TESE DE DOUTORAMENTO

Printing inks for food packaging

Study of the key parameters in the migration of photoinitiators

Asdo.:_____

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PROGRAMA DE DOUTORAMENTO EN INNOVACIÓN EN TECNOLOXÍA E SEGURIDADE ALIMENTARIA

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AUTORIZAN:

A presentación da Tese de Doutoramento titulada "Printing inks for food packaging. Study of the key parameters in the migration of photoinitiators" presentada por D. Miguel Ángel Lago Crespo, alumno do Programa de Doutoramento de *Innovación en Tecnoloxía e Seguridade Alimentari*a. Considerando que reúne os requisitos esixidos no artigo 34 do regulamento de Estudos de Doutoramento, e que como Directores da mesma non incurre nas causas de abstención establecidas na lei 30/1992.

E, para que así conste firman a presente en Santiago de Compostela en Xuño de 2016.

Asdo. Dr. Perfecto Paseiro Losada Asdo. Dra. Raquel Sendón García

Asdo. Dra. Ana Rodríguez Bernaldo de Quirós

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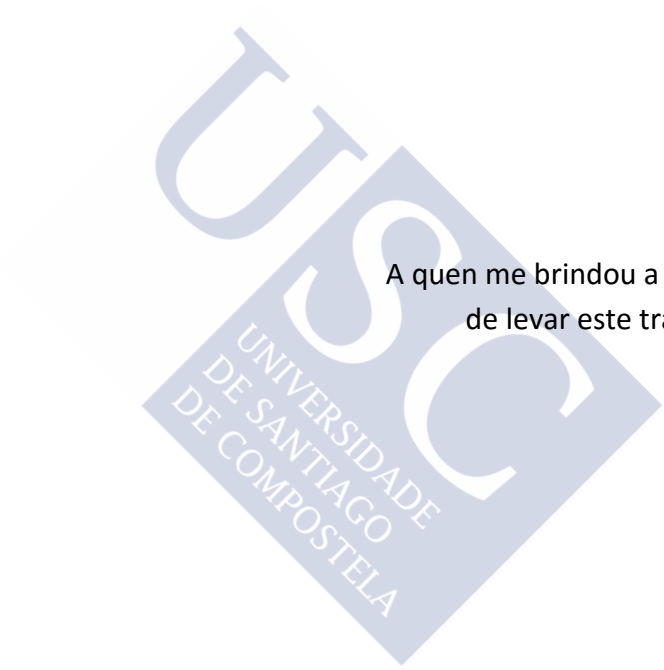

A quen me brindou a oportunidade
de levar este traballo a bo fin

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"O verdadeiro heroísmo está en transformar os desexos en realidades e as ideas en feitos"

Castelao

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RESUMO

Nos últimos 50 anos, as tintas de curado ultravioleta comezaron a substituír ás clásicas tintas baseadas en solventes orgánicos, obtendo envases con mellores impresións e que aparellaban unha menor perigosidade para o consumidor e o medio ambiente. Non obstante, dende que as autoridades sanitarias europeas atoparan no 2005 fotoiniciadores en leite para bebés provintes destas tintas, estas convertéronse nun dos frontes abertos no ámbito da seguridade alimentaria a nivel mundial.

O obxectivo principal desta tese é afondar no coñecemento sobre a migración dos fotoiniciadores e os seus produtos de degradación ó alimento. Para abordar este obxectivo, esta tese divídese fundamentalmente en tres bloques. Primeiramente, preséntase unha revisión sobre os fotoiniciadores dende o punto de vista da seguridade alimentaria, expoñendo o que son, como se produce a súa migración ó alimento, como está a súa lexislación a nivel mundial e cales son as posibles estratexias para a súa detección (cos problemas e peculiaridades que isto supón polo seu mecanismo de acción).

Seguidamente, descríbense diversas metodoloxías analíticas para a determinación tanto de fotoiniciadores como dos seus produtos de degradación por múltiples técnicas, dende as clásicas LC-DAD e GC-MS ata as máis novidosas LC-ESI-MS/MS, UHPLC/ESI-HRMS e DART-HRMS.

O último bloque da tese estuda as cinéticas de migración de 6 dos fotoiniciadores que causaron máis notificacións e alertas sanitarias nos últimos anos. Estes estudos realizáronse a tódalas temperaturas comúns de almacenaxe de alimentos en tódolos simulantes alimentarios previstos a tal efecto pola actual lexislación (agás para alimentos secos), dende o material polimérico máis usado en envasado alimentario, o LDPE. Deste xeito, o modelo matemático desenrolado a partires destes datos permítenos predicir a súa migración ó alimento case en calquera situación.

PALABRAS CHAVE:

Seguridade Alimentaria, Tintas de Curado Ultravioleta, Materiais de Contacto Alimentario, Fotoiniciadores e Migración.

RESUMEN

En los últimos 50 años, las tintas de curado ultravioleta comenzaron a substituir a las clásicas tintas basadas en solventes orgánicos, obteniendo envases con mejores impresiones, que conllevasen una menor peligrosidad para el consumidor y el medio ambiente. Sin embargo, desde que las autoridades sanitarias europeas hallaran en el 2005 fotoiniciadores en la leche para bebés proveniente de estas tintas, éstas se convirtieron en uno de los frentes abiertos en el ámbito de la seguridad alimentaria a nivel mundial.

El objetivo principal de esta tesis es ahondar en el conocimiento sobre la migración de los fotoiniciadores, y sus productos de degradación, al alimento. Para abordar este objetivo, esta tesis se divide fundamentalmente en tres bloques. En primer término, se presenta una revisión sobre los fotoiniciadores desde el punto de vista de la seguridad alimentaria, exponiendo qué son, cómo se produce su migración al alimento, cómo está la legislación a nivel mundial y cuales son las posibles estrategias para su detección (con los problemas y peculiaridades que esto supone debido a su mecanismo de acción).

Seguidamente, se describen diversas metodologías analíticas para la determinación tanto de fotoiniciadores como de sus productos de degradación por múltiples técnicas, desde las clásicas HPLC-DAD y GC-MS a las más novedosas LC-ESI-MS/MS, UHPLC/ESI-HRMS y DART-HRMS.

El último bloque de la tesis estudia las cinéticas de migración de 6 de los fotoiniciadores que causaron más notificaciones y alertas sanitarias en los últimos años. Estos estudios se realizaron a todas las temperaturas comunes de almacenaje de alimentos, en los simulantes alimentarios previstos a tal efecto por la actual legislación (excepto para alimentos secos), desde el material polimérico más usado en envasado alimentario, el LDPE. De este modo, el modelo matemático desarrollado a partir de estos datos, permite la predicción de su migración al alimento casi en cualquier situación.

PALABRAS CLAVE:

Seguridad Alimentaria, Tintas de Curado Ultravioleta, Materiales de Contacto Alimentario, Fotoiniciadores y Migración.

ABSTRACT

In the last 50 years, the UV-curing inks began to substitute the classical solvent-based inks, obtaining better printing packages, safer for the consumers and with less impact for the environment. Nevertheless, since 2005, when the European food safety authorities have found photoinitiators in packaging of milk for babies from these inks, they become in an important food safety issue worldwide.

The main objective of this thesis is going deeper in the knowledge about the migration of photoinitiators and their degradation products into the food. To address this objective, this thesis is divided in three main parts. Firstly, a review about the photoinitiators are presented from the food safety point of view, explaining what they are, how they migrate into the food, what is their legislation over the world and which strategies can be followed for their detection (with the problems and peculiarities that this fact implies due to their action mechanism).

Following, different analytical approaches have been described for the determination of the photoinitiators and their byproducts by multiple techniques, from the classical HPLC-DAD and GC-MS to the novel LC-ESI-MS/MS, UHPLC/ESI-HRMS and DART-HRMS.

The last section of the thesis studies the migration kinetics of 6 of the photoinitiators that produce more health alerts and notifications in the last years. This studies were carried out at all the common temperatures of food storage in all the food simulants present in the current legislation (except for dry foods), from the polymeric material most used in food packaging, LDPE. Thereby, the mathematical model developed from these data allows the prediction of their migration to the foodstuff almost in any case.

KEYWORDS:

Food Safety, UV Curing Inks, Food Contact Materials, Photoinitiators and Migration.

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LIST OF ABBREVIATURES

2-DMEB *– 2-(Diethylamino)ethylbenzoate*
2-HBP *– 2-Hydroxybenzophenone*
2-MBP *– 2-Methyl-benzophenone*
3-MBP *– 3-Methyl-benzophenone*
4-HBP *– 4-Hydroxybenzophenone*
4-MBP *– 4-Methyl-benzophenone*
AAAP's *– α- Aminoalkylacetophenones*
AcA *– Acetic Acid*
AA *– Ammonium Acetate*
AcN *– Acetonitrile*
ADI *– Acceptable Daily Intake*
AECOSAN *– Spanish Agency Consumer Affairs, Food Safety and Nutrition*
AF *– Ammonium Formate*
APCI *– Ambient Pressure Chemical Ionization source*
APPI *– Ambient Pressure Photoionization source*
AQSIQ *– Administration of Quality Supervision, Inspection and Quarantine Department of China*
BDMB *– 2-Benzyl-2-dimethylamino-4-morpholino butyrophenone*
BDK *– 2,2-Dimethoxy-2-phenylacetophenone*
BIS *– Bis(4-diphenylsulphonium)phenylsulphide-bis (hexafluorophosphate)*
BP *– Benzophenone*
BPAcr *– Benzophenone acrylate*
CAA *– Consumer Affairs Agency of Japan*
CFIA *– Canadian Food Inspection Agency*
CFR *– Code of Food Regulations*
CPQ *– d,l-Camphorquinone*
CV *– Cyclic Voltammetry*
DAD *– Diode Array Detector*
DART *– Direct Analysis in Real Time*
DBA *– 9,10-Dibutoxyanthracene*

