percibida por una población). Se estima que el ser humano tiene la capacidad de reconocer cerca de 100.000 olores diferentes, funcionando como un detector selectivo de moléculas odorantes (Duncan et al., 2009; iSens, 2020).

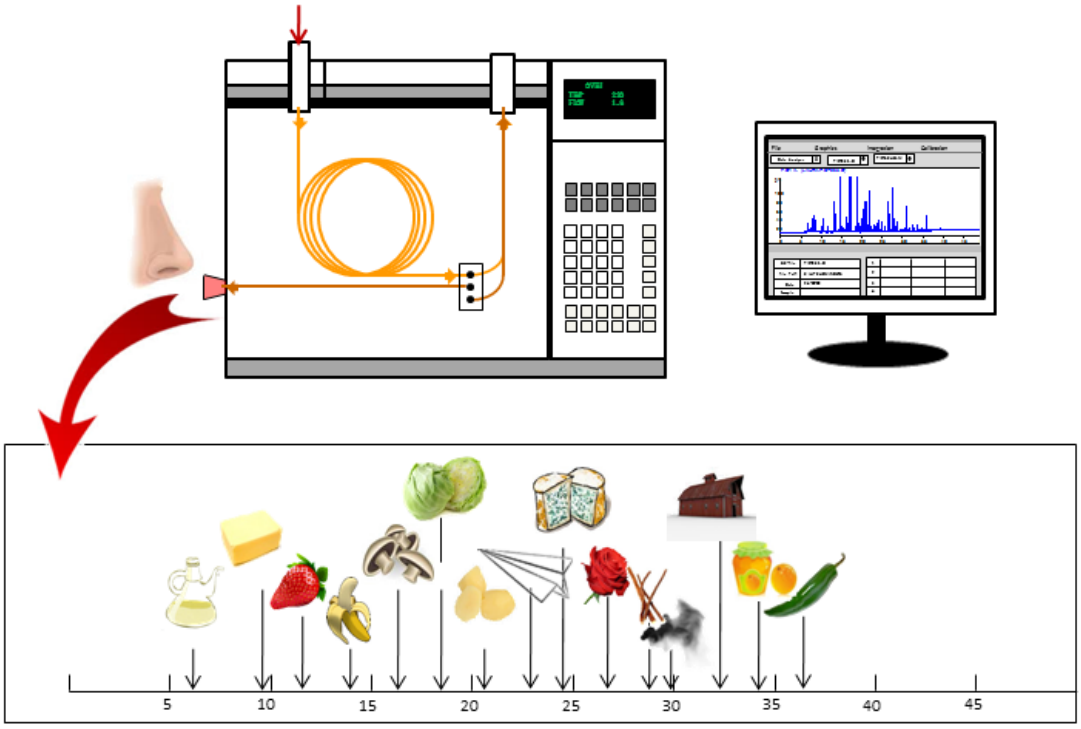


Figura 5. Esquema del análisis cromatografico olfatométrico (iSens, 2020).

7.2.2. Identificación de migrantes no volátiles

La cromatografía líquida de alta resolución HPLC con detector de espectrometría de masas es una de las técnicas más usadas para el análisis de migrantes no volátiles. Sin embargo, la estrategia de identificación es más compleja a la usada en GC-MS, debido a que hay pocas bases de datos bibliográficas con los espectros de masas y son menos reproducibles. Esto es debido a la gran variedad de parámetros que hacen cambiar el espectro dependiendo de las condiciones experimentales de ionización, las fases

móviles, las condiciones de la fuente de iones, los aditivos, entre otras (Paseiro-Cerrato et al., 2019).

Los equipos de UPLC (cromatografía líquida de ultra-alta resolución) acoplados a espectrómetro de masas tándem como el Q/ToF han demostrado ser una herramienta muy útil para la identificación de compuestos migrantes, especialmente los NIAS. El análisis por HPLC-Q/ToF permite obtener en un mismo análisis un espectro con las masas exactas del ion precursor, generado por la adquisición a baja energía; y un segundo espectro donde se observan iones de los fragmentos debido a una adquisición a alta energía (Paseiro-Cerrato et al., 2019).

El protocolo de identificación de la figura 4 para un compuesto no volátil analizado por HPLC-Q/ToF consiste en la generación de fórmulas moleculares empíricas, para posteriormente seleccionar algunas fórmulas candidatas. Luego estas se relacionan con una estructura por medio de bases de datos. Y finalmente se realiza la comparación entre los posibles fragmentos y los iones generados en el espectro de masas de alta energía. Siempre que sea posible, el compuesto debe confirmase con el estándar puro (Nerín, 2016).

7.3. Técnicas de análisis directo

En el campo del envase plástico, las técnicas instrumentales de análisis directo juegan un papel importante, ya que permiten detectar la presencia de una gran variedad de compuestos previamente definidos, de forma simultánea en tiempos cortos de análisis (Li et al., 2013; Lu et al., 2018). Las técnicas de análisis de sólidos a presión atmosférica (ASAP-MS) y análisis directo en tiempo real (DART-MS) acopladas a la espectrometría de masas son dos de las técnicas más usadas para la detección de migrantes específicos provenientes de envases alimentarios (Hoppe et al., 2016; Peters et al., 2019; Smith et al., 2012).

En ambas técnicas, la fuente de ionización del espectrómetro de masas trabaja a presión ambiental y pueden ser usadas tanto para el análisis de muestras sólidas como líquidas (Black et al., 2016; Lu et al., 2018; Uttl et al., 2018). La diferencia entre ambas

radica en la forma como se generan las fuentes de ionización primarias. En ASAP, la fuente de ionización es por medio de electrodos de punta afilada, y en DART por medio de un disco en forma de corona "*corona-to-glow*" (Gross, 2014). Debido a la similitud en el proceso de ionización, los espectros obtenidos por ambas técnicas pueden ser similares, principalmente en ambientes secos (Gross, 2014).

DART-MS se ha usado para el análisis de compuestos provenientes de diversos polímeros. En muestras de polietilenglicol (PEG), polipropilenglicol (PPG) y polimetacrilato de metilo (PMMA), se han detectado los aductos protonados y aductos de amonio de los oligómeros del PEG (desde n:8 hasta n:18); oligómeros del PPG (desde n:17 hasta n:19) y de oligómeros del PMMA (desde n:3 hasta n:11). También, se han reportado diversos aductos de oligómeros provenientes de materiales de polímeros de perfluoropoliéter, poliamidas y polidimetilsiloxano (Bridoux & Machuron, 2013).

Por otro lado, ASAP-MS ha sido ampliamente utilizada para la detección rápida y la identificación de compuestos volátiles migrantes, como oligómeros (Smith et al., 2012), grupos de cetonas (Cossoul et al., 2015), compuestos volátiles orgánicos (Dutra et al., 2011), entre otros, que se encuentran en polímeros destinados al contacto alimentario (Smith et al., 2012)

8. ANÁLISIS SENSORIAL

En términos globales, la evaluación sensorial se define como la disciplina científica que se utiliza para medir, analizar e interpretar las reacciones de los consumidores antes las características físico-químicas de los alimentos y materiales percibidos por los sentidos del olfato, vista, gusto, tacto y oído. En la industria alimentaria se usa con el fin de evaluar el nivel de satisfacción, la similitud entre productos y el impacto que tienen los atributos sensoriales en el consumidor (Ebeler et al., 2017).

En la industria de los envases alimentarios, el análisis sensorial es especialmente utilizado para certificar la idoneidad de los alimentos después de ser envasados y garantizar que el almacenamiento no afecte el producto final. Aunque la evaluación de los envases por medio de los métodos analíticos convencionales suele ser suficiente desde el punto de vista de la seguridad, con los resultados obtenidos no es posible saber cuáles son las sensaciones percibidas por el consumidor acerca del producto final envasado (Saint-Denis, 2018). La implementación de técnicas de análisis sensorial para identificar los compuestos odorantes, puede facilitar la posterior identificación de aquellos compuestos migrantes del envase que alteran la percepción sensorial del alimento envasado (Meilgarard et al., 2015).

El control de calidad de los aromas característicos de un producto se lleva a cabo mediante un análisis sensorial generalmente realizado por un panel entrenado de personas, con entrenamiento y capacitación previa. La evaluación del producto por un panel sensorial puede consistir en la determinación de la presencia, ausencia o cambio en el olor de un producto. La capacidad que tiene una persona de percibir una sustancia química depende, además de las características propias de las sustancias odorantes como la estructura, peso molecular o su concentración, del entrenamiento previo realizado y de la capacidad olfativa del individuo (Civille et al., 2012)

Cuando se realiza un análisis sensorial de un producto se deben controlar varios aspectos antes y durante el desarrollo de la prueba, como evitar estímulos exteriores que puedan llevar a confusiones en los panelistas, y así obtener resultados válidos y objetivos (Saint-Denis, 2018) Otros aspectos importantes son (Ebeler et al., 2017):

- Selección de los panelistas: el panel sensorial debe estar integrado por personas que se encuentran entre un rango de edad amplio. Normalmente, el rango se encuentra entre 18 y 60 años de edad. Se debe tener la misma proporción de hombres y mujeres, sin deficiencias en alguno de sus sentidos.

- Entrenamiento de los panelistas: la realización del entrenamiento debe estar acorde a las necesidades del análisis. Por ejemplo, para la evaluación del olor deben

familiarizarse con una gran variedad de sustancias odorantes. Se debe entrenar el doble de panelistas que requieren las pruebas para evitar tener problemas por deficiencias en la percepción de olores.

- Escalas sensoriales: durante el entrenamiento también se establece la escala de intensidades con la que se va a trabajar, que posteriormente serán los datos que permitirán obtener resultados cuantificables. Cuando se trata de sustancias odorantes, se suele establecer una escala de percepción que va desde un olor perceptible pero poco intenso (valor 1), un olor reconocible con intensidad moderada (valor 2), y un olor muy intenso y fácilmente reconocible (valor 3). También es muy común que se pida una descripción del olor percibido.

- Sala de cata: el lugar donde se realizan las pruebas debe un lugar tranquilo, con buena iluminación, libre de olores y ruido, y condiciones térmicas entre los 20-22 ºC y un 60-70% de humedad relativa.

- Muestras para evaluación: la presentación de las muestras para evaluación difiere significativamente dependiendo del tipo de prueba a realizar. Sin embargo, siempre debe presentarse un único tipo de muestra para todos los panelistas, la temperatura debe ser constante, debe estar codificada y la presentación debe ser aleatoria.

- Pruebas de evaluación: según el tipo de información que se quiere obtener sobre un producto se seleccionará el tipo ensayo sensorial que se deba aplicar. No todos los ensayos sensoriales proveen información total acerca de los atributos sensoriales de un producto.

8.1. Ensayos sensoriales

Los ensayos que se realizan para evaluar la calidad sensorial de un producto están recogidos en la normativa ISO 6658 (Comité tecnico CTN 87, 2019; Meilgarard et al., 2015), y están dividas en tres categorías de pruebas, como son: las pruebas discriminativas, las afectivas y las descriptivas. La elección de una u otra ira en función de los objetivos del estudio sensorial (Saint-Denis, 2018).

8.1.1 Pruebas discriminativas

Las pruebas discriminativas buscan determinar qué tipo de diferencias sensoriales existen entre dos o más muestras. Normalmente se usan para controlar la calidad del producto comparando entre muestras, ya sea de diferentes lotes y/o con muestras de referencia. Son pruebas complejas que requieren ser llevadas a cabo por personal entrenado, preferiblemente miembros de un panel sensorial. Este tipo de pruebas son las más usadas para estudiar los problemas sensoriales causados en los alimentos por compuestos migrantes del envase alimentario (Civille et al., 2012).

Las pruebas discriminativas principales se pueden dividir en dos grupos en función del resultado que se desean obtener (Meilgarard et al., 2015):

- Determinación de diferencias sensoriales entre muestras

❖ Ensayo triangular: en este ensayo se presentan simultáneamente tres muestras, dos de ellas iguales y una diferente, el panelista debe indicar cuál es la muestra diferente

❖ Ensayo dúo- trío: para desarrollar este ensayo se deben presentar tres muestras, en orden, primero se presenta la muestra de referencia y posteriormente las dos muestras codificadas, el panelista debe indicar cual de ellas es igual a la muestra de referencia.

❖ Ensayo de diferencia simple: en este ensayo se presenta dos muestras y el panelista debe decir si son diferentes o iguales.

❖ Ensayo comparación por parejas: durante el ensayo se les presenta dos muestras a los panelistas con un atributo sensorial característico, y estos deben indicar cuál de las muestras tiene mayor intensidad.

❖ Ensayo A no A: En este ensayo se presenta varias muestras al panelista que pueden ser A o diferentes de A, este debe clasificar las muestras como A o diferentes de A.

- Determinación de la variación de un atributo entre muestras

- ❖ Ensayo de comparación por pares: en este ensayo las muestras son entregadas en pares para que sean comparadas entre ellas en base a criterios previamente establecidos.

- ❖ Ensayos de ranking: en este tipo de test se le presenta al panelista un set de muestras y este debe ordenarlos de acuerdo del atributo.

- ❖ Ensayos de comparación múltiple: el ensayo consiste en la evaluación en el aumento de la intensidad de un atributo en relación a una escala numérica previamente estipulada. En este ensayo se suele trabajar con un rango entre 6 y 8 muestras.

8.1.2. Pruebas afectivas

Las pruebas afectivas tienen como objetivo evaluar la preferencia de los consumidores sobre el producto final, en términos de agrado o desagrado. También se les conoce como estudios de consumidores, y suelen requerir el análisis de un número superior de 30 personas sin entrenamientos previos, pero que son potenciales consumidores. De estas es posible reconocer tres tipos de ensayos (Civille et al., 2012).

- Ensayo de preferencia: permite evaluar la preferencia del consumidor entre dos productos con características sensoriales que pueden ser diferentes, aunque no necesariamente. Es decir, que no se busca la capacidad del consumidor para distinguir entre las muestras, sino solamente su opinión.

- Ensayo de satisfacción: permite evaluar las sensaciones que genera el producto en el consumidor. Es muy usado cuando se quiere evaluar más de dos muestras con características sensoriales ligeramente diferentes.

- Ensayo de aceptación: evalúa el deseo o necesidad que tiene una persona de adquirir el producto, sin tener en cuenta el grado de agrado. Suele estar incluido dentro de alguno de los dos ensayos anteriores.

8.1.3. Pruebas descriptivas

Las pruebas descriptivas consisten en describir objetivamente las propiedades sensoriales del producto, sin importar las preferencias personales. Suelen llevarse a cabo con una única muestra que debe ser descrita en totalidad por personal entrenado en diferentes atributos sensoriales. Este tipo de pruebas son muy utilizadas en la industria alimentaria, principalmente durante el desarrollo de nuevos alimentos (Saint-Denis, 2018). Entre las pruebas más destacadas se encuentran el análisis por del perfil del aroma, sabor y textura, el análisis cuantitativo-descriptivo, el perfil de libre elección y el análisis de tiempo-intensidad (Meilgarard et al., 2015).

SECCIÓN II: Objetivos Generales

OBJETIVOS GENERALES

El creciente uso de biopolímeros en el sector del envase alimentario y el aumento en el consumo de alimentos envasados hace que sea necesaria su evaluación en términos de seguridad alimentaria. Principalmente porque los procesos de migración afectan la integridad y las propiedades organolépticas del alimento. Por otro lado, ha aumentado también el uso de métodos de cocinado rápido como el cocinado en el mismo envase. El objetivo principal de esta tesis doctoral está orientado al estudio de la seguridad alimentaria de biopolímeros destinados al contacto alimentario y de materiales para cocinado en envase. El trabajo se ha centrado en la evaluación de los materiales desde un punto de vista químico y sensorial, partiendo del estudio de migración de las sustancias migrantes y sensorialmente activas en diferentes simulantes.

Para lograr este objetivo general, se han planteado los siguientes **objetivos específicos**:

- Optimización de técnicas de tratamiento de muestra que permitan la concentración de los IAS y NIAS presentes en las muestras, con el fin de facilitar el posterior análisis. La HS-SPME y la HF-LPME serán las técnicas evaluadas.

- Desarrollo de metodologías de análisis para la identificación y cuantificación de todos los compuestos migrantes y las sustancias sensorialmente activas presentes en los materiales de estudio. El análisis se llevará a cabo por las técnicas GC-MS y APGC-Q/ToF, utilizadas para el estudio de los compuestos volátiles y semi-volátiles; la técnica GC-MS-Olfatometría, para la evaluación de los compuestos odorantes; y la técnica UPLC-Q/ToF, para el análisis de los compuestos no volátiles.

- Elaboración de protocolos de análisis rápidos mediante las técnicas DART-SVP y ASAP-Q/ToF, que permitan la identificación simultánea de compuestos migrantes volátiles, no volátiles y semi-volátiles presentes en los biopolímeros.

- Comparación de los resultados obtenidos en las diferentes técnicas analíticas.
- Elaboración de librerías de los compuestos migrantes y sensorialmente activos en los materiales de estudio.
- Caracterización sensorial de los principales grupos de odorantes encontrados en las muestras.
- Evaluación del impacto sensorial en los alimentos de los materiales seleccionados.
- Evaluación del riesgo.

Para su consecución se han planteado una serie de objetivos concretos que serán presentados al comienzo de cada uno de los capítulos desarrollados en la sección III, desarrollo experimental.

SECCIÓN III: Desarrollo Experimental

Métodos de pre-concentración en muestras de migración

Capítulo 1: *Analysis of isophthalaldehyde in migration samples from polyethylene terephthalate packaging*

Análisis de materiales destinados al cocinado en envase

Capítulo 2: *Release of volatile compounds from cooking plastic bags under different heating sources*

Análisis de biopolímeros

Capítulo 3: *Determination of volatile non intentionally added substances coming from a starch-based biopolymer intended for food contact by different gas chromatography-mass spectrometry approaches*

Capítulo 4: *Identification of key odorant compounds in starch-based polymers intended for food contact materials*

Capítulo 5: *Rapid and simultaneous determination of polyester oligomer as migrants from biopolymers by Direct Analysis in Real Time mass spectrometry*

Capítulo 6: *Ambient mass spectrometry as a tool for a rapid and simultaneous determination of migrants coming from a bamboo-based biopolymer packaging*

Capítulo 1

Analysis of isophthalaldehyde in migration samples from polyethylene terephthalate packaging

1. RESUMEN

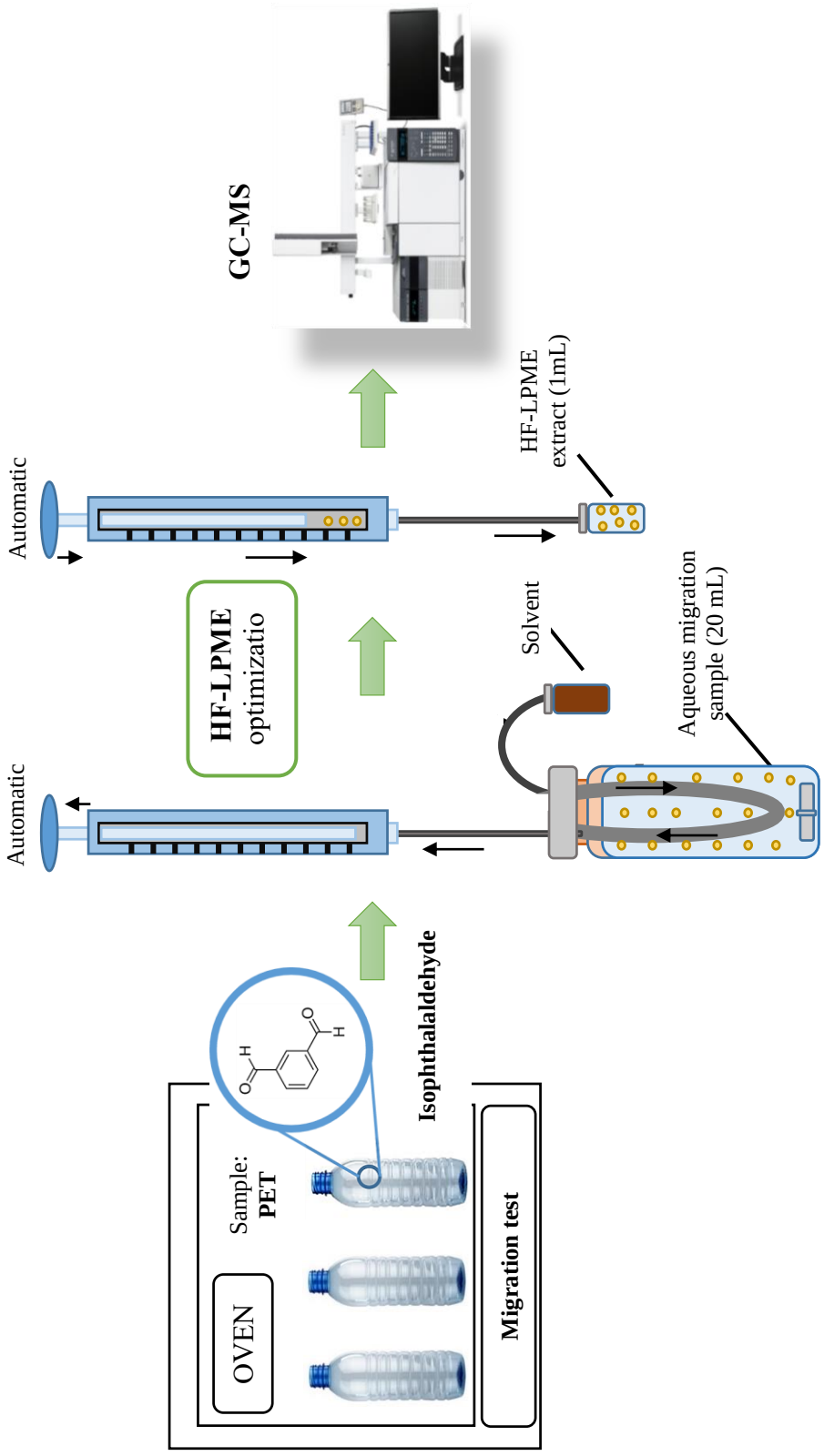
El isoftalaldehído es un compuesto potencialmente migrante, que puede provenir de la oxidación de copolímeros utilizados en la fabricación de materiales para envasado de alimentos. Debido a que es considerado como un NIAS, es necesario determinar su concentración en muestras de migración. En este capítulo, han sido evaluadas diferentes estrategias de preconcentración para el análisis de isoftalaldehído en muestras de migración de materiales de PET. Inicialmente se llevó a cabo la derivatización por SPE, con el agente derivatizantes o- (2,3,4,5,6-pentafluorobencil) hidroxilamina (PFBHA), sin resultados satisfactorios. Posteriormente fueron evaluadas y optimizadas dos técnicas de microextracción: HF-LPME y SPME. La técnica HF-LPME mostró mayor sensibilidad y un factor de enriquecimiento (EF) de 60. LOD y LOQ fueron de 10 ng g^{-1} y 30 ng g^{-1}, respectivamente; y la desviación estándar relativa (RSD) del 6,1%. Finalmente, se realizaron los estudios de migración de un envase de PET para evaluar su seguridad. El contenido de isoftalaldehído se determinó en 2 simulantes alimentarios, etanol al 10% (v/v) y ácido acético al 3% (w/v) utilizando la técnica HF-LPME. También fueron evaluadas diferentes condiciones de tiempo y temperatura en los ensayos de migración. Los resultados obtenidos mostraron un incremento considerable en la concentración de isoftalaldehído con el aumento del tiempo y la temperatura.

2. OBJETIVOS

El objetivo principal de este capítulo fue establecer una metodología para el análisis de isoftalaldehído y cuantificar sus valores de migración en muestras de migración acuosas (etanol 10% y ácido acético 3%) provenientes de envases de PET destinados al contacto alimentario. Para el cumplimiento de este objetivo se realizaron las siguientes tareas:

- Búsqueda de bibliografía del uso y migración de isoftalaldehído en botellas de PET.
- Evaluación de un método de extracción y derivatización para análisis de isoftalaldehído por medio de la técnica SPE.
- Optimización de las técnicas de tratamiento de muestra HF-LPME y SPME para la extracción y concentración simultanea de isoftalaldehído en muestras acuosas.
- Desarrollo de un método de detección y cuantificación de isoftalaldehído mediante GC-MS.
- Realización de los ensayos de migración para los materiales de PET, siguiendo los protocolos establecidos por la legislación.
- Análisis de la influencia de los parámetros tiempo y temperatura en los resultados de migración.

3. ESQUEMA DE TRABAJO



Esquema 1. Diseño experimental del Capítulo 1

4. INTRODUCTION

Polyethylene terephthalate (PET) is one of the most used materials in food packaging due to its good mechanical properties, low cost, transparency and recyclability (Vinicius et al., 2014; Welle, 2014). For this reason, the industries are constantly looking for improving some of these properties, mainly the barrier properties (Michiels, 2017). The most commonly used technologies are aluminium foil addition, copolymerization, laminated, extruded, coextruded, among others (Félix et al., 2011) Copolymerization offers an interesting alternative for improving barrier properties. Different works showed that blending PET with polyamide is effective in reducing gases permeability of the packaging material (Hu et al., 2005; Nand et al., 2012; Özen et al., 2010).

The incorporation of polyamide to PET can involve the presence of new components in the packaging materials, such as isophthalaldehyde, a known substance from polyamide oxidation (Bentayeb et al., 2007; Welle, 2014). When a polymer is intended to be used in food packaging, a risk assessment evaluation must be done to assess consumers' safety. Therefore, the presence of new potential migrants should be checked (Ekinci et al., 2015).

This compound is not present in the positive list of Commission Regulation (EU) No 10/2011 and therefore its concentration in migration samples must be evaluated, that means that analytical methodologies with high sensitivity are required for its quantification (Onghena et al., 2014). But the analysis of aldehydes and in particular isophthalaldehyde is difficult and there is not an analytical methodology for its determination. The analysis of isophthalaldehyde in aqueous simulants requires an extraction step and often derivatization to facilitate the chromatographic analysis (Culleré et al., 2004; Stafiej et al., 2006; Wagner et al., 2003). Microextraction techniques are commonly used because they have shown high sensitivity and reproducibility (Correa et al., 2015; de Bairros et al., 2015; Fashi et al., 2015; Pino, 2007) for many analytes and they require low volumes of extraction solvents.

Solid-phase microextraction (SPME) is one of the most used techniques nowadays because it is easily implemented and automatized and it has high sensitivity (Nawała et al., 2016). Hollow fiber liquid-phase microextraction (HF-LPME) is a technique relatively new, but it has been shown as an efficient technique to enhance the sensitivity and reproducibility (Oliveira et al., 2014; Pezo et al., 2007; Rosero et al., 2014) of many different analytes. Additionally, it is low-cost, clean and it can reach enrichment factors above 400, even though these values depend on the analyte (Charalabaki et al., 2005). In HF-LPME, the hollow fiber is made of a semipermeable inert material that is submerged in the aqueous sample, while the acceptor phase is passing continually inside the fiber. This situation promotes the interactions of analytes present in the aqueous solution with solvent and prevents the two phases from mixing and being contaminated (Payán et al., 2009). Other important parameters that need to be optimized are the extraction volume and the extraction time. Actuality, LPME is an attractive alternative to other conventional extraction techniques, and its use has increased in the last years (Nawała et al., 2016; Oliveira et al., 2014; Payán et al., 2009; Sarafraz et al., 2008; Vinoth et al., 2011).

In the present work, a method for the analysis of isophthalaldehyde in aqueous migration samples was established. For this purpose a derivatization process and two microextraction techniques were studied, following the methods proposed by A. Stafiej (Stafiej et al., 2006). Derivatization with o-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) in solid-phase extraction (SPE), proposed by Ferreira (Culleré et al., 2004) was carried out and analyzed by gas chromatography coupled to mass spectrometry (GC-MS). As none of these methods provided the required sensitivity, HF-LPME and SPME techniques were optimized, and the obtained extracts were analyzed by GC-MS. Finally, isophthalaldehyde was analyzed in migration samples from food packaging materials. The influence of temperature and time in the migration of this compound was also evaluated. Result and discussion about the different methods applied are shown.

5. MATERIALS AND METHODS

5.1 Reagents

Isophthalaldehyde 97% [626-19-7] was from Sigma–Aldrich (Madrid, Spain). O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (99%, PFBHA) was purchased from Fluka (Madrid, Spain). Dichloromethane (DCM) stabilized with 20 µg g^{-1} de amylene from Panreac (Madrid, Spain). Toluene, benzene, p-xylene, chloroform and diethyl ether were from Sigma–Aldrich (Madrid, Spain). Acetonitrile (LC-MS grade), acetic acid and were supplied by Scharlau (Setmenat, Spain). Ultra-pure water was obtained from a Millipore Milli-Q system (Billerica, MA, USA).

5.2. Derivatization with PFBHA by SPE

This process was performed considering the methodology proposed by Culleré (Culleré et al., 2004). In this procedure, the aldehydes are initially retained in the SPE cartridge. Afterwards, a solution of PFBHA is passed inducing the formation of oximes. SPE was carried out using a LiChrolut RP-18 cartridge (200 mg, 6 cc) from Merck (Madrid, España). First, the cartridge was conditioned with 4 mL of dichloromethane, 4 mL methanol and 4 mL of ethanol 10% (v/v). Then, 10 mL of a standard solution of isophthalaldehyde (10 ng g^{-1}) in ethanol 10% (v/v) were passed through the cartridges; immediately 2 mL the PFBHA (1 µg g^{-1}) aqueous solution were added to the cartridge for the derivation process. After 15 min, t 2 mL a 0.5% sulfuric acid solution were passed through the cartridge. Finally, the cartridge was eluted with 2 mL of dichloromethane and the eluate was concentrated to 300 µL by evaporation under nitrogen current and analyzed by GC-MS. The analysis was performed in triplicate.

5.3. Extraction by SPME

For SPME analysis, three types of fibers were evaluated: Polydimethylsiloxane (PDMS 100 µm), Polydimethylsiloxane/ Divinylbenzene (PDMS/DVB 65 µm) and Polydimethylsiloxane/Divinylbenzene/Carboxen (50/30 µm). Afterwards, two extraction parameters were optimized: extraction time (5, 15 and 25 min) and extraction

temperature (60, 80 and 100 ºC). The solutions were conditioned and homogenized at 500 rpm during the extraction procedure. SPME extraction by total immersion mode was carried out automatically in Combi PAL autosampler (CTC Analytics, Zwingen, Switzerland). Later, the fiber was automatically transferred to the injection port of the GC-MS. Optimization was carried out with 10 mL standard solutions of isophthalaldehyde (500 ng g^{-1}) in water for all the experiments. Analyses were performed in triplicate.

5.4. Extraction by HF-LPME

The methodology used was based on the pre-concentration procedure proposed by Pezo (Pezo et al., 2007) using an automatic device with a programmable multi-syringe pump Aladdin AL-8000 from World Precision Instruments (Stevenage, UK). The syringe pump was fitted with six Hamilton (Bonaduz, Switzer- land) microsyringes (100 µL capacity each). Semipermeable hollow fibers consisted of polypropylene hydrophobic membranes Accurel PP 150/330 (0.2 µm pore size, 150 µm wall thickness, 600 mm inner diameter), purchased from Membrana (Wuppertal, Germany) was used for the extraction. In the process, hollow fibers of 11 cm length were used by recommendation of Rosero (Rosero et al., 2014).

According to the previous experience, the extracting solvent selected is critical during the extraction process. For this reason, six different solvents were evaluated: toluene, benzene, dichloromethane, p-xylene, chloroform and diethyl ether. Other variables that were evaluated to determine the optimum extraction conditions were: extraction rate (2.5, 5 and 10 µL/min); solvent volume (50, 75 and 100 µL) and extraction temperature (25 ºC and 50 ºC). The optimization process was carried out with 20 mL isophthalaldehyde aqueous solutions (100 ng g^{-1}). These solutions were homogenized at 300 rpm during the extraction procedure. The extracts were analyzed by GC-MS. Hollow fibers were replaced in all experiments to avoid contamination and carryover effect on the extracts (Ranjbar et al., 2017).

5.5. Analysis by GC-MS

All the analyses were carried out in a Gas Chromatograph 7890N with mass spectrometry detector 5977D (Agilent Technologies. Santa Clara, CA). HP-5MS column was used (30m x 25mm x 0.25µm film thickness). Injection volume was 1 µL and it was carried out in splitless mode. The helium (99,999%) was the carrier gas at a constant flow rate of 2.0 mL/min. Acquisition was performed in SIM mode, selected ions were 105 and 133. These ions were selected according to their relative abundance in the mass spectrum obtained in SCAN mode. The temperature ramp was as follows: initially 50 ºC for 5 min, 10 ºC/min to 300 ºC and held for 5 min.

5.6. Final protocol for isophthalaldehyde analysis by HF-LPME

The protocol for isophthalaldehyde analysis was established according to the optimal conditions for HF-LPME obtained during the optimization. The automatic system previously described in section 5.4 was used. The samples were aqueous acetic acid 3% (w/v) and ethanol 10% (v/v). For a correct extraction, 20 mL of each sample were previously conditioned in a thermostatic bath at 50 ºC. Extraction process was developed in three steps. First, the hollow fiber was completely introduced in the sample forming a U, one end of the fiber was connected to a 2 mL vial with toluene as the extraction solvent and the other end was connected with the needle of a microsyringe. Then, 75 µL of toluene were passed at 2.5 µL/min trough the inside of the fiber while the sample was at constant stirring (300 rpm). During this step, isophthalaldehyde was retained in the toluene. Finally, the HF-LPME extract was automatically transferred to a 2 mL vial, and it was analyzed by GC-MS (Figure 1).

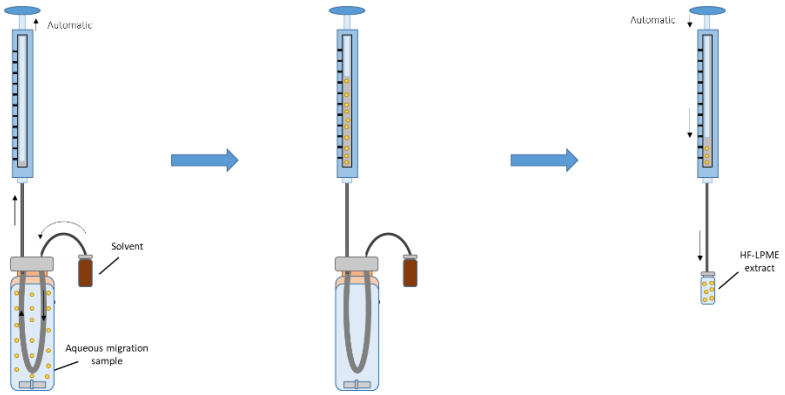


Figure 1. Scheme of the HF-LPME extraction process.

5.7. Determination of isophthalaldehyde in PET migration samples

Sample was supplied by a Packaging Company. Bottles were composed of a PET-PA blend and they were intended to be used to store water, juices or soft drinks without or with low alcohol levels (<10%). The materials used were prototypes and they were not still commercially available. More information cannot be provided due to confidential reasons. The migration assays were carried out in according to the legislation for food contact materials EU/10/2011. The PET bottles were filled with simulant A, ethanol 10% solution (v/v) and simulant B, acetic acid 3% (w/v). For the migration assays, two different conditions were selected: 10 days at 60 ºC and 20, 40 and 80 days at 40 ºC. Experiments were carried out in a thermostatic oven. All the analyses were performed in triplicate and blank samples, consisting of pure simulant, were also analyzed.

6. RESULTS AND DISCUSSION

6.1. Derivatization with PFBHA by SPE

Derivatization with PFBHA by SPE did not provide satisfactory results. No peaks were observed for isophthalaldehyde derivative in the analyzed solutions. The method proposed by Culleré (Culleré et al., 2004) was focused on the analysis of aldehydes with

5-8 carbon atoms with simple structures. However, isophthalaldehyde structure has one aromatic ring, and probably it provided a rigid structure that could interfere in the correct derivation.

6.2. Optimization of SPME analysis

The most important conditions affecting the efficiency of isophthalaldehyde extraction by SPME were independently evaluated. Extraction phase, extraction temperature (60, 80 and 100°C), and extraction time (5, 15 and 25min) were evaluated with experimental parameters. Initially, three SPME fibers with different polarity were evaluated. No peak for isophthalaldehyde was observed when the analysis was performed with the PDMS fiber. Comparing the areas obtained for the other 2 fibers, isophthalaldehyde signal for PDMS/DVB was 36% of CAR/DVB/PDMS signal. CAR/ DVB/PDMS has been previously regarded as a suitable fiber to extract polar compounds such as aldehydes, alcohols or esters (Sagandykova et al., 2017). Thus, CAR/DVB/PDMS fiber was selected as the optimal one for isophthalaldehyde determination. The optimum extraction time was fixed at 15 min because longer times did not show any increase in the peak area. The increase of extraction temperature from 60 to 80°C increased the response of the compound. However, at 100 ºC no increment of the signal was observed. Therefore, 80°C was selected as the optimal extraction temperature. The final extraction process was carried out at 80 ºC for 15 min with a CAR/DVB/PDMS fiber.