***DEAB** – 4,4'-Bis(diethylamino)-benzophenone*
***DETX** – 2,4-Diethyl-thioxanthone*
***DMAB** – 4-(Dimethylamino)-benzophene*
***DMMB** – 2-(4-Methylbenzyl)-2-dimethylamino-1-(4-morpholinophenyl) 1-butanone*
***DMPA** – 2,2-Dimethoxy-2-phenyl acetophenone*
***DOTP** – Di-octyl terephthalate*
***DPV** – Differential Pulse Voltammetry*
***EC** – European Commission*
***EDB** – Ethyl-4-(dimethylamino)benzoate*
***EFSA** – European Food Safety Authority*
***EHA** – 2-Ethylhexyl-4-dimethylamino benzoate*
***EI** – Electron Impact*
***EPA** – US Environmental Protection Agency*
***ESBO** – Epoxidised Soybean Oil*
***ESCO WG** – EFSA Scientific Cooperation Working Group*
***EU** – European Union*
***EuPIA** – European Printing Inks Association*
***EURL** – European Union Reference Laboratory*
***EURL-ECVAM** – European Union Reference Laboratory for Alternatives to Animal Testing*
***FA** – Formic Acid*
***FAO** – Food and Agriculture Organization of the United Nations*
***FCM** – Food Contact Material*
***FCSN** – Food Contact Substance Notification*
***FDA** – Food and Drug Administration*
***FDHA** – Federal Department of Home Affairs of Switzerland*
***FID** – Flame Ionization Detector*
***FLD** – Fluorescence Detector*
***FSA** – Food Standards Agency of United Kingdom.*
***FSC** – Food Safety Commission of Japan*
***FTMS** – Fourier Transform Mass Spectrometry*

HABI's *– Hexaarylbiimidazoles*
HAP's *– Hydroxyacetophenones*
HCPK *– 1-Hydroxycyclohexyl-phenyl-ketone*
HMB *– Hydroxy-methoxy-benzophenone*
HMPP *– 2-Hydroxy-2-methyl propiophenone*
HPTLC *– High Performance Thin Layer Chromatography*
H-SRM *– Highly Selective Reaction Monitoring*
GC *– Gas Chromatography*
GNP *– Gross National Product*
GRAS *– Generally Recognized as Safe*
HC *– Health Canada*
HPLC *– High Performance Liquid Chromatography*
IAC *– Industry and Commerce Department of China*
IARC *– International Agency for Research on Cancer*
ITX *– 2/4-Isopropyl thioxanthone*
JECFA *– Joint FAO/WHO Expert Committee on Food Additives*
JETRO *– Japan External Trade Organization*
LC *– Liquid Chromatography*
LOD *– Limit of Detection*
LOQ *– Limit of Quantification*
LDPE *– Low Density Polyethylene*
MAFF *– Ministry of Agriculture, Forestry and Fisheries of Japan*
MBB *– Methyl-2-benzoylbenzoate*
MBF *– Methylbenzoyl-formate*
MDA *– 4, 4'-Methylenedianiline*
ME *– Ministry of Environment of Japan*
MHLW *– Ministry of Health, Labor and Welfare of Japan*
MK *– Mischler's ketone [4, 4'-Bis(dimethylamino) benzophenone]*
MMMP *– Methyl-1-(4-methylthio)phenyl-2-morpholinopropan-1-one*
MOA *– Ministry of Agriculture of China*
MOH *– Ministry of Health of China*
MPBP *– 4-(4-Methylphenylthio)benzophenone*

MRM *– Multiple Reaction Monitoring*
MS *– Mass Spectrometry*
NIAS *– Non-Intentionally Added Substances*
o-cl-HABI *– 2,2-Bis-(2-chlorophenyl)-4,4',5,5'-tetraphenyl-1,2-biimidazolyl*
OML *– Overall Migration Limit*
OMN 910 *– Omnipol 910®*
OPAc *– Oxyphenylacetic acid 2-[2-hydroxy-ethoxy]ethyl ester*
PAA *– Primary Aromatic Amines*
PBZ *– 4-Phenylbenzophenone*
PHAC *– Public Health Agency of Canada*
PSA *– Primary Secondary Amine*
PTFE *– Polytetrafluoroethylene*
PVC *– Polyvinyl Chloride*
QuEChERS *– Quick, Easy, Cheap, Effective, Rugged, and Safe extraction method*
RASFF *– Rapid Alert System for Food and Feed*
RHO 2074 *– Rhodorsil 2074®*
RSD *– Repeatability Standard Deviation*
SFDA *– State Food and Drug Administration Department of China*
SML *– Specific Migration Limit*
SP 7005 *– Speedcure 7005®*
SP 7010 *– Speedcure 7010®*
SPE *– Solid Phase Extraction*
TCC *– Toxicological Threshold Concern*
THIO *– Diphenyl[(phenylthio)phenyl]sulfonium hexafluorophosphate*
TOR *– Threshold of Regulation*
TPO *– Diphenyl-(2, 4, 6-trimethylbenzoyl) phosphine oxide*
USA *– United States of America*
USDA *– United States Department of Agriculture*
UV *– Ultraviolet*
WHO *– World Health Organization*

1. RESUMO

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Os fotoiniciadores son substancias químicas, que absorben enerxía lumínica, radiación ultravioleta ou visible, pasando a un estado excitado e formando radicais libres. Os radicais libres son especies químicas moi reactivas capaces de iniciar unha reacción de polimerización, a cal progresa rapidamente mediante a propagación dos radicais libres ata a finalización do proceso, cando remata a construción do polímero.

En resumo, os fotoiniciadores transforman a enerxía lumínica en enerxía química, capaz de inicialo proceso de polimerización. Na súa gran maioría, os fotoiniciadores comerciais absorben a enerxía lumínica na rexión ultravioleta sendo utilizados en diversos procesos para o desenrolo de materiais que estarán destinados a entrar en contacto cos alimentos, como recubrimentos, adhesivos, ou o obxectivo desta tese: as tintas de impresión en envases.

Estas tintas de impresión son formulacións con múltiples compoñentes onde os fotoiniciadores constitúen unha pequena porcentaxe que vai dende o 1 ó 20 % do total. O resto da formulación componse principalmente por 3 elementos: pigmentos, un sistema vehicular ou reactivo onde priman os monómeros, oligómeros e prepolímeros e os aditivos, que teñen como obxectivo modular o curado da tinta co fin de acadar o resultado desexado.

O uso destas tintas está moi estendido no eido alimentario, sempre na cara externa dos envases primarios ou en ámbalas caras de envases secundarios, é dicir nas partes do envase que non entran en contacto co alimento. Estes envases alimentarios, substrato da impresión, poden ser de múltiples materiais, sendo os principais: metais, plásticos, papel e cartón.

Aínda que as tintas de impresión non se utilizan nas partes do envase destinadas a entrar en contacto co alimento, no ano 2005, reportouse por parte das autoridades europeas o primeiro caso de presenza dun fotoiniciador en leite para nenos, confirmándose que estas moléculas chegan ó alimento. Dende esa primeira alerta alimentaria, máis dun cento de casos coma este ocorreron, converténdose este feito nun risco serio para o consumidor, incrementándose a preocupación das autoridades con competencias en seguridade alimentaria.

En base a este feito, o terceiro capítulo desta tese de doutoramento introdúcenos no mundo da seguridade alimentaria; analizando os posibles riscos aos que estamos expostos os consumidores polo mero feito de inxerir alimentos. A continuación descríbense os riscos relativos ó uso de materiais de contacto alimentario (FCM), describindo cales son as posibles fontes destes compostos contaminantes. Este capítulo desemboca na presentación dos obxetivos da presente tese no capítulo cuarto.

O quinto capítulo, presenta unha revisión exhaustiva actualizada do problema de seguridade alimentaria que supoñen os fotoiniciadores. Para abordar este complexo problema, o capítulo foi estruturado en catro partes fundamentais. A primeira versa sobre o proceso de fotocurado, clave para entender os diversos tipos de fotoiniciadores que existen na actualidade, saber o porqué do uso en maior ou menor medida de cada un en FCM, comprendendo cara onde se dirixe o mercado na busca de fotoiniciadores con menor risco de migración ó alimento e mellores acabados. A continuación, faise unha descrición de como pode acadar o alimento o fotoiniciador dende a cara externa do envase, habendo tres posibles rutas: por transferencia indirecta, a través da fase de vapor ou por transferencia directa por difusión a través do envase e despois acadando o alimento, ou por *set-off*, o principal mecanismo de migración ó alimento dos fotoiniciadores. Este fenómeno do *set-off*, consiste na transferencia de materia dende a superficie impresa á superficie destinada a estar en contacto co alimento cando os envases son almacenados en pilas ou en rolos.

A terceira parte deste capítulo describe os fotoproductos que se poden xerar no proceso de fotopolimerización de tres fotoiniciadores modelo. Para a determinación da perigosidade destes produtos, levouse a cabo unha análise toxicolóxica en base a clasificación de Cramer, que posibilita amosar o potencial toxicolóxico de cada un destes produtos, obtendo unha visión xeral da capacidade dos fotoiniciadores de xerar compostos potencialmente perigosos para a saúde humana no proceso de curado. E para rematar, este capítulo expón como se regula a día de hoxe lexislativamente a presenza das tintas de curado

ultravioleta en tres dos principais mercados mundiais, como son a Unión Europea, os Estados Unidos de América e o Xapón.

Para completar a revisión sobre o problema de seguridade alimentaria que representan os fotoiniciadores, o sexto capítulo amósanos os métodos analíticos desenrolados ata a data, para a detección de fotoiniciadores, e os seus derivados, en FCM e alimentos. Este apartado da tese, analiza en primeiro termo os métodos extractivos a realizar tanto nos FCM coma nos alimentos. Este pode ser un punto crítico, debido a heteroxeneidade dos substratos (envases ou alimentos) e dos propios fotoiniciadores. Por isto, este capítulo ofrece unha guía de cal pode ser a mellor estratexia en cada caso, dependendo do substrato a extraer e dos fotoiniciadores usados no envase.

Así mesmo, preséntanse as diferentes opcións analíticas tomadas en cada traballo publicado, relacionado cos fotoiniciadores, no eido da seguridade alimentaria. Maioritariamente, os traballos decídense pola cromatografía, tanto líquida como gasosa usando múltiples detectores acoplados, dende os comúns DAD e FLD en cromatografía líquida, como o FID en gasosa, pero sendo as opcións máis completas e dinámicas o uso de detectores MS ou MS/MS. Outras opcións menos comúns son outros tipos de cromatografía, como a HPTLC, ou a voltametría, pero debido as súas limitacións non son técnicas moi usadas, recomendándose o uso das técnicas anteriormente mencionadas. Por último, a técnica máis novidosa en relación á detección de fotoiniciadores e produtos dos mesmos é o DART. Técnica usada con éxito en varios envases alimentarios e que permite un gran avance na identificación destas moléculas, debido a que non necesita de paso previo extractivo, o que leva consigo un gran aforro tanto de tempo como de diñeiro, facendo que sistemas DART/MS-MS ou DART/TOF-MS sexan vistos como unha atractiva alternativa nun futuro próximo.

A continuación, logo de presentar os diversos métodos analíticos desenrolados ata a data para a detección e cuantificación de fotoiniciadores, o séptimo capítulo presenta un método desenrolado con este fin para 14 fotoiniciadores e aminas sinérxicas en envases alimentarios mediante HPLC-DAD e confirmación por LC-ESI-MS/MS. Este método foi desenrolado co

obxectivo de ser un posible método de cribado para análises de rutina, en laboratorios de referencia, para a detección de 14 dos fotoiniciadores que xeraron máis alertas ou notificacións polas autoridades de seguridade alimentaria europeas nos últimos anos.

O método HPLC-DAD desenrolado é un método sinxelo, replicable e que pode ser usado como ferramenta inicial para a identificación de fotoiniciadores de común presenza en FCM, como paso previo á investigación dunha posible migración ó alimento. A sensibilidade e a linearidade do método resultaron ser excelentes no rango de concentracións estudado, acadando LODs de ata 0,31 μg dm^{-2}. Así mesmo, o método LC-ESI-MS/MS permite a confirmación da identidade de cada un dos compoñentes das mostras positivas.

Dentro dos fotoiniciadores analizados incluíuse o BPAcr, un dos primeiros expoñentes da nova xeración de fotoiniciadores poliméricos, formado a partires de varias unidades de BP unidas por estruturas acrílicas. A pesares deste feito, non ten unha estrutura coñecida, e polo tanto, este traballo afonda nos resultados obtidos do estudio por LC-ESI-MS/MS para, tan sequera determinar os produtos formados a partires deste fotoiniciador, o que permite a súa confirmación en mostras reais. Este feito permitiu ofrecer máis información sobre un dos fotoiniciadores poliméricos máis utilizados da actualidade, do cal non se coñece moito ó respecto.

Por último, este método foi probado con éxito en diferentes mostras reais, adquiridas en supermercados locais, entre os que se atoparon envases plásticos, cartóns e latas. Os resultados obtidos supuxeron case un 50 % de mostras positivas en algún dos fotoiniciadores analizados, sendo a BP a máis común nas mostras analizadas, atopándose os fotoiniciadores en concentracións de ata 102,93 μg dm^{-2} de envase.

O oitavo capítulo desta tese foi desenrolado no Center for Food Safety and Applied Nutrition (CFSAN) da Food and Drug Administration (FDA) nos Estados Unidos de América baixo a tutela de D. Tymothy H. Begley e o Dr. Luke Lindahl-Ackerman. Neste capítulo utilizáronse tres técnicas analíticas diferentes: GC-

MS, DART-HRMS e UHPLC/ESI-HRMS, para a detección e identificación de fotoiniciadores e outras moléculas relacionadas coas tintas de impresión en 3 envases diferentes, onde o curado ultravioleta non se rematou de forma intencionada. Para obter máis información acerca da capacidade das tintas de impresión de migrar dende a cara externa á interna, estes envases colocáronse en pilas, podendo deste xeito determinar as moléculas capaces de acadar a cara interna polo fenómeno de *set-off*.

Este estudo tentativamente detectou ou identificou 110 compostos, incluíndo un total de 35 compostos relacionados coas tintas de curado ultravioleta. Destes 35 compostos, 28 amosaron evidencias de *set-off*, entre elas, 16 fotoiniciadores, 7 produtos da escisión dos fotoiniciadores durante o proceso de curado e 5 probables produtos de degración dos mesmos, moitos dos cales amosaron signos de *set-off*. A grande maioría dos compostos identificados ou confirmados na superficie destinada a entrar en contacto co alimento eran monómeros, produtos de degradación, solventes, plastificantes, antioxidantes ou axentes lubrificantes.

O noveno capítulo describe a realización do proceso de fotocurado a escala de laboratorio do fotoiniciador máis usado na actualidade: a BP, e unha das aminas sinérxicas máis utilizadas para a propagación do curado: o EDB. Para isto, prepáraronse diversas mesturas de ámbolos compoñentes, que foron sometidas á luz dunha lámpada ultravioleta durante diversos tempos de exposición, dende 30 segundos a 10 minutos e a diferentes distancias dende a lámpada (50-150 mm). A continuación, as solucións foron analizadas tanto por GC-MS como por HPLC-DAD.

A análise por HPLC-DAD serviu para determinar o efecto sobre a solución de curado, tanto do tempo de exposición como da distancia con respecto á luz ultravioleta. Observouse que a distancia con respecto da fonte UV non é un parámetro crucial no proceso de curado, en tanto ó estudo dos posible fotoproductos xerados durante o curado. Non obstante, a maior tempo de exposición a transformación da BP e do EDB noutros produtos, increméntase obtendo maiores concentracións destes fotoproductos.

Para a análise dos fotoproductos, comparáronse os resultados obtidos por GC-MS coas entradas das librerías de masas dispoñibles, obtendo un total de 23 compostos por este método. Aínda que, á hora de confirmar estes compostos só se puideron obter 13 estándares dos mesmos para corroborar a súa presenza nas solucións irradiadas. Destes 13 estándares, acadouse a confirmación de 4 compostos da fotodegradación das mesturas BP-EDB: benzaldehido, benzocaína, 1,1'-ethenilidenebis-benceno e o éster etílico do ácido benzoico.

Dos 4 compostos confirmados, só 2 están na lista de monómeros autorizados para materiais e obxectos plásticos destinados a contacto alimentario, segundo a regulamentación da Comisión Europea 10/2011, o benzaldehido e o éster etílico do ácido benzoico. Non obstante, o benzaldehido ten a capacidade de deteriorar as capacidades organolépticas do alimento e, ten establecida unha inxestión diaria tolerada de 0-5 mg kg^{-1}, segundo o comité de expertos da FAO/WHO (JECFA).

Para completar o estudo dos fotoproductos fíxose unha pequena busca de fotoiniciadores e os fotoproductos atopados durante o transcurso deste estudo en 13 envases alimentarios plásticos. En primeiro termo, usouse o método de HPLC-DAD descrito no capítulo séptimo para avaliar a presenza de fotoiniciadores nestas mostras, atopándoos no 92 % das mesmas. Tódalas mostras foron a continuación analizadas por GC-MS, non se atopando ningún dos fotoproductos tanto nas mostras con presenza de BP e EDB como nas que non estaban presentes.

Os capítulos do 10 ó 12 están adicados ó estudo das cinéticas de migración dos fotoiniciadores ó alimento por contacto directo. Seleccionáronse 6 fotoiniciadores modelo, escollidos en base ás alertas e notificacións xeradas por parte das autoridades de seguridade alimentaria europeas dos últimos anos: ITX, DETX, BP, EDB, 4-MBP e HCPK. Co fin de estudar as cinéticas da migración destes compostos ó alimento, realizáronse por extrusión filmes de polietileno de baixa densidade (LDPE) con aproximadamente 0,2 % de cada un dos fotoiniciadores por separado. Estes filmes, cunha superficie coñecida, foron somerxidos en tubos protexidos da luz, cun volume coñecido de simulante

alimentario, utilizando aqueles que aparecen na lexislación europea EC 10/2011 (agás do simulante alimentario para alimentos secos): 10 % etanol (v/v), 3 % ácido acético (p/v) e 20 % etanol para substancias hidrofílicas, e 50 e 95 % etanol (v/v) (este último substituto en base a lexislación do aceite vexetal) para alimentos lipofílicos. Estas análises tamén se levaron acabo en auga, un alimento na actualidade, considerado en anteriores lexislacións europeas como simulante de alimentos acuosos. Todos estes estudos foron realizados ás temperaturas comúns utilizadas na conservación de alimentos: conxelación (-18 ^{o}C), refrixeración (4 ^{o}C), temperatura ambiente (20 ^{o}C) e por último realizáronse estudos tamén a 40 ^{o}C, establecendo esta temperatura como o peor caso esperado para un alimento.

Para estudar o proceso de migración, 2 foron os mecanismos seguidos, sendo o tempo de análise 8 horas a 40 ^{o}C, 24-48 horas a 20 ^{o}C, 3-7 días a 4 ^{o}C e 210-335 días a -18 ^{o}C (só en 50 e 95 % etanol (v/v), debido a que o resto dos simulantes a esta temperatura cambian de estado físico). Primeiramente, no caso de 4-MBP, EDB, ITX e DETX, os filmes mantivéronse en contacto cos simulantes alimentarios durante estes intervalos de tempo, para asegurar así acadar o equilibro do sistema filme/simulante alimentario. Durante este tempo, fóronse retirando alícuotas, a tempos preestablecidos, de 0,5 ml de simulante alimentario, que foron analizadas a posteriori por HPLC, co método HPLC-DAD descrito previamente no capítulo cuarto, para cuantificar a concentración de fotoiniciador no simulante a estes tempos. Ó finalizar o estudo, o filme foi retirado, secado con papel de filtro e extraído o fotoiniciador remanente con acetonitrilo a 70 ^{o}C durante 24 horas; esta solución extractiva sería analizada co mesmo método, para acadar a concentración do fotoiniciador remanente no filme no equilibro. Todos estes datos foron tabulados e xeradas as conseguintes curvas de migración.