6.3. Optimization of HF-LPME analysis

In HF-LPME, the selection of the extraction solvent is the most important optimization step because the interaction between the analyte of interest and the organic solvent must be good in order to obtain a high efficiency in the extraction (Pezo et al., 2007). For this reason, the solvent was the first parameter evaluated. The ideal solvent should be insoluble in water and have a low volatility to avoid leakage from the semipermeable PP capillary. Experiments performed with dichloromethane, chloroform and diethyl-ether showed that these solvents expanded the pores of fiber and

considerable leakage was observed. With p-xylene and benzene, the analyses were very irreproducible, being the extraction volume different for all the replicated experiments performed. This could be due to the low density of these solvents in comparison to the other solvents studied. The analyses with toluene were reproducible and no contamination of the aqueous samples was observed. For this reason, toluene was selected as an extraction solvent for the analysis (Correa et al., 2015; Fashi et al., 2015; Pezo et al., 2007; Rosero et al., 2014). The extraction speed and the solvent volume were also essential parameters to promote the correct extraction. The maximum peak area of isophthalaldehyde was obtained at 2.5 µL/min extraction speed. This result agrees with previous experiments that established that low extraction rates increment the interaction between the solvent and the analyte (de Bairros et al., 2015). Regarding the extraction volume, very irreproducible results were obtained using 50 µL of extraction solvent. Using 75 or 100 µL of extraction solvent provided similar results in both cases. Therefore, 75µL were selected as extraction volume. Two extraction temperatures, 25 and 50 ºC, were also evaluated. The highest response was obtained using 50 ºC. This result could be expected since higher temperature increases the kinetic energy of the molecules and this situation facilitates the extraction. The system was not evaluated at higher temperatures because it could cause the evaporation of both the solvent and the analyte (Rosero et al., 2014).

6.4. Comparison between HF-LPME and SPME

To select the optimal methodology for isophthalaldehyde analysis, the analytical characteristics of both extraction methodologies, SPME and HF-LMPE, were determined. Standard aqueous solutions of 20 mL with different isophthalaldehyde concentrations (between 5 and 6000 ng g^{-1}) were prepared and extracted by HF-LPME and SPME according to the pre-established conditions. Initially, a solution of isophthalaldehyde had been prepared at the same concentration in the 3different solvents, water, ethanol 10% and acetic acid 3%, and analyzed by SPME and HF-LPME. The areas were measured and no significant differences were observed among the different solvents. For this reason, the analytical parameters were calculated only in

aqueous solutions. The analytical characteristics are shown in Table 1, including linearity, the limit of detection (LOD) and limit of quantification (LOQ). The linear range was established by linear regression of peak area versus the concentration of isophthalaldehyde. Both methods showed good linearity with R^2 of 0.988 (HF-LPME) and 0.978 (SPME) but in different linear ranges. HF-LPME had lower linear range than SPME. The LOD and LOQ values were calculated according to the formula:

$$LOD = 3\ S_B / m \text{ and } LOQ = 9\ S_B / m$$

were S_B and m are, respectively, the residual standard deviation of the regression line and the slope of the calibration curve (Correa et al., 2015; Hasheminasab et al., 2014).

The LOD obtained in HF-LPME analysis (10 ng g^{-1}) was far below the one obtained by SPME (250 ng g^{-1}), which makes HF-LPME the best method for the analysis of isophthalaldehyde at low concentration levels.

Table 1. Analytical parameters of HF-LPME and SPME sample preparation techniques.

Parameters	HF-LPME	SPME
Linear Range (ng g^{-1})	30 - 540	700 - 5000
R^2	0.988	0.978
LOD (ng g^{-1})	10	250
LOQ (ng g^{-1})	30	700

Figure 2 shows the chromatograms of the extraction of isophthalaldehyde standard by HF-LPME at 2 concentration levels, 10 and 30 ng g^{-1}. Other parameters were evaluated, such as the enrichment factor (EF) and the reproducibility (RSD (%)) of the method, obtaining values of 60 fold and 6.1% respectively. EF was calculated as

the rate between the concentration in the HF-LPME extract and the initial concentration. RSD was calculated by injecting three replicates of each concentration three different days and by different persons (Fashi et al., 2015). The results showed that HF-LPME method is, therefore, more efficient for determination of isophthalaldehyde in migration samples, where low concentrations of the compound are expected.

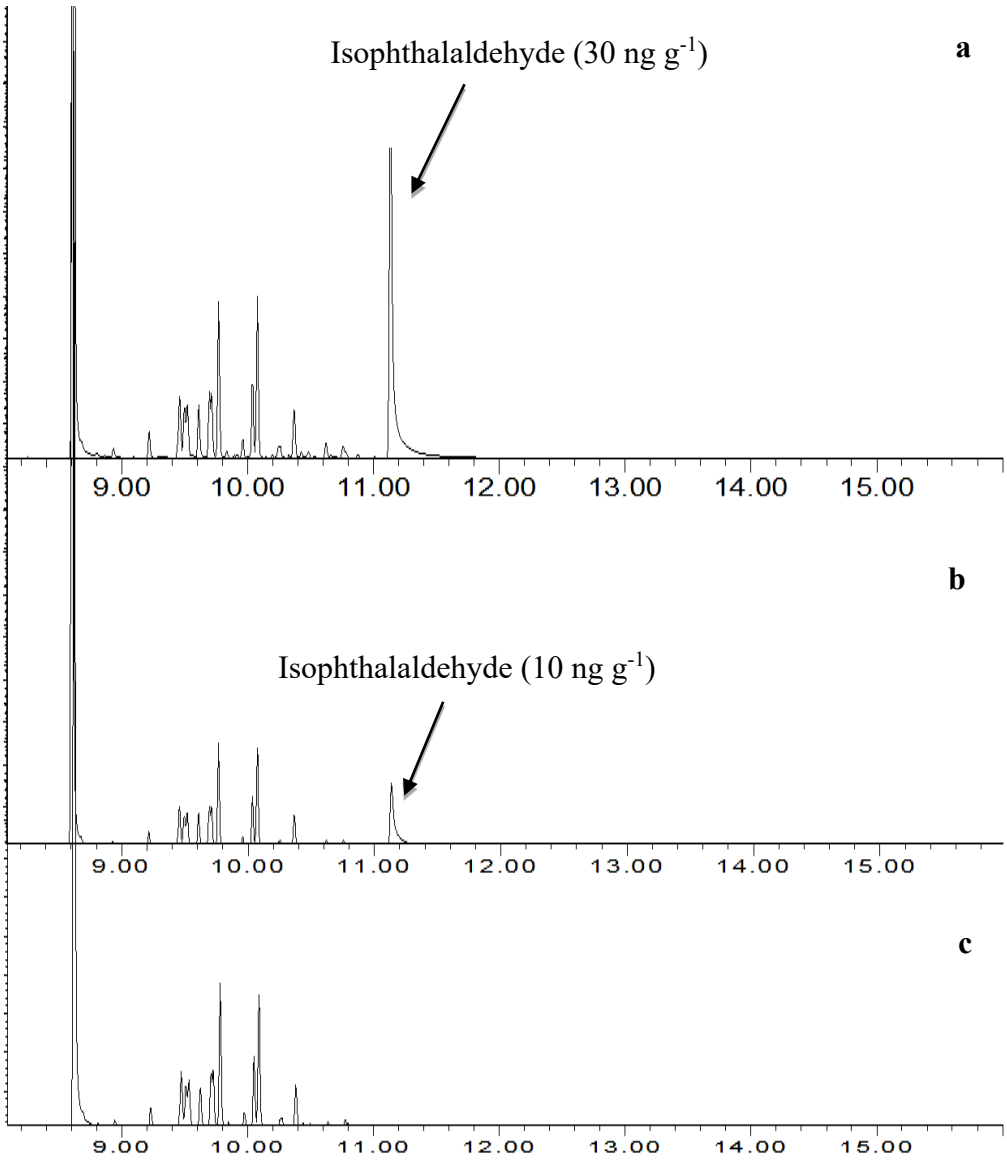


Figure 2. Chromatograms of isophthalaldehyde obtained by HF-LPME and GC-MS analysis: **a)** isophthalaldehyde 30 ng g^{-1} **b)** isophthalaldehyde 10 ng g^{-1} **c)** Blank.

6.5. Determination of isophthalaldehyde in PET migration samples

Migration testing is usually performed during 10 days because after this time the equilibrium of the migrant between the packaging material and foodstuff has been reached and its concentration in the simulant can be measured. Nevertheless, as isophthalaldehyde is a substance coming from polyamide oxidation, its concentration in the packaging can increase over time and therefore migration can keep increasing too. For this reason, migration experiments have been performed for longer periods.

Figure 3 shows the concentration of isophthalaldehyde in migration solutions of acetic acid 3% (w/v) and ethanol 10% (v/v) at different migration test conditions. All the samples obtained concentration values between 54 ± 10 ng g^{-1} and 183 ± 5 ng g^{-1}. In both simulants, migration values increased with time when tests were performed at 40 ºC. The highest concentrations were always observed with ethanol 10% (v/v), being on average 30% higher than in acetic acid. The same figure shows the concentration of isophthalaldehyde in the food simulants when the migration experiment was performed at a higher temperature (60 ºC) but during fewer days. Even the migration time was shorter, the concentration of isophthalaldehyde was higher than in the previous experiments, reaching values of 414 ± 3 ng g^{-1} in acetic acid 3% (w/v) and 514 ± 12 ng g^{-1} in ethanol 10% (v/v). In this case, values in ethanol 10% were also higher than in acetic acid 3% (24%). As it has been described in the introduction section, this compound comes from oxidation processes in the raw material and therefore a higher temperature during migration experiments, not only enhanced the migration phenomena but probably promoted also the oxidation process. In the same way, the increase of migration values in the experiments performed at 40 ºC during 80 days could be due to continuous oxidation in the material that would lead to a progressive release of the compound.

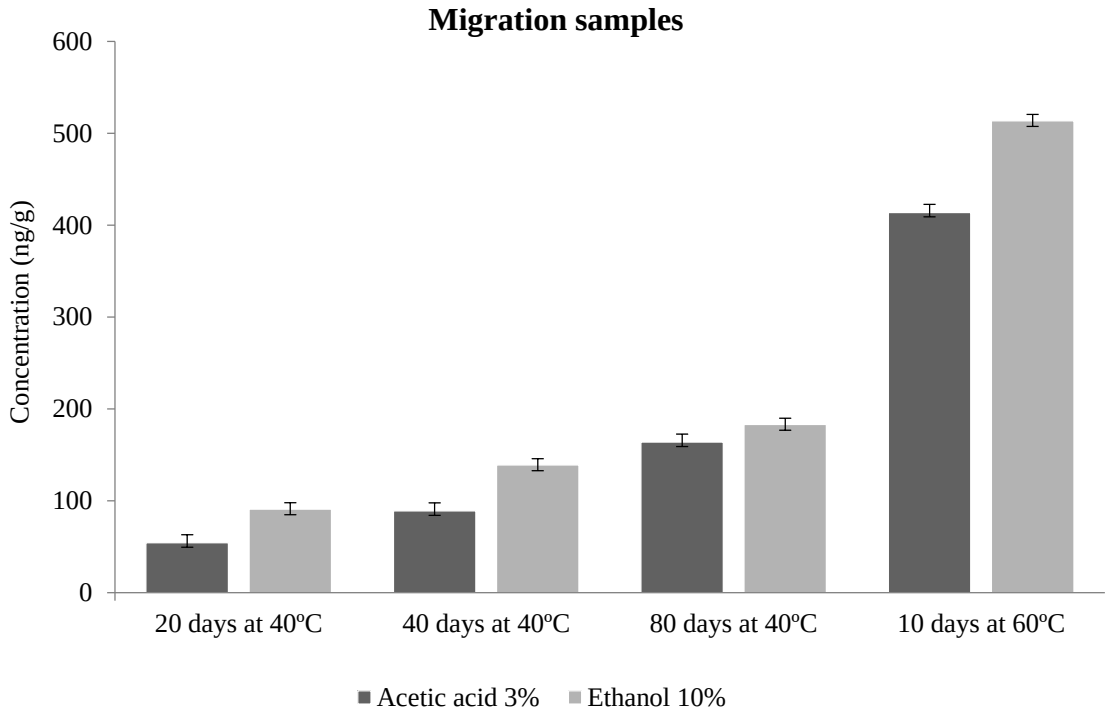


Figure 3. Isophthalaldehyde concentration in migration samples of acetic acid 3 % and ethanol 10% for 20, 40 and 80 days at 40 ºC, and 10 days at 60 ºC.

7. CONCLUSIONS

The results obtained in this study showed that both techniques, SPME and HF-LPME, are suitable for the analysis of isophthalaldehyde in aqueous migration samples. For high concentration levels, the SPME technique can be used, since it has a higher linear range. Nevertheless, for low concentration levels, it is necessary to use HF-LPME technique. The results obtained in the migration tests confirm that isophthalaldehyde can be present in migration from some PET materials to aqueous simulants at concentration levels above 10 ng g^{-1}. Since this compound comes from oxidation processes in the raw material, its release over time could be linked not only to the migration phenomena but also to a continued modification in the packaging material.

Capítulo 2

Release of volatile compounds from cooking plastic bags under different heating sources

1. RESUMEN

Las tendencias de vida actuales van encaminadas al consumo de comida precocinada y al cocinado dentro del envase. El uso a altas temperaturas de materiales destinados al contacto con alimentos, como en la cocción en el envase alimentario, puede conducir a la formación de una gran variedad de compuestos odorantes provenientes del envase que afectan las propiedades organolépticas del alimento. En este capítulo, han sido estudiados los compuestos odorantes provenientes de la cocción a alta temperatura de dos bolsas diferentes de cocinado. La cocción fue realizada mediante un horno convencional y un horno microondas y el análisis se realizó por GC-MS-O. En general se determinó que el calentamiento en horno convencional generó un mayor impacto de los compuestos odorantes sobre el aroma global de las bolsas, en comparación con el calentamiento en microondas. Las familias de aldehídos y cetonas fueron los principales compuestos detectados por olfatometría. También se realizaron ensayos de migración en diferentes simulantes alimentarios (etanol 10% (v/v), etanol 95% (v/v) y aceite vegetal) y en muestras de alimentos (pollo), en las peores condiciones de migración. En las muestras de migración en simulantes solo fueron detectaron compuesto en etanol 10%, donde se identificaron: 1-nonanol por debajo del límite de migración específico establecido en la Normativa Europea, y nonanal y decanal por debajo de 10 ng g^{-1}. En las muestras de migración de pollo fueron detectados en total de 27 compuestos, en su mayoría aldehídos.

2. OBJETIVOS

El objetivo principal de este capítulo fue determinar los compuestos volátiles generados a altas temperaturas en envases plásticos destinados a estar en contacto con el alimento durante su cocinado en horno. Dentro de este objetivo se pueden establecieron dos objetivos específicos:

- Determinar el impacto aromático de los compuestos volátiles detectados, de forma que se pudiera caracterizar el perfil sensorial del material y determinar la posible transferencia de atributos sensoriales negativos al alimento.

- Determinar la migración de los compuestos detectados a simulantes alimentarios/alimentos para garantizar la seguridad de los materiales.

  Para el cumplimiento de estos objetivos se establecieron las siguientes tareas:

  - Entrenamiento de un panel de catadores para la detección olfatométrica de los principales compuestos odorantes.

  - Optimización de la técnica de microextracción en fase sólida por espacio de cabeza (HS-SPME) para extracción y concentración de compuestos volátiles.

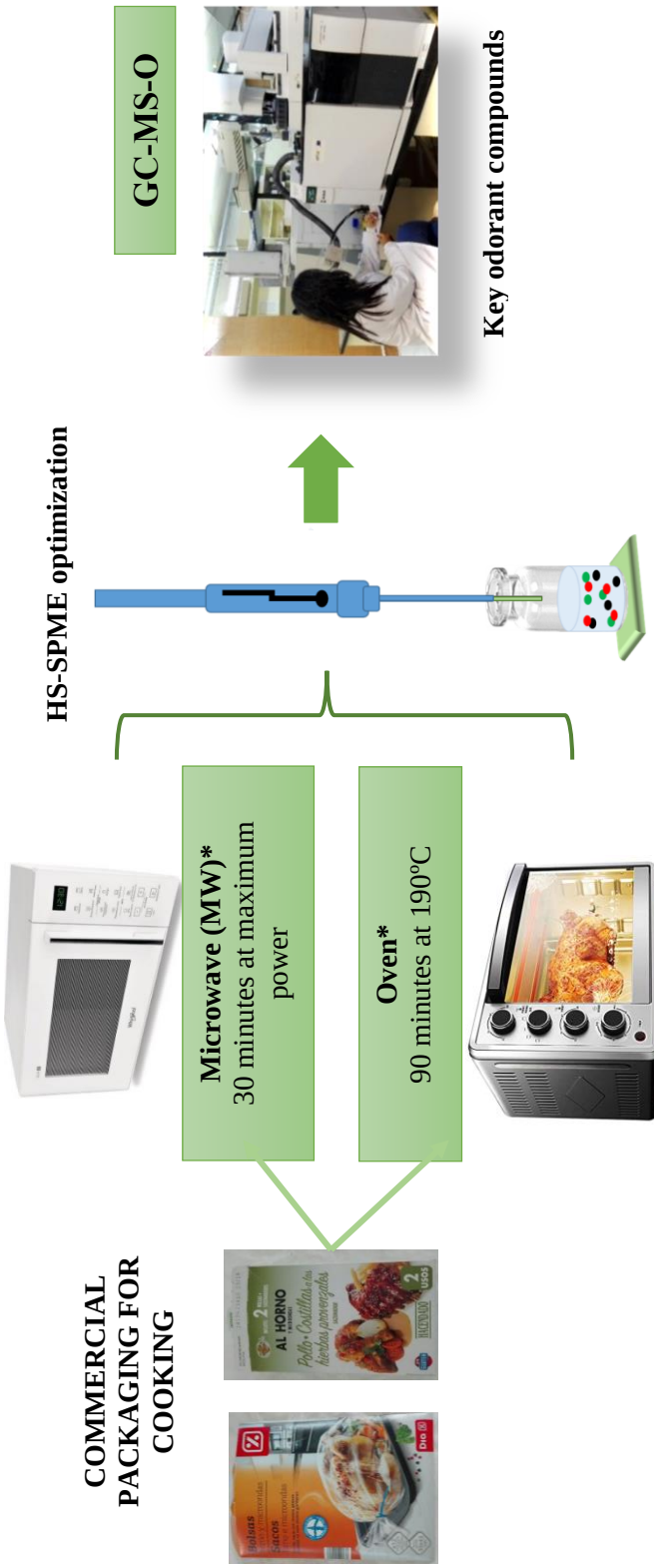
  - Análisis mediante GC-MS-Olfatometría de las muestras de envase sometidas a calentamiento en horno y microondas, y comparación del efecto de la temperatura en ambos escenarios.

  - Realización de los ensayos de migración en simulantes y alimentos, siguiendo los protocolos establecidos por la Legislación.

  - Identificación y elaboración de una librería con los potenciales migrantes odorantes.

  - Cuantificación de los migrantes odorantes en simulantes alimentarios y en muestras de alimentos reales.

3. ESQUEMA DE TRABAJO



Esquema 2. Diseño experimental del Capítulo 2

4. INTRODUCTION

Lifestyle factors can influence the eating habits of consumers and their way of cooking. In-pack cooking has been increasingly used, since it is a fast and clean technique that requires minimum hand manipulation. Since plastic bags used for this purpose are in contact with food, it must be guarantee that there is no transference of compounds that could modified food sensory properties or consumers safety. It is well known that plastic packaging materials are not inert and different interactions between packaging and food can take place. One of the most important process is migration, defined as the transference of compounds from packaging to food (Castle, 2007) . These compounds could modify the sensory food properties or cause damage in consumers' health. For this reason, all packaging materials intended for food contact must fulfil the frame Regulation (EC) 1935/2004 (European-Commission, 2004), whose main principle is that "Any material or article intended to come into contact directly or indirectly with food must be sufficiently inert to preclude substances from being transferred to food, in quantities large enough to endanger human health, or to bring about an unacceptable change in the composition of the food or a deterioration in its organoleptic properties". The most employed materials for the manufacturing of food packaging are plastic polymers, that must also fulfill Regulation EU/10/2011 for plastic materials intended for food contact (European Commission, 2011a). This Regulation establishes a positive list of substances that can be used in the materials manufacturing as well as the migration test that must be done before the commercialization of the materials.

Some research studies have been focused on the identification and quantification of migrants that could have adverse effects on human health;(García et al., 2019). Other authors have focused their work in the study of the sensory impact of migrating compounds on the packaged food and the determination of the main odor-active compounds (Vera et al., 2020). These studies have been performed mostly by gas chromatography-olfactometry.

These studies have been mainly performed in polyolefins (polyethylene and polypropylene), where carbonyl compounds, such as aldehydes and ketones, coming from oxidation processes, were the main responsible for off-flavors (Bravo et al., 1992; Hopfer et al., 2012; Sanders et al., 2005; Wrona et al., 2017). Bravo et al studied the odor-active compounds produced by thermal oxidation of polyethylene (Bravo et al., 1992), determining that the thermal processing in the presence of oxygen could lead to the formation of by-products with high aroma impact. Compounds responsible for the wax-like flavor of polyolefins were mainly saturated and unsaturated aldehydes and ketones (C6-C9) such as hexanal, 1-hepten-3-one, octanal, 1-nonen-3-one, nonanal, E-2-nonenal and diacetyl. Subsequently, Sanders et al. determined that 8-nonenal was the main contributor to the "plastic" off-odor in polyethylene packaging (Sanders et al., 2005). According to the studies performed by several authors (Dzięcioł, 2012; Hopfer et al., 2012; Miskolczi et al., 2006), high temperature, the presence of oxygen during processing or the presence of antioxidants are key factors in the formation of odor-active oxidation species in plastic polymers. The irradiation of polypropylene has also shown to influence the formation of odor-active compounds (Tyapkova et al., 2009). In the case of polyethylene terephthalate (PET), studies have been focused in migration of odorants from PET bottles to water, being acetaldehyde the main responsible for off-flavours detected in bottled water (Bach et al., 2012). In addition to conventional polymers, odor-active compounds have also been studied in emerging packaging materials such as starch (Osorio et al., 2019) or polylactic acid (PLA) (Ubeda et al., 2019).

The release of odor compounds from the packaging and its transference to food will be influenced by external factors such as the temperature, and in this way the new eating and cooking habits should be considered. Then, in-pack cooking heats not only the food but also the packaging materials, and the high temperatures could cause modifications in the composition of the packaging that will affect both, the safety and quality of the packaged food. The new cooking methods are increasingly used nowadays and this fact makes necessary to guarantee consumers health and food sensory properties when they are used. One of this new methods is in-pack cooking, where PET is commonly used as packaging material due to the high temperatures that are reached

during cooking, especially in the oven. Previous works that have studied the effect of high temperatures in the migration from PET food packaging materials, most of them focused on the migration of PET oligomers (Alin et al., 2013; López-Cervantes et al., 2003) or antimony (Haldimann et al., 2013). However, as far as the authors know, the effect on the release of volatile compounds, and specially odorants, had not been determined. In this work, the effect of two heating sources (oven and microwave) in the release of odorant compounds from two different PET cooking bags, has been studied by GC-MS-O The volatile migrants generated during this process have also been determined both in food simulant and in a real food sample.

5. MATERIALS AND METHODS

5.1. Samples

Two different kinds of plastic bags designed for in-pack cooking were studied (B1 and B2), they were purchased in two different supermarkets. These cooking bags were intended for cooking different types of meat, such as chicken or pork, as well as fish. They were made of polyethylene terephthalate. For oven cooking, the packaging instructions recommended not to exceed a maximum temperature of 200 ºC and cooking times from 45 to 90 min depending on the food weight. In the case of microwave cooking, 800 W and times from 25 to 35 min were recommended.

5.2. Reagents and SPME fibers

Methanol (LC-MS quality) from Scharlau Chemie (Sentmenat, Spain) and dichloromethane from Panreac (Barcelona, Spain) were used. Hexanal, m-xylene, 1-octen-3-one, octanal, (E)-2-octenal, 1-nonanol, nonanal, sotolon, (E)-2-nonenal, estragol, decanal, undecanal,tridecanal, heptanal, 1-octanol, (E)-2-decenal and tridecane were bought to Sigma-Aldrich (Barcelona, Spain). SPME fibers (PDMS 100 µm, DVD/CAR/PDMS 50/30 µm and CAR/PDMS 75 µm) were provided by Supelco (Bellefonte, PA, USA).

5.3. Analysis by GC-MS-Olfactometry of the odorants released from cooking bags

5.3.1. Protocol for the heating of cooking bags

For this study, cut-offs of 2.5 x 2.5 cm of the cooking bags (B1 and B2) were introduced inside 20 mL glass vials and closed with screw caps in order to avoid volatiles losses during the heating process. These vials were kept at the most extreme cooking conditions described in the packaging: 190 ºC (to assure that temperatures did not exceed 200 ºC) during 90 min for oven heating and 800 W during 35 min for microwave heating.

5.3.2. Selection of the methodology for the analysis of the main odorants

Two different protocols were tested over the bag samples, in order to select the best methodology for the determination of the main odorants generated during the samples heating: liquid extraction and direct analysis by HS-SPME. All the analyses were performed with the samples before and after oven heating (BH and AOH), following the protocol described in section 5.3.1., and the GC-MS chromatograms were compared.

In liquid extraction, the samples were cut in small pieces and 3 consecutive extracted with 3, 2 and 2 mL of the extraction solvent during 1 hour in an ultrasounds bath. Once the extractions were performed, extracts were mixed and evaporated under a gentle nitrogen current up to 1 g. Two extraction solvents were tested, dichloromethane and methanol. Finally, 1 □L of the extract was analyzed by GC-MS. In direct analysis by HS-SPME the samples were directly analyzed in the 20 mL vials where they were oven heated by HS-SPME. Three kinds of fibers with different polarities were tested: PDMS, DVD/CAR/PDMS and CAR/PDMS. Experiments were performed in triplicate.

5.3.3. Analysis of the odorants release from cooking bags by SPME-GC-MS-Olfactometry

According to the results obtained in the former experiment, the analysis of the odorants from cooking bags was performed by HS-SPME-GC-MS-O using a DVD/CAR/PDMS SPME fiber.

Materials 1 and 2 were analyzed before heating (BH) and after being oven heated (AOH). Material 1 was also analyzed after being heated in the microwave (AMH). Samples were directly analyzed in the 20 mL vials where they were heated, without opening them, in order to avoid volatiles losses. Blanks of the vials, with and without heating, were also analyzed.

For the SPME extraction, samples were first equilibrated at 80 ºC during 15 min and then, extracted at 80 ºC during 20 min. Samples were heated in the heater module of a Combipal autosampler from Agilent. SPME fiber was desorbed at 250 ºC for 2 min in splitless mode.

For the analysis, a gas chromatograph 7820A GC system coupled in parallel to a mass spectrometer 5977B MSD from Agilent Technologies (Santa Clara, CA, USA) and an olfactory detection port from Phaser GL Sciences (Germany) were used. The column was a HP-5MS (30m x 25mm x 0.25µm film thickness) from Agilent. The oven temperature ramp was as follows: initially 40 ºC for 5 min, 10 ºC/min to 300 ºC and held at 300 ºC for 10 min. MS analysis was performed in SCAN mode from m/z 50 to 450. For the olfactometry, the transfer line was heated at 200 ºC and the sniffing port was purged with humidified air. Olfactometries were performed by 5 trained panelists that described the aroma perceived and its intensity in a scale from 1 (low intensity) to 3 (high intensity), middle values were also allowed. All the panelists performed the analysis of all the samples. They were previously trained using the same methodology than Osorio et al (Osorio et al., 2019). In order to compare the aroma impact of the compounds perceived, the modified frequency percentage (MF %) was calculated according to the following equation:

$$MF\ (\%) = \sqrt{F(\%)\ x\ I\ (\%)} \qquad \text{Equation 1}$$

Where F is the frequency of perception and I the average intensity, both expressed as percentage. Compounds with MF% values above 50% were considered relevant for the global aroma of the material (Osorio et al., 2019; Wrona et al., 2017).

5.3.4. Identification of odorant compounds

The identification of a detected compound was initially performed by comparison of its mass spectrum with those reported in NIST v2.2 library. A candidate was *confirmed by NIST*, when the match value between the mass spectrum of the compound and the candidate proposed (values from 0 to 1000) was above 800. The retention index (RI) of the detected compounds was also calculated. For this purpose, a solution of alkanes from C_7 to C_{40} was injected in the same conditions than the sample and the RI was calculated. A candidate was *confirmed by RI* if when the relative difference between its calculated RI value and RI value from the bibliography was less than 5%. Bibliography databases consulted were [www.flavornet.org] or [www.thegoodscentscompany.com]. These databases were also consulted in order to know the aroma description of the candidates previously reported in the literature. Only compounds with similar descriptions to those detected by the panelists were taken into account for the identification.

Finally, when the standard of the compound was available, it was injected in the same conditions as the sample. When there was a good match of retention time and mass spectra between the compound and the standard, the candidate was considered *confirmed by standard*.

5.4. Analysis of migration from cooking bags

5.4.1. Migration assays

Migration assays were performed in food simulants as well as in real food. For the migration assays with food simulants, the following simulants were used: ethanol

10% (simulant A), vegetal oil (sunflower oil) (simulant D2) and ethanol 95% as simulant D2 substitute. The selection was based on the Regulation EU/10/2011 and according to the intended uses of these cooking bags, such as chicken or fish. First, a cut-off of 1 x 5 cm of the sample materials (1 and 2) was immersed in a 20 mL vial containing 9 g of the food simulant. Then, the vial was closed with a screw cap and introduced in the oven for carrying out the migration test. Since according to the results obtained in the analysis of the odorants, oven cooking provided a higher release of volatiles, this cooking method was chosen for migration experiments. Migration test with vegetal oil simulant was performed at the worst case conditions, 190 ºC during 90 min. Migration test with ethanol 10% were performed at 100 ºC during 90 min. These conditions were selected according to Regulation EU/10/2011 that establishes that when migration temperatures exceed 100 º C, contact temperature for ethanol 10% must be replaced by a test at 100 ºC. In the case of ethanol 95%, substitute of simulant D2, the migration conditions were selected according to the Guidelines on testing conditions for articles in contact with foodstuffs, 6 hours at 60 ºC. A blank of the simulants was also carried out, submitting them to the same time-temperature conditions.

For the migration assays with food, chicken breasts were purchased in a retail store. In order to have a homogeneous sample, a chicken breast was cut in 3 similar size pieces; one of them was cooked in the oven without the plastic bags (blank), and the others wrapped in the cooking bags 1 and 2. The cooking conditions were selected according to the meat weight (190 ºC, 20 min).

5.4.2. Analysis of migration samples by HS-SPME-GC-MS

In migration to food simulants experiments, the migration vials were withdrawn from the oven, opened, and the cooking bag samples were removed from the migration solution. Vials were immediately closed and migration solutions were analyzed by HS-SPME-GC-MS. In the case of ethanol 95% food simulant, solutions were previously diluted 10 fold with water and an aliquot of 9 g introduced in the 20 mL vials, for its analysis. In migration to chicken samples, 2.50 g of chicken (migration samples or

blanks) were cut in small pieces and introduced in 20 mL glass vials. Then, the vials were closed with screw caps for its analysis by HS-SPME-GC-MS.

The analysis of food simulants and chicken was performed under the same conditions used for the analysis of cooking bags described in section 5.3.2.

5.4.3. Determination of migrants

For determining the migrants present in the different simulants two strategies were followed. First, in order to check the appearance of new peaks or a significant increment in the peaks already present in the blank, chromatograms of migration solutions and migration blanks were overlaid and visually compared. Then, due to the complexity of some matrixes, such as vegetal oil and chicken, a targeted analysis was performed. This analysis was focused on the volatiles released from cooking bags after being heated. For this purpose, chromatograms of the cooking bags obtained by HS-SPME-GC-MS before and after being oven heated were overlaid and visually compared. A list with the compounds released during heating was created (Table 2). This list included 72 compounds, its retention time, its identification based on NIST library match value, and the masses used for its confirmation. The listed compounds were searched in vegetal oil and chicken migration samples and blanks, and the areas of the peaks were measured. A t-student test was performed in order to know if there were significant differences between blanks and migration samples.

In food simulants, significant differences between samples and blanks were only found in ethanol 10%. The quantification in this simulant was performed by external calibration. Calibration curves were prepared in ethanol 10% and analyzed by HS-SPME-GC-MS following the procedure described in section 5.3.3. Table 2 shows the analytical parameters of the analysis. All the analyses were done at triplicate.

For the analysis of chicken migration, a calibration curved was built spiking chicken blank samples (chicken cooked in the oven without cooking bag at 190 ºC – 20 min) at different concentration levels. For this purpose, aliquots of 2.5 g of chicken were

cut in small pieces, introduced in 20 mL vials and spiked with 20 µL of the spiking solutions. Vials were closed with screw caps and analyzed by HS-SPME-GC-MS.

5.5. Sensory analysis

Panelists were 4 men and 11 women with ages between 22 and 65 years old and with experience in sensory test. Sensory test were only performed on odor (orthonasally) in all cases. The tests were performed in a room with no odor interferences.

First, panelists performed a triangle test where the differences in the aroma perception of cooking bags before and after the oven heating process were evaluated. For this test, cut-offs of the plastic bags (2.5 x 2.5 cm) were introduced in glass vials (20 mL) that were closed with screw caps. Half of them were introduced in the oven at the previously described conditions, 190 ºC during 90 min (AOH) and the other half was not heated (BH). Afterwards, samples were codified with a 3 digits code and presented to the panelists for the triangle test. Each panelist performed 2 triangle tests (2 AOH + 1 BH, 1 AOH + 2 BH). Finally, the heated samples were presented to the panelists for a free description of the main aroma notes perceived.

6. RESULTS AND DISCUSSION

6.1. Identification of the odorants released from the cooking bags after heating

6.1.1. Methodology selected for the analysis of the main odorants

The results obtained by the tested methodologies showed that there were clear differences in the volatile compounds composition of cooking bags before and after heating. These differences were detected in a higher extent in direct analysis by HS-SPME rather than with liquid extraction. Among the 3 different fibers used, DVD/CAR/PDMS provided the most intense peaks. Probably, because this fiber includes 3 different adsorbents and allows the extraction of compounds with different polarities. For this reason, this fiber was selected for further experiments. Figure 1 shows

a chromatogram obtained by HS-SPME-GC-MS of a cooking bag before and after being heated in the oven.

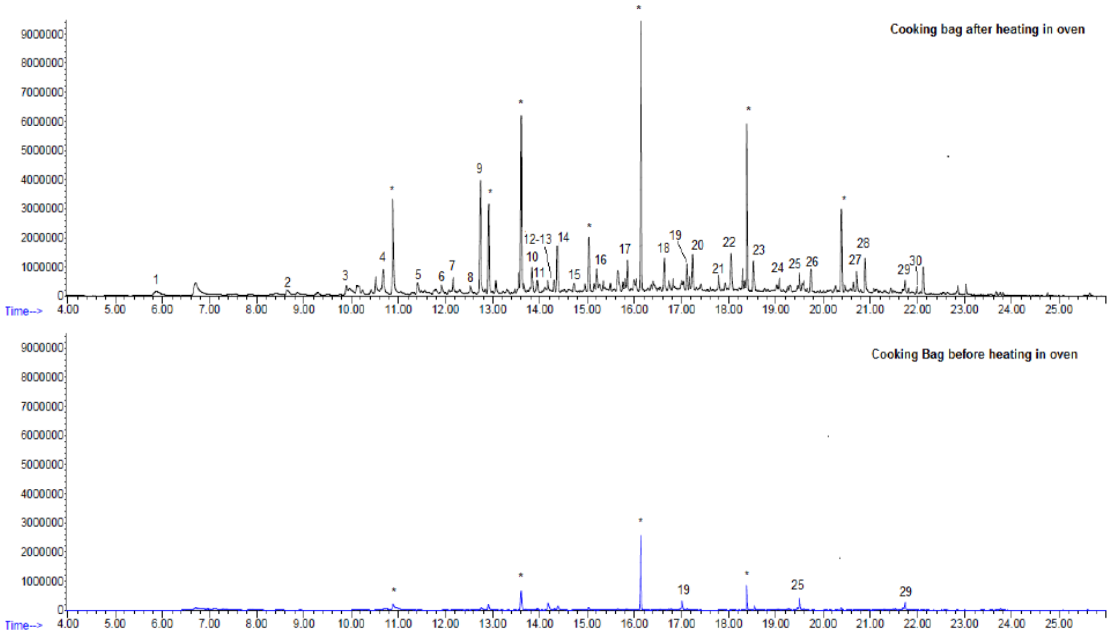


Figure 1. Chromatogram obtained by SPME-GC-MS of cooking bag 1 before and after being submitted to oven heating. Compounds: (1) hexanal; (2) heptanal; (3) 2-heptenal; (4) 2-pentyl furan; (5) 2-ethyl-1-hexanol; (6) 2-octenal; (7) 1-octanol; (8) 2-nonanone; (9) nonanal; (10) 1-nonanol; (11), 1-methyl-cyclododecene; (12) 2-decanone; (13) dodecane; (14) decanal; (15) benzothiazole; (16) 2-decenal; (17) undecanal; (18) 2-undecenal; (19) tretradecane; (20) dodecanol; (21) tetradecanol; (22) dodecanal; (23) tridecanal; (24) nonylciclohexane; (25) cetene; (26) tetradecanal; (27) 2-pentadecanone; (28) pentadecanal; (29) 1-octadecene; (30) octadecanal; *siloxanes.

6.1.2. Identification of the odorants released from the cooking bags during oven cooking

The triangle sensory test allows knowing if there are perceivable differences between 2 samples. A small probability value (p-value < 0.05) evidences the existence of significant differences between them. The results obtained from the cooking bags (before and after oven heating) showed significant differences among them. 28 out of

30 of the answers provided by the panelists were correct providing a p-value <0.001 (Meilgaard et al., 2006). These differences were described by the tasters with the notes: plastic, burnt, rubber, burnt oil, closed and old.