No caso de BP e HCPK, o mecanismo seguido para a determinación da cinética de migración foi lixeiramente distinto, utilizando 10 filmes para cada combinación de fotoiniciador-simulante alimentario-temperatura, sendo retirados cada un a tempos preestablecidos para analizar a concentración de

fotoiniciador no simulante alimentario por HPLC-DAD. Para coñecer a concentración inicial de fotoiniciador no sistema, tomouse como valor a media das extracción totais con acetonitrilo de varios filmes. Do mesmo xeito que co resto de fotoiniciadores, xeráronse as curvas de migración dende o filme ó alimento.

Para evitar toda esta experimentación, que consume moitos recursos, a presente lexislación europea permite a xeración de modelos de predición da migración baseados en evidencias científicas. Para facer isto, tódolos datos experimentais obtidos foron axustados a un modelo matemático baseado nas solucións propostas por Crank á segunda lei de Fick, que describe os fenómenos de difusión como a migración. Deste xeito, obtivéronse os valores dos dous parámetros considerados fundamentais para a descrición da migración para cada un dos sistemas fotoiniciador-simulante alimentario-temperatura: D, o coeficiente de difusión do fotoiniciador que define o movemento do migrante; e, o coeficiente de partición polímero/alimento $K_{P/F}$, que define o equilibro termodinámico do proceso de migración, é dicir, a proporción entre a concentración do fotoiniciador no polímero e no simulante alimentario cando se acada o estado de equilibro.

Os datos da migración obtidos referendan, que a migración dos fotoiniciadores, como a doutros migrantes, está supeditada as propiedades do sistema polímero-migrante-simulante alimentario. De xeito xeral, nestes estudos a maior peso molecular menor D, e a maior temperatura maior D. Con respecto ó $K_{P/F}$, a maior valor de log $K_{o/w}$ maior é a afinidade polos simulantes de alimentos lipofílicos e viceversa.

Así mesmo, atopáronse 3 feitos salientables nos ensaios cinéticos realizados:

- pH do alimento (simulante alimentario), pode influír enormemente na difusión dos fotoiniciadores como o EDB, debido a que un pH baixo como o do ácido acético 3 % (p/v), o EDB está maioritariamente na súa forma protonada, que é máis afín polo simulante alimentario que a súa forma neutra, obtendo

resultados semellantes de migración neste simulante alimentario a simulantes máis lipofílicos.

- Existen pequenas diferenzas entre os resultados de migración en auga (alimento acuoso) e o seu correspondente simulante: 10 % etanol (v/v). No caso de BP e HCPK, hai un maior ratio de migración dende o LDPE ó auga que ó simulante alimentario. Isto non quere dicir que non sexa un simulante axeitado, pero análises máis exhaustivos deben ser realizados co fin de esclarecer este comportamento.

- Existe relación entre a porcentaxe de etanol do simulante alimentario e os valores acadados de D nos experimentos levados a cabo. Atopouse unha certa linearidade entre ámbolos parámetros, sendo moito máis clara a temperaturas elevadas que a temperaturas de refrixeración (aínda que no caso da BP tamén se fai patente esta linearidade a temperaturas de refrixeración).

Por último, comprobouse a relación entre os valores de D determinados para cada sistema fotoiniciador-simulante alimentario, e a temperatura á que se realizou a análise. Isto foi realizado mediante a aplicación da ecuación de Arrhenius, que determina se existe linearidade entre estes dous parámetros. Se existe linearidade entre os dous parámetros, pódese extrapolar o D de cada sistema no rango de temperaturas de estudo. Os resultados reflectiron que existe linearidade en tódolos sistemas analizados, con valores do coeficiente de determinación $R^2 > 0.95$ no 93 % dos casos e de $R^2 > 0.92$ no 100 % dos casos analizados entre as temperaturas de 4 e 40 ^oC. Non obstante, atopouse que nos estudos realizados a -18 ^oC a linearidade pérdese, pero demostrándose o importante feito de que a migración destas moléculas continúa, inclusive a tan baixas temperaturas, continuando o risco para a saúde dos consumidores.

Para completar a análise da linearidade entre D e a temperatura, comprobouse tamén a ecuación proposta por Piringer, que establece unha relación entre D e o tipo de polímero e a masa molecular do migrante. Esta ecuación non determina o D real, pero si un coeficiente de difusión no polímero sobreestimado (D_P*). Este valor pode ser usado como D, debido a que a súa

sobreestimación resulta útil, xerando un marxe de seguridade para o consumidor. Os resultados obtidos demostraron que esta ecuación efectivamente sobreestima a migración dos fotoiniciadores na maioría dos casos; non obstante, no simulante alimentario 50 % etanol (v/v) esta ecuación subestima a migración do 4-MBP a temperaturas menores de 14,2 ^{o}C. Así mesmo ocorre en 95 % etanol (v/v) na BP, onde comeza a subestimación da migración a partires dos 7,7 ^{o}C e no 4-MBP a partires dos 16,7 ^{o}C. Debido a estes resultados, os valores obtidos nos estudos desta tese confirman que esta ecuación é útil para a predición da migración, pero a súa utilización debe facerse con precaución.

2. SUMMARY

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The photoinitiators are chemical substances that can absorb UV or visible radiation, achieving an excited state to generate free radicals. The free radicals are very reactive chemical species able to initiate a polymerization reaction due to the fast spread of them until the end of the process, when the polymer is already finished.

To sum up, the photoinitiators transform light energy in chemical energy that it is able to initiate the polymerization process. Mostly, the commercial photoinitiators absorbs the light energy in the UV region, being used in different processes for the development of food contact materials, as can be adhesives, coatings, … and the aim of this thesis: the UV curing inks.

The UV curing inks are solutions with many components, where the photoinitiators are in percentages between 1 and 20 %. The rest of the formulation is basically 3 other elements: pigments, a vehicular or reactive system composed mainly by monomers, oligomers and pre-polymers, and finally, the additives which main objective is modulate the photocuring process in order to achieve the desired coating.

The UV curing inks are commonly used in the outer surface of food packagings or in both surfaces of secondary packages, not destined to be in contact with the food. These food packagings, printing substrata, can be of different materials, being mainly metal, plastic and paper/cartonboards.

Although the UV curing inks are not used in the package surface destined to enter in contact with the food, in 2005, it was notified by European food safety authorities the first case of the presence of photoinitiators in infant baby formulas, confirming that these compounds can achieve the food and contaminate them. Since that case, more than a hundred food alerts as this one were reported. This number demonstrates that this fact is a serious hazard for the consumers' health.

In this context, the third chapter of this thesis stated the photoinitiators issue in the current Food Safety scenario. The global Food Safety scenario is settled, defining how it is managed in the main markets of the world. Then the

possible risks that can affect the consumers' health due to the simple fact of eat packaged food are described. Also, the possible sources of these main food contaminants are defined. To finish this section, in the fourth chapter, the objectives of this thesis are presented.

The fifth chapter presents a comprehensive review of the food safety problem related to the use of photoinitiators in food packagings. In order to a better understanding of this complex issue, the chapter was structured in four sections. The first section describes the photopolymerization process, essential to perceiving the significant differences between the three main types of photoinitiators and why is used each one more or less in FCM. This fact also helps us to understand the recently released new UV curing formulations and where it is headed the market in the future.

Then, there is a description of the migration process to understand how molecules that are on the outer surface of the package can reach the food. There are three different routes: direct transfer, the molecules can go through the package by diffusion reaching the surface of the foodstuff or, by indirect transfer, through the vapor phase after diffuse through the package and, finally by *set-off*, the main migration process of photoinitiators. The *set-off* effect consists in the transfer of material from the outer surface of the package to the inner one when the package is stored in reels or stacks.

The third section describes, in three common examples, the photoproducts derived from the process of photopolymerization of three common photoinitiators (or photoinitiator systems). To determine the risk posed by these compounds, it was carried out a toxicological analysis based on Cramer's classification. This classification allows determine the toxicological profile of each one of these products. This fact allows a global vision of the toxicity of a single compound, which can creates during the photopolymerization process many other potentially hazardous molecules for the human's health, that otherwise, were not expected to be in the final coating.

Finally, the chapter ends with the description of the regulations and legislations related to the UV curing inks issue in the main markets of the world: EU, USA and Japan.

In order to complete the food safety review of the previous chapter, the sixth chapter presents all the analytical methods developed to date to determine and quantify photoinitiators and derivatives in foodstuffs and FCM. Firstly, the extraction methods used by the different authors in foodstuffs and FCM are discussed. This is a critical point due to the substrata and photoinitiators heterogeneity. For this reason, this chapter offers a guide to select the better extraction methodology for them.

All the different analytical options used by the different authors are also presented and discussed. The great majority of the works have chosen as analytical technique for the determination and/or quantification of photoinitiators the chromatography, gas or liquid. A wide range of detectors were coupled to these techniques, usually FID for GC and DAD or FLD in LC; however, the most complete options are the MS or MS/MS detectors. Other options much less common are other chromatographic techniques as HPTLC or even voltammetry; nonetheless, due to their limitations they are not recommended.

The most novel technique used in the detection of photoinitiators and their byproducts is the DART. It was successfully used in different food packages and which great advance is that allows to avoid the extraction step. This fact achieves a great afford of money and time, being DART/MS-MS or DART/TOF-MS promising options in a near future.

After present the photoinitiators food safety issue and the analytical methods developed for their detection, the seventh chapter presents a novel method for the determination and quantification of 14 photoinitiators in food packagings by HPLC-DAD and confirmation by LC-ESI-MS/MS. This method was developed with the objective of being a routine method for the determination

of the photoinitiators that have appeared in the alerts and food notifications reported by RASFF in the last years.

This HPLC–DAD method is simple, reliable, and could be useful as a screening tool for common photoinitiators in FCM, previous to an analysis of the foodstuff. Sensitivity and linearity were excellent in the range of concentrations studied, achieving LODs of 0.31 μg dm^{-2}. Furthermore, the LC-ESI-MS/MS method enables confirmation of the identity of substances in positive samples.

One of the analyzed photoinitiators is the BPAcr. This is one of the first polymeric photoinitiators used in food packaging. Its structure is based in acrylate unities linked to BP molecules; however, the exact structure is unknown. This work is one of the first that deal with novel components of the UV curing inks and, thanks to the LC-ESI-MS/MS analysis the mass products formed from the scission of the BPAcr are known allowing its confirmation in real samples.