In order to know the volatiles responsible for these aroma notes, samples were analyzed by HS-SPME-GC-MS-O. Table 1 shows the compounds detected in the olfactometries of both cooking bags (B1 and B2) after being submitted to the oven heating (AOH). Only odorous compounds with MF% values above 30% in at least, one of the samples are shown in the table. Regarding the materials before being submitted to high temperatures, olfactometries did not show odor regions with MF% over 30% and therefore no data have been included in the table. Very similar results were found for both materials, B1 and B2. The results showed a total of 28 different odor regions where the largest proportion corresponded to aldehydes and ketones.

Table 1. Compounds detected by GC-O-MS, experimental and bibliographic retention index (RI_{exp} and RI_{bib}), aroma descriptors and aroma group (AG) (1: Chemical; 2: Green, aldehyde; 3: Roasted; 4: Mushroom; 5: spicy, liquorice; 6: pleasant, sweet); modified frequency (MF%) in cooking bags (B1 and B2) after oven (AOH) or microwave (AMH) heating.

#	rt	Candidates	CAS	RI_{exp}	RI_{bib}	Aroma descriptors	AG	MF % B1 AOH	MF % B2 AOH	MF % B1 AMH
1	6.55	3-methyl-2-pentanol	565-60-6	772	768	Gas, solvent, chemical	1	50.9	61.1	44.7
2	7.11	Hexanal✓*	66-25-1	795	801	Lemon, green	2	11.8	30.6	<10.0
3	8.25	m-Xylene*	108-38-3	843	866	Chemical, plastic, moisture	1	56.1	65.3	<10.0
4	8.87	Methyl furanthiol	28588-74-1	869	868	Roasted corn	3	56.9	11.5	16.3
5	9.11	2-4-Dimethyl thiazole	541-58-2	879	878	Chemical	1	62.3	61.1	<10.0
6	10.10	Methyl dihydrofuranthiol	26486-13-5	924	936	Roasted corn, food, bread	3	57.7	67.3	44.7
7	11.23	**1-octen-3-one✓***	4312-99-6	981	980	Mushroon	4	**88.2**	**89.0**	**81.6**
8	11.68	**Octanal✓***	124-13-0	1004	1006	Lemon, green	2	**71.4**	**89.0**	32.7
9	12.77	2-octenal✓*	2363-89-5	1068	1060	Chemical, green, sweet	2	58.9	53.0	18.3
10	13.03	**3,5-octandienone**	38284-27-4	1083	1095	Mushroom	4	**89.7**	**84.2**	49.0
11	13.32	**Nonanal✓***	124-19-6	1100	1104	Cucumber, green, pine	2	**94.3**	**89.0**	61.3
12	13.50	**Sotolon***	28664-35-9	1112	1113	Curry, marple syrup	5	**94.3**	**89.0**	<10.0
13	14.13	**Isoborneol✓**	124-76-5	1152	1158	Green, moisture	2	**71.4**	**73.6**	**83.7**
14	14.25	Unknown		1160		Unpleasant, chemical	1	39.1	61.2	<10.0
15	14.35	**(E)-2-nonenal✓***	60784-31-8	1167	1160	Chemical, lipstick, modelling clay	1	**77.6**	**86.6**	36.5
16	14.57	Unknown		1181		Cucumber, green	2	37.3	55.9	<10.0
17	14.85	**Estragol✓***	140-67-0	1199	1200	Liquorice, sweet	5	**76.4**	**76.4**	11.5
18	15.01	**Decanal✓***	112-31-2	1210	1209	Green, cucumber	2	**84.9**	**84.2**	<10.0
19	15.57	Unknown		1250	--	Sweet, pleasant	6	50.9	**70.7**	38.7

20	15.72	**(E)-**2-decenal✓	730-46-1	1261	1261	Flowery, lipstick	6	61.0	28.9	56.6
21	15.83	**g-octalactone**	104-50-7	1269	1261	Coconut, sweet	6	**71.6**	**76.4**	<10.0
22	16.43	**Undecanal✓***	112-44-7	1291	1291	Flowery, lemon, aldehyde	2	64.5	**76.4**	<10.0
23	17.20	**(E)-2-Undecenal**	2463-77-6	1372	1366	Aldehyde, cucumber	2	**73.1**	**73.6**	54.2
24	17.32	**4,5-epoxydec-2-enal**	134454-31-2	1381	1380	Liquorice	5	59.3	**73.6**	34.6
25	17.42	**(E,Z)-3,6-nonadien-1-ol✓**	28069-72-9	1389	1386	Unpleasant, metallic	1	**71.4**	**89.0**	<10.0
26	17.65	**Dodecanal✓***	112-54-9	1407	1420	Sweet, flowery	6	55.0	**79.1**	23.1
27	18.52	Unknown		1477		Unpleasant, old, powder	1	64.5	40.8	<10.0
28	19.00	Tridecanal✓*	10486-19-8	1516	1511	Coconut, sweet	6	43.0	35.4	16.3

✓Confirmed by NIST. *Confirmed with standard injection.

Compounds with the highest MF% values (above 80%) and therefore, the maximum responsible for the notes detected after the heating process were: 1-octen-3-one (*mushroom*), octanal (*lemon, green*), 3,5-octadien-one (*mushroom*), nonanal (*cucumber, green*), sotolon (*curry*), (E)-2-nonenal (*chemical, lipstick*), decanal (*cucumber, green*) and (E,Z)-3,6-nonadienol (*unpleasant, metallic*). Ketones and aldehydes have been described in previous works as compounds coming from polyolefin oxidation (Bravo et al., 1992; Hopfer et al., 2012; Sanders et al., 2005) and 1-octen-3-one was also found in irradiated polypropylene (Tyapkova et al., 2009). Some of these compounds have been detected also in non-polyolefin polymers. (E)-2-nonenal has been linked to the autooxidation of fatty acids present in PVC lubricants (Wiedmer et al., 2017) and sotolon was described by Osorio as one of the main responsible for aroma of starch-base (Osorio et al., 2019) and PLA based films (S. Ubeda et al., 2019). Compounds with MF% values above 70%, and therefore with also a considerable impact in the final aroma, were: isoborneol, estragol, γ-octalactone, undecanal, (E)-2-undecenal, 4,5-epoxydec-2-enal and dodecanal. γ-Octalactone had been previously defined as a product of polyolefin oxidation (Hopfer et al., 2012) and 4,5-epoxydec-2-enal had been detected in packaging labels (Landy et al., 2004) and polypropylene (Tyapkova et al., 2009).

Figure 1 shows a chromatogram obtained by HS-SPME-GC-MS of cooking bag 1 before and after being submitted to oven heating. As it can be observed, the number of peaks detected significantly increased after submitting the material to high temperatures, which was expected, due to the possible degradation processes linked to high temperatures (Bravo et al., 1992; Dzięcioł, 2012; Hopfer et al., 2012; Miskolczi et al., 2006).

A total of 72 volatile compounds were identified in cooking bags after heating, they are displayed in Table 2. Aldehydes were the major compounds, even though ketones, alcohols and alkenes also increased due to high temperatures.

Table2. Compounds whose signal in HS-SPME-GC-MS analysis increased when cooking bags were oven heated, retention time (RT), NIST match value, quantification ion (QI) and confirmation ions (CI1 and CI2) used for its determination and its presence in migration to vegetal oil (simulant D2) and chicken.

	t_R	Candidate	NIST Match	QI	CI 1	CI 2	Vegetal Oil	Chicken
C1	5.88	Hexanal	839	56.1	72.1	82.1	X	X
C2	8.65	Heptanal	913	70.1	55.1	81.1	X	X
C3	8.86	Oxime-, methoxy-phenyl-_	879	133.0	151.0	86.0		
C4	9.90	(E)-2-Heptenal	952	83.1	70.1	97.0	X	X
C5	9.97	Benzaldehyde	855	105.0	77.0	51.0	X	X
C6	10.12	2H-Pyranmethanol, tetrahydro-2	778	113.1	95.1	59.1		
C7	10.24	Formic acid, heptyl ester	770	70.1	56.1	83.1	X	X
C8	10.42	1-Octen-3-ol	828	57.1	99.1	85.1	X	X
C9	10.68	Furan, 2-pentyl-	802	81.1	138.1	57.1	X	X
C10	11.41	1-Hexanol, 2-ethyl-	816	57.1	83.1	98.1		
C11	11.78	2(3H)-Furanone,5-heptyldihydro-	942	85.0	57.1	100.0	X	
C12	11.92	(E)-2-Octenal	893	70.1	83.1	97.1		X
C13	12.06	Acetophenone	951	105.1	77.1	120.1		
C14	12.16	1-Octanol	884	56.1	69.1	84.1	X	X
C15	12.53	2-Nonanone	821	58.1	71.1	142.1		
C16	12.74	Nonanal	942	57.1	98.1	114.1	X	X
C17	13.29	2-Oxepanone	824	55.1	84.1	114.1		
C18	13.65	(E)-2-Nonenal,	924	55.1	70.1	96.1	X	X
C19	13.72	α-Campholenal	743	108.1	95.0	85.1		
C20	13.83	1-Nonanol	923	56.1	70.1	97.1	X	X
C21	13.94	1-methyl-cyclododecene	746	97.1	68.1	180.2		
C22	14.11	Ethanol, 2-(2-butoxyethoxy)-	782	57.1	128.1	75.0		
C23	14.17	2-Decanone	770	58.1	71.1	156.1	X	X
C24	14.29	Dodecane	929	57.1	71.1	170.2	X	X
C25	14.37	Decanal	936	57.1	70.1	128.1	X	X
C26	14.71	Benzothiazole	792	135.0	108.0	69.0		
C27	14.93	NI (RI: 1239)		85.1	55.1	147.1	X	
C28	15.14	γ-Dodecalactone	800	85.0	57.1	69.1		
C29	15.20	(E)-2-Decenal	935	70.1	55.1	136.1	X	X
C30	15.27	Octan-2-one, 3,6-dimethyl-	776	55.1	83.1	154.1		
C31	15.34	1-Decanol	884	70.1	83.1	97.1		
C32	15.67	(E,Z)-2,4-Decadienal	836	81.0	95.1	180.2		X
C33	15.76	Tridecane	886	57.1	71.1	85.1		X
C34	15.81	Formamide, N,N-dibutyl-	876	72.1	114.1	157.1		
C35	15.85	Undecanal	966	57.1	82.1	126.1	X	X
C36	16.00	(E,E)-2,4-Decadienal	910	81.1	152.1	95.1		X

C37	16.28	NI (RI: 1338)		57.1	71.1	182.2		
C38	16.35	Trimethyl-cyclohex-2-en-1-ol	740	84.0	125.1	97.1		
C39	16.40	Heptylcyclohexane	834	83.1	55.1	182.2		
C40	16.63	2-Undecenal	813	70.1	83.1	121.1		X
C41	16.74	1-Octanol, 2-butyl-	898	57.1	83.1	169.2		
C42	16.82	Alcane (RI: 1368)		71.1	97.1	127.1		
C43	17.01	NI (RI: 1392)	892	55.1	83.1	111.1		
C44	17.04	2-Dodecanone	864	58.1	71.1	184.2		
C45	17.11	Tetradecane	956	57.1	85.1	198.2	X	X
C46	17.23	Dodecanal	971	57.1	82.1	140.2	X	X
C47	17.40	Epiglobulol	827	161.1	189.1	204.1		
C48	17.61	Alcane (RI: 1438)		57.1	85.1	183.2		
C49	17.78	NI (RI 1451)	885	83.1	69.1	183.2	X	
C50	18.05	Dodecanol	931	55.1	71.1	140.1		
C51	18.29	1-Pentadecene	944	83.1	97.1	111.1		X
C52	18.33	2-Tridecanone	849	58.1	71.1	85.1		
C53	18.51	Tridecanal	927	57.1	82.1	154.2		X
C54	19.02	NI (RI: 1550)		85.1	197.2	71.1		
C55	19.07	n-Nonylcyclohexane	873	83.1	55.1	210.2		
C56	19.49	Cetene	920	83.1	97.1	224.2		
C57	19.56	NI (RI: 1595)		58.1	149.0	177.1		
C58	19.59	Hexadecane	930	57.1	71.1	226.2	X	
C59	19.74	Tetradecanal	940	57.1	82.1	168.2		X
C60	20.26	Cyclopentane, undecyl-	822	69.1	83.1	224.3		
C61	20.46	1-Tetradecanol	933	55.1	83.1	168.1		
C62	20.64	NI (RI: 1689)		71.1	197.1	212.1		
C63	20.71	2-Pentadecanone	887	58.1	71.1	226.2		
C64	20.89	Pentadecanal	969	57.1	82.1	182.2		X
C65	21.44	NI (RI: 1765)		83.1	55.1	185.1		
C66	21.73	1-Octadecene	917	83.1	97.1	252.4		
C67	21.81	Octadecane	850	57.1	71.1	97.1	X	
C68	21.98	Hexadecanal	935	57.1	82.1	196.2		X
C69	22.85	2-Heptadecanone	892	58.1	71.1	254.2		
C70	23.04	Heptadecanal	900	57.1	82.1	96.1		
C71	24.75	Henicosane	800	57.1	85.1	296.1	X	
C72	25.65	Docosane	800	57.1	71.1	310.3		

t_R: retention time (min). **X** in bold represent those compounds that showed a significant increment in migration samples compared to migration blanks (t-student test, $p<0.01$); NI: non identified; RT: retention time (Min)

6.1.3. Identification of the odorants released from the cooking bags during microwave cooking

Since cooking bags were also intended to microwave cooking, the odorants released during this process were studied. Table 1 shows MF% values when the cooking bag 1 was submitted to microwave heating (B1_AMH). The results showed a lower intensity of the odorous compounds compared to those released after oven heating. These results were expected, since the temperature reached in MW was not as high as in the conventional oven. The same influence of the heating source had been previously observed in the migration of different photoinitiators to Tenax® (Ji et al., 2019). Only 5 compounds obtained MF% values above 50%: 1-octen-3-one, nonanal, isoborneol, (E)-2-decenal and (E)-2-undecenal; and only 2 out of them obtained MF% values above 80%, 1-octen-3-one and isoborneol.

If the sum of the MF% is considered, the value obtained for the samples submitted to the temperatures of the oven (1793 and 1872) is more than twice the value of samples submitted to the microwave heating (704). Therefore, the release of off-flavors from the plastic bag will be much higher when bags will be used for oven cooking.

6.1.4. Changes in the sensory notes of cooking bags during cooking

The compounds detected in the olfactometries were classified in 6 categories according to their aroma: chemical-solvent (1); green-aldehyde-lemon (2); roasted-burnt (3); mushroom (4); spicy-liquorice (5) and pleasant-sweet-flowery (6). The sum of MF% values was calculated for each category in order to evaluate the differences in the different aromas among samples. The results are shown in figure 2. The three samples showed a similar profile, although the bags submitted to microwave heating obtained a lower intensity in the descriptors. In all cases, the notes green-aldehyde-lemon and chemical-solvent had a predominant role in the aromas produced due to high temperatures. It has to be taken into account that other compounds with similar aromatic

notes with low MF% values, could enhance the perception of these descriptors. This is the case of other aldehydes produced during heating.

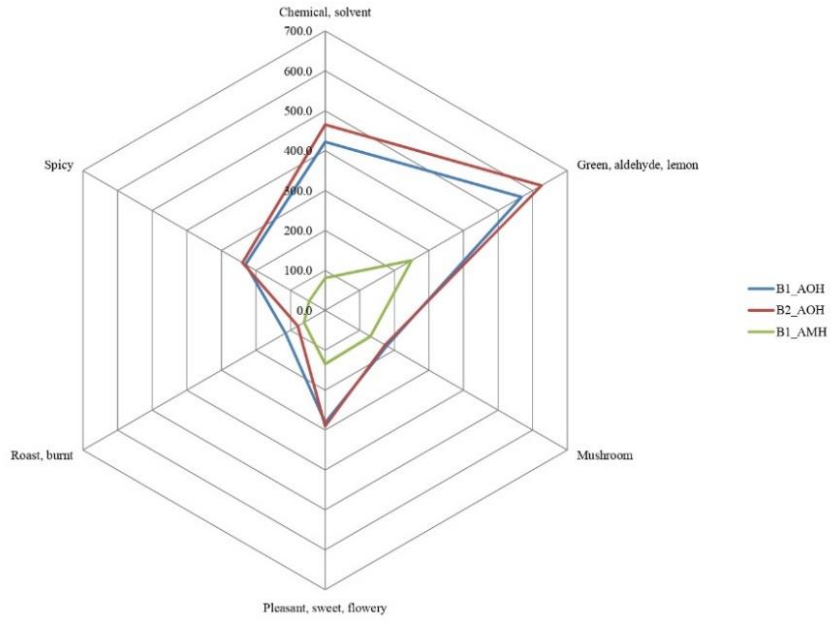


Figure 2. Spider graph of the main aroma categories perceived during GC-O in cooking bags after being submitted to oven heating (B1_AOH, B2_AOH) or microwave heating (B1_AMH).

Figure 3 shows the areas of the aldehydes detected by HS-SPME-GC-MS in bag 1 before and after oven heating. As it can be seen, there was a significant increment in all aldehydes detected, and a similar profile was observed in bag 2. All these aldehydes will also probably contribute to the green-aldehyde-lemon global notes.

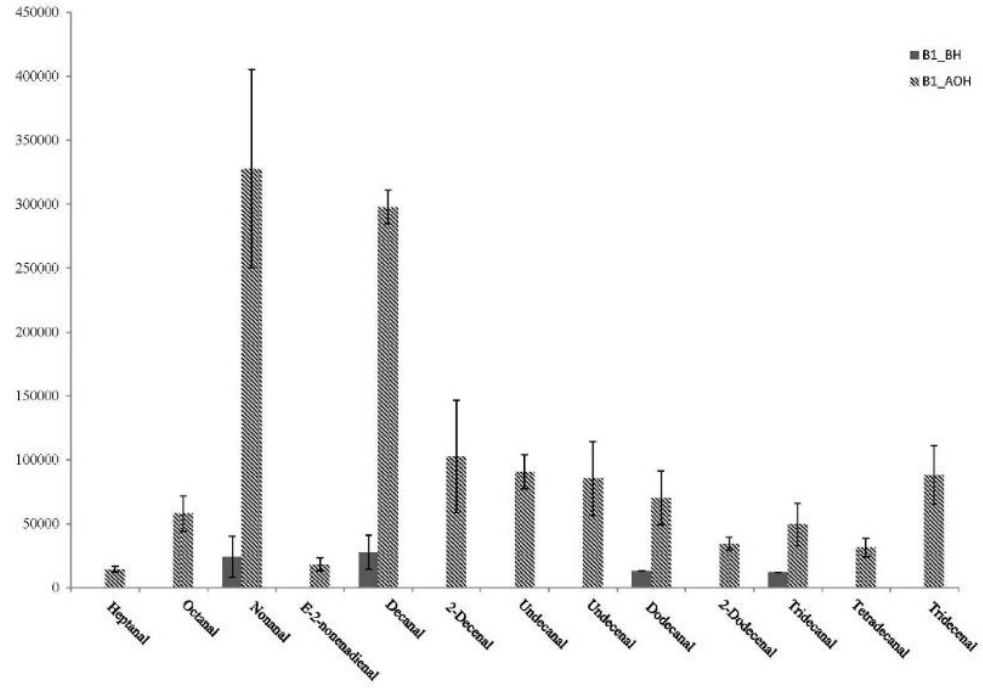


Figure 3: Intensity of aldehydes in cooking bag 1 before and after being submitted to oven heating (B1_BH and B1_AOH).

6.2. Migration from cooking bags to food simulants

The study of migration from the cooking bags to the different food simulants was focused not only in the odorant compounds but also in the determination of other volatile migrants, since, depending on their toxicity and concentration, its presence in food could be a risk for human's health. As it was reported in section 5.4.3, the presence of all the volatile compounds released from cooking bags was checked in vegetal oil and chicken. Table 2 shows the list of the 72 targeted compounds, their retention time, NIST match value, quantification and confirmation ions.

In simulant A (ethanol 10%), 3 compounds were found in migration, nonanal, 1-nonanol and decanal. Results from migration are shown in table 3. Nonanal and decanal are not listed in Regulation EU/10/2011, therefore they should not migrate at detectable quantities, what means at quantities above 10 ng g^{-1}. In both cases, the values found

were below 2 ng g^{-1}. 1-nonanol is listed in the Regulation with no SML, and it was found in both cases below 5 ng g^{-1}. According to the literature, the detection threshold of these compounds in water solutions is: 2.8 ng g^{-1} for nonanal (Czerny et al., 2008), 5 ng g^{-1} for decanal (Rychlik et al., 1998) and 0.1 µg g^{-1} for 1-nonanol (Sheftel, 2000). Since the concentration values found in migration were in all cases below these values, no changes in the food aroma are expected. In ethanol 95%, no significant differences between migration samples and migration blanks were observed. Since ethanol 95% migration samples were 10 fold water diluted previous to its analyses, it can be stated that the concentration of nonanal, 1-nonanol and decanal in migration was below 10 times the limit of detection calculated for ethanol 10% solutions (nonanal < 0.50 ng g^{-1}; 1-nonanol < 10.0 ng g^{-1} and decanal LOD < 0.10 ng g^{-1}). Table 4 shows the analytical parameters of the analysis. All the analyses were done at triplicate.

Table 3. Analytical parameters and migration values from cooking bags 1 and 2 (B1 and B2) to simulant A (Ethanol 10%) (n=3).

Migrant	**Linear range (ng g^{-1})**	**Correlation coefficient R^2**	**LOD (ng g^{-1})**	**Ethanol 10% (ng g^{-1})**	
				B1	**B2**
Nonanal	0.15 – 57.0	0.998	0.05	1.4 ± 0.18	0.58 ± 0.02
1-Nonanol	3.0 – 95.0	0.997	1.0	3.8 ± 0.33	< 1.0
Decanal	0.03 – 59.0	0.998	0.01	0.49 ± 0.01	0.31 ± 0.04

Table 4. Analytical parameters of the calibration curves performed in spiked chicken and analyzed by SPME-GC-MS (n = 3)

Compound	Linear range (ng g-1)	Correlation coefficient (R^2)	LOD (ng g-1)
Hexanal	5.1 – 77	0.993	1.5
Heptanal	9.0 – 127	0.973	3.0
(E)-2-Octenal	30.0 - 141	0.990	10
1-Octanol	6.0 - 180	0.978	2.0
Nonanal	9.0 - 150	0.975	3.0
(E)-2-Nonenal	15.0 - 130	0.987	5.0
1-Nonanol	9.0 - 280	0.995	3.0
Decanal	10 .0- 150	0.978	3.5
(E)-2-Decenal	24.0- 240	0.977	8.0
Tridecane	3.0 - 210	0.982	1.0
Undecanal	18.0 - 200	0.983	6.0

In simulant D2 (vegetal oil) when chromatograms of migration samples and migration blank were overlaid, no visual differences were observed. The main compounds detected in the chromatogram were aldehydes, such as hexanal, (E)-2-heptenal, (E)-2-octenal, (E)-2-decenal or 2,4-decadienal. Due to the complexity of the matrix a target analysis of the 72 compounds detected in the cooking bags was performed. This analysis showed the presence of 24 compounds (Table 2), most of them also aldehydes. A bar chart of the average areas of these compounds in migration samples (cooking bags 1 and 2) and blank is shown in figure 4. t-student test was performed in the area values in order to detect significant differences between samples and blank. The results from the statistical test did not reflect significant differences (p-value > 0.05), and consequently, the contact with the cooking bag was not the origin of these compounds in the vegetal oil (Cao et al., 2017). In the case of aldehydes, it has to be taken into account the high aldehydes content of vegetal oil, especially if it is submitted it to high temperatures (Katragadda et al., 2010). This fact was observed for aldehydes such as heptanal, (E)-2-nonenal or (E)-2-undecenal.

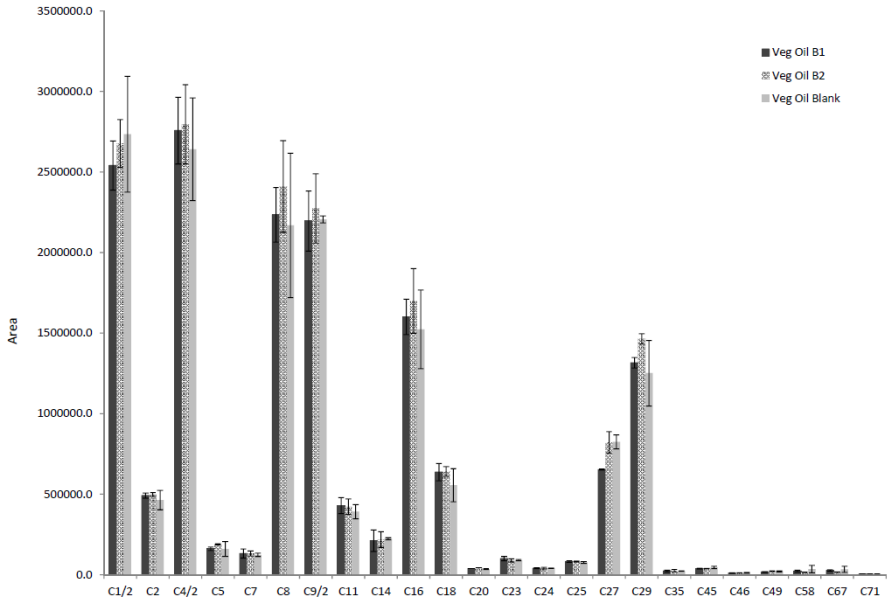


Figura 4: Bars diagram of the peak areas of the compounds detected in migration to vegetal oil from cooking bags (Veg Oil B1 and Veg Oil B2) and vegetal oil blank (Veg Oil Blank).

6.3. Migration from cooking bags to chicken

A chromatogram of migration to chicken samples, aldehydes were also the main compounds detected in this sample. In this case, nonanal was the aldehyde with the highest intensity, followed by hexanal and 1-octen-3-ol. In the targeted analysis, 28 compounds were detected, 27 out of them showed significant differences (p-value < 0.01) between samples and blank, a bar chart of the average areas of these compounds is shown in figure 5. The migrants were quantified and the concentration values are shown in table 5. Migration values were recalculated according to the EU/10/2011 Regulation rate, 6 dm^2 / 1 Kg food. None of the migrants, except for benzaldehyde, 1-octanol and 1-nonanol, were in the positive list of the Regulation and therefore they should not be detectable, it means that concentrations should be below 10 ng g^{-1}, what is not fulfilled in most cases. It has to be taken into account that migration experiments were performed in the most adverse conditions and that the contact between chicken and

cooking bags was quite high compared to a real in-pack chicken cooking where bigger pieces are cooked.

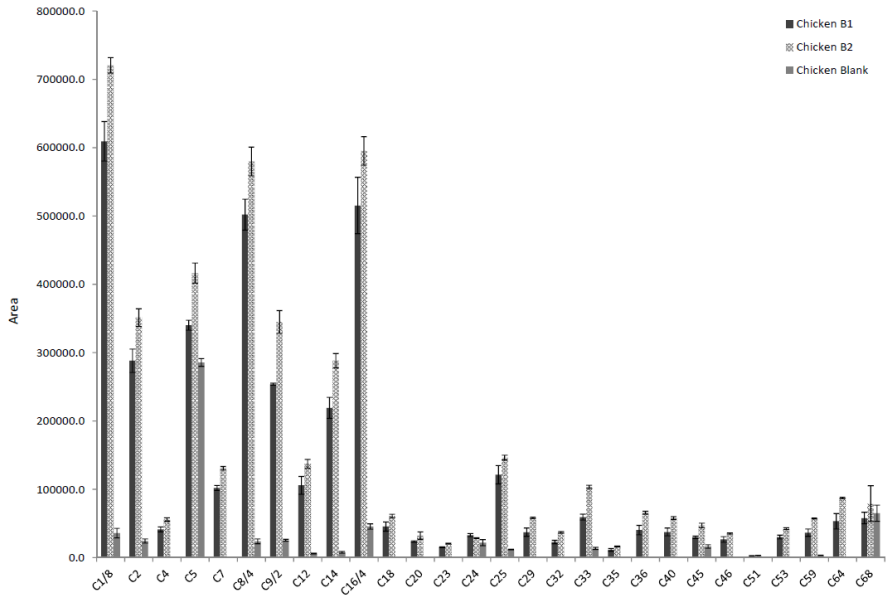


Figure 5: Bars diagram of the peak areas of the compounds detected in migration to chicken from cooking bags (Chicken B1 and Chicken B2) and Chicken (n=3). (Areas of compounds C1, C8, C9 and C16 were divided by 2, 4 or 8 in order to scale them).

Table 5. Compounds detected in migration to chicken from cooking bags 1 and 2 (B1 and B2); their migration values; and the standards used for their quantification (QS) and their specific migration values (SML) on EU/10/2011 Regulation.

	Compound	**CAS Nº**	**QS**	**B1 (ng g^{-1})**	**B2 (ng g^{-1})**	**EU/10/2011**
C1	Hexanal	66-25-1	C1	48.4 ± 2.3	64.0 ± 1.0	--
C2	Heptanal	111-71-7	C2	12.4 ± 0.7	27.5 ± 1.0	--
C4	(E)-2-Heptenal,	18829-55-5	C12	16.6 ± 1.4	23.6 ± 1.0	--
C5	Benzaldehyde	100-52-7	C2	24.8 ± 0.5	43.1 ± 1.5	No SML
C7	Formic acid, heptyl ester	112-23-2	C2	17.3 ± 0.6	24.2 ± 0.5	--
C8	1-Octen-3-ol	3391-86-4	C14	257 ± 11.6	299 ± 10.8	--
C9	Furan, 2-pentyl-	3777-69-3	C2	64.9 ± 0.4	109 ± 5.2	--
C12	(E)-2-Octenal	2548-87-0	C12	22.8 ± 2.8	38.2 ± 1.8	--
C14	1-Octanol	111-87-5	C14	18.7 ± 1.3	27.9 ± 1.0	No SML
C16	Nonanal	124-19-6	C16	19.8 ± 1.6	37.3 ± 1.3	--
C18	(E)-2-Nonenal	18829-56-6	C18	16.5 ± 2.4	22.9 ± 0.9	--
C20	1-Nonanol	143-08-8	C20	5.10 ± 0.21	7.10 ± 1.27	No SML
C23	2-Decanone	693-54-9	C25	1.37 ± 0.04	1.84 ± 0.07	--
C24	Dodecane	112-40-3	C33	<1.0	< 1.0	--
C25	Decanal	112-31-2	C25	12.0 ± 1.3	15.1 ± 0.4	--
C29	(E)-2-Decenal	3913-81-3	C29	16.2 ± 2.6	26.1 ± 0.4	--
C32	(E,Z)-2,4-Decadienal	2363-88-4	C29	9.47 ± 1.07	16.1 ± 0.5	--
C33	Tridecane	629-50-5	C33	<1.0	1.56 ± 0.04	--
C35	Undecanal	112-44-7	C35	3.57 ± 0.65	5.38 ±0.20	--
C36	(E,E)-2,4-Decadienal	25152-84-5	C29	17.7 ± 3.0	29.8 ± 1.0	--
C40	2-Undecenal	2463-77-6	C29	16.4 ± 2.5	26.0 ± 0.9	--
C45	Tetradecane	629-59-4	C33	<1.0	< 1.0	--

C46	Dodecanal	112-54-9	C35	9.01 ± 1.4	12.0 ± 0.3	--
C51	1-Pentadecene	13360-61-7	C29	<1.0	< 1.0	--
C53	Tridecanal	10486-19-8	C35	10.3 ± 0.9	14.6 ± 0.5	--
C59	Tetradecanal	124-25-4	C35	12.5 ± 1.8	19.8 ± 0.2	--
C64	Pentadecanal	2765-11-9	C35	18.4 ± 3.9	30.4 ± 0.3	--

7. CONCLUSIONS

The high temperatures used during oven cooking lead to the formation of high impact aroma compounds such as aldehydes and ketones in the cooking bag. In the case of microwave cooking, the formation of high impact aroma compounds was considerably less. Its sensory description was related to green-aldehyde-lemon and chemical-solvent notes. The presence of these compounds could modify the initial perception of the consumers once the cooking bag is opened, and produce a disgusting effect to them. When migration was evaluated, the results showed in food simulants did not show risks for consumers' health. In the case of migration to chicken, the results reveals that compounds generated from cooking bags due to the extreme temperatures reached in the oven could be transferred to food and therefore a control of these materials is needed.

Capítulo 3

Determination of volatile non intentionally added substances coming from a starch-based biopolymer intended for food contact by different gas chromatography-mass spectrometry approaches

1. RESUMEN

El rápido crecimiento de la producción de biopolímeros como materiales destinados al contacto con alimentos (FCM) debe ser respaldado por la mejora continua de la evaluación de los materiales, a fin de garantizar la seguridad de los alimentos. En este capítulo, ha sido evaluado el perfil de migración de los compuestos volátiles y semi-volátiles provenientes de biopolímeros base almidón. Para el desarrollo experimental se utilizaron muestras en forma pellets, almidón puro (material prima) y prototipos de utensilios de cocina (platos y vasos). El estudio de migración se realizó en base al protocolo secuencial para materiales de uso repetido. El análisis de los compuestos se efectuó mediante dos técnicas de cromatografía de gases-espectrometría de masas, la primera utilizando ionización electrónica con un analizador de masas de un solo cuadrupolo (GC-EI-MS); y la segunda por APGC-Q/ToF. Inicialmente se optimizó el proceso de extracción con tres formas diferentes de los pellets: gránulos sin modificación (esféricos), gránulos estrujados a alta presión (lentejas) y gránulos molidos criogénicamente (polvo). En total, en la extracción se detectaron veintiuno compuestos volátiles y semi-volátiles, de los cuales cinco únicamente se identificaron por EI y dos únicamente por APGC. El análisis de la migración se realizó con los vasos y platos utilizando varios simulantes alimentarios: etanol al 10% (v/v), ácido acético al 3% (w/v), etanol al 95% (v/v), isooctano y aceite vegetal. En total se identificaron y cuantificaron catorce compuestos en migración específica, y se estableció su nivel de toxicidad de acuerdo con la estructura molecular. Los resultados obtenidos mostraron una disminución en la migración de los compuestos volátiles y semi-volátiles con el uso repetido.

2. OBJETIVOS

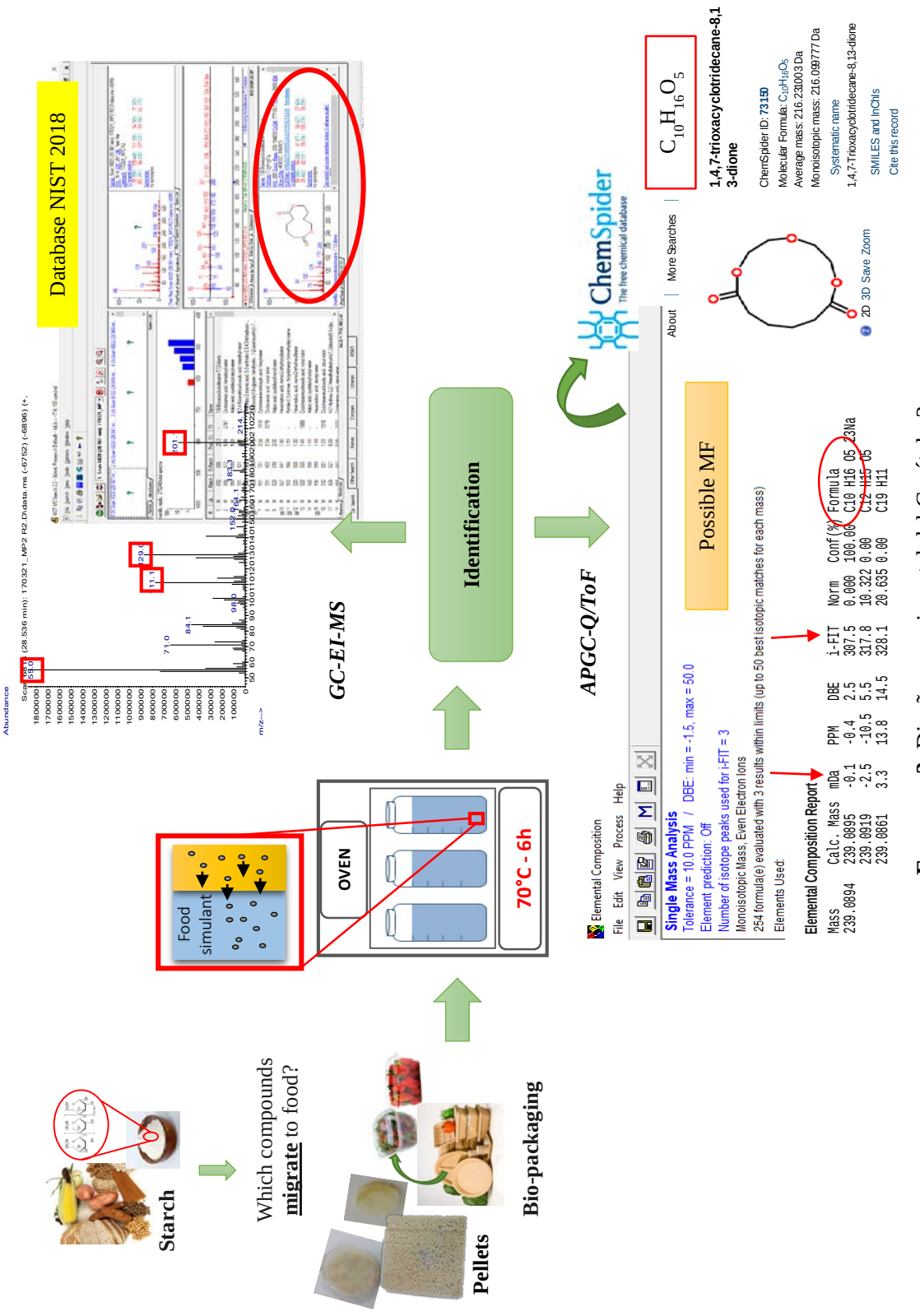
El objetivo principal de este capítulo fue la búsqueda de nuevas estrategias analíticas que pudieran garantizar la seguridad de los alimentos que se encuentran en contacto con biopolímeros. Para ello, fueron objetivos específicos de este estudio:

- Determinar y cuantificar los principales NIAS volátiles y semi-volátiles provenientes de muestras de migración de un biopolímero base almidón
- Comparar los resultados obtenidos mediante diferentes estrategias de identificación: GC-EI-MS y APGC-Q/ToF.