To complete this chapter, different types of printed packaging, including plastic, cardboard, and cans were surveyed for the occurrence of PIs. For almost 50 % of samples, amounts of the substances selected were quantifiable, being benzophenone the most frequently detected. The quantities of photoinitiators arrived to 102.93 μg dm^{-2}.

The eighth chapter was carried out under the direction of Mr. Timothy H. Begley and Dr. Luke Lindahl-Ackerman in the Center for Food Safety and Applied Nutrition (CFSAN) in the Food and Drug Administration (FDA) in Maryland, USA. In this chapter three different techniques: Direct Analysis in Real Time High Resolution Mass Spectrometry (DART-HRMS), Gas Chromatography-Mass Spectrometry (GC-MS) and Ultra-High Pressure Liquid Chromatography/ Electrospray Ionization-HRMS (UHPLC/ESI-HRMS) were used to detect and identify print related molecules from the food-contact and print surfaces of 3 different packages with under-cured prints. To obtain more information about the possible migration of photoinitiators and print related compounds from the

outer to the inner surface, the packages were stored in piles in order to study the *set-off* phenomenum.

This approach tentatively identified or confirmed 110 compounds, including 35 print related molecules. The majority of the compounds identified on food-contact surfaces were packaging monomers/byproducts, solvents/plasticizers, antioxidants/degradants, or slip agents/lubricants. Of these, 28 showed evidence of *set-off*. The identity of 16 PIs, 7 known scission products, and 5 probable PI degradants were confirmed, most showing signs of *set-off*. Of the print related molecules, at least 5 are novel print contaminants such as 4-morpholin-4-yl-benzaldehyde or 3-phenyl-2-benzofuran-1(3H)-one.

The ninth chapter describes the realization of a photopolymerization process at a laboratory scale. The chosen photoinitiator system was comprised by the most used photoinitiator, BP, and one of the most common amine synergist, EDB. For the study, different mixtures of both components were under a UV light source in order to initiate the photopolymerization process. The influence of two variables was studied: the distance from the UV source, testing distances from 50 mm to 150 mm; and, the time of exposure to the UV source, from 30 to 600 seconds. To study their influence, the different solutions were studied by HPLC-DAD, with the method developed in the fourth chapter and also by GC-MS.

The HPLC-DAD analysis helped to determine the effect of the two variables studied. Related to the possible photoproducts, the distance to the UV source was not a critical parameter. However, the time of exposure to the UV source it was a critical parameter. Longer times of exposure increased the number of by-products formed in the process, increasing also the concentration of these new-formed molecules.

To analyze the photoproducts generated during the photopolymerization tests, the results obtained by GC-MS were compared with the entries of the MS libraries available, obtaining 23 compounds by this method. However, in order to confirm the presence of these molecules in the solutions only 13 standards

were available for this purpose. After analyze these 13 molecules, 4 compounds were confirmed: benzaldehyde, benzocaine, 1, 1'-ethenylidenebis-benzene and the benzoic acid methyl-ester.

Of the confirmed compounds only 2 are in the positive list of authorized monomers for plastic materials and articles intended to come into contact with food of the Regulation EC 10/2011: benzaldehyde and benzoic acid methyl-ester. Nevertheless, there is a risk that the migration of benzaldehyde deteriorates the organoleptic characteristics of the food in contact and then, that the final product does not comply with current legislation. Also, the JECFA has established for benzaldehyde an acceptable daily intake of 0-5 mg kg^{-1}.

A small survey (13 samples) was carried out in order to search photoinitiators and the photoproducts confirmed in the previous test. In the HPLC-DAD analysis, photoinitiators were found in the 92 % of the samples. However, in the GC-MS analysis, no one of the photoproducts was found in the samples with BP and EDB.

The study of the migration kinetics of the photoinitiators to the food is the aim of the last three chapters (10 to 12). With this objective, six model photoinitiators were selected on the basis of the alerts and notifications of the European authorities in the last years: ITX, DETX, EDB, BP, 4-MBP and HCPK. As photoinitiator sources, six films of LDPE were extruded with 0.2 % of each photoinitiator. These films, with a known surface, were introduced in tubes protected from the light, with a known volume of food simulant. The experiments were carried out in all the food simulants proposed by the European legislation EC 10/2011 (except the simulant for dry foods: MPPO): 10, 20, 50 and 95 % ethanol (v/v) and 3 % acetic acid (w/v). Also, in order to compare with previous legislations were the food simulant for hydrophilic foods were distilled water (currently considered as a food), the experiments were also carried out in this simulant.

All the studies were performed at the common temperatures of storage: -18 ^{o}C for freezing storage, 4 ^{o}C for refrigeration storage, 20 ^{o}C as ambient

temperature and 40 ºC as the worst case expected for a foodstuff storage. The time of analysis was 8 hours at 40 ºC, 24-48 hours at 20 ºC, 3-7 days at 4 ºC and 210-335 days at -18 ºC (only in 50 and 95 % ethanol (v/v) due to the other food simulants are not liquid at this temperature).

To study the migration process, two different procedures were followed. The first procedure was used to study the migration kinetics of 4-MBP, EDB, ITX and DETX. The films were immersed in the food simulant and at preset times, aliquots of 0.5 ml of the food simulant were removed, in order to be analyzed by the HPLC-DAD method described in the chapter four, to know the photoinitiator concentration in the food simulant. This procedure continues until the equilibrium is reached, when the film is removed from the food simulant and extracted the remnant photoinitiator with acetonitrile at 70 ºC for a day. This extractive solution was analyzed by HPLC-DAD to know the quantity remnant in the film. Finally, with the data obtained the migration curves, for each photoinitiator from the film into food simulant, were constructed.

In the case of BP and HCPK, the procedure was slightly different. Ten films were used for each combination of food simulant-photoinitiator-temperature. At preset times, one of the films was taken and the food simulant of the tube was analyzed in order to know the concentration of photoinitiator at this time in the food simulant. In this case, to calculate the total quantity of photoinitiator in the film, different films were extracted with the same procedure explained above and after their analysis by HPLC-DAD the mean value was considered as the total quantity of photoinitiator in each of the films. The migration curves for each photoinitiator from the film into food simulant were constructed as previously.

To avoid carry out all these time-consuming and expensive migration assays, the current legislation allows its calculation applying generally recognized diffusion models based on scientific evidence. For that purpose, the data obtained with the six photoinitiators were adjusted to a mathematical model based on the solutions proposed by Crank to the Fick's Second Law, which describes the diffusion phenomena as migration.

This modelling allows the calculation of the two capital parameters that describe the process of migration in the systems food simulant-photoinitiator-temperature: D, the diffusion coefficient that defines the mobility of the migrant, and $K_{P/F}$, the partition coefficient between the polymer and the food, which defines the thermodynamic equilibrium of the migration process (the concentration of migrant in the polymer and in the food when the equilibrium is reached).

The migration data obtained confirm that the migration of photoinitiators, as other migrants, it is highly influenced by the properties of the system food simulant-photoinitiator-temperature. Generally, higher Mw of the migrant means lower D values and higher temperatures mean higher D values. With respect to $K_{P/F}$, higher values of log $K_{o/w}$ mean more affinity for the lipophilic foodstuffs and vice versa.

However, three remarkable facts were found in the kinetic migrations assays:

- The pH of the food/food simulant. This parameter can have high influence in the diffusion of photoinitiators as EDB, due to at low pH as it is the case of acetic acid 3 % (w/v), the EDB is mainly in its protonated form, having a higher affinity for this food simulant than the neutral form, resulting in a faster migration into the food simulant, reaching values of D similar to those obtained in lipophilic food simulants.

- There are little differences between the migration data in water and the food simulant for aqueous foods: 10 % ethanol (v/v). The migration of BP and HCPK is quantitatively bigger into water than into the food simulant from LDPE. This fact does not mean that 10 % ethanol (v/v) is not the appropriate food simulant for water; however, further studies should be done in order to explain this behavior.

- There is a clear relation between the percentage of ethanol in the simulant and the D values obtained. There is linear relation between both

parameters, being clearer at high temperatures; however, in some photoinitiators, this relation is clear even at refrigeration temperatures (BP).

Finally, under the assumption that D is dependent of the temperature, the linearity between these parameters was checked based on Arrhenius equation. If exists linearity between both parameters, the values of D can be calculated in the range of temperatures studied. The results showed that there is linearity in all the systems studied, with coefficients of determination $R^2 > 0.95$ in 93 % of the cases and $R^2 > 0.92$ in the rest of the assays between 4 and 40 ^oC. At freezing temperatures the relation between temperature and D is not linear; however, even at this low temperature, the migration of photoinitiators continues, being a risk for the consumers' health.

To complete the analysis of the linearity between D and the temperature, the Piringer equation was also checked. This equation establishes a relation between D, the polymer and the Mw of the migrant. This equation does not determine the real D, but it is an overestimated diffusion coefficient in the polymer (D_P*). The value of D_P* can be used as D, in order to overestimate the migration creating a safety margin for the consumer. The results obtained showed that this equation overestimates the migration of most of all the systems studied; nonetheless, underestimates the migration of 4-MBP in 50 % ethanol (v/v) at temperatures under 14.2 ^oC. The same happens in 95 % ethanol (v/v) for the migration of BP under 7.7 ^oC, and for 4-MBP from 16.7 ^oC. These results showed that this equation is very useful to overestimate the migration; however, it should be used carefully.