  Para el cumplimiento de este objetivo se establecieron las siguientes tareas:

  - Revisión bibliográfica de los principales compuestos migrantes provenientes de biopolímeros base almidón.
  - Optimización del proceso de extracción de los biopolímeros.
  - Desarrollo de un método de detección y cuantificación en los equipos GC-EI-MS y APGC-Q/ToF.
  - Elaboración de una librería de los potenciales NIAS volátiles.
  - Cuantificación de los NIAS volátiles identificados en los ensayos de migración secuencial.
  - Evaluación del riesgo de los materiales.

3. ESQUEMA DE TRABAJO



Esquema 3. Diseño experimental del Capítulo 3

4. INTRODUCTION

A number of surveys concerning starch as a principal raw material for the production of biodegradable plastics are in progress. Due to its renewability, cheapness, and physical properties, starch has been embraced by the food industry to be employed as a food contact material (Canellas et al., 2015; Muller et al., 2017; Nerín et al., 2018). Today, thermoplastic-like starch (TPS), together with polylactic acid (PLA), are the main research routes for the manufacturing of biodegradable materials (Vilpoux et al., 2010). Starch itself is not thermoplastic, but in the presence of plasticizers and reagents with hydrophilic character, high temperatures (90 – 180 °C) and shearing, it melts and fluidizes, enabling its use in injection, extrusion and blowing equipment, such as those used for synthetic plastics (Lourdin et al., 1999). Environmental pollution and disposal problems of traditional packaging materials, can be overcome by TPS films, which are made mainly from starch finished with different thermoplastic polyesters (Peters et al., 2019).

Nevertheless, the components in polymer packaging materials are not completely inert. Several studies have warned about potential toxic effects of some packaging components such as bisphenol A or phthalates (endocrine-disrupting chemicals) in plastic toys and in cosmetics in contact with plastic packaging (Andaluri et al., 2018; Galonnier et al., 2018). A correlation between the use of some multilayer plastic bags for artificial insemination has also been correlated to reproductive failures (Nerin et al., 2014). In the case of packaging intended for food contact, plastic components can migrate into the food. This mass transfer is considered as a potential source of pollution because the migrants could alter the food composition, deteriorate the organoleptic properties, and even incur a human health risk (Aznar et al., 2019; Biedermann et al., 2018; García Ibarra et al., 2018; Noureddine et al., 2019; Rodríguez Rojas et al., 2019; Szczepańska et al., 2018; Wang et al., 2019).

The rapid growth of polymer technology in the field of food contact materials (FCMs) needs to be sustained by continuous improvement in material testing, in order

to ensure the safety of foodstuff (Cheng et al., 2018). High resolution mass spectrometry is one of the most powerful tools when dealing with the analysis of Non-Intentionally Added Substances (NIAS), which are defined in the European Union (EU) Regulation No 10/2011 (European Commission, 2011a) as "impurities in the substances used, or reaction intermediates formed during the production process or decomposition or reaction products". It is not surprising that these substances have been recognised as a major challenge in material suitability testing, since the main concern about NIAS is the lack of toxicological data to be used for determining a specific migration limit (Cheng et al., 2018; Nerin et al., 2013). The main reasons that make the identification process remarkably complex and time-consuming are the increasing complexity of materials and the absence of commercial standards for structure confirmation. Furthermore, there is a consistent lack of information about the real composition of the different ingredients and materials employed for polymer manufacturing, as the material composition is usually strictly confidential.

Before performing a migration study, a screening analysis of the packaging material is often required to identify the chemicals that are more likely to migrate into the food. Particular emphasis should be given to NIAS having a molecular weight below 1000 Da, since it is generally recognized (Piringer et al., 2000) that heavier compounds have lower diffusion coefficients. Migration tests are run under specific conditions of time and temperature, selecting the food simulants according to the intended use of the material (Félix et al., 2012; Vera et al., 2014; Wagner et al., 2018).

With respect to semi-volatile and volatiles analyses, a GC coupled to a quadrupole mass spectrometer equipped with electron ionization (EI) using 70 eV is typically employed (GC-EI-MS), since this ion source is fairly capable of ionizing virtually any organic compound in a robust and reproducible way, and it allows the analyst to make use of scientific libraries for comparing acquired spectra with references (Yanhao Zhang et al., 2019). However, the identification process becomes almost impossible when the compound of interest is not listed in the library, or when the sensitivity of the single quadrupole MS is not sufficient for reliable mass

confirmation. At this stage, atmospheric pressure gas chromatography (APGC) coupled to high resolution mass spectrometry (HRMS) can become an interesting alternative to traditional GC-EI-MS (Canellas et al., 2012; Cheng et al., 2016; Cherta et al., 2015; ten Dam et al., 2016; Zhang et al., 2019). APGC is a "soft" ionization technique, similar in nature to atmospheric pressure chemical ionization (APCI). With respect to Chemical Ionisation (CI), APGC offers the advantage of simplicity and flexibility, as the ionisation would take place even in the absence of a chemical modifier, such as methane and ammonia, commonly employed in CI. Furthermore, APGC is compatible with higher carrier gas flow rates, and there is no need of breaking the vacuum when general maintenance of the ion source is required. In literature, two main ionization processes have been described for APGC in some detail (Aznar et al., 2015; Domeño et al., 2012; Hasheminasab et al., 2014; Mohammadi et al., 2017; Portolés et al., 2010). In brief, ionization is affected by the source environmental conditions. When operating under "dry" conditions, the high make-up and auxiliary gas flow rates provide substantial amounts of nitrogen, which determines the formation of a nitrogen plasma. Radical cation species react with the analytes via charge transfer, and give rise to the $M^{+\cdot}$ molecular ion. Alternatively, ionization can take place indirectly through proton transfer reactions, forming $[M+H]^+$ ions. Protonation is enhanced when an excess of water or other modifiers are present in the system. In essence, the analyte proton affinity, or gas phase basicity, determines if the modifier will facilitate or suppress the protonation reactions. By opportunely setting the gases flow in the source, it is also possible to run the instrument in a mixed mode configuration, where both charge transfer and protonation are observed. However, this will result in a compromise in sensitivity for both forms of ionization. The chemical structure of the target molecules can provide useful insight on the most suitable configuration for the analysis.

The work here in aims to determine the main volatile NIAS coming from a novel starch-based biopolymer and to explore new applications of APGC in support of GC-EI-MS, by means of using the latest discoveries in the APGC ionization. The potential

new ionization processes, such as the multi-adduct formation, will be used in order to increase the confidence in the structure elucidation of the unknown compounds. In addition, specific migration assays were carried out, in order to check the compliance of some prototype samples to European legislation.

5. MATERIALS AND METHODS

5.1. Reagents

Ethylhexyl adipate 99% (CAS: 103-23-1), diethyl phthalate >99% (CAS: 84-66-2), bis (2ethylhexyl) sebacate 97% (CAS: 122-62-3), 1,4-trioxa- cyclotridecane-8,13-dione (CAS: 1675-54-3) 98%, 11-eicosenamide 98% (CAS: 10586-57-9), isopropyl palmitate 90% (CAS: 142-91-6), palmitamide 99% (CAS: 629-54-9), octadecanamide 85% (CAS: 124-26-5), hexachlorobenzene 99% (CAS: 118-74-1), adiponitrile 99% (CAS: 111-69-3), furfural 99% (CAS: 98-01-1), methyl palmitate >99% (CAS: 112-39-0), dipropyl phthalate 98% (CAS: 131-16-8), and furaneol >99% (CAS: 3658-77-3) were purchased from Sigma–Aldrich (Madrid, Spain). Docosanoic acid ethyl ester 99% (CAS: 5908-87-2) was supplied by LGC Standards (Barcelona, Spain). Ethanol absolute (HPLC grade), methanol (LC-MS grade), acetic acid, dichloromethane, toluene, dimethyl sulfoxide, and isooctane (HPLC grade) were supplied by Scharlau (Setmenat, Spain). Hexane was from Fischer Chemicals (UK). Ultra-pure water was generated by a Millipore Milli-Q system (Billerica, MA, USA). Commercial sunflower oil was used for the migration assays. The nitrogen evaporator was a TECHNE sample concentrator (Cole-Parmer Ltd., UK). The ultrasonic generator was a Branson 3510 (frequency applied 40 Hz).

5.2. Sample characteristics

Biopolymers based on starch and polylactic acid (PLA) were supplied by a polymer manufacturing company for this study. The company is protected by a non-

disclosure agreement and additional information about the sample can not be provided. The samples were provided in two different forms: raw starch (powder), pellets of two different compositions, and prototypes of retail samples (cups and dishes).

5.3. Analysis by GC-EI-MS

Analyses were carried out on a GC system (Agilent 7890N, Santa Clara, CA, USA) equipped with an electron ionization (EI) ion source operating at 70 eV, and coupled with a quadrupole mass spectrometry detector (5977D, Agilent) operating in SCAN mode (scan range 50-350 *m/z*). The autosampler was a Combi PAL (CTC Analytics, Zwingen, Switzerland). The chromatographic separation was performed on a HP-5MS column of 30 m x 0.25 mm i.d. x 0.25 µm film thickness, injecting 1 µL at 250 ºC in splitless mode. The oven temperature program was: 50 ºC held for 5 min, ramp 10 ºC/min to 300 ºC and held for 5 min, with 4 min solvent delay. Helium was used as carrier gas at a constant flow of 1 mL/min. The transfer line heater was set at 280 ºC.

5.4. Analysis by APGC-Q/ToF

In parallel, analyses were performed using a 7890A GC system (Agilent, Santa Clara, CA, USA) equipped with an Agilent 7683B autosampler, and coupled to a hybrid quadrupole/time-of-flight mass spectrometer (Xevo G2-XS QToF, Waters Corporation, Manchester, UK), operating in sensitivity mode. The chromatographic separation was performed on a DB-5MS capillary column, 30 m x 0.25 mm i.d. x 0.25 µm film thickness. The oven temperature program was: 50 ºC for 2 min, 10 ºC/min ramp to 300 ºC and held for 10 min. 1 µL was injected in pulsed splitless mode at 250 ºC (pulse time 1.2 min, pulsed pressure 32 psi). Helium was used as carrier gas at a constant flow of 1 mL/min.

The ion source was operating in APCI+ mode, with a Corona current of 2.2 µA. The sampling and extraction cone voltages were 30 and 3 V, respectively. The cone and auxiliary gas flows were 140 and 225 L/h, respectively. The make-up gas was N_2

at 300 mL/min and 300 ºC, while the source temperature was 150 ºC. When operating under "wet" conditions, 50:50 v/v H_2O:MeOH was introduced in the ion source as chemical modifier and the cone gas flow decreased to 50 L/h. 100 pg/µL solution of hexachlorobenzene (HCB) in hexane was used for installation checks, and for monitoring the source environmental conditions. The acquisition was performed in MS^E mode, maintaining 6 eV collision energy in function 1, while a collision energy ramp 20-30 eV was used in function 2. The scan time was 0.5 s, and the acquisition range was 50-650 *m/z*. Sodium formate was used for routine mass calibration, whilst real-time mass correction was performed using a persistent column bleed peak (lock-mass 207.0324 *m/z*, $C_5H_{15}O_3Si_3$).

5.5. Data processing

Data generated by GC-EI-MS were acquired and processed with MSD ChemStation software (v. F.01.03, Agilent). Library search was performed on NIST Standard Reference Database (2018), where only Match Values greater than 700 were considered. APGC-Q/ToF data were acquired and processed using UNIFI Scientific Information System (Waters Corporation). The general screening approach for the analysis of NIAS is described elsewhere (Nerin et al., 2013), and was opportunely implemented here. Where possible, unidentified peaks in EI were submitted for an accurate mass analysis of the related molecular ions and their adducts on APGC, keeping the maximum mass error threshold at 2 mDa. Binary Compare feature of UNIFI was used to locate the components coming from the actual sample, by a direct comparison to the extracted blank sample data. In this way it was possible to trace also the compounds not visible in the total ion chromatogram (TIC). The Elucidation toolset of UNIFI was used to obtain *in silico* fragmentation of the most significant candidates after a screening check of the ChemSpider database. When feasible, the candidates where confirmed by standard injection under the same analytical conditions.

A confidence level was attributed to each candidate. Compounds presenting a NIST Match Value above 700, and supported by the accurate mass of the molecular

ion and it's adducts (when possible) were labelled as *Tentative*. In addition to the previous conditions, compounds showing fragmentation pathways described by *in-silico* fragmentation, or by the analyst's expertise, were labelled as *Confident*. Compounds whose retention time and mass spectra match those of an authentic standard, were labelled as *Confirmed*. Finally, compounds that did not fulfil any of the previous conditions were labelled as *Unknown*.

5.6. Sample extraction protocol

First, pellet samples were crushed to obtain flat lentils, 0.5 g of lentils were extracted three times with 2.5 mL of methanol in an ultrasonic bath at 40 ºC for 1 hour. The total extraction solution (7.5 mL) was concentrated to 1 mL under a gentle nitrogen flow at room temperature before injection. For consistency, each sample and solvent blank were extracted and analysed in triplicate.

5.7. Migration assays

The migration assays were carried out on prototype samples (cups) in accordance with the European legislation on food contact materials (Regulation No 10/2011/EU) (European Commission, 2011a). Two migration assays were performed: overall migration and specific migration. In overall migration the objective is to ensure that food contact materials will not bring about an unacceptable change in the composition of the food. For this purpose, overall migration values must be, according to EU legislation, below 60 mg kg^{-1}. Overall migration is the measurement of the total mass transferred from the FCM during the exposure to a food simulant. It was assessed with ethanol 10% (v/v), acetic acid 3% (w/v), ethanol 95% (v/v), isooctane, and sunflower oil, at 70 ºC for 2 hours. In specific migration, the amount of every single component that migrates from the food contact material is evaluated, and these values should be below the specific migration values (SMLs) established in the EU legislation. Specific migration was assessed with ethanol 10% (v/v), acetic acid 3% (w/v) and ethanol 95% (v/v). Contact time and temperature were 6 hours and 70 ºC, respectively. In both cases, migration test was performed by single-side contact, filling

the cups with the corresponding simulants. Since the FCMs under study are intended for repeated use, the migration tests were performed three consecutive times (European Commission, 2011a). The experiments were carried out in a thermostatic oven. The tests were performed in triplicate for each sample and blank. After migration, the detected analytes were semi-quantified against an external calibration, by using a range of standard compounds diluted in methanol. GC-EI-MS was used for quantitation purposes.

6. RESULTS AND DISCUSSION

6.1. Optimization of solvent extraction

Different solvents were tested, but some of them such as dichloromethane, hexane, toluene or dimethyl sulfoxide seemed to dissolve the polymer. For this reason, methanol was selected as the final extraction solvent. The extraction was tested on the original pellet samples (spherical) and after submitting them to a mechanical transformation such as crushing (lentils) or grinding (powder). The aim was to check if an increase of the contact area improved the extraction efficiency. According to the results, a more efficient extraction of the compounds was achieved with lentils as well as with powder (Figure 1). Since extraction efficiency was similar in both cases, lentils were selected since the extraction process was easier to handle. Two extraction times were evaluated: 30 min and 60 min. The maximum total area of the peaks was obtained with 60 min of extraction (Figure 1).

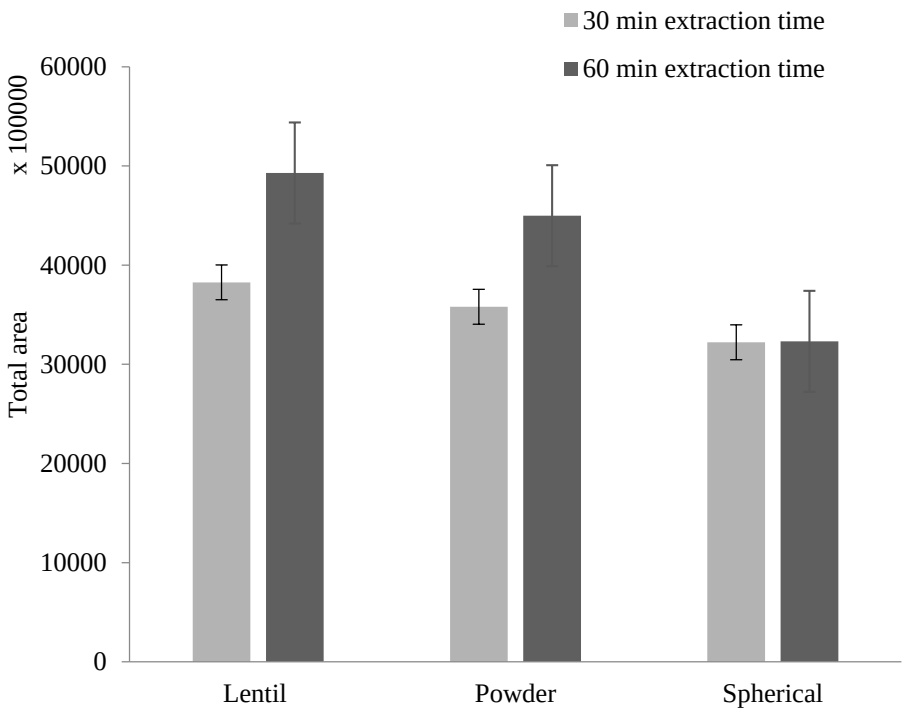


Figure 1. Total peaks area of the compounds detected by GC-EI-MS of the extracted samples obtained from three forms of pellet (spherical, lentils and powder) at two extraction times, 30 and 60 minutes. Error bars representing standard deviation.

6.2. Advances in APGC ionization pathways

Even though charge transfer and protonation are considered the prevalent ionization pathways in APGC, in this work potential new ionization processes that appeared to be dependent on the source environment were investigated. Generally, GC-APCI is well known to generate molecular weight diagnostic $M^{+\cdot}$ and $[M+H]^+$ ions (Hourani et al., 2012; Jin et al., 2016). However, a few studies demonstrated that in ambient corona discharge, the addition of N and O to hydrocarbons via ion/molecule reactions is possible (Ayrton et al., 2018). In this work, we carried out an ionization experiment by injecting individual 100 pg μL^{-1} methanolic solutions of adiponitrile, furfural, methyl palmitate, and dipropyl phthalate into the APGC-Q/ToF system, under the instrumental conditions detailed in chapter 2.4. In addition to $M^{+\cdot}$, $[M+H]^+$ and [M-

H]$^+$, it appeared that adiponitrile, furfural and methyl palmitate present the common adduct [M+CH_3]$^+$, whilst the dipropyl phthalate spectrum showed the ions [M+N]$^+$, [M+O]$^+$, [M+NO]$^+$ and [M+H+NO]$^+$. Nitrogen atom insertion into a C-C molecule backbone was reported by Li et al. (B. Li et al., 2017) as taking place in field-assisted ionization. Somewhat related plasma-induced ambient reactions can occur in the APGC source, where the voltage applied to the corona pin can give rise to N_3^+, $N_2^{+\cdot}$, and $N_4^{+\cdot}$, which might then react with the analyte molecules, forming the kinetically favored nitrenium ions [M+N]$^+$ and iminium cations [M+N-H]$^+$ (thermodynamic product) by neutral hydride loss. As a matter of fact, a certain level of moisture is always present in the ambient ionization chamber. When ionization takes place in a nitrogen atmosphere with traces of oxygen at low corona current, nitrogen addition is strongly favored over oxygen addition. At higher currents, the opposite is true (Ayrton et al., 2018). Oxygen is known to produce ozone in corona discharges (Yehia et al., 2008) and is likely responsible for the oxidation processes, including dipolar insertion and protonation to [M+3O+H]$^+$, and sequential loss of one or two molecules of water, obtaining [M+2O-H]$^+$ and [M+O-3H]$^+$. The [M-H+O]$^+$ species can be associated with O-O bond cleavage and rearrangement to generate ketones or C-C bond heterolysis resulting in smaller chain aldehydes (Ayrton et al., 2018). In the proximity of the corona discharge needle, the NO^+ species can form (Dzidic et al., 1976) and react with the analyte molecules to give [M+NO]$^+$ ions. As well as water and dioxygen, the ambient ionization chamber can also contain hydrocarbons in a lesser extent, which might lead to the formation of [M+CH_3]$^+$ ions. These processes are determined by the atmospheric composition of the ion source, gases flow rate, corona pin position with respect to the sampling cone orifice, and by the chemical structure and reactivity of the target molecule.

6.3. Identification of NIAS present in a starch-based biopolymer

The recent advances in the understanding of APGC ionization were used for identification purposes. After the optimized extraction of the polymer, a total of 21 compounds were detected. Glycerol, tetradecanoic acid, ethyl ester, 2-palmitoglycerol,

and 9,12-octadecadienoic acid, methyl ester were only detected with EI; whilst tris(2,4-ditert-butylphenyl)phosphite and the unknown peaks at 26.2, and 28.8 minutes where detected by APGC exclusively. This occurrence is further evidence that the two ionization techniques are complementary for non-targeted analysis (Domeño et al., 2012; Pintado-Herrera et al., 2014). Table 1 shows the accurate mass of the most abundant adducts in APGC, retention times, and molecular formulae.

Table 1. List of compounds identified by APGC-Q/ToF and GC-EI-MS in the raw starch and pellet samples.

t_R	*m/z* $M^{+\cdot}$	Molecular Formula	Candidate Name (#CAS)	APGC major adducts	Label	Raw starch	Pellet
15.5	-	$C_3H_8O_3$	Glycerol (56-81-5)[a]	-	Confident		x
16.4	201.1120[§] (-0.1)	$C_{10}H_{16}O_4$	AA-BD (777-95-7)	$[M+H]^+$ $[M+N]^+$ $[M-H+O]^+$ $[M+NO]^+$	Confident		x
17.2	232.1817 (-0.5)	$C_{16}H_{24}O$	1,2,3,4-Tetrahydro-1-methoxy-1,6-dimethyl-4-(1-methylethyl) naphthalene (60698-94-4)	$[M+N]^+$ $[M+O]^+$ $[M+H+2O]^+$	Confident		x
20.4	272.1385[§] (-0.2)	$C_{10}H_{22}O_8$	2, 3-bis- (2-hydroxyethyl)glucitol (114413-68-2)	$[M+H]^+$	Tentative		x
20.8	-	$C_{16}H_{32}O_2$	Tetradecanoic acid, ethyl ester (124-06-1)[a]	-	Tentative	x	x
21.2	299.2955[§] (0.5)	$C_{19}H_{38}O_2$	Isopropyl palmitate (142-91-6)	$[M+H]^+$	Confirmed	x	x
22.1	265.2760 (-0.4)	$C_{18}H_{35}N$	Stearonitrile (638-65-3)	$[M+H]^+$ $[M-H+O]^+$ $[M+NO]^+$	Confident		x
22.4	310.3106[§] (-0.4)	$C_{20}H_{39}NO$	11-Eicosenamide (10586-57-9)	$[M+H]^+$	Confirmed	x	x
22.8	255.2548 (-0.9)	$C_{16}H_{33}NO$	Palmitamide (629-54-9)	$[M-H]^+$ $[M+H]^+$ $[M+CH_3]^+$ $[M-3H+O]^+$	Confirmed	x	x

22.9	-	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid, methyl ester (2462-85-3)[a]	-	Tentative	x	x
24.0	273.1696[§] (-0.1)	$C_{14}H_{24}O_5$	mono-2-ethyloxoexyl adipate (134998-72-4)	$[M+H]^+$ $[M+H_2O]^+$ $[M+H+H_2O]^+$	Confident		x
24.6	284.2947[§] (-0.1)	$C_{18}H_{37}NO$	Octadecanamide (124-26-5)	$[M+H]^+$ $[M-3H+O]^+$ $[M-H+NO]^+$	Confirmed		x
25.5	-	$C_{19}H_{38}O_4$	2-Palmitoylglycerol (23470-00-0)[a]	-	Tentative		x
26.2	312.3252 (-0.9)	$C_{20}H_{42}NO$	Unknown[b]	$[M-H]^+$ $[M-2H+O]^+$ $[M+CH_3]^+$ $[M+NO]^+$	-		x
28.8	412.3689 (-1.1)	$C_{29}H_{48}O$	Unknown[b]	$[M+O]^+$ $[M-H+O]^+$ $[M-H+2O]^+$	-		x
29.1	400.2085 (-0.6)	$C_{20}H_{32}O_8$	$(AA)_2$-$(BD)_2$ (141850-18-2)	$[M+H]^+$ $[M-H+O]^+$ $[M-2H+N]^+$ $[M+NO]^+$	Confident		x
29.2	424.4268 (-0.7)	$C_{28}H_{56}O_2$	Myristyl myristate (3234-85-3)	$[M+H]^+$ $[M-H+O]^+$ $[M+NO]^+$	Confident		x
31.5	452.4577 (-1.1)	$C_{30}H_{60}O_2$	Cetyl myristate (2599-01-1)	$[M+H]^+$ $[M-H+O]^+$ $[M+NO]^+$	Confident		x
33.6	420.1783 (-0.1)	$C_{22}H_{28}O_8$	TPA-AA-$(BD)_2$	$[M+H]^+$ $[M+NO]^+$ $[M+NO+O]^+$ $[M-H+O]^+$	Confident		x
34.8	480.4893 (-0.8)	$C_{32}H_{64}O_2$	Palmitoil ester related	$[M+H]^+$ $[M-2H+O]^+$ $[M+H+NO]^+$	Unknown		x
36.0	646.4510 (0.1)	$C_{42}H_{63}O_3P$	Tris(2,4-ditert-butylphenyl)phosphite (31570-04-4)[b]	$[M+H]^+$	Confident		x

t_R, APGC retention time (min) (EI when the compound is not detected by APGC). *m/z* $M^{+\cdot}$, radical cation molecular ion accurate mass-to-charge (mDa error). ([§]), accurate mass of the protonated molecular ion; ([a]) detected only by GC-EI-MS; ([b]) detected only by APGC-Q/ToF. AA, adipic acid; BD butane-1,4-diol; TPA, terephthalic acid.

Some of the identified compounds might come from the degradation of the material, which is caused by oxidation reactions taking place at the surface of the polymer in contact with air and moisture. These radical reactions involve the formation of hydroporoxyl species, which will then undergo β-cleavage and fragmentation, rearrangement, and cyclization, leading to secondary products, such as alcohols and carbonyl compounds with lower molecular weight (MW) (Porter et al., 1995). These compounds further promoted the formation of lactones and furanones (Caillé et al., 2017; Ferreira et al., 2003), such as 4-hydroxy-3,5-dimethyl-2-furanone, detected after migration to ethanol 95% (Table 2). Polymer manufacturers are very conscious of the issues that oxidation generates. Therefore, the intentional addition of authorised antioxidants to the polymer is a common procedure. Indeed, in the pellet samples tested, the antioxidant tris(2,4-ditert-butylphenyl)phosphite, also called Irgafos 168 (B. Li et al., 2017), was detected at the very end of the chromatogram via APGC.

Table 2. Results of specific migration from the prototype samples to ethanol 95% (v/v), expressed as mg of analyte per kg of food simulant.(± standard deviation).

t_R	Candidate	# CAS	Cramer Class	Specific migration (mg kg^{-1})			QS
				First	Second	Third	
11.4	2-Acetyl-2,3,5,6-tetrahydro-1,4-thiazine **(M1)**	958999-62-7	III	31.08 ± 0.97	16.51 ± 1.50	10.20 ± 0.80	e
15.3	AA-BD	777-95-7	I	2.77 ± 0.15	1.30 ± 0.07	1.00 ± 0.1	e
19.1	Docosanoic acid, ethyl ester	5908-87-2	I	1.55 ± 0.05	1.17 ± 0.13	< 0.60	d
19.5	1,4-Benzenedicarboxylic acid, diethyl ester	636-09-9	I	< 1.63	< 0.53	< 0.53	b
20.8	4-hydroxy-3,5-dimethyl-2-Furanone **(M2)**	22621-29-0	III	27.31 ± 1.63	18.29 ± 2.20	16.03 ± 2.56	f
24.5	Hexadecanoic acid, dodecyl ester	42232-29-1	I	< 0.57	< 0.57	<0.19	a
24.8	Hexadecanoic acid, octadecyl ester	2598-99-4	I	1.93 ± 0.30	< 0.57	< 0.57	a
25.2	Hexadecanoic acid, hexadecyl ester	540-10-3	I	0.68 ± 0.12	<0.57	< 0.19	a
26.9	Tetradecanoic acid, dodecyl ester	2040-64-4	I	< 2.58	< 2.58	< 2.58	c
27.6	$(AA)_2$-$(BD)_2$	141850-18-2	I	< 0.81	< 0.27	< 0.81	e
28.2	Myristyl myristate	3234-85-3	I	8.28 ± 0.29	2.82 ± 0.14	< 2.58	c
29.5	Tetradecanoic acid, hexadecyl ester **(M3)**	2599-01-1	I	18.76 ± 0.95	7.17 ± 0.42	6.34 ± 1.36	c
29.9	TPA-AA-$(BD)_2$	-	III	0.77 ± 0.02	0.36 ± 0.02	< 0.27	e
30.7	Hexadecanoic acid, hexadecyl ester **(M4)**	540-10-3	I	14.92 ± 0.61	4.53 ± 0.31	3.33 ± 0.40	a

t_R: retention time (min). QS: Quantitation Standard
M 1-4: Compounds with the highest migration values
a: ethylhexyl adipate; b: diethyl phthalate; c: bis(2-ethylhexyl) sebacate; d: docosanoic acid ethyl ester; e: 1,4-trioxa- cyclotridecane-8,13-dione; f: furaneol
AA: adipic acid; BD: butane-1,4-diol; TPA: terephthalic acid

The addition of lubricants in starch-based biopolymers is a common practice. A typical lubricant is soybean oil, which is mainly composed of fatty acids, such as myristic and palmitic acids (Muller et al., 2017; Nerin et al., 2013; Schieberle, 1995; Watanabe et al., 2007). In Table 1, it can be observed that nine of the identified compounds are myristic and palmitic acid derivatives. Esters could be formed by the reaction between fatty acids and alcohols coming from material degradation. Eicosenamide, palmitamide, and octadecanamide were also detected in the methanol extract, which might be intentionally added as slip agents (Lv et al., 2009; Vera et al., 2018) for reducing the friction coefficient of the material. In the first half of the chromatogram, clusters of small and broad peaks were detected. By the analysis of the EI spectra, the most abundant fragment ions were indicative of polyalcohols. However, the low response in both EI and APGC did not allow us to reliably identify such components. At 15.5 minutes, a high fronting-peak was identified as glycerol in EI. The high concentration of glycerol can be explained by its function as the principal plasticizer. Other potential plasticizers detected were 2,3-bis-(2-hydroxyethyl)glucitol and MEOHA (mono-2-ethyloxoexyl adipate). It is worth noting that MEOHA can be considered a proper NIAS, as it was found to be an oxidative metabolite of DEHA (2-ethylexyl adipate), which is widely used as a plasticizer for food contact plastics (Silva et al., 2013).

Three oligomers were also identified: [AA-BD], [$(AA)_2$-$(BD)_2$], and [TPA-AA-$(BD)_2$] (where AA: adipic acid; BD: butane-1,4-diol; and TPA: terephthalic acid). These compounds were found to be degradation products of polymeric resins (Watanabe et al., 2007). According to Canellas et al. (Canellas et al., 2015), [AA-BD] and [$(AA)_2$-$(BD)_2$] can also lead to the formation of high MW cyclic oligomers and lactones. [TPA-AA-$(BD)_2$] could come from the degradation of the copolymer polybutylene adipate terephthalate (PBAT), which is commonly used in the manufacture of starch-based materials in order to increase its mechanical and barrier properties (Bheemaneni et al., 2018; Olivato et al., 2012). In Figure 2, the APGC mass spectrum of [TPA-AA-$(BD)_2$] is shown, highlighting principal fragments and adducts under “dry conditions”.

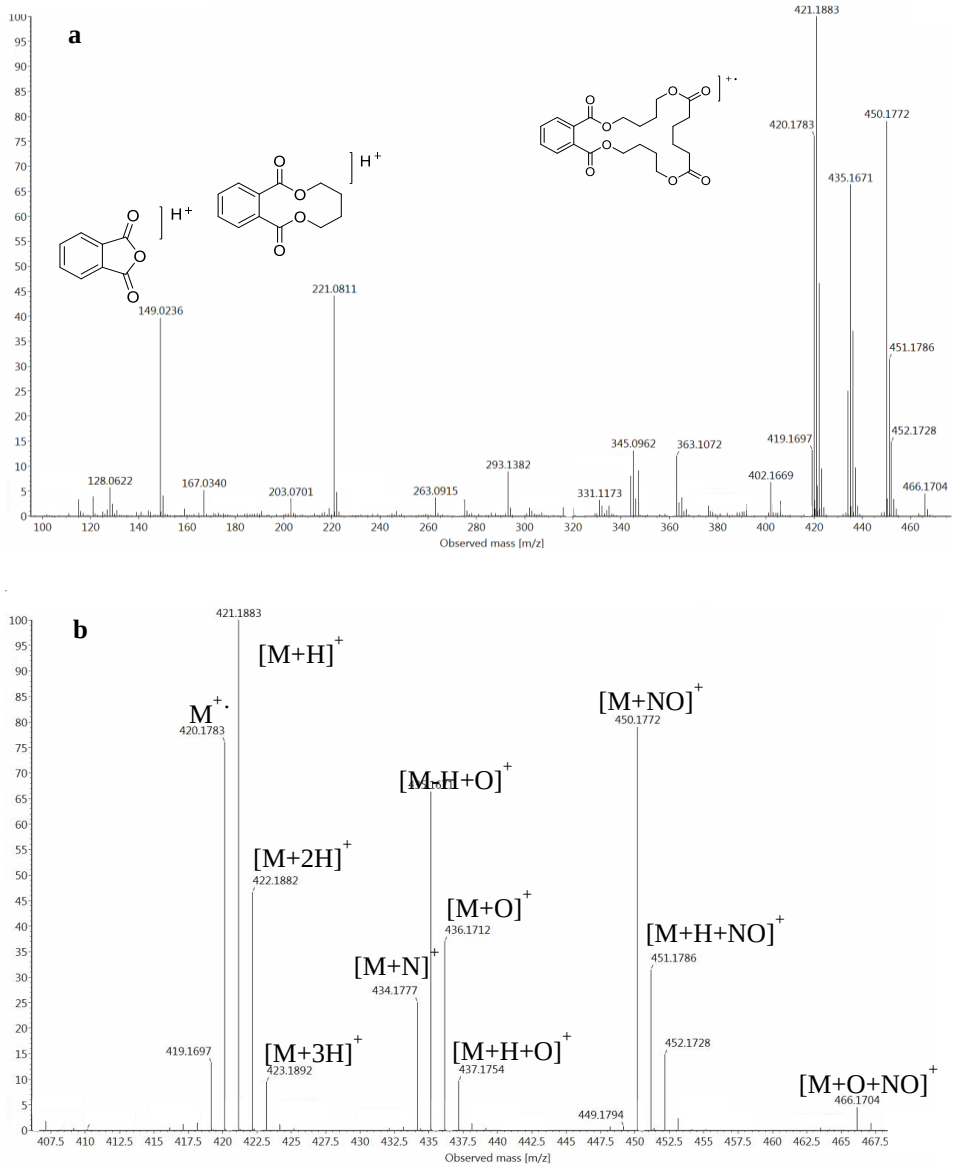


Figure 2. a) APGC-Q/ToF mass spectrum of the oligomer TPA-AA-$(BD)_2$, showing molecular ion and principal fragments' structures; **b)** detailed mass spectrum of TPA-AA-$(BD)_2$, highlighting adduct ions.

6.4. Migration study

Overall migration from the cup samples was 5.9 ± 2.6 mg kg^{-1} in ethanol 10%; 23.5 ± 2.6 mg kg^{-1} in acetic acid 3%; and 26.5 ± 3.1 mg kg^{-1} in sunflower oil. At the end of the overall migration experiment, physical changes were not observed in any of the samples. The results showed that overall migration was below the Overall Migration

Limit (OML) of 60 mg kg^{-1}, established by Regulation EU No 10/2011 (European Commission, 2011a) for the simulants tested and therefore the material fulfilled the requirements. Overall migration was also performed in vegetal oil substitutes, ethanol 95%, and isooctane. However, they seemed to overestimate the overall migration with migration values above 60 mg kg^{-1} (100.0 ± 5.1 mg kg^{-1} and 143.4 ± 15.6 mg kg^{-1} respectively). The main reason is that probably isoctane and ethanol 95% interact with the biopolymer and partially dissolve it, providing a higher mass transferred during the test.

With regards to specific migration tests, no compounds were detected in ethanol 10% and acetic acid 3%. By contrast, 14 different compounds were found in ethanol 95%. Among them, four were previously detected in the pellet samples. Table 2 shows the concentrations of the detected analytes in ethanol 95% after each migration test. They were quantified by external calibration using the indicated standard compounds. All the standards showed good linearity with R^2 greater than 0.987 and limits of detection (LODs) between 0.19 and 5.55 mg kg^{-1} (Table 3).

Table 3. Analytical parameters of the GC-MS method used for the quantification of the migrating compounds.

Analyte	Equation	R^2	RSD % slope	RSD % intercept	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Lineal range (mg kg^{-1})
Ethylhexyl adipate	y = 64351x + 8242.4	0.9901	1.2	11	0.19	0.57	0.75 - 15.60
Diethyl phtalate	y = 57834x + 63370	0.9870	1.1	7.0	0.53	1.63	1.21 - 8.02
Bis (2ethylhexyl) sebacate	y = 95808x - 167227	0.9897	1.2	3.5	0.86	2.58	2.60 - 21.96
Docosanoic acid ethyl ester	y = 63873x - 33359	0.9963	4.9	12	0.60	1.80	0.75 - 2.70
1,4-trioxa- cyclotridecane-8,13-dione	y = 26590x - 26298	0.9964	2.1	6.5	0.27	0.81	1.05 - 14.89
Furaneol	y = 7354.4x - 91378	0.9983	2.9	10	5.55	16.66	17.01 - 33.95

RSD %: Percentage of Relative Standard Deviation

The legislation states that compounds migrating from the material to the food should not pose a risk to human health (Skjevrak et al., 2005). After cross-checking the EU positive list of Regulation No 10/2011/EU (European Commission, 2011a), the identified compounds appeared not to be listed molecules. Therefore, the Cramer rules were applied to each compound for a theoretical evaluation of toxicity (Dewhurst et al., 2013). The toxicity level is established according to the molecular structure of the compound evaluated. The compounds can be classified within one of three different categories: class I (low toxicity), class II (medium toxicity), class III (high toxicity). Three compounds, 2-Acetyl-2,3,5,6-tetrahydro-1,4-thiazine, TPA-AA-$(BD)_2$ and 4-hydroxy-3,5-dimethyl-2-furanone, were found to be Class III-substances. The maximum concentration was found for 2-Acetyl-2,3,5,6-tetrahydro-1,4-thiazine (31.08 ± 0.97 mg kg^{-1}All other identified compounds were found to be Class I, obtaining tetradecanoic acid, hexadecyl ester the Maximum concentration value (18.76 ± 0.95 mg kg^{-1}). According to the Threshold of Toxicological Concern (TTC) concept, depending on the Cramer class group of a compound, a maximum daily intake (TTC value) can be established in order to assure that there is no health risk for the consumer. TTC values for Class I, II and III are 30, 9.0 and 1.5 mg kg^{-1} bw/day (EFSA, 2012). Assuming an average bodyweight of 60 kg and a daily consumption of 1 kg food that has been in contact with the packaging, the maximum migration values to assure consumers health would be 1.8, 0.54 and 0.09 mg kg^{-1} (for Class I, II and III, respectively). The results showed migration values above these limits for many volatiles after the first use of this material.

The four compounds presenting the highest concentration after migration were 2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine (M1), 4-hydroxy-3,5-dimethyl-2-furanone (M2), tetradecanoic acid, hexadecyl ester (M3) and hexadecanoic acid, hexadecyl ester (M4). The concentration of these compounds was plotted for three consecutive experiments simulating repeated use (Figure 3). The results showed that the migration of volatile NIAS decreased with the repeated use of this material, being 31.08 ± 0.97, 27.31 ± 1.63, 18.76 ± 0.95 and 14.92 ± 0.61 mg kg kg^{-1}. the maximum concentration found in migration respectively.

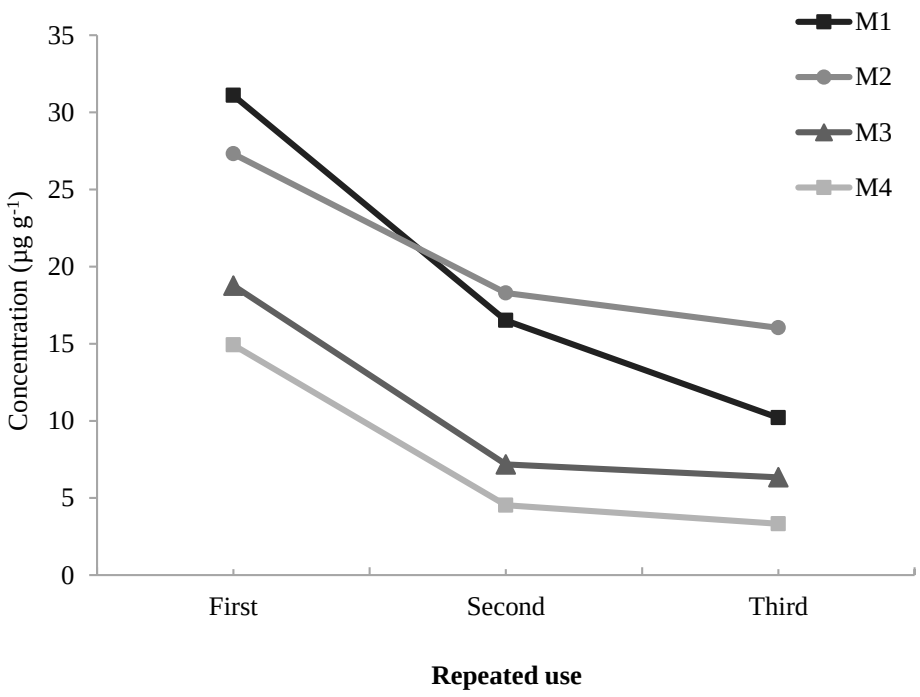


Figure 3 Repeated migration values for the four compounds presenting the highest concentration in ethanol 95%: M1 (2-acetyl-2,3,5,6-tetrahydro-1,4-thiazine), M2 (4-hydroxy-3,5-dimethyl-2-furanone), M3 (tetradecanoic acid hexadecyl ester), and M4 (hexadecanoic acid, hexadecyl ester).

7. CONCLUSIONS

After a strong solvent extraction of the starch-based material, 21 compounds were detected, 14 of which were identified with a certain level of confidence, and four confirmed by standard injection. GC-EI-MS and APGC-Q/ToF systems were demonstrated to be complementary for identifying extractables and leachables. Notably, APGC coupled to high resolution mass spectrometer provides the accurate mass of the molecular ion, not always visible in the EI spectrum, as well as its fragments and adducts formed at the GC-MS interface. APGC-Q/ToF has shown to be an extremely useful tool in the process of NIAS identification and therefore and its use will help to determine potential migrants from food contact materials and improve its risk assessment. In this work, for the first time the recent advances in APGC ionization pathways were applied to the structure elucidation of unknown compounds coming from a novel biopolymer.

The results showed that a range of compounds were intentionally added to the polymer as lubricants, plasticizers, slip agents, and antioxidants. Among NIAS, three oligomers were identified. Specific migration in ethanol 95% (v/v) showed migration values above those recommended according to the TTC approach, suggesting a re-formulation of the material to be needed if being employed to contain foods with a lipophilic character. Further investigations on the non-volatile leachable fraction and a toxicological study on the major migrant components should also be addressed to ensure the safety of consumers.

Capítulo 4

Identification of key odorant compounds in starch-based polymers intended for food contact materials

1. RESUMEN
2. OBJETIVOS
3. ESQUEMA DE TRABAJO
4. INTRODUCTION
5. MATERIALS AND METHODS
6. RESULTS AND DISCUSSION
7. CONCLUSIONS

1. RESUMEN

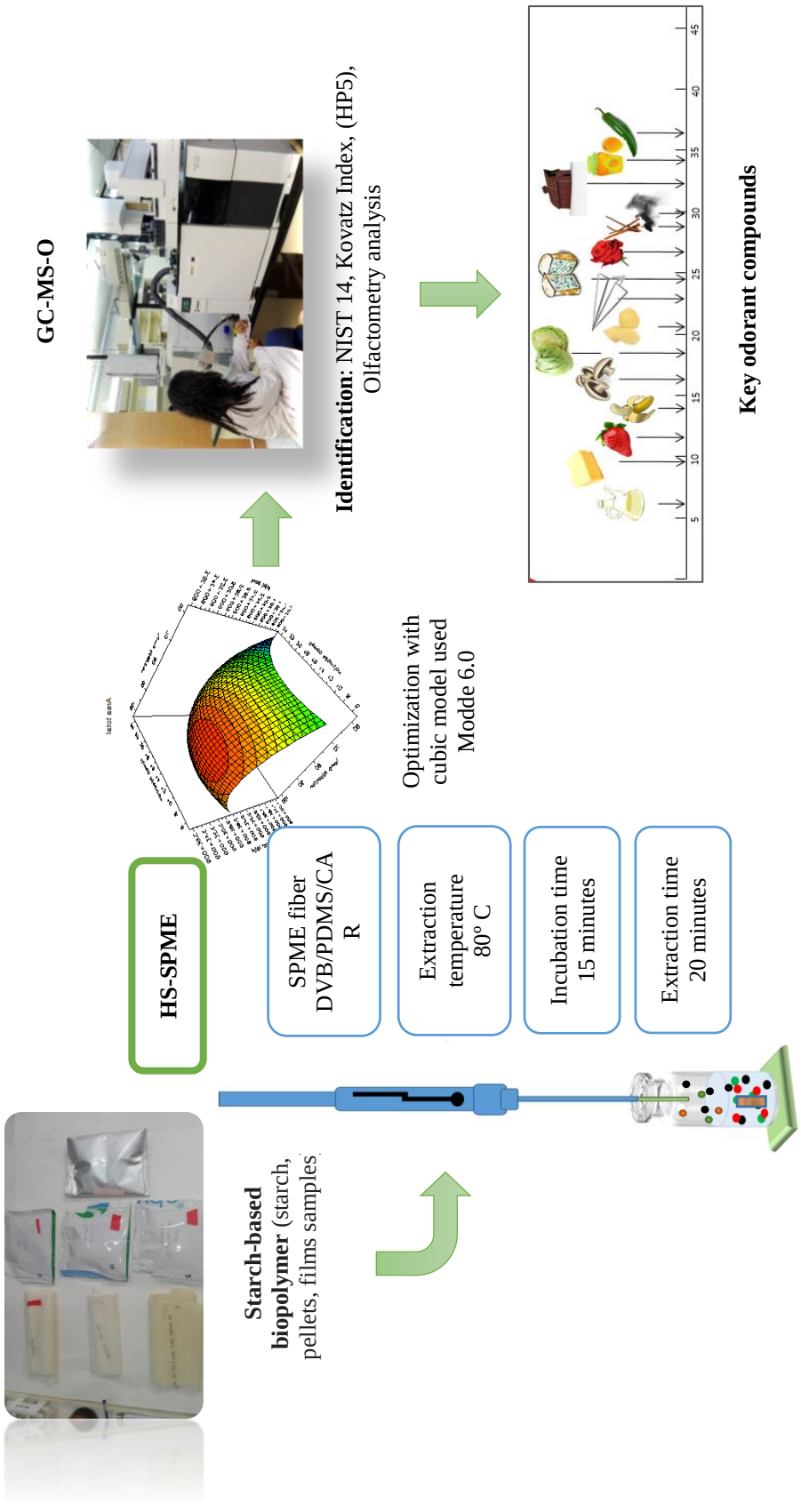
Los bioplásticos base almidón están siendo ampliamente utilizados para la fabricación de pellets y películas destinados a la producción de utensilios de cocina. Por lo tanto, los atributos sensoriales de los bioplásticos deben evaluarse para garantizar que la integridad de las propiedades organolépticas de los alimentos no sea afectada. En este capítulo, han sido caracterizados los perfiles del aroma de cuatro bioplásticos base almidón. Para ello fueron estudiados el almidón, como materia prima, un pellet y en cuatro películas. La técnica de HS-SPME y la GC-MS-O fueron utilizados para la extracción y la identificación de los compuestos volátiles odorantes. En total, se detectaron treinta y cinco compuestos odorantes, de los cuales se determinó que los alcoholes, fenoles y aldehídos son los compuestos con el mayor impacto en el aroma global de los bioplásticos base almidón. Los compuestos eugenol, 2-fenoxietanol, (Z) y (E) 2-nonenal fueron identificados como los odorantes de mayor valor de frecuencia modificada (MF%) en la muestra de almidón. En tres de las cuatro películas, los valores MF% superiores al 60% los tuvieron ocho compuestos, trimetilamina, 1-octen-3-ona, sotolon, (Z) y (E) -2-nonenal, p-vinilguaiacol, eugenol y 1-undecanol, que fueron establecidos como los compuestos de impacto aromático. Finalmente, un panel sensorial evaluó las películas para buscar la presencia, ausencia y la intensidad en notas sensoriales, como tostado, dulce/frutal, verde, floral, desagradable, graso y especiado. Los resultados demostraron que la calidad de todas las muestras disminuía cuando aumentaban las notas de tostado y especiado.

2. OBJETIVOS

Comprender el efecto que puede tener un material de envase sobre la calidad sensorial de un alimento no es una tarea sencilla. Por ello, el objetivo principal de este capítulo fue la caracterización del perfil aromático de biopolímeros base almidón (pellets y películas) destinados a la fabricación de utensilios de cocina y envases alimentarios mediante la determinación de los compuestos con mayor impacto aromático. Para el cumplimiento de este objetivo se establecieron las siguientes tareas:

- Optimización de la técnica de microextracción en fase sólida por espacio de cabeza (HS-SPME) para extracción y concentración de compuestos volátiles.
- Entrenamiento de un panel de catadores para análisis olfatométrico y de un panel sensorial para el reconocimiento de atributos sensoriales.
- Desarrollo de un método de detección olfatométrico mediante GC-MS-O para la identificación de los compuestos odorantes.
- Identificación y elaboración de una librería con los potenciales migrantes odorantes para cada tipo de material.
- Evaluación de los atributos sensoriales y su correlación con los odorantes identificados previamente.

3. ESQUEMA DE TRABAJO



Esquema 4. Diseño experimental del Capítulo 4

4. INTRODUCTION

Biopolymers are increasingly used in food packaging, because they are abundant, renewable, environmentally friendly, biodegradable, and biocompatible. In addition, their mechanical properties such as durability, flexibility, high gloss, clarity, and tensile strength are often suitable for being used as packaging materials (Mittal et al., 2014; Othman, 2014; Rhim et al., 2013; Zhang et al., 2014). Biopolymers include two different categories: synthetic and natural. Synthetic biopolymers can be produced by microorganisms such as the polyhydroxy-alkanoates (PHA) or by conventional chemical synthesis, such as polylactic acid (PLA), polycaprolactone (PCL) and polyvinyl alcohol (PVA) (Rhim et al., 2013). Natural polymers are developed using proteins such as gelatin, gluten or alginate, or carbohydrates such as cellulose, chitosan and starch as a raw material (Othman, 2014; Oun et al., 2017). The starch-based polymers have been one of the most used for manufacturing bio-films, because starch is low cost, safe and abundant (Averous et al., 2014; Zhang et al., 2014). Their characteristics may be different depending on their origin, because they can come from different sources such as tubers (potato and cassava), cereal grains (corn, rice and wheat) or agro-food waste(Averous et al., 2014; González et al., 2011; Ribba et al., 2017).

One important feature of starch-based biopolymers as food contact materials is the odor they can release, that affects the quality of packaged food. The manufacturing process as is a critical source where the raw materials suffer extreme conditions, such as high temperatures, pressure and extrusion, among others, and different odorant compounds can be formed during this process (Wypych, 2013). The formation of these compounds can be enhanced because of the presence of amino acids and fatty acids in the polymer that easily degrade into different small compounds (Khlestkin et al., 2018; Samavati et al., 2014; Wypych, 2013). Therefore, the analysis of odorant compounds is especially important since the organoleptic properties of food could negatively modified.

The objective of the present study was to characterize the aromatic profile of starch-based biopolymers intended for food packaging and evaluate the presence of

negative sensory attributes that could affect the organoleptic properties of food. For this purpose, the volatile compounds profile of 1 pellets sample and four different films coming from starch, were analyzed. The analysis was performed by headspace solid phase microextration (HS-SPME) technique and gas chromatography coupled simultaneously to a mass spectrometer and a sniffing port. (GC-MS-O). Retention index (RI), mass spectra and aromatic description were used to identify the compounds (Feng et al., 2015; Pino et al., 2012). In addition, a sensory panel evaluated the olfactory attributes of packaging samples that were subsequently correlated to the odorant compounds previously identified.

5. MATERIALS AND METHODS

5.1. Reagents

Standards (E)-2-nonenal [18829-56-6]; 2-Phenoxyethanol [122-99-6]; p-vinylguaiacol [7786-61-0]; trimethylamine [75-50-3]; 1-undecanol [112-42-5]; sotolon [28664-35-9]; 1-octen-3-one [4312-99-6]; benzaldehyde [100-52-7]; (E)-cinnamaldehyde [14371-10-9]; decanal [112-31-2]; limonene [138-86-3]; dodecanal [112-54-9]; ethyl isovalerate [108-64-5]; ethyl octanoate [106-32-1]; toluene [108-88-3]; eugenol [97-53-0]; furfural [98-01-1]; linalool [78-70-6]; nonanal [124-19-6]; octanal [124-13-0]; and vanillin [121-33-5] were from Sigma–Aldrich (Madrid, Spain). Methanol (UPLC-MS grade) was supplied by Scharlau (Setmenat, Spain). Standard of n-alkanes (C8 to C20) (40 mg/L each, in hexane solution) and the SPME fibers were purchased to Supelco (Bellefonte, PA, USA). Ultra-pure water was obtained from Millipore Milli-Q system (Billerica, MA, USA).

5.2. Samples

In this work, different starch-based biopolymers for food packaging were supplied by a Packaging Company. They were provided in different forms: 1 sample of starch powder, 1 sample of starch-based pellets and 4 samples of starch-based films

(BP1, BP2, BP3 and BP4). Film BP2 was manufactured using the pellet samples from this study. BP1, BP2 and BP3 came from the starch powder analyzed in this work. BP4 had a different origin.

5.3. Analysis by GC-MS-O

The analyses of the volatile compounds were carried out using a gas chromatograph 7890N coupled through a split valve to a mass spectrometry detector 5977D (Agilent Technologies. Santa Clara, CA) and a sniffing port (Phaser, GL Sciences, Germany). A HP-5MS column (30m x 25mm x 0.25µm film thickness) from Agilent technologies was used. Detector was set in SCAN acquisition mode (45 - 350 *m/z).* The oven temperature ramp was as follows: initially 40 ºC for 5 min, 15 ºC/min to 300 ºC and held at 300 ºC for 10 min. The SPME fiber was transferred manually to the injection port and desorbed at 250 ºC for 2 min in splitless mode.

The transfer line of the olfactometer was heated at 200 ºC and the sniffing port was purged with humidified air. Olfactometry analyses were carried out by six trained panelists. Sniffing time was approximately 20 min and each judge carried out a maximum of one session a day. During the olfactometry, the panelists described the odors perceived and its intensity in a scale from 1 (low intensity) to 3 (high intensity). Fractional values were also allowed.

5.4. Experimental design optimization of HS-SPME-GC-MS-O analysis

The pellets sample and the film BP2 were used for the optimization process because they showed the highest intensity of global odor. A maximum total area of the integrated peaks was the selection criteria for the optimal conditions.

5.4.1. Selection of the most appropriate SPME fiber.

Three different fibers were evaluated: polydimethylsiloxane (PDMS 100 µm), polydimethylsiloxane/divinylbenzene (PDMS/DVB 65 µm) and polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR 50/30 µm). Prior to the analysis, the samples were conditioned during 2 min at 80 ºC. Afterwards, they were

extracted at the same temperature during 15 min and under agitation at 500 rpm. Injection and analysis were carried out using the conditions described in section 5.3.

5.4.2. Selection of extraction conditions

Optimization was performed using an experimental design with the software package Modde 6.0 from Umetrics (Umea, Sweden). The experiments were carried out with the selected fiber. Two variables selected were: extraction time (5, 15 and 25 min) and extraction temperature (60, 80 and 100 ºC). The cubic model was implemented. This model consisted of 17 different experiments, where values of parameters (high, medium, low) were combined each other. The experiments were carried out in randomized order.

5.5. Identification of odorous compounds and determination of its impact on aroma

For the identification of the main aroma active compounds different strategies were used. The experimental mass spectra were compared to the mass spectra of NIST 2018 Standard Reference Database and only matches above 800 were considered acceptable. In addition, the retention indexes (RI) were calculated using a C8–C20 n-alkane standard solution (Jeleń et al., 2013; Wrona, Vera et al., 2017; Zellner et al., 2008) and compared with those found in the literature or chemical databases such as NIST and flavornet. Only those compounds with differences in RI below 1% were considered. The odor described by the panelists was also compared with the compilation of aroma compounds from databases, such as [www.flavornet.org] and [www.thegoodscentscompany.com] and previous bibliography (Barata et al., 2011; Feng et al., 2014; Vera et al., 2012; Wrona et al., 2017). Additionally, those compounds whose standards were available, were confirmed by the comparison of retention time and mass spectrum of the identified peak and the standard.

For determining the aromatic impact of the compounds identified, their modified frequency (MF%) was calculated according to the following equation (Vera et al., 2012)

$$MF\ (\%) = \sqrt{F(\%)\ x\ I\ (\%)}$$

where F (%) is the percentage of the sniffers that detected the smell (100 x number of sniffers that detected the odorant/total number of sniffers) and I (%) is the average percentage of intensity (100 x average I/3). The compounds with a MF% higher than 50% were considered as key compounds of the sample base aroma (Wrona et al., 2017).

5.6. Sensory evaluation

A quantitative descriptive analysis (QDA) was carried out in all samples. The analysis was performed following the methodology proposed by Yunzi Feng et al. (Feng et al., 2014). The samples were evaluated according to the descriptors defined in section 5.5. The sensory evaluation was carried out by twelve judges (20 to 45 years, 8 female, 4 male), from the sensory panel. The panel was trained considering the most important attributes present in the sensory properties of starch-based polymer samples based on the olfactometry results. Eight different descriptors were evaluated in a scale from 0 "very low intensity" to 5 "very high intensity": toasted, sweet/fruity, green, flower, distasteful, fat, earth and spices. Global intensity of the samples was also evaluated using the same scale. Previously, a sensory training had been carried out, using compounds with similar descriptions to the evaluated descriptors: eugenol (spicy), ethyl isovalerate (fruit), ethyl octanoate (orange), limonene (citrus), vanillin (vanilla), linalool (flower), and 1-octen-3-one (mushroom). The panelists also indicated the quality of the samples in a scale 0-5, where 5 was "like very much" and 0 was "dislike very much". Sensory tests were carried out in a conditioned room.

6. RESULTS AND DISCUSSION

6.1. Optimization of HS-SPME analysis

The results from CAR/DVB/PDMS fiber showed the highest efficiency in the extraction of volatile compounds compared to PDMS and PDMS/DVB phases. CAR/DVB/PDMS had been previously regarded in the laboratory as a suitable fiber to

extract compounds such as aldehydes, alcohols or esters (Vera et al., 2014; Vera et al., 2012).

For the optimization of the HS-SPME extraction conditions, the 2 parameters with the highest relevance in extraction according to the literature were evaluated, extraction temperature and extraction time (Vera et al., 2012; Wrona et al., 2017). Since pellets as well as BP2 film were solid samples, the salting effect was not evaluated.

Figure 1 shows the 3-D response surface plot obtained from the experimental design performed in order to determine the optimal conditions for the HS-SPME extraction. The maximum total area of the peaks detected in the chromatogram was the response used for the selection of the best conditions. The results showed that optimal extraction conditions were: PDMS/DVB/CAR 50/30 µm fiber, extraction time 15 min and extraction temperature 90 ºC.

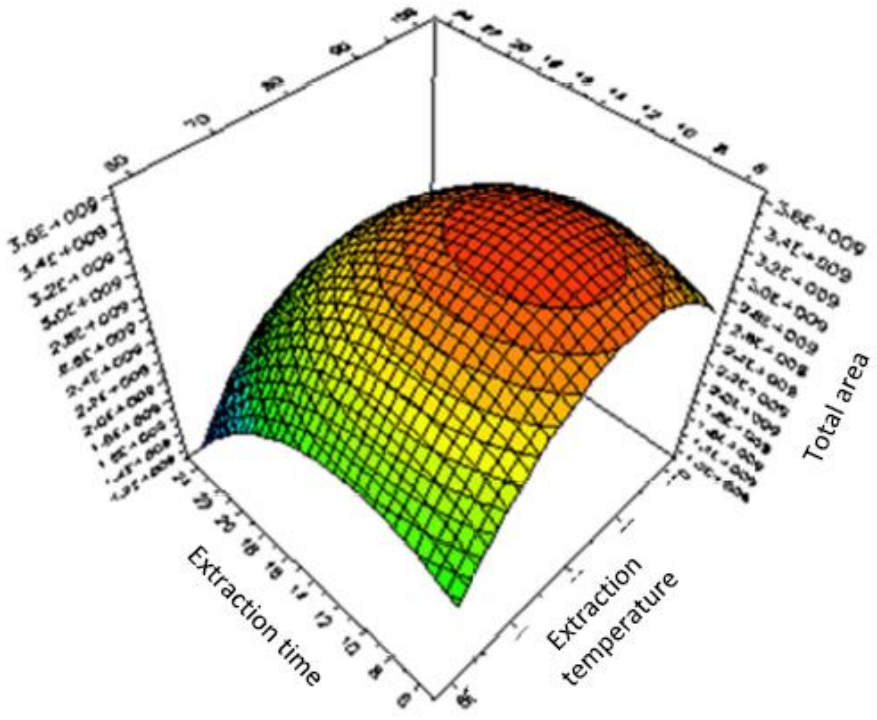


Figure 1. Response surface plot for the optimal conditions of HS-SPME analysis.

6.2. Identification of odorous compounds

Table 1 shows the odor active compounds detected by GC-MS-O in the samples with MF% values above 20%. A total of 35 compounds were detected, and among them, 17 showed MF% values over 50% (marked in bold letters). They were classified according to its functional group (FG) into 8 categories: FG 1-amides and amines (5), FG 2- acids (2), FG 3- alcohols and phenols (5), FG 4- aldehydes (10), FG 5- esters (2), FG 6- hydrocarbons (3), FG 7-ketones (3) and FG 8- others (5); and according to their aroma group (AG) (Barata et al., 2011; Capone et al., 2013) into 8 categories: AG 1 - toasted (5), AG 2 - fruity and sweet (1), AG 3 - flower (6), AG 4 - chemical and distasteful (9), AG 5 - fat (1), AG 6 - spices (4), AG 7 - green (6) and AG 8 - earth (3).

Table 1: Identified odorous compounds with its retention index (RI) and its odor description perceived by the different assessors. Its modified frequency (MF%) by HS-SPME extraction in starch, pellet and four different types of films (BP1, BP2, BP3, BP4). AG. aroma group: 1 - toasted, 2 - fruity and sweet, 3 - flower, 4 - chemical and distasteful, 5 - fat, 6 - spices, 7 - green and 8 - earth. FG: functional group: 1 - amides and amines; 2 - acids, 3 - alcohols and phenols, 4 - aldehydes, 5 - esters, 6 - hydrocarbons, 7 - ketones.

t_R	Reference RI	RI	Name	# CAS	Odor description	AG	FG	MF% Starch	MF% Pellet	MF% BP1	MF% BP2	MF% BP3	MF% BP4
2.30	369	491	Trimethylamine* [a]	75-50-3	Fish	4	1			**85.6**	**81.6**	**85.6**	**70.7**
8.44	829	823	Furfural*	98-01-1	Sweet, bread	1	4	25.8	11.5	8.2	8.2	23.1	11.5
9.10	865	862	m-Xylene*	108-38-3	Plastic	4	6	28.3	16.3	11.5	11.5	16.3	
9.95		904	Ni		Flower, sweet,	3			36.5	37.4	8.2	11.5	20.0
10.15		915	Ni		Fat, oily	5			25.8	28.3	42.4	42.4	14.1
11.40	960	958	Benzaldehyde*	100-52-7	Almond, burnt	1	4	28.3	23.1				
11.30	979	976	1-Octen-3-one* [a]	4312-99-6	Mushroom	8	7		**73.0**	**74.8**	**52.9**	**85.6**	**73.0**
11.80	1005	1001	Octanal*	124-13-0	Lemon, green	7	4	25.8	11.5		34.6	28.3	34.6
12.57	1012	1013	2-Acetylpyridine	1122-62-9	Popcorn	1	7	32.7	30.6		14.1		
13.06	1080	1079	2,5-dimethyl-3-ethylpyrazine	13360-65-1	Roast, mushroom	8	1	16.3	**63.2**	44.7	28.3	40.0	20.0
13.09		1083	Ni		Mushroom	8				25.8	25.8	28.3	14.1
13.25		1094	Ni		Burned	1			**69.3**	**65.3**	**73.0**	23.1	28.3
13.43	1100	1099	Undecane*	1120-21-4	Alkane	4	6	16.3	23.1	16.3		11.5	32.7
13.48	1104	1104	Nonanal*	124-19-6	Fat, citrus, green	7	4	28.3		46.9	**52.9**	**51.0**	
13.56	1110	1108	Sotolon* [a]	28664-35-9	Spices, licorice	6	5	16.3	**100.0**	**87.6**	**93.1**	**73.0**	

14.21	1149	1152	(Z)-2-Nonenal* [a]	60784-31-8	Cucumber, fruit	7	4	**81.6**		**77.5**	**86.4**	**58.9**	**83.7**
14.33	1160	1159	(E)-2-Nonenal* [a]	18829-56-6	Cucumber, green	7	4	**85.6**	36.5	**65.3**	**74.8**	**69.3**	**76.6**
14.52	1170	1171	Benzoic acid	65-85-0	Almond, sweet	1	2	38.3		44.7	36.5	**71.2**	**73.0**
14.60	1175	1177	δ-Valerolactam*	675-20-7	Piper, green	7	1	32.7	36.5	32.7	**56.6**	**65.3**	
14.98	1204	1205	Decanal*	112-31-2	Flower	3	4	28.3	28.3	44.7	44.7	11.5	16.3
15.23	1221	1220	2-Phenoxyethanol	122-99-6	Pleasant, green	7	3	**79.6**	44.7	49.0	**61.1**	**56.6**	**73.0**
15.34	1223	1228	Benzothiazole	95-16-9	Gasoline, rubber	4	1		36.5	23.1		16.3	
15.70	1255	1254	Caprolactam*	105-60-2	Distasteful	4	1	11.5	11.5	51.0	34.6	36.5	
15.83	1272	1268	Nonanoic acid	112-05-0	Fat, distasteful	4	2	14.1	42.4	**56.6**	30.6	32.7	32.7
16.05	1283	1280	(E)-Cinnamaldehyde*	14371-10-9	Cinnamon, sweet	6	4	28.3	25.8	32.7	28.3	20.0	40.0
16.42	1304	1306	Undecanal	112-44-7	Sweet, flower	3	4	16.3	34.6	11.5	40.0	32.7	
16.67	1323	1322	p-vinylguaiacol* [a]	7786-61-0	Clove, curry	6	3	32.7	**65.3**	**65.3**	**73.0**	**58.9**	**65.3**
16.90	1344	1340	Triacetin	102-76-1	Fat, chlorine	4	5	28.3		46.2	**51.0**	**58.9**	11.5
17.15	1364	1364	Eugenol*	97-53-0	Clove, honey	6	3	**73.0**		**81.6**	**75.3**	**81.6**	
17.30	1371	1374	1-Undecanol	112-42-5	Fruity, mandarin	2	3		32.7	**63.2**	**69.3**	**52.9**	**63.2**
17.63	1400	1399	Tetradecane*	629-59-4	Alkene	4	6	25.8	49.0	36.5	40.0	32.7	32.7
17.73		1409	Ni		Distasteful	4		16.3	**80.0**	32.7	40.0	34.6	
17.85	1419	1419	Dodecanal*	112-54-9	Flower	3	4	30.6		16.3	23.1	32.7	11.5
18.27	1448	1451	Geranyl acetone	3796-70-1	Flower	3	7			32.7		16.3	20.0
18.42	1463	1466	1-Dodecanol	112-53-8	Flower	3	3	25.8	16.3	28.3	32.7	28.3	

t_R: retention time (min). N.i: No identified. Marked in bold letters values over 50%.

* Verified with standard

[a] Odorant compounds selected as aroma-impact

The compounds with the highest aromatic role in starch-based films (MF% values above 60% in at least 3 of the 4 films) were: trimethylamine, 1-octen-3-one, sotolon, (Z) and (E) 2-nonenal, p-vinylguaiacol, eugenol and 1-undecanol. Except for trimethylamine, all of them had been previously detected in pellets and/or starch. Its presence in films could be due to degradation processes during the film manufacturing. trimethylamine, present in all the films, had a distasteful fish aroma and could lead to off-flavours in the packaged food. 1-octen-3-one, sotolon and 1-undecanol were only detected with MF% values above 20% in pellets. This fact suggests that they could have been formed either during the pellets manufacturing process due to temperature and pressure conditions, or present in the other raw materials used for its manufacture (Caillé et al., 2017). Sotolon and p-vinylguaiacol were also detected in the starch but their MF% was lower than in the films. However, other compounds such as eugenol, 2-phenoxyethanol, (Z) and (E) 2-nonenal were already present in starch at high MF%, what suggests that these compounds were characteristic of the raw starch and not produced during the production of the biopolymer. Most of the key aromatic compounds in films belonged to spices or green aromatic groups. Other compounds with a clear aromatic impact in at least 2 films were: nonanal, α-valerolactam, 2-phenoxyethanol, triacetin and a non-identified compound with RI 1094.

Figure 2a shows a bar chart of total MF% for each aroma group in the 4 films and figure 2b shows the results for each functional group. Aroma groups “chemical and distasteful” (AG 4), “spices” (AG 6) and “green” (AG 7) were the main aroma descriptors in the films. All the films showed a similar pattern. Only film BP4, which came from a different starch, obtained lower MF% values for almost all the descriptors. Sotolon, p-vinylguaiacol and eugenol were the main responsible for the differences in MF% between BP4 and the other films. The type of starch used and the temperature conditions used during the extrusion process can favor the formation of these compounds (Espert et al., 2005; Wypych, 2013). Regarding the functional groups, “alcohol and phenols” (FG 3) and “aldehydes” (FG 4) were the chemical families with the highest impact in aromatic profile of starch based films. The different films showed

also a similar pattern regarding the functional groups. In this case, BP4 showed lower MF% values especially for “amides and amines” (FG 1) and “esters” (FG 5).

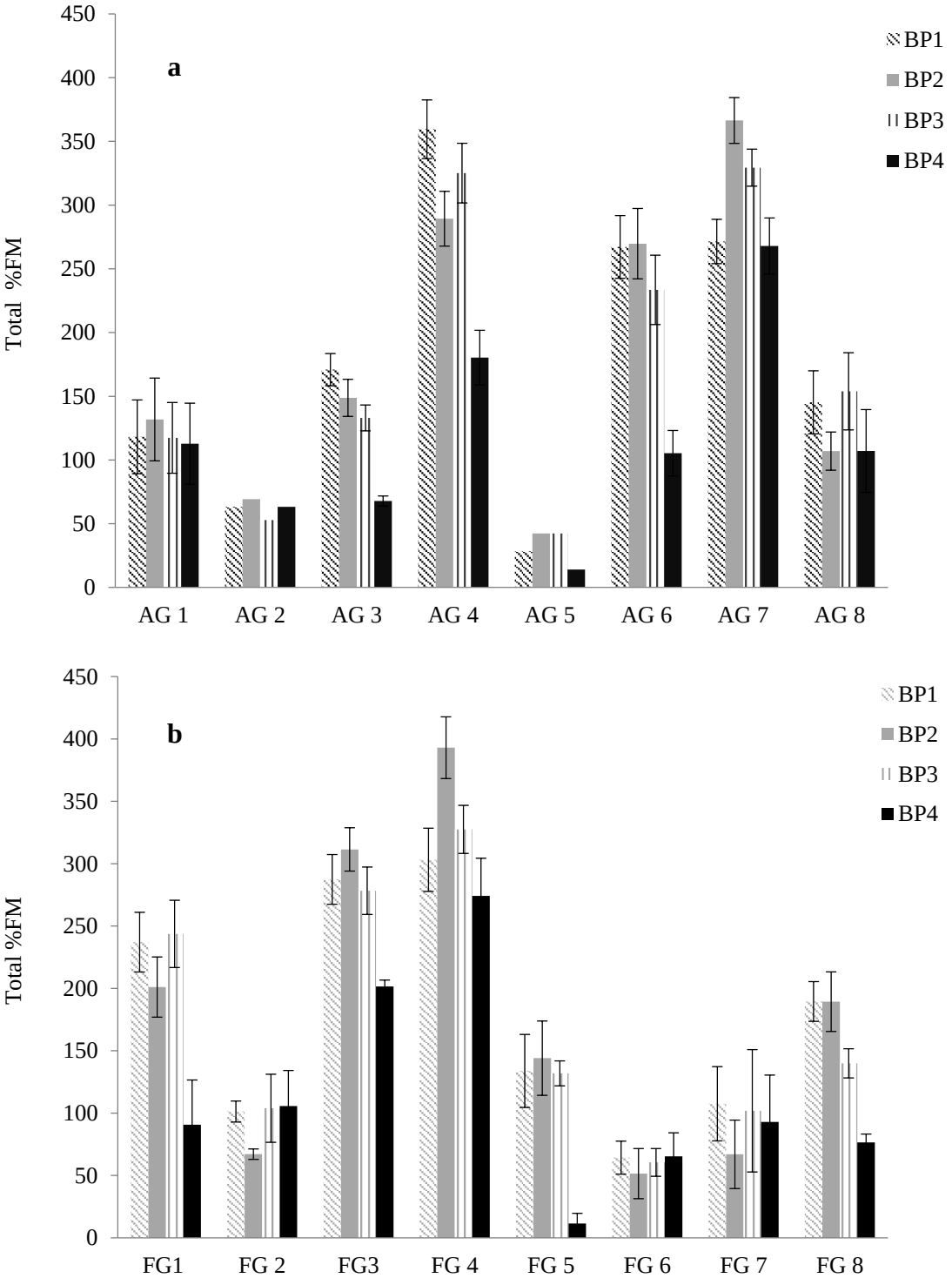


Figure 2. Bars chart of the addition of MF% values for the 4 starch-based films for **a)** aroma descriptors and **b)** principal functional group.