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3. INTRODUCTION

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3.1. FOOD SAFETY

What is in your meal? Where did the ingredients come from? Were they properly handled from every stage, from farm to plate? Are you at risk eating …? All these questions and many more can be sum up in only one: Is this food safe? This is the aim of a 2015 WHO campaing, being the key question nowadays, which has a different answer depending on the audience (WHO, 2015). This question has considerations beyond food safety, being also related to food security, and even food quality. To a complete comprehension of the main question, it should be stated the difference between those terms:

- **Food Security**. "It exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (World Food Summit, 1995). To meet the Food Security definition, a foodstuff should comply with four requirements (FAO, 2008):
    - Physical availability.
    - Economical and physical access.
    - Food utilization, understanding this as "the way the body makes the most of various nutrients in the food".
    - Stability in the time of the previous requirements.
- **Food Safety**. This term "refers to all those hazards, whether chronic or acute, that may make food injurious to the health of the consumer". It should not be confused with Food Quality, term that sometimes is overlapped to Food Safety in the consumers' minds (Joint FAO/WHO, 2003).
- **Food Quality**. It includes "all other attributes that influence a product's value to the consumer. This includes negative attributes such as spoilage, contamination with filth, discoloration, off-odors and positive attributes such as

the origin, color, flavor, texture and processing method of the food" (Joint FAO/WHO, 2003).

In this sense, food safety standards should be settled in order to "protect, and promote, the health of the consumers" (EC, 2000). These standards prevent the consumers from several risks, understanding the risk as "a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard". The risk analysis has been widely recognized as the fundamental methodology to stablish the food safety standards thanks to their "three interconnected components — risk assessment, risk management, and risk communication — that provides a systematic methodology for the determination of effective, proportionate and targeted measures or other actions to protect health" (Joint FAO/WHO, 1997; EC, 2002).

The possible hazards that can affect the consumers' health are usually categorized in three different groups: biological, chemical and physical hazards (Joint FAO/WHO, 1995). The biological hazards are usually called microbiological hazards due to this subgroup constitutes the main biological hazard, being several different infections caused by them. The chemical hazards are a more heterogeneous group where enters phytosanitary residues, industrial chemicals as cleaners or oils. Also in the chemicals enter additives, technological adjuvants or even chemicals migrated from the food contact materials. Finally, the physical hazards are glass, stones, wood, plastic, metals and others that can accidentally contaminate the foodstuff (Seward II, 2003; AECOSAN, 2015).

3.1.1. FOOD SAFETY AGENCIES

Governments have understood that the Food Safety is a capital issue nowadays. With this idea in mind, many organizations and institutions have been created all over the world in order to protect the consumers' health from different food hazards. In Europe, the Regulation EC 178/2002 has stablished in 2002 the EFSA, with the objective of reinforce the European feed and food safety system, "taking on the role of an independent scientific point of reference

in risk assessment and in so increase the consumers' confidence in the feed and food" (EC, 2002).

In the USA, two agencies are sharing the competences in Food and Feed Safety: the FDA and the USDA. The FDA was created earlier in the 1906 by the Federal Food and Drugs Act and, it "is responsible for protecting the public health by assuring the safety, efficacy and security of human and veterinary drugs, biological products, medical devices, our nation's food supply, cosmetics, and products that emit radiation". The FDA is responsible of more than 80 % of the USA food supply; however, USDA oversees most of poultry and meat products (European Parliament, 2015; FDA, 2015).

Most of the developed countries have their own agencies to protect the consumers' health. In Canada, three federal agencies coordinate all the food safety issues: CFIA, HC and PHAC (Keener *et al.*, 2014). In Japan, three ministries (MAFF, MHLW and ME) share with the CAA agency the risk management issues; however, the FSC undertakes the risk assessment (FSC, 2015). Nevertheless, other countries collaborate between each other as Australia and New Zealand that have in common the Food Standards Agency (Food Standards, 2015).

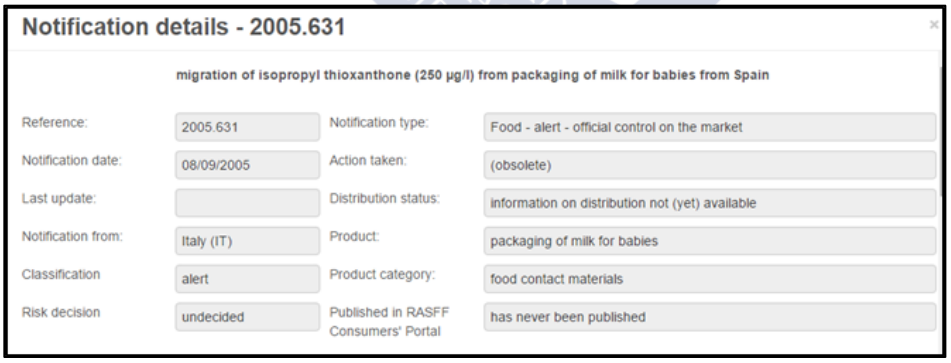
But it lacks China, one of the biggest food markets of the world, who is the origin country of 13 % of the European food safety notifications, becoming a serious concern for third countries (RASFF, 2015a). The food safety has experimented in China great improvements since 2009 with the new Food Safety Law; this law defines better than previously the responsibilities of the five agencies involved in the food safety issues: MOH, MOA, AQSIQ, IAC and SFDA. However, there are still many problems in the Chinese food safety regulations: higher maximum amounts for contaminants than the international limits, some regulations are not consistent or contradictory and also, even the relation between the different agencies are not very clear yet or the information does not flow correctly between them (Jia & Jukes, 2013).

At international level, FAO and WHO have stablished in 1963, the Codex Alimentarius, or "Food Code", organization that "develops harmonized

international food standards, which protects consumers' health and promote fair practices in food trade". This guidelines are the pattern for new food safety legislations in all countries, advising that "when formulating national policies and plans with regard to food, Governments should take into account the need of all consumers for food security and should support and, as far as possible, adopt standards from the Codex Alimentarius or, in their absence, other generally accepted international food standards", usually EFSA or FDA standards (Joint FAO/WHO, 2006; Codex Alimentarius, 2015).

3.2. RISKS FOR CONSUMERS' HEALTH

There are many risks for the consumers' health. However, new manufacturing procedures and other innovations in food science have dropped their number. In order to provide an analysis of the common risk nowadays, the RASFF presents annual reports of the food safety notifications reported through the year. These notifications "report on risks identified in food, feed or food contact materials that are placed on the market in the notifying country or detained at an EU point of entry at the border with an EU neighboring country" (RASFF, 2015a).



Notification details - 2005.631

migration of isopropyl thioxanthone (250 µg/l) from packaging of milk for babies from Spain

Reference:	2005.631	Notification type:	Food - alert - official control on the market
Notification date:	08/09/2005	Action taken:	(obsolete)
Last update:		Distribution status:	information on distribution not (yet) available
Notification from:	Italy (IT)	Product:	packaging of milk for babies
Classification	alert	Product category:	food contact materials
Risk decision	undecided	Published in RASFF Consumers' Portal	has never been published

Figure 3.1: First RASFF notification related to photoinitiators (RASFF, 2015b).

The notifications are distributed in: alerts (when represents a serious risk and rapid actions are needed), border rejections (food, feed or FCM are not allowed to enter in the UE territory due to be considered as risks), and

information for attention (not need for a rapid action) or to follow-up (adding information to a previous notification). Figure 3.2 shows the RASFF notifications of the last five years. As can be seen, the number of notifications is decreasing (from 3812 in 2011 to 2984 in 2015), decreasing also the numbers of border rejections and informations; however, the number of alerts it was increased more than 20 % in comparison with 2011 or even more if it is compared with 2013 (RASFF, 2012, 2013, 2014, 2015b, 2016).

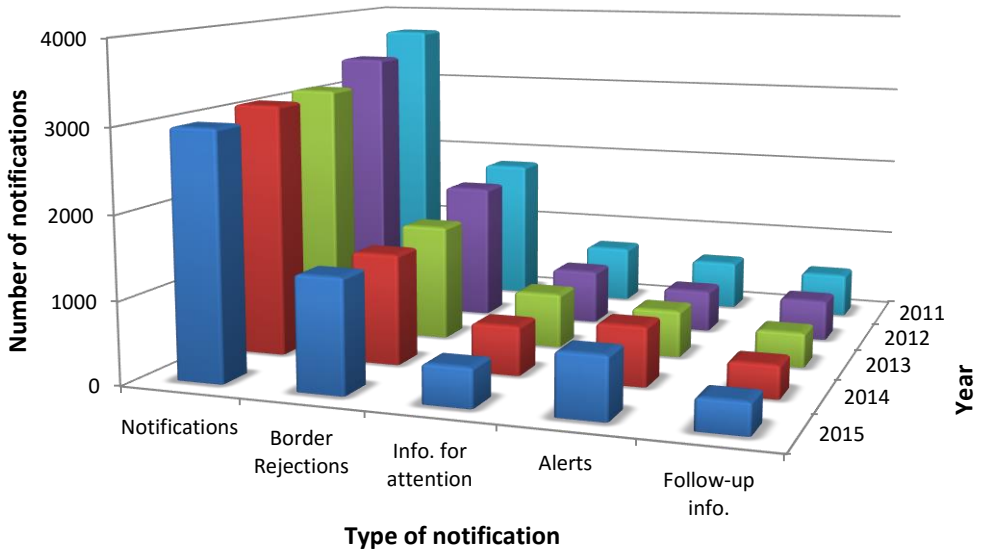


Figure 3.2: Evolution of RASFF notifications during the last 5 years.

The 2015 notifications showed that the most common hazards are the pathogenic microorganisms (745 notifications), mycotoxins (495), pesticide residues (405) and heavy metals (219). These four hazards constitute 62.5 % of the notifications; however, a low percentage of the pesticide residues (5.9 %) and mycotoxins notifications (14.9 %) are alerts (notifications of high risk). Other hazards as allergens (83.2 %), biotoxins (66.7 %) or biocontaminants (52.3 %) create comparatively more alerts (RASFF, 2016).

The same fact happens if we classify the notifications by food category, where fruits and vegetables (634), nuts, nuts products and seeds (477) and fish and fish products (297) are the categories that cause more notifications.

However, if we considered the alerts, the report shows that the notifications of milk and milk products (81.4 %), ices and desserts (60.0 %) and soups and prepared dishes (57.1 %) create more percentage of alerts (RASFF, 2016).

A geographical study of the notifications (figure 3.3) shows that more than 40 % of the notifications are generated by products which origin is Asia. Only the sum of the notifications created by Chinese, Indian and Turkish products generate 30.0 % of the RASFF notifications of 2015 (946 notifications). However, the own European products generate > 35 % of the total notifications highlighting that the hazards not only come from markets with weaker food safety legislations (RASFF, 2016).