Figure 3 showed a radial chart comparing MF% values in the raw starch and in the film BP2 for the different functional groups (3b) and aroma groups (3a). As it can be seen, aldehydes, with green aromas, are the compounds with the highest aromatic impact in these materials. They can come from the degradation of amino acids or formed by the beta oxidation and decarboxylation of fatty acids (Feng et al., 2014; Gao et al., 2010; Steinhaus et al., 2007). Figure 3b also shows that the aromatic impact of the amides and amines and also of the no identified compounds increased in the film versus the raw material. The presence of amides and amines in the biomaterials is a significant sign of deterioration, maybe caused by the exposure to the light and the environment (Wypych, 2013), which is more intense.

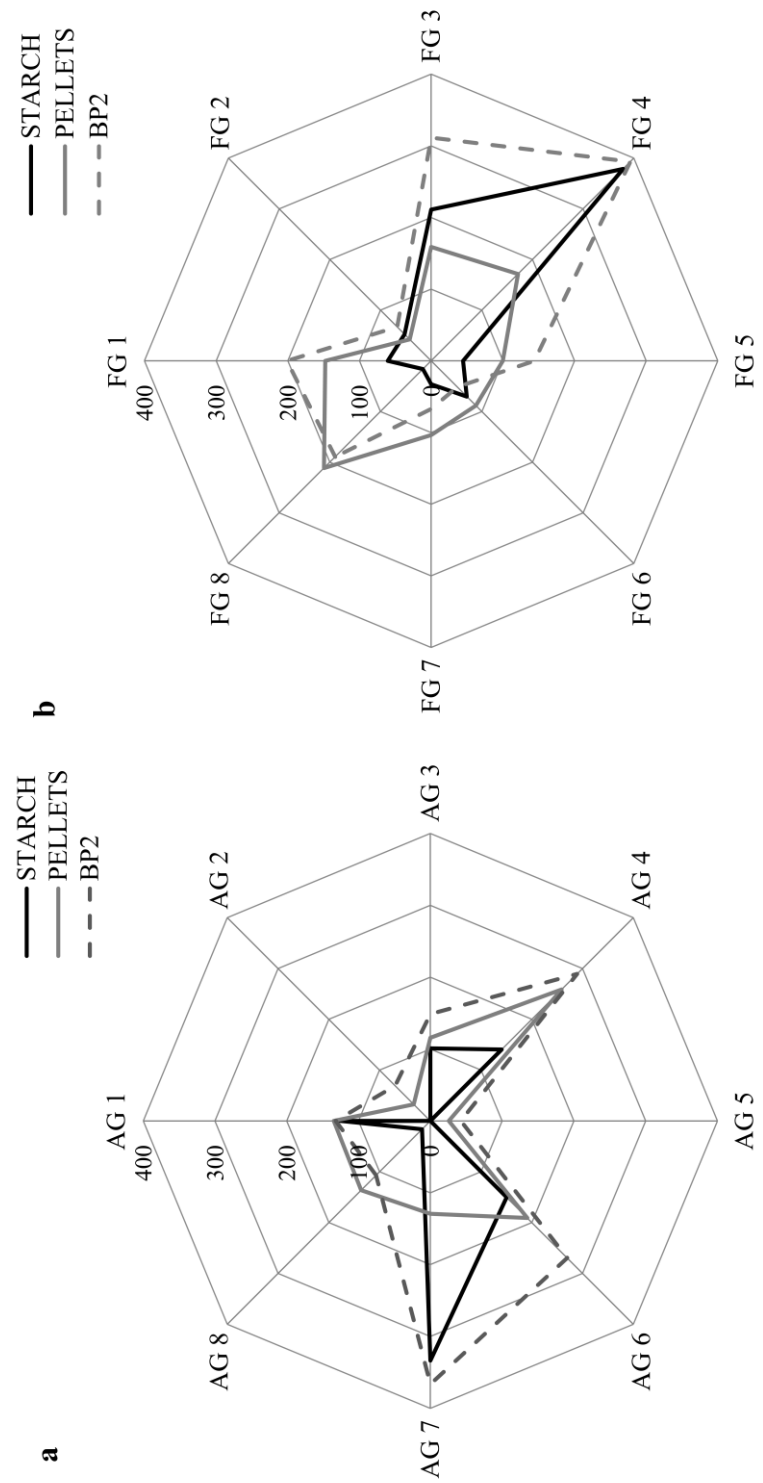


Figure 3. Radial chart of the comparison between the starch, pellets and the film BP2 for **a)** aroma descriptors and **b)** principal functional group. Axes represent the addition of MF% values.

6.3. Sensory analysis

The overall quality as well as the intensity of the 8 aroma descriptors defined in the materials and methods section were evaluated by a trained panel. Figure 4a showed the intensity of the descriptors in the 4 films. Only 4 descriptors were detected by the panel: “fat”, “sweet”, “toasted” and “spices”, being “spices” and “toasted” the most intense ones for most of the films. Only BP4 showed a low intensity for these descriptors. This film was manufactured with a different starch and probably this was the reason of the differences in the aroma perception. In addition, this film showed the highest overall quality, probably because these 2 descriptors were not considered by the panel as appropriate for a packaging material. According to the olfactometry, the highest differences in these aroma groups between BP4 and the other films corresponded to sotolon, eugenol and caprolactam, which obtained lower values in BP4. In the sensory evaluation of starch (Figure 4b), the intensity of these descriptors was lower than in the film. No more descriptors were detected in starch samples.

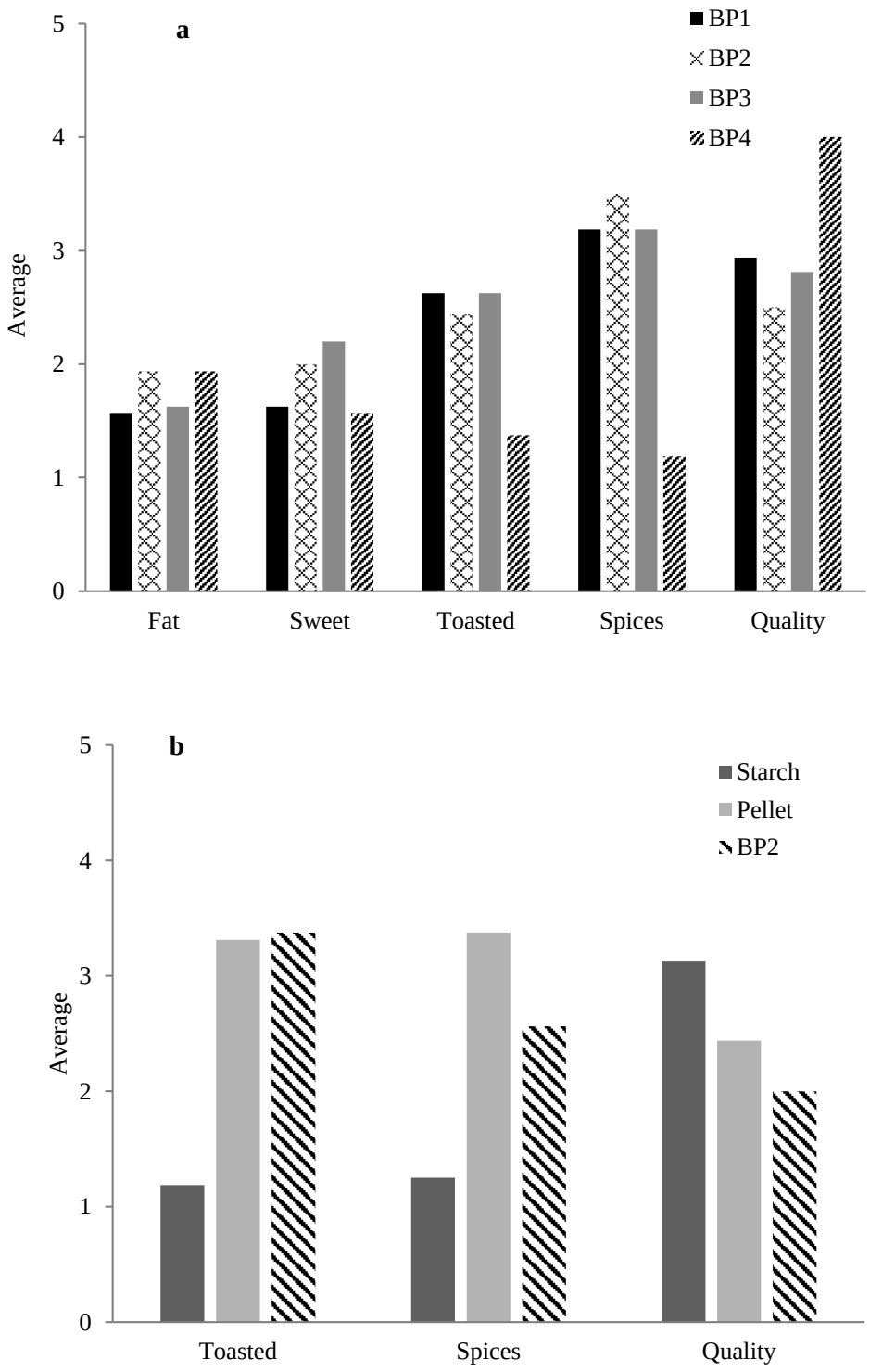


Figure 4. Bars chart of the sensory test for comparison between a) the 4 starch-based films and b) starch, and film BP2.

7. CONCLUSIONS

The main odorous compounds present in different starch-based biopolymers intended for food packaging have been identified. Compounds such as trimethylamine, 1-octen-3-one, sotolon, (Z) and (E)-nonenal, eugenol or p-vinyguiacol were the main responsible of starch-base films aroma, that were mainly perceived with descriptors belonging to the aroma groups “chemical and distasteful”, “spices” or “green”. The final perception of the films by the panelists was linked to toasted and spicy notes. The three films coming from the same starch-based biopolymer showed a similar aromatic profile, what highlights the role of the initial starch in the final aroma of the packaging material. These compounds could be transferred to packaged food and for this reason migration studies would be necessary.

Capítulo 5

Rapid and simultaneous determination of polyester oligomer as migrants from biopolymers by direct analysis in real time mass spectrometry

1. RESUMEN

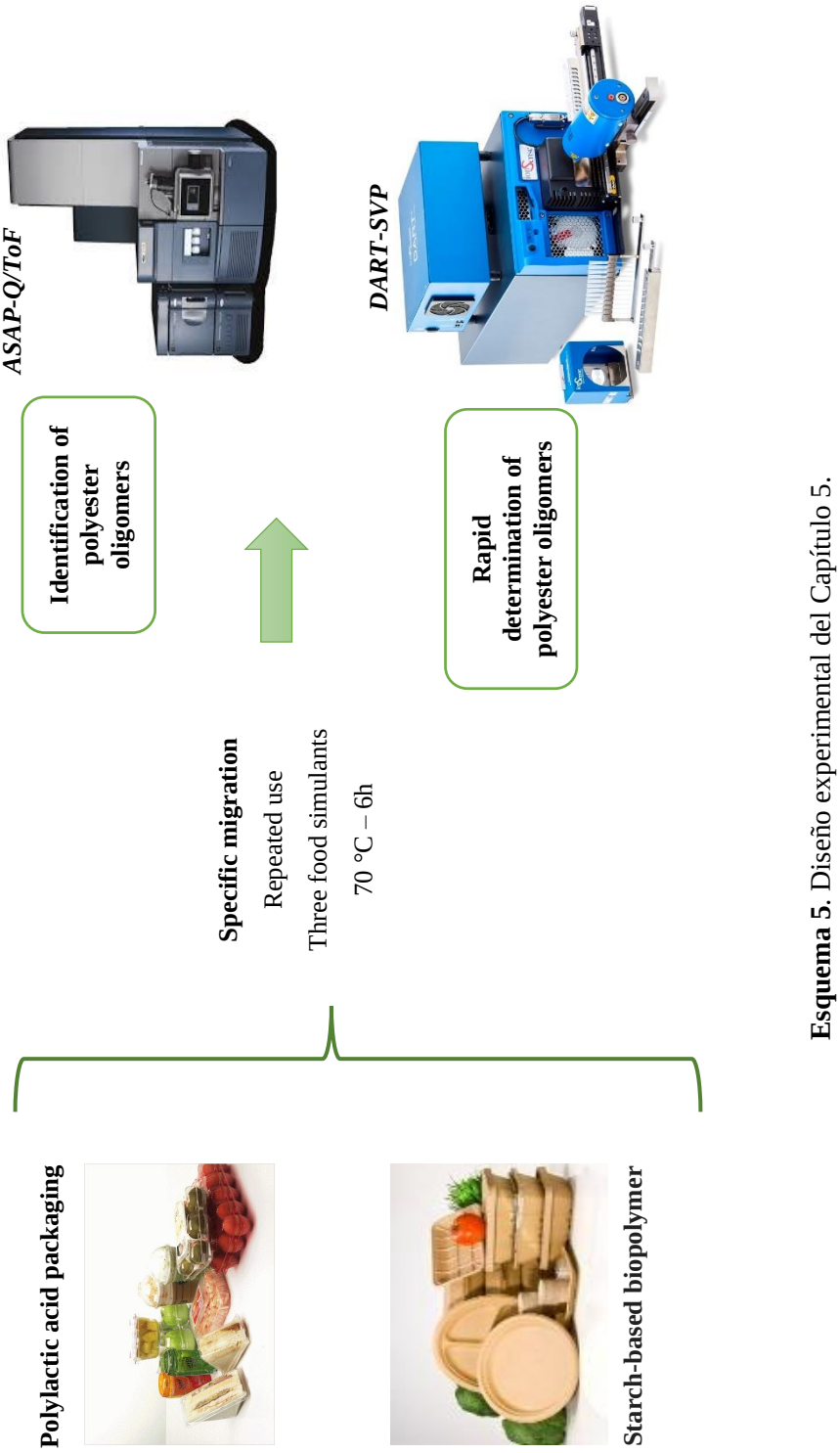
Los biopolímeros a base de ácido poliláctico (PLA) y almidón tienen muchas ventajas, como son renovables y de bajo costo. Sin embargo, suelen necesitar la adición de resinas de poliésteres para mejorar sus propiedades mecánicas. En este capítulo, se ha estudiado la migración de oligómeros de poliéster provenientes de dos biopolímeros, base de PLA y almidón. Las muestras de migración en los tres simulantes fueron analizados por HPLC-Q/ToF, que permitió la identificación de las principales series de oligómeros en cada material. En las muestras de PLA fueron detectados 23 oligómeros de poliéster compuestos por combinaciones entre ácido adipico [AA] y cuatro tipos de polioles: propilenglicol [PG], dipropilenglicol [DPG], 2,2-dibutil-1,3-propanodiol [DBPG] e isobutanol [i-BuOH]. De ellos fueron identificados 14 oligómeros con estructura lineal y 5 estructura cíclica. En las muestras del biopolímero base almidón fueron detectados 14 oligómeros, que en la literatura han sido reportados como provenientes del poliéster de poli (co-tereftalato de adipato de butileno) poliéster (PBAT), de los cuales 12 tienen estructura cíclica y 2 estructura lineal. Posteriormente, las muestras de migración fueron analizadas mediante dos técnicas de ionización ambiental: DART-SVP y ASAP-Q/ToF. Con estas metodologías fue posible detectar simultáneamente los principales oligómeros migrantes y sus aductos de una forma muy rápida y eficaz. Las técnicas prometen ser buenas herramientas de apoyo a la evaluación de la seguridad y el cumplimiento legal de los biopolímeros destinados al contacto alimentario.

2. OBJETIVOS

Las resinas de poliéster adicionadas a biopolímeros pueden dar lugar a la formación de oligómeros de poliésteres. Los oligómeros son considerados NIAS que pueden migrar a los alimentos y por tanto es necesaria su evaluación. Por este motivo, el objetivo principal de este capítulo fue establecer metodologías de análisis para la detección rápida y simultanea de oligómeros de poliésteres, provenientes de muestras de migración de un material de PLA y un biopolímero base almidón. Estas metodologías estaban basadas en las técnicas DART-SVP y ASAP acopladas a un espectrómetro de masas. Para el cumplimiento de este objetivo se establecieron las siguientes tareas:

- Búsqueda bibliográfica acerca de las familias de oligómeros de poliéster presentes en diversos biopolímeros.
- Realización de los ensayos de migración secuencial para los materiales de uso repetido, PLA y base almidón, siguiendo los protocolos establecidos por la legislación.
- Elucidación de las familias de oligómeros de poliéster migrantes implementado las estrategias de identificación por UPLC-Q/ToF.
- Desarrollo de diferentes métodos mediante DART-SVP y ASAP-Q/ToF para la detección de los aductos de oligómeros de poliéster provenientes de biopolímeros.
- Elaboración de una librería de los aductos detectados de cada uno las series de oligómeros de poliéster migrantes.
- Comparación de la idoneidad de las metodologías de detección rápida de oligómeros de poliésteres.

3. ESQUEMA DE TRABAJO



Esquema 5. Diseño experimental del Capítulo 5.

4. INTRODUCCIÓN

The packaging industry is constantly looking for more environmentally friendly materials, that also have the mechanical characteristics of conventional packaging materials, such as its flexibility, strength or thermal stability (Tsochatzis et al., 2020). For this reason, the demand for biopolymers has increased in the last years, especially for polylactide (PLA) and starch-based polymers (Aznar et al., 2019; Osorio et al., 2019). This is because they are biodegradable and/or compostable under industrial conditions, and come from renewable resources (Geueke, 2014), making them suitable candidates to replace conventional plastics in the packaging sector (Badía et al., 2011). For these materials, the addition of a biodegradable aliphatic-aromatic (co)polyester(BPES) is necessary, in most cases to improve their physicochemical properties (Badía et al., 2011). For food contact materials (FCMs), polybutylene adipate terephthalate (PBAT), and polybutylene succinate terephthalate (PBST) are widely used as biodegradable polyesters (Badía et al., 2011).

Polyesters are manufactured by the polymerization of aliphatic diols, aliphatic dicarboxylic acids and\or aromatic dicarboxylic acids during a polycondensation reaction (Geueke, 2014; Pietropaolo et al., 2018). During the manufacturing process, oligomers can be also formed. These oligomers are considered non intentionally added substances (NIAS), and could potentially migrate from the FCM to the food, compromising the consumer´s safety. Even though pure biopolymers are only regulated by the frame regulation 1935/2004/CEE, if they include some conventional materials or resins, the European Plastics Regulation, Regulation (EU) No 10/2011, should be applied. This legislation establishes a positive list with authorized substances in the manufacturing process, as additives and monomers, and their specific migration limits (SMLs) (European Commission, 2011a). The maximum allowed concentration in migration for any substances not included in the positive list must be lower than 0.01 mg kg-1 food or food simulant (European Commission, 2011a).

In previous works, some polyester oligomers from PLA and starch-based biopolymers were identified by UPLC-Q/ToF (Aznar et al., 2019; Osorio et al., 2019).

The identification of other polyester oligomers is difficult because they are not included in any database. The use of hyphenated techniques, that combine chromatographic separation and high resolution mass spectrometry, is very useful to achieve this purpose. Once the compounds are identified, other techniques such as ambient mass spectrometry (AMS) can be used in order to detect its presence in new samples. AMS techniques are commonly used for direct and rapid analysis of compounds present in solid or liquid samples (Black et al., 2016; Lu et al., 2018), since they allow a rapid confirmation of the presence of target compounds. They have been previously used in the study of different food packaging materials, for example, in the analysis of non-visible set-off components (Karim Bentayeb et al., 2012) and the quantitative determination of BPA (Castro et al., 2019).

In the present study, the two AMS techniques used were Direct analysis in real-time (DART) and Atmospheric pressure solid analysis probe (ASAP). Both provided a direct sample analysis at ambient condition, a fast scan time and easy operation.

DART is one of the most popular ambient pressure ionization methods. In this technique, the sample is vaporized and afterwards the molecules are ionized by excited helium molecules. Then, the ionized vapour is introduced in the detector for its analysis. In the ionization process, different adducts are commonly formed, such as $[M+H]^+$ or $[M+NH_4]^+$ (Black et al., 2016; Castro et al., 2019). The formation of adducts is promoted by the molecular weight, volatility or polarity of the species present in the samples (Gross, 2014). This technique has been successfully implemented for the detection of different analytes, such as the determination of BPA from thermal printing receipts and tickets (Castro et al., 2019); forensic screening (Drury et al., 2018) or food quality and safety control (Kerpel dos Santos et al., 2018) among others. ASAP is also an ambient pressure ionization method for analyzing volatile or semi-volatile compounds (volatility below 500 ºC) coming from liquids or solid materials (Carrizo et al., 2016). This technique was successfully applied for the detection of nicotine and their metabolites (Carrizo et al., 2015) or for polyaromatic hydrocarbons (Carrizo et al., 2015). Even though both techniques have a similar operating principle, in ASAP the sample is

introduced directly into the ionization chamber, improving the general sensitivity. except for the heaviest compounds where sensitivity decrease (Carrizo et al., 2016).

The aim of this work was to explore a direct method based on AMS techniques for the screening of polyester oligomers present in the migration samples from PLA and starch biopolymers used in food packaging. The structural elucidation of the linear or cyclic polyester oligomers detected was based on their parent ion exact mass and their fragmentation mass spectra. This analysis was performed by UPLC-Q/ToF. Subsequently, DART-SVP-MS and ASAP-MS techniques were used as a tool to assess the presence of all polyester oligomers in a very short analysis time.

5. MATERIALS AND METHODS

5.1. Chemicals and Reagents

Methanol (UHPLC-MS grade), ethanol absolute (HPLC grade) and acetic acid (HPLC grade) for the UPLC-Q/ToF analysis and ASAP were supplied by Scharlab (Barcelona, Spain). Ethanol absolute (HPLC grade) for the analysis in DART-SVP supplied by Merck (Darmstadt, Germany). Ultra-pure water was obtained from a Millipore Milli-Q system (Billerica, MA, USA).

5.2. Samples

Biopolymers based on polylactic acid (PLA) and starch were supplied by a polymer manufacturing company for this study. Additional information about the sample cannot be provided. Samples were in the form of cups and dishes.

5.3. Migration assays

The migration tests were established in accordance with the European legislation on food contact materials (Regulation No 10/2011/EU) (European Commission, 2011a). Three simulants were evaluated: ethanol 10% (v/v) (simulant A), acetic acid 3% (w/v)

(simulant B) and ethanol 95% (v/v) (simulant D2 substitute). Migration assays carried out during 10 days at 70 ºC. The assays were carried out by total immersion of the sample (5 cm x 2 cm) into 20mL of the simulant.

5.4. Analysis by UPLC-Q/ToF

Chromatographic separation of the oligomers present in the migration solutions was performed using an Acquity UPLC from Waters Corporation (Milford, MA, USA) with a UPLC BEH C18 column of 1.7 μm particle size (2.1 × 100 mm). The chromatography parameters were: 35 °C column temperature, 0.3 mL min^{-1} column flow, and 10 μL injection volume. The gradient elution was carried out with two mobile phases: (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid. The separation started at 98/2 (phase A/phase B), and at eight min it was changed to 0/100 (phase A / phase B) with two additional minutes at the final composition.

A time-of-flight Waters Xevo G2 QTOF mass spectrometer from Waters Corporation (Milford, MA, USA) with an ESI probe was coupled UPLC system. The following parameters were used: ESI+ (positive ionization mode); sensitivity (analyser mode); 3.0 kV (capillary voltage); 30 V (sampling cone voltage); 3 V (extraction cone); 150 °C (source temperature); 20 L/h (cone gas flow rate); and 500 L/h (desolvation gas flow rate) at 450 °C (desolvation temperature). The acquisition was carried out in MS^E (acquisition mode), at low and high collision energy (CE) in the collision cell, in a mass range between 50 and 1000 m/z.

5.5. Analysis by DART-SVP

Direct analysis in real-time coupled to standardised voltage and pressure (DART-SVP) 100 model ion source (IonSense, Saugus, MA) was operated with helium (grade A) in running mode and nitrogen in standby mode, with 3,5 L min^{-1} helium flow, temperature 150 – 450 °C, and ion-source grid voltage 350 V. DART-SVP was coupled to an Acquity QDa single quadrupole mass spectrometer (Waters Corporation, Manchester, UK), operated in positive ion mode via a vapour interface (IonSense, Saugus, MA), with desolvation line temperature 250 °C, heat block temperature 350 °C,

interface voltage 4.5 kV and 50 – 1000 m/z. scan range. The mass spectrometer was controlled using MassLynx v4.1 SCN888 (Waters Corporation, Wilmslow, UK). Data were analyzed by MassLynx v4.1. The injection was carried out manually, where 3 μL of the migration solution was pipette-spotted directly onto the Quick Strip card. Then the Quick Strip card was mounted on the sampling rail for analysis

5.6. Analysis by ASAP-Q/ToF

The atmospheric solids analysis probe (ASAP) was coupled to a time-of-flight Waters Xevo G2 QTOF mass spectrometer, Xevo G2 QTOF (Waters Corporation, Milford, MA, USA). The following mass spectrometer parameters were used: API+ (positive ionization mode), source temperature at 120ºC, desolvation temperature at 450 ºC, desolvation flow 650 L/h, current corona at 5 □A. Three cone voltage were evaluated: 30 V, 50 V and 70 V, and 30 V was finally selected. The acquisition was carried out in the mass range between 50 –1000 m/z. Samples were directly introduced into the ASAP dipping previously a solid glass capillar in the migration samples. A blank, introducing the glass capillary in the migration blank was also performed. The analysis was acquired in SCAN continuous mode (scan time 0.5 s).

5.7. Data processing

The UPLC-Q/ToF and ASAP-Q/ToF mass data were analysed with MassLynx software V 4.1 from Waters (Milford, MA, USA). In both techniques, the mass spectra obtained in function 1 provided information about the elemental composition of the precursor ion and the mass spectra in function 2 provided information about the fragment ions. The identification methodology was optimized in previous works (Aznar et al., 2019; Osorio et al., 2019). The DART-SVP mass spectrums were acquired with MassLynx SCN888T software and processed with MassLynx software V 4.1.

6. RESULTS AND DISCUSSION

6.1. Identification of polyester oligomers

The polyester oligomers found in migration samples from PLA and starch based biopolymers are described in tables 1 and 2 respectively. The tables also show their retention time, their accurate mass, the adduct detected ($[M+H]^+$ or $[M+Na]^+$), their molecular formula and the simulant where they were detected.

The analysis of PLA migration sample revealed that the polyester resin used was composed by one kind of polyacid, adipic acid [AA] and four different kinds of polyols: propylene glycol [PG], dipropylene glycol [DPG], 2,2-dibutyl-1,3-propanediol [DBPG] and isobutanol [i-BuOH]. In the manufacturing of polyesters, it is common the use of a polyacid and various polyols (Bradley, 2010; Hoppe et al., 2016). Table 1 shows the presence of nineteen different polyester oligomers in the migration samples from PLA, where thirteen of them were cyclic and six were linear. The main monomers found were [AA-PG], [AA-DPG], [AA-DBPG], and [i-BuOH-AA-i-BuOH]. Their respective dimers, trimers, tetramers or different combinations among them were also observed. Simulant D2 was the simulant with the highest number of oligomers (nineteen oligomers), followed by simulant B (fourteen oligomers) and simulant A (twelve oligomers). Therefore, these compounds had a higher tendency to migrate to fat food.

In this analysis, five series of oligomers were found. The first series corresponds to cyclic oligomers with the structure $[AA\text{-}PG]_n$ (n = 1 to 5). The second series is similar to the first one but with the addition of a water molecule and opening the ring $H\text{-}[AA\text{-}PG]_n\text{-}OH$ (n = 2 to 5), resulting in a linear oligomer. Other series of linear oligomers found were: $H\text{-}[AA\text{-}DPG]\text{-}[AA\text{-}PG]_n\text{-}OH$ (n = 0 to 3), $H\text{-}[AA\text{-}DBPG]\text{-}[AA\text{-}PG]_n\text{-}OH$ (n = 0 to 3) and finally, [i-BuOH-AA- i-BuOH]-$[AA\text{-}PG]_n$ (n = 2 to 3).

Table 1. List of polyester oligomer detected in migrations samples from PLA by UPLC-Q/ToF and DART-SVP.

UPLC-Q/ToF								Main adduct- DART-SVP*				
t_R	Mass	Adduct	MF	Candidate oligomer	Simulant A	Simulant B	Simulant D2	*m/z*	Adduct	Int	Adduct $[M+NH_4]^+$	Int
5.79	187.1375	$[M+H]^+$	$C_9H_{14}O_4$	[AA-PG] *(cyclic)*	X	X	X	187.07	$[M+H]^+$	4.7		
5.07	285.0988	$[M+Na]^+$	$C_{12}H_{22}O_6$	H-[AA-DPG]-OH (*linear)*	X	X	X	245.21	$[M-H_2O+H]^+$	4.0		
8.00	339.2166	$[M+Na]^+$	$C_{17}H_{32}O_5$	H-[AA-DBPG]-OH (*linear)*	X	X	X	299.33	$[M-H_2O+H]^+$	39.3		
6.65	395.1667	$[M+Na]^+$	$C_{18}H_{28}O_8$	2[AA-PG] *(cyclic)*	X	X	X	373.33	$[M+H]^+$	10.6	390.36	1.6
6.04	413.1787	$[M+Na]^+$	$C_{18}H_{30}O_9$	H-2[AA-PG]-OH *(linear)*	X	X	X	373.33	$[M-H_2O+H]^+$	10.6		
6.29	471.2228	$[M+Na]^+$	$C_{21}H_{36}O_{10}$	H-[AA-DPG]-[AA-PG]-OH (*linear)*	X	X	X	431.43	$[M-H_2O+H]^+$	7.8		
8.27	525.3069	$[M+Na]^+$	$C_{26}H_{46}O_9$	H-[AA-DBPG]-[AA-PG]-OH (*linear)*	X	X	X	485.54	$[M-H_2O+H]^+$	60.5		
7.32	581.2668	$[M+Na]^+$	$C_{27}H_{42}O_{12}$	3[AA-PG] *(cyclic)*	X	X	X	559.49	$[M+H]^+$	16.1	576.50	3.7
7.06	599.2734	$[M+Na]^+$	$C_{27}H_{44}O_{13}$	H-$[AA\text{-}PG]_n$-OH *(linear)*	X	X	X	559.49	$[M-H_2O+H]^+$	16.1	594.47	3.5
8.44	653.3636	$[M+Na]^+$	$C_{32}H_{54}O_{12}$	[i-BuOH-AA- i-BuOH]-2[AA-PG] *(linear)*			X	631.57	$[M+H]^+$	3.7	648.60	6.1
6.92	657.3203	$[M+Na]^+$	$C_{30}H_{50}O_{14}$	H-[AA-DPG]-2[AA-PG]-OH (*linear)*	X	X	X	617.51	$[M-H_2O+H]^+$	18.5		
8.40	711.5505	$[M+Na]^+$	$C_{35}H_{60}O_{13}$	H-[AA-DBPG]-2[AA-PG]-OH (*linear)*[a]		X	X	671.67	$[M-H_2O+H]^+$	100.0		
7.70	767.3526	$[M+Na]^+$	$C_{36}H_{56}O_{16}$	4[AA-PG] *(cyclic)*	X	X	X	745.70	$[M+H]^+$	8.8	762.76	8.1
7.22	785.3586	$[M+Na]^+$	$C_{36}H_{58}O_{17}$	H-4[AA-PG]-OH *(linear)*	X	X	X	745.70	$[M-H_2O+H]^+$	8.8	780.74	1.2
8.60	839.4518	$[M+Na]^+$	$C_{41}H_{68}O_{16}$	[i-BuOH-AA- i-BuOH]-3[AA-PG] *(linear)*			X	817.83	$[M+H]^+$	4.8	834.90	6.6
7.62	843.3873	$[M+Na]^+$	$C_{39}H_{64}O_{18}$	H-[AA-DPG]-3[AA-PG]-OH (*linear)*			X	803.77	$[M-H_2O+H]^+$	5.3		
8.49	897.4931	$[M+Na]^+$	$C_{44}H_{74}O_{17}$	H-[AA-DBPG]-3[AA-PG]-OH (*linear)*		X	X	857.89	$[M-H_2O+H]^+$	41.4		
7.98	953.4359	$[M+Na]^+$	$C_{45}H_{70}O_{20}$	5[AA-PG] *(cyclic)*			X	931.05	$[M+H]^+$	14.1	948.08	26.2
7.63	971.4451	$[M+Na]^+$	$C_{45}H_{72}O_{21}$	H-5[AA-PG]-OH *(linear)*			X	931.05	$[M-H_2O+H]^+$	14.1		

t_R: retention time (min) in ethanol 95%. MF: Molecular formula. Simulant A: ethanol 10% (v/v). Simulant B: acetic acid 3% (w/v) Simulant D2: ethanol 95% (v/v). Int: relative intensity. [a] Oligomer from figure 2a. * Oligomers detected in specific migration samples to Simulant D2.

Table 2. List of polyester oligomer detected in migrations samples from starch-based biopolymer by UPLC-Q/ToF and DART-SVP.

UPLC-Q/ToF								Main adduct- DART-SVP*			
t_R	Mass	Adduct	MF	Candidate oligomer	Simulant A	Simulant B	Simulant D2	Adduct $[M+H]^+$	Int	Adduct $[M+NH_4]^+$	Int
7.50	221.1270	$[M+H]^+$	$C_{12}H_{12}O_4$	[TPA-BD] *(cyclic)*			X	221.13	5.0		
5.50	223.1412	$[M+Na]^+$	$C_{10}H_{16}O_4$	[AA-BD] *(cyclic)*			X	201.15	4.2	218.22	1.3
5.55	313.1660	$[M+Na]^+$	$C_{14}H_{26}O_6$	[AA-BD]-[BD] *(lineal)*	X	X	X	291.27	11.8	308.30	3.9
7.00	423.1984	$[M+Na]^+$	$C_{20}H_{32}O_8$	2[AA-BD] *(cyclic)*	X	X	X	401.39	13.1	418.44	18.5
6.40	441.2093	$[M+H]^+$	$C_{24}H_{24}O_8$	2[TPA-BD] *(cyclic)*	X	X	X	441.38	1.6	458.46	3.3
7.50	443.1684	$[M+Na]^+$	$C_{22}H_{28}O_8$	[TPA-BD]-[AA-BD] *(cyclic)*	X	X	X	421.35	12.6	438.42	15.7
6.64	513.3770	$[M+Na]^+$	$C_{24}H_{42}O_{10}$	2[AA-BD]-[BD] *(lineal)*	X	X	X	491.51	7.5	508.54	14.4
7.77	623.3054	$[M+Na]^+$	$C_{30}H_{48}O_{12}$	3[AA-BD] *(cyclic)*	X	X	X	601.50	27.6	618.54	58.2
8.09	643.4090	$[M+Na]^+$	$C_{32}H_{44}O_{12}$	[TPA-BD]-2[AA-BD] *(cyclic)* [a]			X	621.47	50.0	638.53	100.0
8.34	663.3835	$[M+Na]^+$	$C_{34}H_{40}O_{12}$	2[TPA-BD]-[AA-BD] *(cyclic*			X	641.47	24.3	658.51	61.4
8.75	683.3540	$[M+Na]^+$	$C_{36}H_{36}O_{12}$	3[TPA-BD] *(cyclic)*			X	661.50	5.5	678.52	16.3
7.98	823.4092	$[M+Na]^+$	$C_{40}H_{64}O_{16}$	4[AA-BD] *(cyclic)*			X	801.80	7.7	818.81	12.5
8.23	843.5593	$[M+Na]^+$	$C_{42}H_{60}O_{16}$	[TPA-BD]-3[AA-BD] *(cyclic)*			X	821.73	16.3	838.77	28.5
8.48	863.3457	$[M+Na]^+$	$C_{44}H_{56}O_{16}$	2[TPA-BD]-2[AA-BD] *(cyclic)*			X	841.73	8.6	858.77	18.0

t_R: retention time (min) in ethanol 95%. MF: Molecular formula. Simulant A: ethanol 10% (v/v). Simulant B: acetic acid 3% (w/v) Simulant D2: ethanol 95% (v/v). Int: relative intensity.

[a] Oligomer from figure 2b. * Oligomers detected in specific migration samples to Simulant D2.

Table 2, shows the oligomers found in migration from the starch-based polymer. A total of fourteen oligomers composed by butanediol [BD] and two different kinds of diacids, terephthalic acid [TPA] or adipic acid [AA], were detected. Some of these oligomers were previously reported by different authors as coming from poly(butylene adipate co-terephthalate) polyester (PBAT) (Aznar et al., 2019; Osorio, Aznar, et al., 2019). Twelve were cyclic oligomers and two were linear oligomers. All of them were found in simulant D2; since in simulant A and B, the same six oligomers were observed. Four series of cyclic oligomers with the following structures were detected: $[TPA\text{-}BD]_n$ (n = 1 to 3); $[AA\text{-}BD]_n$ (n = 1 to 4) and $[TPA\text{-}BD]_m$-$[AA\text{-}BD]_n$ (m/n = 1 to 3). In addition, a series of linear oligomers was also observed: $[AA\text{-}BD]_n$-[BD] (n = 1 to 2).

Previous works showed that the polyester oligomer series showed a common fragmentation spectra, which confirmed the similarity of their structures (Canellas et al., 2015). The common masses of the identified oligomers in PLA were 187.1375 and 111.0682 m/z, that correspond to the formula $C_9H_{15}O_4$ ($[AA\text{-}PG]_1$) and $C_6H_7O_2$ (monomer [AA]), respectively. Figure 1a shows the high collision energy spectra of the H-[AA-DBPG]-$[AA\text{-}PG]_2$-OH linear oligomer (8.40_711.5505), where the two masses reported in the literature can be observed.

In starch-based migration samples, the common masses of PBAT oligomers were 111.0768 and 149.0565 m/z that correspond to the monomers formula [TPA] ($C_8H_4O_3$) and [AA] ($C_6H_7O_2$), respectively (Aznar et al., 2019; Elena Canellas et al., 2015). Figure 1b shows high collision energy spectra of $[TPA\text{-}BD]_1$-$[AA\text{-}BD]_2$ cyclic oligomer (8.09_643.4090), where the PBAT common masses are also present.

In both cases, the polyester oligomers were selected because in the analysis, their adduct showed the highest intensity of the DART-SVP spectrum

Figure 1. High collision energy spectra for **a)** [AA-DBPG]-[AA-PG]$_3$ oligomer from PLA and **b)** [TPA-BD]-[AA-BD]$_2$ polyester oligomer from starch-based biopolymer.

6.2. DART-SVP analysis of polyester oligomers in migration samples

The ability to implement AMS techniques as a rapid methodology to determine the polyester oligomers coming from biopolymers was investigated. Table 1 shows the main adducts of the polyester oligomers detected in PLA migration samples by DART-SVP, as well as their m/z and relative intensity. All the oligomers were previously

detected and identified in migration samples by UPLC-Q/ToF. Figure 2a shows a DART-SVP mass spectrum of a simulant D2 migration sample from the PLA sample. The structure and adducts of the candidate oligomers can be also observed in that figure.

The highest intensity in the mass spectrum was observed for m/z 671.67 (100 %), corresponding to the adduct $[M\text{-}H_2O+H]^+$ of the linear oligomer with structure H-[AA-DBPG]-[AA-PG]$_2$-OH. The following masses with high intensity were 485.54 (60.5 %), 857.89 (41.4 %) and 299.33 (39.3 %).

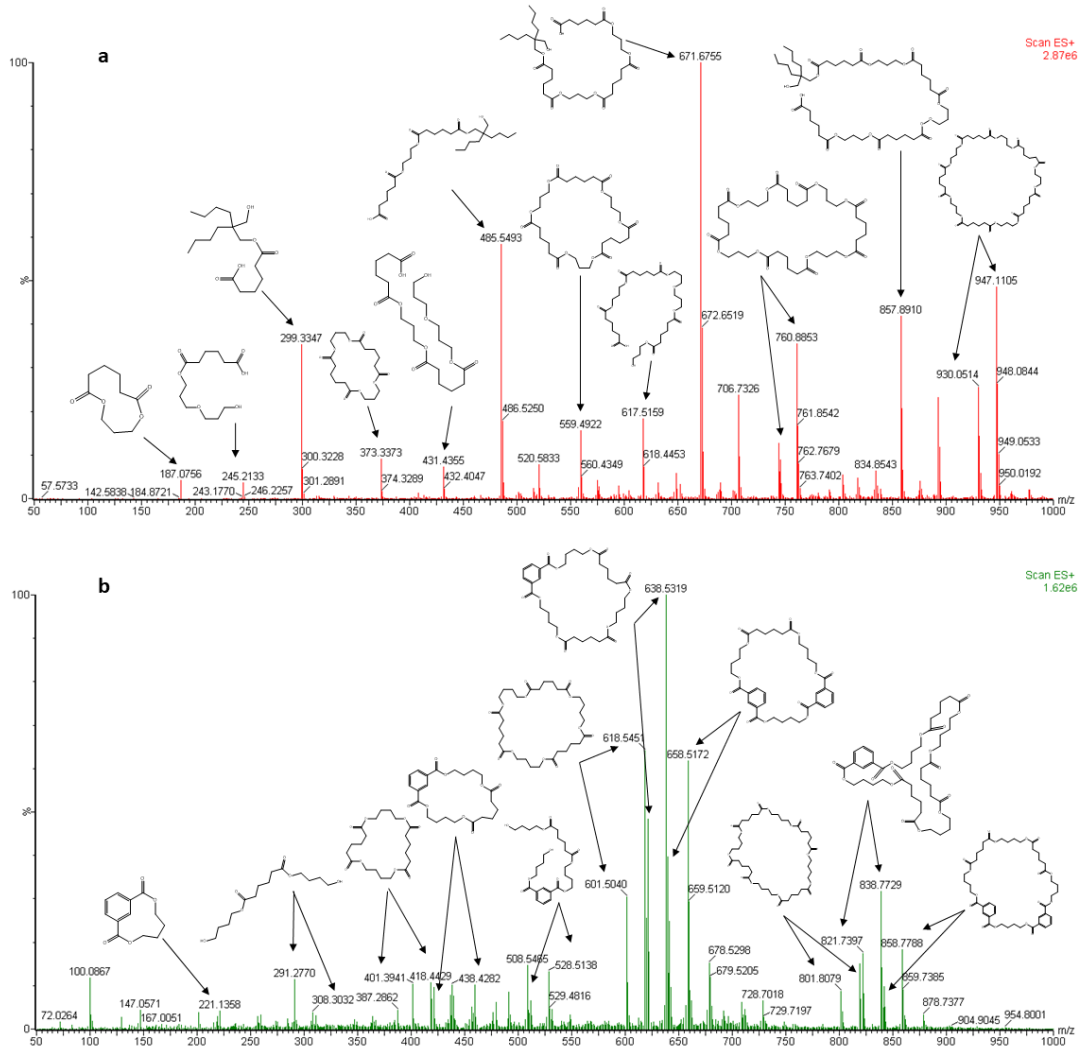


Figure 2. DART-SVP spectrum of migration assay in EtOH 95 % from **a)** PLA and **b)** starch-based biopolymer. The adducts for each of the polyester oligomers are linked to tables 1 and 2.

Similarly, table 2 shows the main adducts of PBAT oligomers ($[M+H]^+$ and $[M+NH_4]^+$) detected in migration samples of starch-based biopolymer by DART-SVP, and its relative intensity and m/z. In addition, six adduct masses were only detected by DART-SVP in the same migration samples of simulant D2 and their masses and the candidate polyester oligomers proposed are shown in table 3. The structure and molecular formula of the six oligomer candidates could be calculated because the monomer units used in the manufacturing PBAT polyester were known. Combining the monomers [TPA], [AA] and [BD] and the knowledge obtained from previous work carried out in our research group (Aznar et al., 2019; Canellas et al., 2015; Osorio, et al., 2019), six polyester oligomers candidates were proposed. This strategy was previously used by E. Brandly (Bradley, 2010) to identify other oligomers. Figure 2b shows a DART-SVP mass spectrum and the structure of the candidate oligomers present in the starch-based biopolymer sample. The highest intensity in the mass spectrum was observed for m/z 638.53 (100 %), corresponding to the adduct $[M+NH_4]^+$ of the cyclic oligomer with structure [TPA-BD]-$[AA-BD]_2$. The following masses with high intensity were 658.51 (61.4 %), 618.54 (58.2 %) and 621.47 (50.0 %).

Table 3. Polyester oligomer candidates detected only in specific migration samples of ethanol 95% (v/v) from from starch-based biopolymer by DART-SVP.

Candidate Name	MF	Main adduct			Other adducts
		m/z	**adduct**	**Int**	
[TPA-BD]-[BD] *(lineal)*	$C_{16}H_{22}O_6$	311.25	$[M+H]^+$	4.0	
[TPA-BD]-[AA-BD]-[BD] *(lineal)*	$C_{26}H_{38}O_{10}$	528.51	$[M+NH_4]^+$	12.9	$[M+H]^+$ (5.4)
$[TPA-BD]_2$-[BD] *(lineal)*	$C_{28}H_{34}O_{10}$	531.45	$[M+H]^+$	3.6	$[M+NH_4]^+$ (3.0)
$[AA-BD]_3$-[BD] *(lineal)*	$C_{34}H_{58}O_{14}$	691.66	$[M+H]^+$	4.3	$[M+NH_4]^+$ (3.1)
[TPA-BD]-$[AA-BD]_2$-[BD] *(lineal)*	$C_{36}H_{54}O_{14}$	728.70	$[M+NH_4]^+$	6.4	$[M+H]^+$ (4.1)
$[TPA-BD]_3$-[AA-BD] *(cyclic)*	$C_{46}H_{52}O_{16}$	878.74	$[M+NH_4]^+$	3.3	$[M+H]^+$ (1.6)

MF: Molecular formula. Int: relative intensity.

In DART, high polarity compounds generate adducts such as $[M+H]^+$, $[M-H+H_2O]^+$, $[M-H+O]^+$ or $[M+NH_4]^+$; and medium polarity compounds form adducts as $[M^*]^+$, $[M+H]^+$ or $[M-H+O]^+$ (Cody, 2009; Gross, 2014). In PLA and in starch-based samples, the adducts $[M+H]^+$, $[M-H+H_2O]^+$ and $[M+NH_4]^+$, $[M+H]^+$, were observed, respectively. Detection of these adducts is very common in the analysis of oligomers in different polymers by the DART technique (Bridoux et al., 2013). The identified adducts were carried out considering the possible interaction between the molecular ions of polyester oligomers and the species detected in the environment (oxygen, water and ammonia).

The adducts that showed the highest intensity in PLA and in starch-based samples were H-[AA-DBPG]-$[AA-PG]_n$-OH (n = 1 to 4) and the $[TPA-BD]_m$-$[AA-BD]_n$ (m/n = 1 to 3) , respectively. Their high intensity could be attributed to their higher concentration than other compounds in the samples. It has to be also taken into account that adducts detected in DART could come from oligomers belonging to oligomer series with similar structures, and that, therefore, have common fragments (Figure 1).

This fact can be specially observed for those DART masses corresponding to the monomers, such as [AA-PG] (187.08), [AA-DPG] (245.21) and [AA-DBPG] (299.33). If these masses are extracted in the UPLC-Q/ToF chromatogram, it can be observed that they are also present in heavier oligomers that contain the former structures. Figure 3(a-b-c) shows the intensity of the 3 monomers in all the oligomers of the chromatogram where they were detected. Therefore, the presence of the masses 187.08, 245.21 and 299.33 in a DART spectrum will inform the analysts about the presence of oligomers containing the monomers [AA-PG], [AA-DPG] and [AA-DBPG] respectively. With this information, the formula of different possible combinations within them to form oligomers can be calculated and the presence of their masses in the DART spectrum can be checked.

Figure 3(d-e) shows the intensity of the monomers [AA-BD] (201.15) and [TPA-BD] (221.13) in various PBAT polyester oligomers previously detected by UPLC-Q/ToF. Therefore, the presence of masses 201.15 and 221.13 in the DART spectrum

would indicate that the sample contains PBAT polyester oligomers. As it was described for polyesters present in PLA based polymers, by calculating the formula of the different monomers combination, it would be possible to obtain their masses and look for them in the DART spectrum.

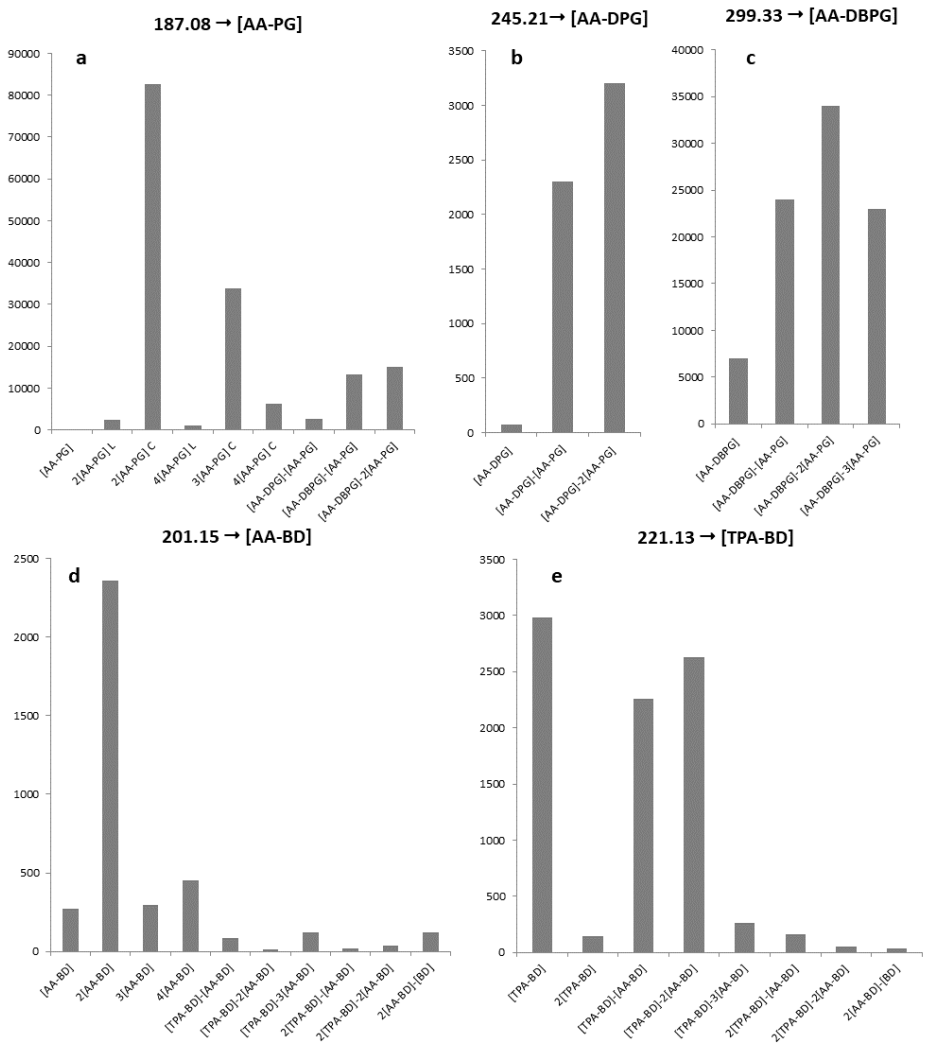


Figure 3. Intensity of the monomers in all the oligomers of the chromatogram where they were detected **a)** monomer [AA-PG] (187.08); **b)** monomer [AA-DPG] (245.21); **c)** monomer [AA-DBPG] (299.33); **d)** monomer [AA-BD] (201.15) and **e)** monomer [TPA-BD] (221.13).

6.3. ASAP-Q/ToF analysis of polyester oligomers in migration samples

The mass spectra in figure 4 correspond to a migration sample of PLA analyzed by ASAP-Q/ToF (a.1) and DART-SVP (a.2), and a migration sample of a starch-based biopolymer analyzed by ASAP-Q/ToF (b.1) and DART-SVP (b.2).

In the ASAP spectrum of PLA, figure 4a.1, the mass with the highest intensity was 111.0530 m/z that corresponds to [AA] monomer. Also, the masses of oligomers [AA-PG] (187.0716 m/z), H-[AA-DBPG]-[AA-PG]-OH (299.2458 m/z) and $[AA\text{-}PG]_2$ or H-$[AA\text{-}PG]_2$-OH (373.2156 m/z) could be observed. Furthermore, these masses were detected in the DART mass spectrum of the same sample (Figure 4a.2 (a.2) but at lower intensity).

In the ASAP mass spectrum of starch-based biopolymer in figure 4b.1, masses 121.0363 and 229.2376 m/z had the highest relative intensity in the spectrum. These and other characteristic masses of the PBAT oligomers were not detected in DART-SVP. Unidentified masses could correspond to volatile and semi-volatile compounds present in the sample (Osorio et al., 2019). They were not identified because it was not the objective of this work. On the other hand, the masses 201.1273 m/z, 221.0965 m/z, 421.2215 m/z and 621.3349m/z corresponded to the oligomers [AA-BD], [TPA-BD], [TPA-BD]-[AA-BD], and [TPA-BD]-$[AA\text{-}BD]_2$, respectively, previously detected in DART analysis.

Protonation and charge transfer are very common ionization mechanisms in ASAP, but the mechanism mainly depends on the polarity of the analyte (Carrizo et al., 2015; Smith et al., 2012). Polar molecules have a high affinity for protons, therefore polyester oligomers (polar molecules) tend to form protonated adducts in ASAP (Smith et al., 2012). In both samples, the masses of the detected oligomers corresponded to their $[M+H]^+$ adducts. Unlike DART technique, $[M+NH_4]^+$ adducts are not common in ASAP.

Finally, figure 4 showed that, for both biopolymers, the adducts coming from the polyester oligomers had a higher intensity in DART than those in ASAP mass spectra. On the other hand, small masses showed a higher intensity in ASAP analysis than in

DART analysis and therefore this technique would be suitable for the analysis of smaller molecules. Therefore, the DART-SVP method has better sensitivity determination of polyester oligomer high molecular weight in migration samples from biopolymers, but ASAP could be a good alternative to analyze volatile or semi-volatile oligomers with volatility below 500 ºC (Carrizo et al., 2015).

It can be pointed out that ASAP spectrum provides higher intensities of the lowest masses, such as 111.0530 or 129.0638, that would confirm the presence of AA in the monomer (Hoppe et al., 2016).

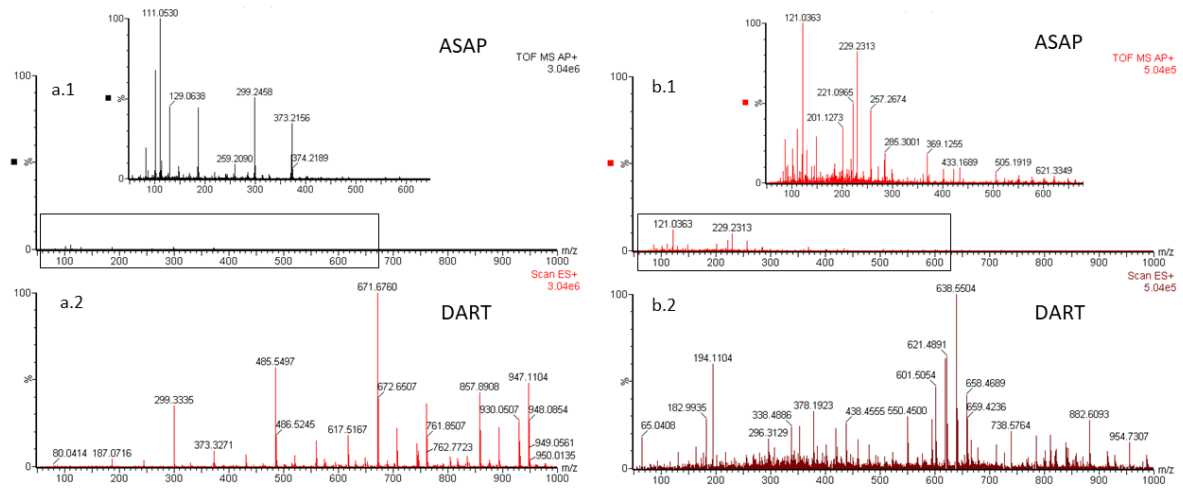


Figure 4. Comparison between **(a.1)** DART-SVP spectrum and **(a.2)** ASAP-Q/ToF spectrum of PLA migration sample in ethanol 95%. **(b.1)** DART-SVP spectrum and **(b.2)** ASAP-Q/ToF spectrum of starch-based biopolymer migration sample in ethanol 95%.

7. CONCLUSIONS

Although biopolymers such as starch or PLA intended for food contact are considered ecological alternatives to conventional polymers, they are not pure biopolymers and polyester resins are commonly blended with them to improve their mechanical properties. Several polyester oligomers were found in migration samples

from PLA and starch-based biopolymers, showing that polyester resins have a critical role in the evaluation of the material.

Polyester oligomers were detected by DART-SVP analysis, in a mass range between 50 and 1000 m/z, in a unique analysis of 1.5 minutes duration for each replicate. In ASAP-Q/ToF, only polyester oligomers with small mass molecular were observed. Therefore, volatiles and semi-volatiles polyester oligomers and their monomer could be analyzed by this technique.

These results suggest that both DART-SVP and ASAP-Q/ToF techniques are powerful techniques for rapid and simultaneous determination of polyester oligomer present in biopolymers samples.

Capítulo 6

Ambient mass spectrometry as a tool for a rapid and simultaneous determination of migrants coming from a bamboo-based biopolymer packaging

1. RESUMEN

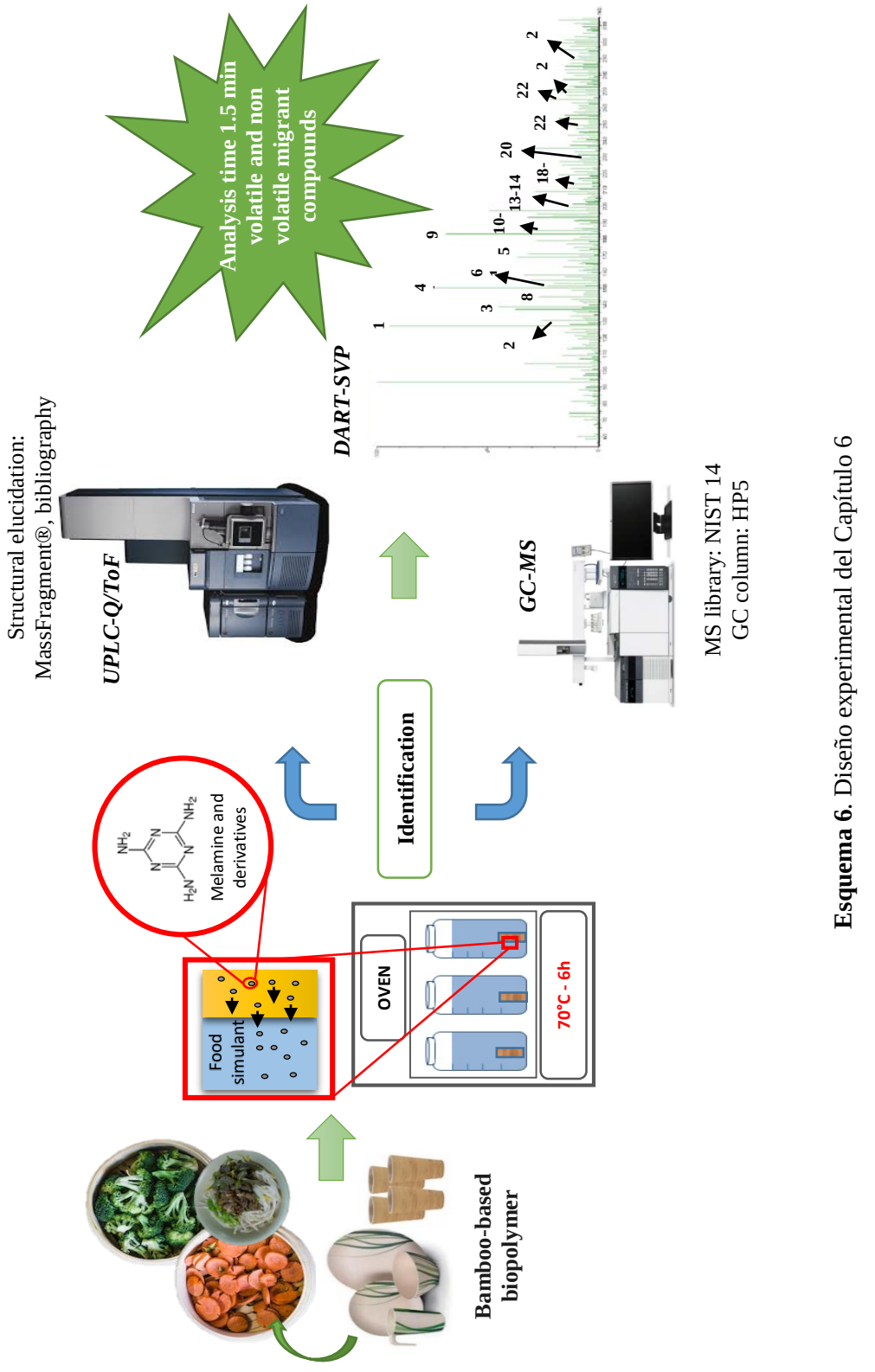
Los nuevos biopolimero base bambú han tenido una importante implementación en la fabricación de una variedad utensilios de cocina. Sin embargo, las mayoria de estos materiales no ofrencen garantias de seguridad para el consumidor. En este capitulo, se ha estudiado la migración de los compuestos provenientes de utensilios comerciales de cocina (plastos y jarras), fabricados apartir de un biopolímero base bambú. Los ensayos de migración se realizaron siguiendo el protocolo secuencial para materiales de uso repetido, utilizando tres simulantes etanol 10% (v/v), ácido acético 3% (w/v) y etanol 95% (v/v). Los compuestos migrantes se estudiaron desde tres perspectivas diferentes. Los compuestos volátiles y semivolátiles se analizaron por GC-MS. En total se detectaron veinticinco compuestos, entre los que se identificaron varios fitosteroles en etanol 95% (v/v) que pueden provenir de la carga de bamboo. Los compuestos no volátiles se identificaron y cuantificaron mediante UPLC-Q/ToF. En el proceso se detectados doce migrantes no volatiles, principalmente melamina y sus derivados, que provienen de resinas poliméricas presentes en el biopolímero. Posteriormente se realizó la cuantifiación de la melamina y sus derivados y la migración de melamina fue superior a 50 mg/kg en la tercera prueba de migración de uso repetido secuencial. Finalmente, las muestras de migración se analizaron por la tecnica DART-SVP. Con esta tecnica fue posible la detección simultáneamente, de forma rápida y efectiva, de los principales aductos de los compuestos migrantes volátiles y no volátiles. Esta metodolgía se muestro como una herramienta prometedora para evaluar y garantizar la seguridad de los biopolimeros base bambú destinados al contanto con alimentos.

2. OBJETIVOS

El objetivo principal de esta capitulo se estableció con la intención de verificar y evaluar la complementariedad de las metodologías analíticas para la evaluación de envases emergentes establecidas en esta tesis.Este objetivo consistió en la determinación de los compuestos migrantes provenientes de utensilios de cocina comerciales, fabricados con un biopolímero base bambú, mediante diferentes técnicas analíticas: GC-MS, UPLC-Q/ToF y DART-SVP y la comparación de los resultados obtenidos mediante cada técnica. Para el cumplimiento de este objetivo se establecieron las siguientes tareas a desarrollar:

- Búsqueda de antecedentes y bibliografía relacionada con envases de bambú y materías primas usadas en la fabricación.
- Identificación de los compuestos volátiles y semi-volátiles mediante GC-MS.
- Determinación del nivel de toxicidad teórica de los compuestos volátiles y semi-volátiles detectados en migración de acuerdo con su estructura molecular.
- Elucidación de los compuestos no volátiles implementado las estrategias de identificación en UPLC/Q-ToF.
- Elaboración de una librería de los potenciales migrantes.
- Cuantificación de los compuestos migrantes no volátiles legislados.
- Detección de los compuestos migrantes y sus aductos mediante DART-SVP.

3. ESQUEMA DE TRABAJO



Esquema 6. Diseño experimental del Capítulo 6

4. INTRODUCCIÓN

There is a great demand for biopolymers in the sector of food contact materials manufacturing. This is because biopolymers are a green alternative to the traditional packaging materials used in the food industry, especially those which are abundant, renewable, biodegradable, and biocompatible (Khlestkin et al., 2018). Biopolymers can be classified into two groups, polymers produced from biological products that can be extracted from biomass or produced in fermentation processes, such as carbohydrates, proteins or lipids; and biodegradable and/or compostable polymers (Aznar et al., 2019; Geueke, 2014; Maisanaba et al., 2018). The addition of additives to the biopolymers is necessary in order to improve their physico-chemical properties. These additives can be for example: polyols, used as non-volatile plasticizers (e.g., glycerol, glycol, and sorbitol); and resins, that mold the material (e.g., urea, melamine, melamine formaldehyde, and amines) (Eckardt et al., 2018; Geueke, 2013). Surprisingly, melamine and other resins are neither biodegradable or come from natural sources (Chien et al., 2011; Geueke, 2013).

In recent years, food packaging industries have been highly innovative in the production of new biopolymers derived from various natural resources, such as starch, cellulose, bamboo, and chitosan. Among them, bamboo-base biopolymers are of enormous importance. This fibre is attractive as a natural-based raw material, since it is economical, eco-friendly and biodegradable (Jena, 2018; Xie et al., 2019). Bamboo plants are easily available due to its very short life cycle and because bamboo timber can be harvested multiple times from a single planting, which makes its use very sustainable. Bamboo-based biopolymers have better mechanical properties than other biomaterials, such as a good impact resistance, high durability and crack resistance, and good flexural properties (Xie et al., 2019). As any food packaging material or food contact article, the biopolymers must be evaluated to confirm their acceptability and safety. The bamboo-base biomaterials can contain other resins and also non intentionally added substances (NIAS), such as impurities from raw materials, degradation compounds or novel substances coming from the reaction between different reagents

(Xu et al., 2020). These compounds, as well as those additives intentionally added, are present in the packaging and could migrate to the food.

Migration tests are required in order to guarantee the consumers safety, food preservation and organoleptic properties (Bradley, 2010). These tests require specific conditions of temperature, time and simulants, which should be selected according to the intended use of the material. In the specific migration tests, compounds previously identified in the material as well as new formed compounds can be detected (Nerin et al., 2013). Pure biopolymers are not specifically included in the European plastics regulation and only the frame regulation 1935/2004/CEE applies. However, if they contain some resin or conventional plastic, the regulation on plastics should be applied. European legislation on food contact plastics (Regulation No 10/2011/EU) establishes a positive list with substances that can be used in the manufacturing process and also their specific migration limits (Osorio et al., 2019).

Since the migrants can be volatile as well as non-volatile compounds, different technologies should be applied in order to have a global view of the safety of the material. Gas chromatography can be applied to the separation of the most volatile and semi-volatile compounds (Biedermann et al., 2019) and liquid chromatography to the less volatile ones. Both of them coupled to mass spectrometry detection will allow the identification of the NIAS present in the packaging material. Ambient mass spectrometry techniques can be also used to get a quick confirmation of the presence/absence of the identified compounds.

Ambient mass spectrometry (AMS) or ambient ionization techniques (AMI) are ionization sources used in mass spectrometry for direct and rapid analysis of compounds present in solid or liquid samples (Black et al., 2016; Lu et al., 2018). Additionally, AMS has been utilized as screening method in food safety analysis, criminal investigation, environmental test and other different analytical objectives (Gross, 2014; Lennert et al., 2019; Zhang et al., 2018). DESI-MS (Desorption ElectroSpray Ionization Mass Spectrometry) was the first AMS technique developed. Since then, other techniques such as direct analysis in real time (DART), extractive electrospray ionization (EESI),

desorption atmospheric pressure chemical ionization (DAPCI), dielectric-barrier-discharge ionization (DBDI) or low-temperature plasma (LTP) have been designed (Barnett et al., 2018). AMS techniques had been previously used in the study of food packaging materials by other groups. For example, Bentayeb et al. determined the compounds present due to print set-off process (Karim Bentayeb et al., 2012; ten Dam et al., 2016) while Aznar et al. analyzed the distribution of ink components in a packaging material and Rothenbacher et al. studied the plasticizers in poly(vinyl chloride) (PVC) (Aznar et al., 2016).

Direct analysis in real time (DART) is one of the most popular ambient pressure ionization methods. In this technique, the molecules are ionized by excited helium molecules and adducts, mainly protonated/deprotonated molecular ions, are commonly formed (Black et al., 2016). Some of its main advantages are: the possibility of direct sampling at ambient conditions; to generate ions from liquid or solid samples; to be simple to operate and to have a rapid analysis speed (acquisition time < 60 s) compared to HPLC and GC-MS methods (Nerin et al., 2013). DART-SVP technique has diverse applications such as food quality and safety control, drug screening, contamination analysis and environmental monitoring (Barnett et al., 2018; Djelal, et al., 2017; Lennert et al., 2018).

In the present study, the migration from a bamboo-based biopolymer intended for food contact to different food simulants was evaluated. Volatile, semi-volatile and non-volatile compounds were firstly identified by GC-MS and UPLC-Q/ToF. Additionally, DART-SVP was used as a tool to assess the presence of all potential migrants in a very short analysis time.

5. MATERIALS AND METHODS

5.1. Reagents

Melamine 99% [108-78-1] (IUPAC name: 1,3,5-triazine-2,4,6-triamine) was from Sigma–Aldrich (Madrid, Spain). Ethanol absolute (HPLC grade), methanol (HPLC-MS grade), acetic acid (HPLC grade) were supplied by Scharlau (Setmenat, Spain) for the analysis in GC-MS and UPLC-Q/ToF. Ethanol absolute (HPLC grade) supplied by Merck (Darmstadt, Germany) for the analysis in DART-SVP. Ultra-pure water was obtained from Millipore Milli-Q system (Billerica, MA, USA). The nitrogen evaporator was a TECHNE sample concentrator (Cole-Parmer Ltd., UK). SPME fibers were purchased at Supelco (Bellefonte, PA, USA).

5.2. Samples

Bamboo-based biopolymer samples were purchased at a local supermarket in the form of cups, dishes and jugs. All of these were monolayer materials.

5.3. Migration assays

Migration assays were carried out by total immersion of the sample (5 cm x 1 cm) into 20 mL of the simulant. The process was established in accordance with the European legislation on food contact materials (Regulation No 10/2011/EU) (European Commission, 2011a). Three simulants were evaluated: ethanol 10% (v/v) (simulant A), acetic acid 3% (w/v) (simulant B) and ethanol 95% (v/v) (simulant D2 substitute). Overall migration studies were performed during 2 hours at 70 ºC. The overall simulant solutions were evaporated to dryness at 110 ºC. Overall migration was calculated by weighting the dry residue. Specific migration was performed during 6 hours at 70 ºC. Since the food contact materials of this study were intended for repeated use, the migration tests were performed three consecutive times. The assays were carried out in triplicate for each sample and a blank.

5.4. Analysis by GC-MS

The analyses of volatile and semi-volatile compounds were carried out using a gas chromatograph 6890N equipped with an electron ionization ion source operating at 70 eV, and coupled to a quadrupole mass spectrometry detector 5975D (Agilent Technologies. Santa Clara, CA). It was set in SCAN acquisition mode (50 - 450 m/z). The transfer line heater was set at 280 ºC. The autosampler was a Combi PAL (CTC Analytics, Zwingen, Switzerland). The injector was used in two modes: liquid injection (migration samples: ethanol 95% (v/v)) and SPME injection (aqueous migration samples: acetic acid 3% (w/v) and ethanol 10% (v/v)). The stationary phase of polydimethylsiloxane/divinylbenzene/carboxen (PDMS/DVB/CAR 50/30 µm) was used for the SPME extraction. The migration samples were conditioned for 2 min at 80 ºC. Subsequently, the volatile and semi-volatile compounds were extracted at 80 ºC and 500 rpm agitation during 15 min. SPME fiber was desorbed at 250 ºC for 2 min. One µL of liquid samples was injected at 250 ºC in splitless mode. The chromatographic separation was performed on a HP-5MS column (30 m length × 0.25 mminner diameter × 0.25 µm film thickness). The oven temperature program was: 40 ºC held for 3 min, ramp 10 ºC/min to 300 ºC and held for 2 min. Helium was used as carrier gas at a constant flow of 1 mL/min. The SPME extraction process in biopolymer samples was optimized in a previous work (Osorio et al., 2019).

5.5. Analysis by UPLC-Q/ToF

The analyses of the non-volatile compounds present in migration solutions were performed using an ACQUITY HPLC chromatograph (Milford, MA, USA). Flow rate was 0.3 mL/min. Injection volume was 10 µL. An HPLC BEH C18 column (Waters) of 2.1 × 100 mm, 1.7 µm particle size was used at a temperature of 35 °C. Two mobile phases were used for the separation: (A) water with 0.1% formic acid and (B) methanol with 0.1% formic acid. Chromatography was performed using the following gradient elution: initial composition 98/2 (phase A/ phase B), and at eight min it was changed to 0/100 (phase A/ phase B) with two additional minutes at the final composition.

HPLC system was coupled to a Xevo G2 Q/ToF mass spectrometer from Waters (Milford, MA, USA) with an ESI probe. The conditions of analysis were: positive ionization (ESI+), sensitivity mode, capillary voltage 3.0 kV, sampling cone voltage 30V, extraction cone 3 V, source temperature 150 °C, desolvation temperature 450 °C, cone gas flow rate of 20 L/h, and desolvation gas flow rate of 500 L/h. Acquisition was carried out in MSE mode in a mass range between 50 and 1200 m/z. The chromatogram was acquired at low and high collision energy (CE) in the collision cell.

5.6. Analysis by DART-SVP

A direct analysis in real time (DART) couple to Standardised Voltage and Pressure (SVP) 100 ion source (IonSense, Saugus, MA) was coupled to an Acquity QDa single quadrupole mass spectrometer (Waters, Wilmslow, UK) via a Vapur interface (IonSense, Saugus, MA). The DART-SVP and Acquity QDa were operated in positive ion mode. The DART-SVP was operated with helium (grade A) in running mode and nitrogen in standby mode. The DART-SVP was operated at several different temperatures between 150 ºC and 450 ºC, the ion-source grid voltage was set to 350 V. The mass spectrometer was operated with the desolvation line temperature set at 250 ºC, heat block 350 ºC, interface voltage 4.5 kV and 50-1000 Da scan range. The mass spectrometer was controlled using MassLynx v4.1 SCN888 (Waters, Wilmslow, UK). Data were analyzed by MassLynx v4.1.