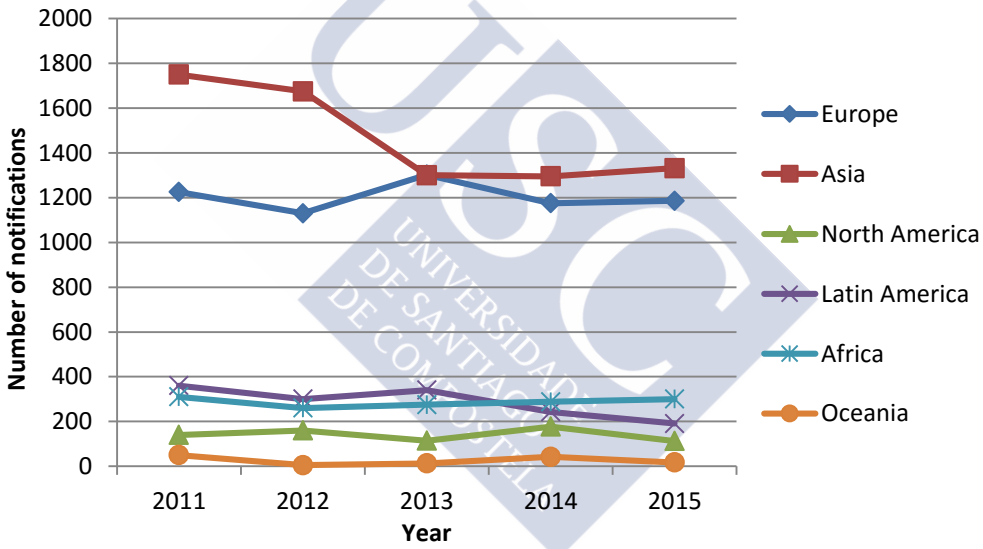


Figure 3.3: Geographical evolution of the RASFF notifications during the last 5 years.

3.3. FOOD CONTACT MATERIALS

The 5.1 % of the 2015 RASFF notifications were related to Food Contact Materials (152 notifications). In the last years, FCM constitute a common category in the RASFF notifications as can be seen in figure 3.4. However, it seems that a decreasing tendency is appearing in the last years, from 8.2 % to 5.1 % in the last five years.

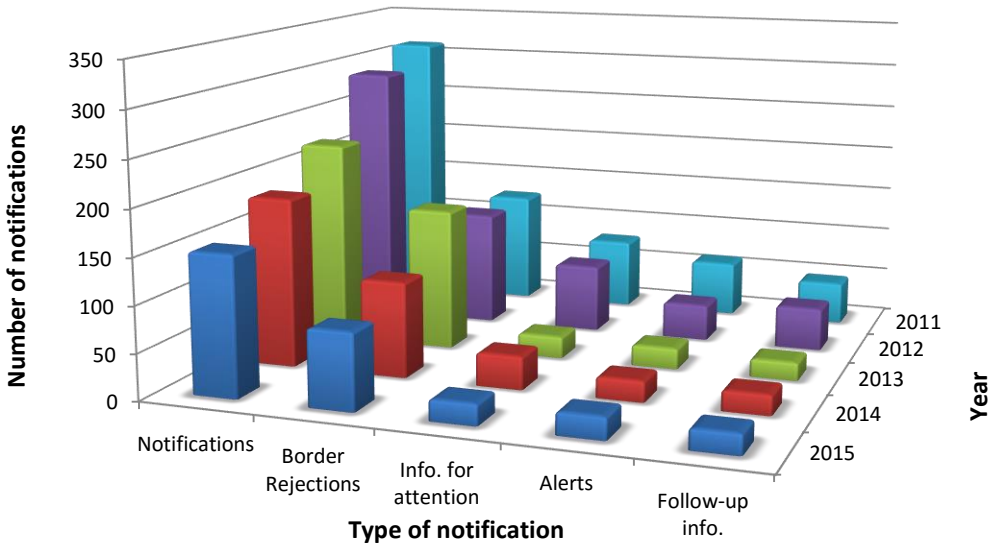


Figure 3.4: Evolution of the FCM RASFF notifications in the last 5 years.

The RASFF notifications related to FCM have a main characteristic: their heterogeneity. This fact is due to differences in the materials that can be used in the manufacturing of the food packaging, mainly plastics, metals, and paper/cartonboards. Many of the hazards derived from the use of one material are almost exclusive of this material, being a common example the migration of heavy metals from the metal of cans or kitchenware.

With these hazards in mind, the use of these materials is regulated in Europe by the Regulation EC No. 1935/2004 on materials and articles intended to come into contact with food. This regulation establishes a total of seventeen groups of materials and articles intended to come into contact with food. Due to the different characteristics of each one of the groups, the regulation establishes that “specific measures may be adopted or amended” for each one of them, or for the combination of them where appropriate (EC, 2004). Nevertheless, at the present time, specific measures were only taken for five groups of materials: plastic materials, rubbers and elastomers, ceramics, regenerated cellulose and active and intelligent packages.

3.3.1. PRINCIPAL HAZARDS RELATED TO FCM

3.3.1.1. Heavy Metals

Chromium is present in 39 RASFF notifications related to migration of heavy metals from FCM in 2015. Nonetheless, the migration of other heavy metals as cadmium, nickel, manganese or lead generates > 9 notifications each one in the last year (RASFF, 2015b). In particular, the migration of lead is a serious hazard in children, being recommended in the 2014 RASFF Annual Report to avoid the use of decorated items for drinking (RASFF, 2015a).

3.3.1.2. Primary Aromatic Amines

PAAs are used as starting materials in the production of articles intended to be in contact with food due to their low price and high reactivity. However, some of these compounds can remain in the material and migrate into the food. Due to the high toxicity of these amines, the specific migration limits (SMLs), established by the European legislation for these compounds are very low (< 10 µg L^{-1}) (Paseiro-Cerrato *et al.*, 2012). In 2015, 18 notifications were reported by RASFF (RASFF, 2015b).

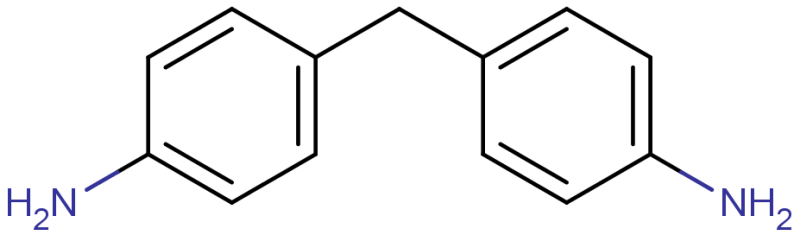


Figure 3.5: 4,4'-Methylenedianiline (MDA) has been one of the most common PAAs in FCM notifications.

3.3.1.3. Melamine and formaldehyde

The use of melamine-formaldehyde resins in the manufacture of tableware (colloquially called "melaware") is very common. Both molecules, and some melamine analogues as cyanuric acid, ammelide and ammeline, can migrate into the food, especially in the case of acidic foods at high temperatures (EFSA, 2010).

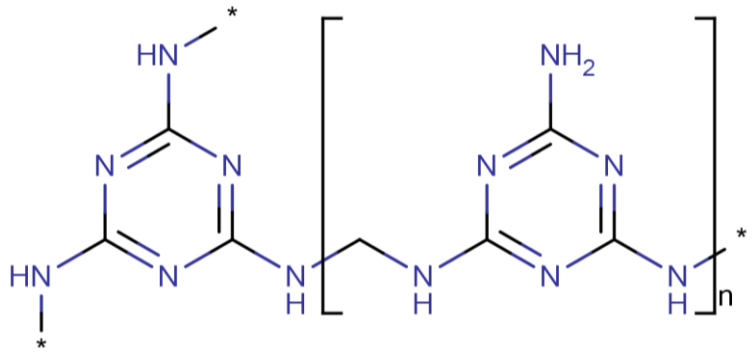


Figure 3.6: Melamine-formaldehyde polymer.

3.3.1.4. Phthalates

This chemical family is used in the manufacture of PVC or other vinyl flexible plastics. In the recent years, most of the RASFF notifications are related to Chinese/Thailand products packaged in glass jars, being the lid gaskets the source of contamination. However, there is no notifications of phthalates in European countries, due to the progressive substitution of phthalates for other plasticizers as epoxidised soybean oil (ESBO), although it has also generated three notifications in the last two years (RASFF, 2012, 2015b).

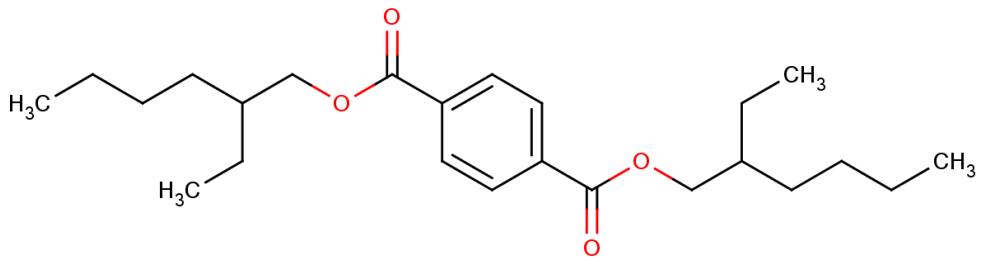


Figure 3.7: Di-octyl terephthalate (DOTP), the phthalate that have generated more notifications in 2014.

3.3.1.5. Overall Migration

In order to protect the consumers' health, the Regulation (EC) No 10/2011 establishes that, according to the Article 3 (1) (b) of Regulation (EC) No 1935/2004 and the good manufacturing practices; "it is feasible to manufacture plastic materials in such a way that they are not releasing more than 10 mg of substances per 1 dm^2 of surface area of the plastic material". So, in this basis, an Overall Migration Limit (OML) was established in 10 mg dm^{-2}, being

generated during 2014 21 notifications related to FCM that exceed this limit (RASFF, 2015b).