Manual sample injection mode was used. Three microliters of the migration solution were pipette-spotted directly onto the Quick Strip card in the position one. Then, Quick Strip card was simply mounted on the sampling rail. Further, the sample came in contact with the He stream from the DART ion source outlet. Even though the three simulants were tested, only data from ethanol 95% (v/v) are included in the manuscript. In the other two simulants its high water content provided low quality mass spectra. In DART-SVP, atmospheric moisture is ionized by helium in the 2^3S state with extremmely high efficiency. However, the formation of protonated water clusters is likely to occur from the water present in the samples, while metastable helium atoms

are unlikely to survive. For this reason, it has found that moisture should be minimized to avoid competing reactions (Cody, 2009).

5.7. Data processing

In GC-MS analysis, the chromatograms and mass spectra were acquired and processed with MSD ChemStation software version F.01.03 from Agilent Technologies. NIST Standard Reference Database (2018) was used as mass spectra library for compounds identification. Only Match values above 800 were considered acceptable for a candidate confirmation.

In UPLC-Q/ToF, the mass data were analysed with MassLynx software v4.1 from Waters (Milford, MA, USA). Data were acquired in MS^E mode, where 2 funtions (low and high energy) were acquired simultaneously. Mass spectra obtained in function 1 provided information about the elemental composition of the precursor ion and mass spectra in function 2 provided information about the elemental composition of the fragment ions. In UPLC-Q/ToF the identification is a more complex process, since there are not spectral libraries. In this case, a structural elucidation of the molecules is necessary. For this purpose, the first step was to determine the elemental composition of the precursor ion, those formulas with the lowest mass error (always below 10 ppm) and the highest isotopic fit were selected. These formulas were introduced in a chemical databases, such as [www. chemspider.com] or [www.scifinder.com] and the possible hemical structures were searched. The criteria for selecting a candidate were based on previous knowledge about the sample and a chemical background. Finally, the MassFragment® software was used to evaluate if the fragment ions detected in function 2 could fit with the breakage of the proposed candidate. Those candidates with the best match were selected as final candidates (Nerin et al., 2013). In the case of the analysis by DART-SVP, the aim was to determine possible adducts of compounds previously identified by GC-MS or UPLC-Q/ToF. The DART-SVP mass spectrums were acquired with MassLynx SCN888T software and processed with MassLynx software v4.1.

6. RESULTS AND DISCUSSION

The results showed that overall migration was much higher than in other polymers, close to the Overall Migration Limit (OML) of 60 mg kg^{-1} established by Regulation EU/10/2011 (European Commission, 2011a) in some simulants. The values found for the different simulants were as follows: 50.6 ± 9.2 mg kg^{-1} in ethanol 10% (v/v); 13.2 ± 3.2 mg kg^{-1} in acetic acid 3% (w/v) and 48.5 ± 4.4 mg kg^{-1} in ethanol 95% (v/v). Specific migration results are shown below for each technique.

6.1. Volatile and semi-volatile compounds present in migration from a bamboo-based biopolymer

Table 1 shows the compounds identified with their retention times, their molecular formula and their presence in the different simulants after the migration tests. A total of twenty-five compounds were identified by comparing their mass spectra with the NIST Standard Reference Database (2018). The identified compounds were checked in the EU/10/2011 positive list (European Commission, 2011a), to confirm if they were authorized and their specific migration limit. Many compounds detected were non-listed substances and among them, only a 2,6,10-trimethyl dodecane had a NOAEL value (1000 mg kg^{-1}*day) (Agency United States Environmental Protection, 2019). Therefore, their theoretical toxicity level was established according to the TTC (threshold of toxicological concern) and Cramer rules for each compound (Dewhurst et al., 2013; Szczepańska et al., 2018). Table 1 shows the theoretical classification of toxicity for the non listed compounds found.

Table 1. Volatile and semi-volatile compounds detected by GC-MS in migration samples from Bamboo-based biopolymer.

	t_R	MW	Candidate Name	# CAS	MF	Cramer Class	Food simulant A	Food simulant B	Food simulant D2
10	4.40	164.0473	Phenacyl formate	55153-12-3	$C_9H_8O_3$	I			X
2	7.43	132.0786	3,3-Dimethoxy-2-butanone	21983-72-2	$C_6H_{12}O_3$	I	X		
3	7.65	151.0633	Methyl N-hydroxybenzenecarboximidate	67160-14	$C_8H_9NO_2$	III		X	X
4	10.95	142.1722	Decane	124-18-5	$C_{10}H_{22}$	I	X		
5	10.98	142.1722	3,5-dimethyloctane	15869-93-9	$C_{10}H_{22}$	I		X	
6	12.17	170.2035	2,3-dimethyldecane	17312-44-6	$C_{12}H_{26}$	I	X		
7	12.73	128.1535	2,4-dimethylheptane	2213-23-2	C_9H_{20}	I	X		
8	13.66	170.2035	2-methyl undecane	7045-71-8	$C_{12}H_{26}$	I		X	
9	13.78	212.2504	2,6,10-trimethyl dodecane	3891-98-3	$C_{15}H_{32}$	I		X	
10	13.86	198.2348	4,6-dimethyl dodecane	61141-72-8	$C_{14}H_{30}$	I		X	
11	13.98	226.2661	Hexadecane	544-76-3	$C_{16}H_{34}$	I		X	
12	14.52	170.2035	3,6-dimethyl decane	17312-53-7	$C_{12}H_{26}$	I		X	
13	14.57	184.2191	2,4-dimethyl undecane	17312-80-0	$C_{13}H_{28}$	I		X	
14	14.66	184.2191	4,6-dimethyl undecane	17312-82-2	$C_{13}H_{28}$	I		X	
15	15.27	198.2348	Tetradecane	629-59-4	$C_{14}H_{30}$	I		X	
16	16.47	340.3705	Eicosane, 1-propoxy	281211-96-9	$C_{23}H_{48}O$	I	X	X	
17	16.93	156.1878	2,4,6-trimethyl octane	62016-37-9	$C_{11}H_{24}$	I		X	
18	17.07	212.2504	2,6,11-trimethyl dodecane	31295-56-4	$C_{15}H_{32}$	I		X	
19	17.18	268.313	Nonadecane	629-92-5	$C_{19}H_{40}$	I		X	
20	19.02	380.4382	Heptacosane	593-49-7	$C_{27}H_{56}$	I		X	
21	22.92	256.2402	(S)-12-methylmethy tetradecanoic ester	62691-05-8	$C_{16}H_{32}O_2$	I	X		
22	26.03	400.3705	3β-Ergost-5-en-3-ol	4651-51-8	$C_{28}H_{48}O$	III			X
23	27.27	412.3705	Stigmasterol	83-48-7	$C_{29}H_{48}O$	II			X
24	28.87	414.3862	Clionasterol	83-47-6	$C_{29}H_{50}O$	III			X
25	30.21	440.4018	Arundoin	4555-56-0	$C_{31}H_{52}O$	III			X

t_R: retention time (min). MW: molecular weight. Simulant A: ethanol 10% (v/v). Simulant B: acetic acid 3% (w/v) Simulant D2: ethanol 95% (v/v).
X: compound detected in the simulant.

6.2. Non-volatile compounds present in migration from a bamboo-based biopolymer

Table 2 shows the non-volatile compounds found in migration solutions from the bamboo-based biopolymer, their retention time, their accurate mass, the adduct detected ($[M+H]^+$ or $[M+Na]^+$) and their molecular formula. A total of twelve compounds were detected.

The amino acid valine was detected in all food simulants. The presence of valine in bamboo shoots was previously reported by different authors (De Silva et al., 2019; Nongdam et al., 2014). C. Nirmala et al, reported it as one of the most abundant amino acids in the composition of Phyllostachys manii (juvenile bamboo shoots) (Nirmala et al., 2018). Triethanolamine, commonly used as surfactant, was also detected. None of these compounds was present in the EU/10/2011 positive list (European Commission, 2011a). Triethanolamine has a NOAEL value 300 mg kg^{-1}*day and that of valine is 628 mg kg^{-1}*day (Agency United States Environmental Protection, 2019).

It was also observed that melamine and eight melamine derivatives were identified in all food simulants. This is because in the manufacture of biopolymers it is common to use a polymeric resin, such as melamine, to improve the mechanical properties of the material.

Melamine has been reported as a common component in the manufacture of laminates, coatings and mainly, kitchenware utensils (Geueke, 2013; Q. Li et al., 2019). Melamine derivatives can also be formed from the reaction of residual melamine and food simulants (Bradley, et al., 2011; Chien et al., 2011; Karthikraj et al., 2018) or during the bamboo based biopolymer manufacture.

Table 2. List of compounds non-volatile detected by UPLC-Q/ToF in migration from bamboo-based samples (Repeated use).

#	t_R	Mass (*m/z*)	Adduct	MF	Candidate Name_# CAS	Simulant A	Simulant B	Simulant D2
1	0.65	214.9170	$[M+Na]^+$	$C_2N_4O_3S_2$	Unknown	X	X	
2	0.73	150.1137	$[M+H]^+$ $[M+Na]^+$	$C_6H_{15}NO_3$	Triethanolamine_102-71-6	X	X	
3	0.79	118.0865	$[M+H]^+$	$C_5H_{11}NO_2$	Valine_72-18-4	X	X	X
4	0.80	127.0732	$[M+H]^+$	$C_3H_6N_6$	Melamine_108-78-1	X	X	X
5	0.89	157.0840	$[M+H]^+$	$C_4H_8N_6O$	N-Hydroxymethylmelamine_937-35-9_**(MD 1)**		X	X
6	0.90	185.1156	$[M+H]^+$	$C_6H_{12}N_6O$	N-Hydroxypropylmelamine_91313-29-0 **(MD 2)**			X
7	0.94	157.0835	$[M+H]^+$	$C_4H_8N_6O$	N-Hydroxymethylmelamine_937-35-9 **(MD 3)**	X	X	
8	1.04	139.0726	$[M+H]^+$	$C_4H_6N_6$	Methylene melamine_85946-83-4 **(MD 4)**		X	
9	1.13	169.0826	$[M+H]^+$	$C_5H_8N_6O$	N-(4,6-Diamino-1,3,5-triazin-2-yl)acetamide_16274-60-5 **MD 5)**	X	X	
10	2.65	277.1375	$[M+H]^+$	$C_8H_{12}N_{12}$	2,4,6-Pyrimidinetriamine, 5,5'-azobis_ 63436-10-2 **(MD)**	X	X	
11	2.83	307.1478	$[M+H]^+$	$C_9H_{15}N_{12}O$	Propanamide, N-(4,6-diamino-1,3,5-triazin-2-yl)-2-[(4,6-liamino-1,3,5-triazin-2-yl)amino]_1421766-78-0_**(MD 7)**		X	
12	4.54	311.0979	$[M+H]^+$	$C_{14}H_{26}N_6O_2$	Glycine, N-[4-[(1,1-dimethylethyl)amino]-6-(ethylamino) 1,3,5-triazin-2-yl]-N-propyl _2037785-60-5 **(MD 8)**	X	X	X

t_R: retention time (min). Simulant A: ethanol 10% (v/v). Simulant B: acetic acid 3% (w/v) Simulant D2: ethanol 95% (v/v). X: compound detected in the simulant. X: compound detected in the simulant. MD: melamine derivate.

Melamine is listed in the Regulation EU/10/2011 with a specific migration limit (SML) of 2.5 mg kg^{-1}. For this reason, melamine as well as its derivatives were quantified in all food simulants. Since the material evaluated was intended for repeated used, according to EU/10/2011 operating guidelines, the migration was evaluated over three consecutive migration experiments. The results are presented in figure 1. Melamine was the compound with the highest migration values. In all cases, the migration values were above 2.5 mg kg^{-1} in the first, second and third migration experiments, especially in acetic acid 3% (w/v). Therefore, this bamboo-based biopolymer did not comply with the SMLs established by the Regulations (EU) No 10/2011 and No284/2011. According to these results, the material cannot be considered as bamboo, but melamine with bamboo filler. Then, the Regulation 284/2011 concerning melamine kitchenware tools applies (European Commission, 2011b). Regarding the food simulants, the highest values were found in the second migration of acetic acid 3% (w/v) (144.3 ± 1.1 mg kg^{-1}), followed by ethanol 10% (48.8 ± 3.4 mg kg^{-1}) and ethanol 95% (v/v) (4.3 ± 1.0 mg kg^{-1}), suggesting an important effect of acidity and a high water content in the migration process. The same pattern was observed for all the melamine derivatives analyzed. N-Hydroxymethylmelamine (MD 1) was the melamine derivative with the highest migration values.

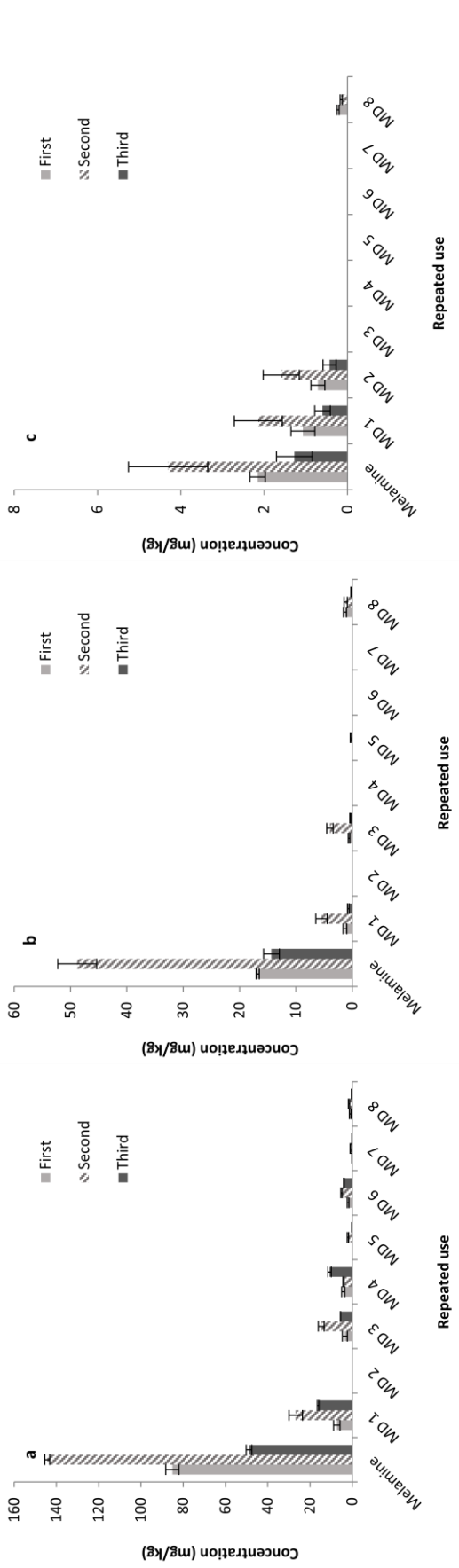


Figure 1. Bar chart of migration values of melamine and its derivative compounds from bamboo-based biopolymer in three consecutive migration assays. **a)** acetic acid 3% (w/v), **b)** ethanol 10% (v/v) and **c)** ethanol 95% (v/v). MD codes are linked to table 2.

The acidic medium of the acetic acid simulant could accelerate the hydrolysis reaction of the melamine resin and as a result, provide high migration values (Bradley et al., 2011; Geueke, 2013). The results also showed that the migration of melamine and its derivatives increased in the second migration experiment and decreased in the third one. This behavior could be attributed to the possible variation in the internal structure of the polymer caused by the simulants, which could have modified the diffusion of the compounds though the polymer and thus affecting the migration. Therefore, the addition of the polymeric resin to the bamboo biopolymer improved its mechanical properties but at the same time, entailed a risk to consumer's health. In addition, melamine is neither a biopolymer nor biodegradable, what means that these materials, promoted as "bamboo food contact materials" can be considered as a fraud to consumers.

6.3. DART-SVP analysis of migration samples

DART-SVP is an electric discharge ambient ionisation technique where excited helium atoms start gas-phase reactions. These reactions result in ions created from atmospheric water-vapor or other gaseous compounds present (Gross, 2014; Navare et al, 2010). As the chemical ionization is the same as in APCI (atmospheric pressure chemical ionization) (Gross, 2014), the formation of adducts according to polarity, molecular weight or volatility of the species present in the samples is very common.

In this study, the ability to implement a DART-SVP method to quickly determine the main migrants and their adducts from bamboo-base biopolymer was investigated. Figure 2 shows a mass spectrum of an ethanol 95% (v/v) migration sample. Table 3 shows a total of twenty-six masses detected in the DART mass spectrum and that matched with adducts of compounds that were previously detected in migration solutions by GC-MS or UPLC-Q/ToF. The range of m/z values was between 127.0733 and 291.1033. Only peaks with relative intensity higher than 1% of the base peak were considered. The table shows the main adduct ion detected as well as its m/z and relative intensity. In addition, other adducts identified and its relative intensity for each of the compounds are shown. The presence of other adducts confirmed the candidates proposed.

Regarding the ions detected, adducts such as $M^{\cdot+}$, $[M+H]^+$, $[M+N]^+$, $[M-H+O]^+$ and $[M+H-H_2O]^+$ were observed with major relative intensity. Oxygen, water and ammonia species were detected in the environment (blank spectrum mass), therefore the different adducts identified were the interaction products between gaseous species of the atmosphere and molecular ions from the sample. Other adducts detected were $M^{\cdot+}$, $[M+H]^+$, $[M-H+O]^+$, $[M+H+O]^+$, $[M+NH_4]^+$ and $[M-H+O_2]^+$.

In this technique the analytes generate different adducts according to the polarity of specie: $[M+H]^+$, $[M-H+O]^+$, $[M+NH_4]^+$ adducts for medium polar to polar analytes; and $M^{\cdot+}$, $[M-H+H_2O]^+$, $[M+H]^+$, $[M-H+O]^+$ adducts for non-polar analytes (Cody, 2009; Gross, 2014). The results suggested that the polarity was an important parameter in the formation of adducts from the migration samples of bamboo-based biopolymer. Melamine and it derivatives formed mainly adducts such as $[M+H]^+$, $[M-H+O]^+$ and $[M-H+O_2]^+$ because these compounds have a high polarity. These adducts had high relative intensity (between 10% - 100%) probably because they were at higher concentration than other compounds in the samples (section 6.2). On the other hand, some compounds with low polarity, such as alkanes, formed $[M+N]^+$ and $[M-H+O]^+$ adducts. However, they were in the adduct group with low relative intensities, between 1-10%. Probably, the high volatility of small molecules promotes a higher number of interactions with the different reactive species and therefore a higher variety of adducts (Bentayeb et al., 2012; Kerpel dos Santos et al., 2018). Among the masses found in DART MS spectrum with relative intensities above 10%, 10 out of them could not be associated to any of the previously identified compounds by GC-MS or UPLC-Q/ToF.

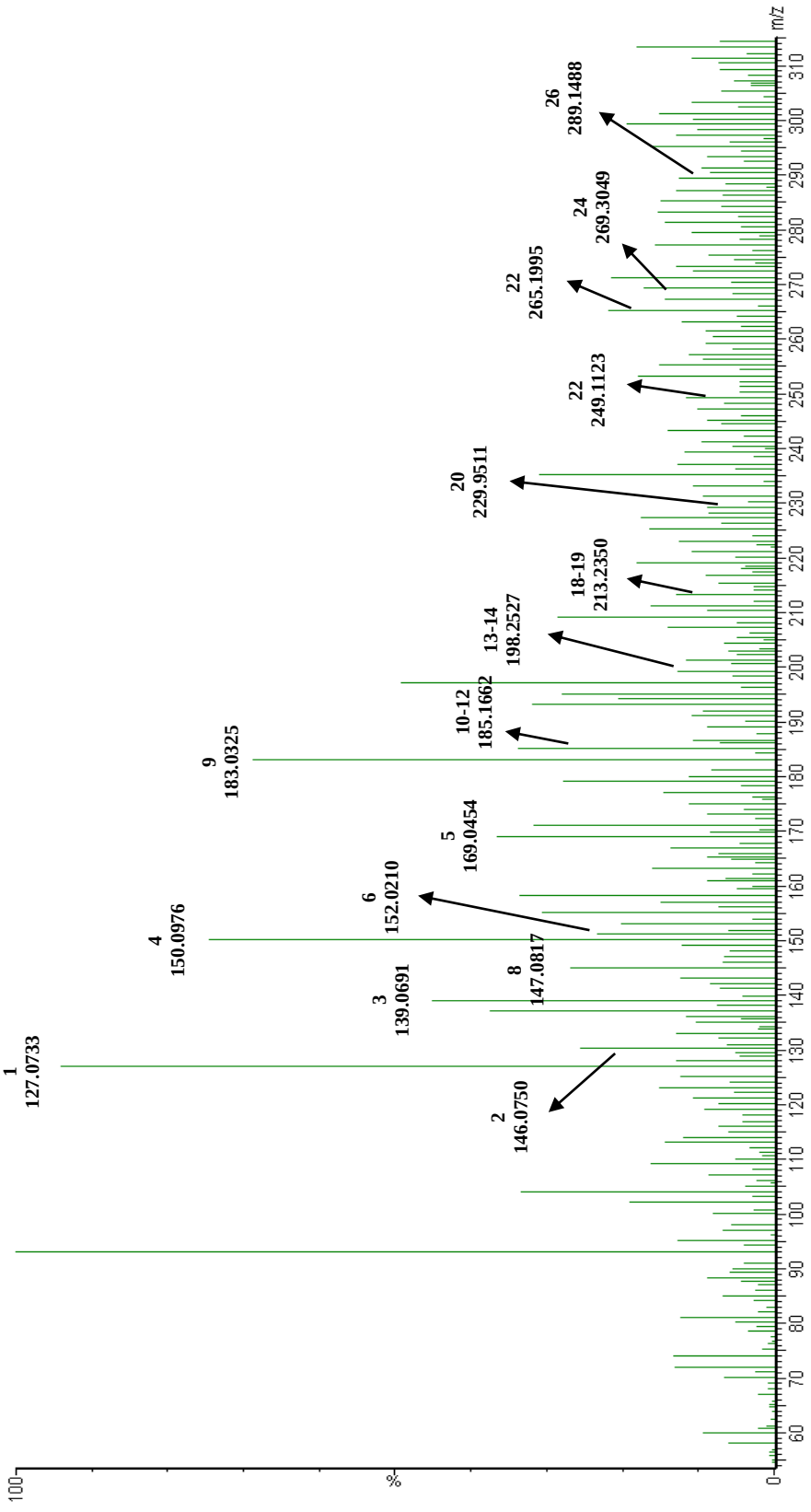


Figure 2. DART-SVP mass spectrum of a migration solution (ethanol 95%) from a bamboo-based biopolymer sample. Numbers in brackets are linked to table 3.

Table 3. List of compounds detected in specific migration to ethanol 95% (v/v) from Bamboo-based samples by DART-SVP.

#	MW	Candidate Name	MF	Main adduct			Other adducts (Int)
				m/z	Ion	Int	
1	127.0732	Melamine[b]	$C_3H_6N_6$	127.07	$[M+H]^+$	87.7	$[M-H+O_2]^+$ (3.9)
2	132.0786	3,3-Dimethoxy-2-butanone[a]	$C_6H_{12}O_3$	146.07	$[M+N]^+$	6.0	$[M-H+O]^+$ (6.0)
3	139.0726	Methylene melamine[b]	$C_4H_6N_6$	139.06	$[M+H]^+$	29.9	$[M-H+O_2]^+$ (7.8); $[M-H+O]^+$ (4.0); $M^{\cdot+}$ (3.2); $[M+NO]^+$ (2.7); $[M+N]^+$ (2.6)
4	150.1137	Triethanolamine[b]	$C_6H_{15}NO_3$	150.09	$[M+H]^+$	100.0	$[M-H+O]^+$ (8.1)
5	151.0633	Methyl N-hydroxybenzenecarboximidate[a]	$C_8H_9NO_2$	169.04	$[M+NH_4]^+$	7.8	$[M+H+O]^+$ (2.7)
6	156.1878	2,4,6-trimethyl octane[a]	$C_{11}H_{24}$	152.02	$[M+H]^+$	2.6	
7	157.0835	N-Hydroxymethylmelamine[b]	$C_4H_8N_6O$	139.06	$[M+H-H_2O]^+$	29.9	$[M+H]^+$ (3.9); $[M+H+O]^+$ (1.3)
8	164.0473	Phenacyl formate [a]	$C_9H_8O_3$	147.08	$[M+H-H_2O]^+$	2.7	
9	169.0826	N-(4,6-Diamino-1,3,5-triazin-2-yl)acetamide	$C_5H_8N_6O$	183.03	$[M-H+O]^+$	19.3	$[M+H]^+$ (7.8); $M^{\cdot+}$ (2.7); $[M+NH_4]^+$ (2.5)
10	170.2035	2,3-dimethyldecane[a]	$C_{12}H_{26}$	185.16	$[M-H+O]^+$	13.3	$[M+H]^+$ (7.5); $[M+NH_4]^+$ (2.1)
11	170.2035	2-methyl undecane[a]	$C_{12}H_{26}$	185.16	$[M-H+O]^+$	13.3	$[M+H]^+$ (7.5); $[M+NH_4]^+$ (2.1)
12	170.2035	3,6-dimethyl decane[a]	$C_{12}H_{26}$	185.16	$[M-H+O]^+$	13.3	$[M+H]^+$ (7.5); $[M+NH_4]^+$ (2.1)
13	184.2191	2,4-dimethyl undecane[a]	$C_{13}H_{28}$	198.25	$[M+N]^+$	4.8	$[M+NH_4]^+$ (2.5); $[M+H+O]^+$ (1.6)
14	184.2191	4,6-dimethyl undecane[a]	$C_{13}H_{28}$	198.25	$[M+N]^+$	4.8	$[M+NH_4]^+$ (2.5); $[M+H+O]^+$ (1.6)
15	185.1156	N-Hydroxypropylmelamine[b]	$C_6H_{12}N_6O$	185.16	$[M+H]^+$	13.3	$[M+NO]^+$ (2.3)
16	198.2348	4,6-dimethyl dodecane[a]	$C_{14}H_{30}$	198.25	$M^{\cdot+}$	4.8	$[M+H-H_2O]^+$ (2.3); $[M-H+O]^+$ (2.0)
17	198.2348	Tetradecane[a]	$C_{14}H_{30}$	198.25	$M^{\cdot+}$	4.8	$[M+H-H_2O]^+$ (2.3); $[M-H+O]^+$ (2.0)
18	212.2504	2,6,10-trimethyl dodecane[a]	$C_{15}H_{32}$	213.23	$[M+H]^+$	2.0	
19	212.2504	2,6,11-trimethyl dodecane[a]	$C_{15}H_{32}$	213.23	$[M+H]^+$	2.0	
20	214.9171	Unknown[b]	$C_2N_4O_3S_2$	229.95	$[M-H+O]^+$	2.0	$M^{\cdot+}$ (1.8)
21	226.2661	Hexadecane[a]	$C_{16}H_{34}$	240.26	$[M+N]^+$	3.1	
22	249.1123	Glycine, N-[4-[(1,1-dimethylethyl)amino]-6-(ethylamino)-1,3,5-triazin-2-yl]-N-propyl [b]	$C_{10}H_{12}N_6O_2$	265.19	$[M-H+O]^+$	17.1	$[M+NO]^+$ (3.1); $M^{\cdot+}$ (2.6) $[M+NO]^+$ (2.4)
23	256.2400	(S)-12-methylmethy tetradecanoic ester[a]	$C_{16}H_{32}O_2$	239.26	$[M+H-H_2O]^+$	6.1	$[M-H+O]^+$ (3.6); $[M+H+O]^+$ (3.5); $[M-H+O_2]^+$ (3.3); $[M+N]^+$ (2.6)
24	268.3130	Nonadecane[a]	$C_{19}H_{40}$	269.30	$[M+H]^+$	3.9	$[M+H+O]^+$ (3.8)
25	277.1395	2,4,6-Pyrimidinetriamine, 5,5'-azobis [b]	$C_8H_{12}N_{12}$	291.10	$[M-H+O]^+$	2.5	$M^{\cdot+}$ (2.0)
26	307.1478	Propanamide, N-(4,6-diamino-1,3,5-triazin-2-yl)-2-[(4,6-diamino-1,3,5-triazin-2-yl)amino] [b]	$C_9H_{15}N_{12}O$	289.14	$[M+H-H_2O]^+$	2.1	

MW: molecular weight. MF: Molecular formula. Int: relative intensity. [a] Compound identified by GC-MS. Average atomic mass ("*m/z* $M^{+\cdot}$") was calculated using the MassLynx software. [b] Compound identified by UPLC-Q/ToF.

For 5 of these masses, new candidate compounds were proposed (Table 4). The candidates were selected according to its elemental composition, adducts detected, and to bibliography of bamboo composition (D. De Silva et al., 2019; Nirmala et al., 2018; Nongdam et al., 2014). Other researchers have found in the composition of young bamboo shoots different amino acids, minerals, polyphenols, and/or vitamins, such as serine, coniferyl alcohol, caffeic acid, threonine, paracoumaryl alcohol, histidine and protocatechuic acid, among others (De Silva et al., 2019; Nirmala et al., 2018; Nongdam et al., 2014; Zhu et al., 2019). Some of the expected adducts for these compounds coincide with the masses detected. However, for 5 of the masses detected in the mass spectrum with intensity values above 10%, it was not possible to propose a candidate: 93.05 (26.8%), 145.09 (15.0%), 130.22 (15.0%), 295.28 (14.5%) and 192.88 (11.5%).

Table 4. Adducts detected only by DART-SVP in specific migration samples in ethanol 95% (v/v) from Bamboo-based samples.

m/z $M^{+\cdot}$	Candidate Name	MF	Main adduct			Other adducts
			m/z	Ion	Int	
150.0681	Paracoumaryl alcohol	$C_9H_{10}O_2$	151.14	$[M+H]^+$	15.4	$[M+H+O]^+$ (5.3)
154.0266	Protocatechuic acid	$C_7H_6O_4$	137.16	$[M+H -H_2O]^+$	12.0	$[M+N]^+$ (2.7); $[M+NO]^+$ (4.8)
155.1546	L-Histidine	$C_6H_9N_3O_2$	155.14	$M^{+\cdot}$	9.9	$[M-H+O_2]^+$ (2.5)
180.0423	Caffeic acid	$C_9H_8O_4$	197.16	$[M+H+O]^+$	21.0	
180.0786	Trans-coniferyl alcohol	$C_{10}H_{12}O_3$	197.16	$[M+H+O]^+$	21.0	

MF: Molecular formula. Int: relative intensity. Average atomic mass ("*m/z* $M^{+\cdot}$") was calculated using the MassLynx software.

7. CONCLUSIONS

Even though some of the compounds found in migration came from bamboo, such as phytosterols, most of the migrants came from the melamine added to it in order to improve the biopolymer properties. Not only melamine but several melamine derivatives were found in migration above the limits established in European legislation. Consequently, this material does not comply with the EU legislation. In fact, the material cannot be identified as bamboo, but as melamine with bamboo filler. As melamine is neither a biopolymer not biodegradable material, the promotion of these kitchenware materials as bamboo can be considered as a fraud to consumers.

Regarding to DART-SVP experiments, the analysis allowed detecting the volatile, semi-volatile and non-volatile compounds from migration samples of the bamboo-based biopolymer in a unique analysis of 1.5 minutes duration for each replicate. This technique offers great advantages for rapid detection and routine analysis, without requiring sample preparation.

All the compounds identified using DART-SVP were detected in the initial part of this study, where GC-MS and UPLC-Q/ToF methodologies were used. Melamine and its derivatives form the adducts with greater relative intensity. These results demonstrate that the DART-SVP technique is a very useful tool for direct target analysis, where in a few minutes can provide data about the main migrants present in samples.

SECCIÓN IV: Conclusiones Generales

CONCLUSIONES GENERALES

A continuación, se muestran las conclusiones generales más relevantes de esta tesis doctoral:

1. Para evaluar el impacto químico de un material destinado al contacto con alimentos, se ha demostrado que el desarrollo de metodologías analíticas con alta resolución son herramientas de gran utilidad. En ese supuesto, la cromatografía acoplada a espectrometría de masas ha probado ser una herramienta muy poderosa. Las técnicas APGC-Q/ToF y GC-EI-MS para la identificación de los compuestos volátiles y semi-volátiles son técnicas complementarias, porque la espectrometría de masas de alta resolución permite complementar la información obtenida por EI. Por otro lado, la técnica UPLC-Q/ToF ha evidenciado ser una herramienta muy útil en el proceso de identificación de NIAS, permitiendo obtener la masa exacta del ion molecular y de los aductos formados, además del patrón de fragmentación que facilita el proceso de elucidación estructural de compuestos.

2. Evaluar el impacto sensorial de un material destinado al contando con alimentos, es de gran relevancia para poder definir los posibles defectos que el envase pudiera generar en las propiedades organolépticas del alimento. La técnica HS-SPME, que permite obtener un extracto representativo del aroma, combinada con la técnica GC-MS-O para la detección e identificación de los compuestos volátiles odorantes han mostrado ser una combinación apropiada para la determinación de este impacto sensorial.

3. Los biomateriales compostables o biodegradables son una alternativa más ecológica a los plásticos convencionales, que generan grandes problemas ambientales de contaminación. A pesar de ello, se ha evidenciado que los biopolímeros destinados al contacto con alimentos también desprenden sustancias que migran a los alimentos y que pueden ser tóxicas. Estas sustancias afectan no sólo a la toxicidad sino también

a las características organolépticas (calidad) del alimento, por lo que deben estudiarse en profundidad.

4. Para la determinación de compuestos migrantes provenientes de biopolímeros se logró establecer un protocolo de análisis mediante la técnica de análisis directo DART-SVP, para la determinación simultanea de compuestos volátiles, semi-volátiles, no volátiles, y sus aductos en tiempos de análisis cortos (1.5 min). No obstante, hay que tener en cuenta que, para lograr una correcta detección con esta metodología es necesario tener previo conocimiento de la muestra y los posibles analitos presentes, de lo contario el análisis del compuesto, y principalmente de los aductos, puede llegar a ser muy complicado. Por ello, la técnica DART-SVP se plantea como un primer parámetro de evaluación del material para detectar la presencia o ausencia de migrantes conocidos, es decir, como "targeted analysis".

5. El proceso de optimización de las técnicas de tratamiento de muestra por micro-extracción en fase líquida (HF-LPME) y micro-extracción en fase sólida (SPME), para la obtención de compuestos volátiles de muestras de migración acuosas, mostraron una buena reproducibilidad y exactitud. Sin embargo, el uso de disolventes de alta toxicidad y la semi- automatización de la HF-LPME suponen una pequeña desventaja para el análisis de estas muestras frente a la SPME, que no requiere el uso de disolvente y está completamente automatizada. Por lo tanto, se estableció que, entre estas dos técnicas, la SPME es la técnica de tratamiento de muestra más conveniente para la determinación global de analitos provenientes de simulantes alimentarios acuosos (inmersión), y la determinación de compuestos volátiles odorantes (espacio de cabeza) de los materiales para contacto alimentario estudiados.

6. En los estudios realizados con las muestras de biopolímeros base almidón y PLA se detectaron e identificaron varios oligómeros de poliéster, comprobando que la composición de estos materiales, además del biopolímero, se había adicionado un poliéster con el fin de mejorar las propiedades del material. También, en el análisis de migración, los compuestos con mayor intensidad tenían su origen en la

composición del poliéster, sugiriendo que debería evaluarse en mayor profundidad el efecto final que pueden tener estos sobre los alimentos.

7. La detención principalmente de oligómeros de poliéster, sus fragmentos y aductos mediante la técnica DART-SVP, sugirió que esta metodología es una herramienta de gran provecho para la detección directa de oligómeros de poliéster, y podría plantearse como una posible metodología de detección directa para otras familias de oligómeros presentes en diversos materiales.

8. En el análisis de migración del biopolímero comercial base bambú es importante resaltar la importancia de la identificación y cuantificación de melanina y sus derivados en el mismo. Los valores de migración que se encontraron superaban por mucho el límite de migración especifica (SML) establecido por la legislación. La composición de este material debería ser por tanto reevaluada para poder garantizar el cumplimento de la normativa y asegurar la calidad para el consumidor.

9. Se determinó que los principales compuestos odorantes percibidos en los materiales estudiados pertenecían a las familias de grupos funcionales de los aldehídos, alcoholes y cetonas, y se relacionaban con notas sensoriales como verde o desagradable. Además, se verificó que no todos estos compuestos migraban a los simulantes, y por tanto no necesariamente generarían un efecto negativo en los atributos sensoriales de los alimentos. No obstante, sería conveniente realizar estudios de migración con alimentos y evaluar el efecto directo que tienen sobre estos, considerando que la técnica de cocinado también puede influir en la variación del perfil sensorial.

10. Se han establecido una variedad metodologías analíticas que se complementan entre sí y que son una gran herramienta para la evaluación de sustancias que pueden estar presentes en los materiales emergentes destinados al contacto alimentario. La presencia de estas sustancias, como NIAS, oligómeros y compuestos odorantes, en el material puede dar lugar a su migración al alimento y comprometer la integridad del mismo y del consumidor. Para evitar esto, sería recomendable evaluar la

composición y los procesos de producción de estos materiales, tratando de evitar o disminuir al máximo la presencia de precursores de dichas sustancias.

Gracias al desarrollo de este trabajo, se podrían plantear perspectivas de investigación futuras, encaminadas a la evaluación de métodos directos que permitan realizar un primer análisis para descartar o confirmar la presencia de familias de NIAS, y posteriormente realizar análisis de confirmación y cuantificación con las metodologías analíticas previamente establecidas. Además, sería de gran valor realizar el análisis del impacto sensorial de diferentes muestras fabricadas con el mismo tipo de biopolímero, con el fin de obtener un perfil aromático característico de ese biopolímero, y facilitar el análisis acerca del efecto que tienen sus compuestos odorantes sobre las propiedades organolépticas del alimento.

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