3.3.1.6. Photoinitiators

These components of the UV curing inks have generated a total of 147 notifications in the last decade (2005-2015) (RASFF, 2015b). They are the substances that absorb the energy from the UV light in order to start the photopolymerization process in the UV curing inks. 2/4-isopropyl thioxanthone (ITX) and benzophenone (BP) have generated more than a hundred notifications in this time; however, their incidence have decreased in the last years due to the use of alternative packaging systems and the use of new photoinitiators.

This thesis was focused in the food safety problem generated by photoinitiators, and for this reason, a deeper review of them is presented in chapters 5 and 6.

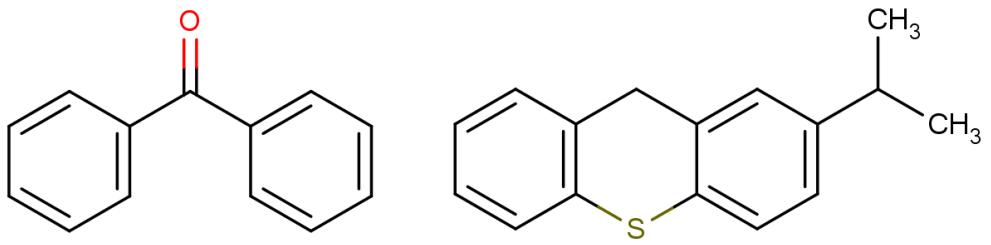


Figure 3.8: Benzophenone (BP) and 2/4-isopropylthioxanthone (ITX).

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4. OBJECTIVES

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This thesis has been carried out in the framework of the project MIGRATIN "Printing inks for food packaging. Study of the migration of photoinitiators into the food", that has financed part of the studies. The presence of photoinitiators in foods has been a matter of concern in the last years; however, some questions remain unanswered. For this reason, this project and, by extension, this thesis, was focused in this food safety problem. In order to obtain a more in-depth knowledge of this issue, this thesis has three main objectives:

- The realization of a comprehensive review about photoinitiators in order to understand the peculiarities of this food safety problem. Special emphasis should be placed in two aspects:
    - The mechanism of migration of photoinitiators into the foodstuffs
    - The current analytical methods used for their determination in FCM and foodstuffs.
- The development of different methodologies and analytical methods to:
    - Identify and quantify photoinitiators in FCM.
    - Determine the *set-off* migration of photoinitiators, by-products and other print related molecules.
    - Determine the degradation products of the photocuring process of model photoinitiators and amine synergists
- Study the migration kinetics of six model photoinitiators, at all the common temperatures of food storage, into water and into the food simulants present in the current legislation (except for dry foods), from LDPE. The obtained data were applied to a mathematical model, obtaining the migration key parameters: partition and diffusion coefficients. These parameters allow the prediction of the photoinitiators' migration into foodstuffs.

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5. PHOTOINITIATORS: A FOOD SAFETY REVIEW

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5.1. ABSTRACT

UV inks are considered safer than the classical inks; however, despite being on the outer surface of the packaging material, their components can migrate into foodstuffs and can give rise to contaminations. The photoinitiators are a part of printing inks formulation, being an important class of migrant, for which there have been more than 100 incidents of packaged food reported through RASFF (Rapid Alert System for Food and Feed) alerts in the EU.

In this review the process of photopolymerization is explained in depth to provide an insight into the complexity of the process, and the diversity of potential contaminants together with their degradation products. The critical factors affecting the migration process itself are reviewed, together with analytical methods and the current legislation in the UE and other parts of the world.

Keywords: photoinitiator, photopolymerization process, migration, food safety and food contact materials.

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5.2. INTRODUCTION

The energy of UV light can be used in the formation of polymeric materials. This fact was exploited by researchers leading to a huge development in laboratories and industry since 70's. From this decade the UV photocuring process has allowed the development of new photoinitiators, which have been used in biocompatible processes and in a wide variety of materials like coatings, varnishes, graphic arts, high-speed printing, metal decorating, adhesives, laminates, printed circuit boards, imaging, dentistry and cosmetics (Allen, 1996; Decker, 1996; Green, 2010).

The main advantages of UV photocuring over other curing methods are: the speed of the process and the high polymerization rates reached under UV irradiation that allows the transformation of liquid resins into solid and insoluble polymers in short times; moreover, it is a process carried out at room temperature and, unlike the classical solvent-based inks, the printing process occurs in a relative absence of residues (Fouassier, 1995; Decker, 1996, 2002; Papilloud & Baudraz, 2002a; Arsu, 2006a; Rothenbacher *et al.*, 2007).

The UV photocuring process has been extensively discussed by authors like Fouassier (1995), Allen (1996), Decker (1996) and many others in different textbooks and reviews. Nevertheless, most of these works were focused on the fundamentals of the UV photocuring and its industrial applicability, and considering that over 90 % of all manufactured foodstuffs are sold in printed package, the attention to food contact materials (FCM) is very scarce and even less to its food safety (Leks-Stepien, 2011).

Taking into account the above mentioned and that more than a hundred cases of photoinitiators presence in foodstuffs were reported by the European authorities (RASFF, 2014) more information about the whole photocuring process and migration of these molecules is needed.

This comprehensive review tries to fill this gap addressing four main themes: a) description of the photocuring process with the main types of photoinitiators used in FCM and the novel options; b) discussion about the migration processes from the package surface to the foodstuffs with the analytical methods developed for their determination in foods and packages; c) study of three photopolymerization cases and the toxicological study of the photoproducts created in the process; and d) description of the current legislations for UV inks in USA, Europe and Japan.

5.3. PHOTOCURING PROCESS

The UV photocuring process is based on the absorbance of UV irradiation. The substance responsible for capturing that energy from photons is called photoinitiator (the monomer does not usually absorb that energy). In response to the photons, the photoinitiator produces reactive species that can initiate the process of polymerization (Arsu *et al.*, 2009; Green, 2010).

The overall procedure of polymer formation after receiving UV energy by the photoinitiator has four stages: initiation, propagation, chain transfer and termination (Ledwith, 1977; Fouassier & Rabek, 1993b; Fouassier, 1998; Andrezejewska *et al*., 2006; Arsu *et al.*, 2009; Green, 2010; Lalavèe *et al.*, 2011), represented in figure 5.1.

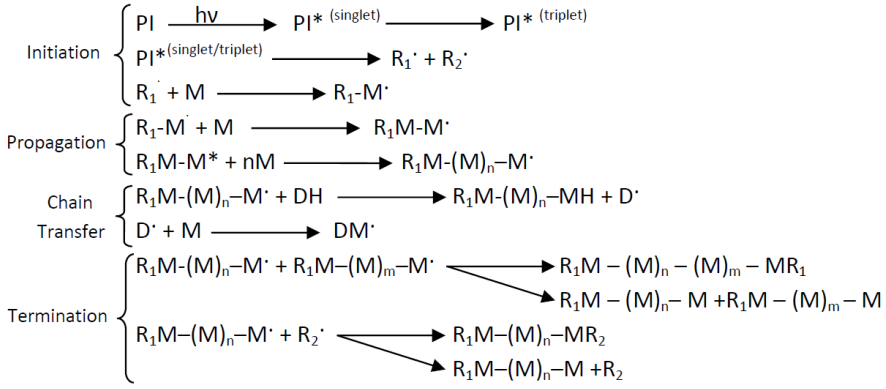

Figure 5.1: General UV photocuring steps. PI: photoinitiator, M: monomer, D: donor; R: radical.

The proper selection of the photoinitiator is a critical step in the photocuring process due to the fact that the photochemical and photophysical properties of each photoinitiator have an impact on the final polymer (structure, cured speed, by-products, etc.). Regarding this, several photoinitiator features should be addressed before making a selection for the overall formulation (Ledwith, 1977; Allen, 1996; Fouassier, 1998; Allen *et al.*, 1999a; Arsu *et al.*, 2009; Green, 2010):

- Radical formation is enhanced when the chosen photoinitiator has a maximum absorption peak at the wavelength emitted by the UV lamp and a high quantum yield.
- The solubility of the photoinitiator affects the stability of the formulation.
- High storage stability and easy handling of the molecule.
- The photoinitiator should not stimulate the generation of odors, volatile compounds or toxic photoproducts. Neither the photoinitiator should cause any yellowing or discoloration on the final surface of polymer.
- Two essential aspects must be minimized: the migration of photocuring components or by-products and, the economic cost of the process.

These are the main aspects to assess when selecting a photoinitiator for a specific formulation, being the last two features of the most important taking into account the safety of FCM. However, there are other factors that can affect the photocuring process like the oxygen inhibition, the film thickness, the use of pigments, etc. All of them are important factors that also affect the properties of the final coating. Accordingly its characteristics must to be defined and then the above mentioned aspects should be adjusted to obtain the desired coating.

5.3.1. PHOTOINITIATOR TYPES

There are two main types of photoinitiators: free-radical and cationic photoinitiators. Other photoinitiators like the anionic photoinitiators will not be

considered in this review due to the scarce information related to its use in food packaging materials.

5.3.1.1. Free-radical photoinitiators

These are the classical photoinitiators, the most studied and their reaction mechanisms are well established. There are two main types of radical photoinitiators: Type I, which are one component photoinitiators α- or β-cleavable and Type II, two component photoinitiators, based on a hydrogen transfer reaction between a photoinitiator and a co-initiator.

5.3.1.1.1. Type I

When incident UV light is absorbed by the type I photoinitiators, a homolytic cleavage process starts once the molecules reach the excited singlet or triplet state. This cleavage creates free radicals, regularly from Norrish type I reactions (photochemical cleavage or homolysis of aldehydes and ketones arising two free radical intermediates), which can induce the polymerization process.

The cleavage occurs at any weak bond, but usually takes place at α position of carbonyl group (α-cleavage), and less probably is the β-cleavage. Frequently, some type I photoinitiators, like methyl-1-(4-methylthio)phenyl-2-morpholinopropan-1-one (MMMP) may suffer the two types of scission (with α-cleavage predominance) (Fouassier, 1995; Arsu *et al.*, 2009; Green 2010). The possible scissions and the main chemical families of type I photoinitiator are represented in figures 5.2 and 5.3:

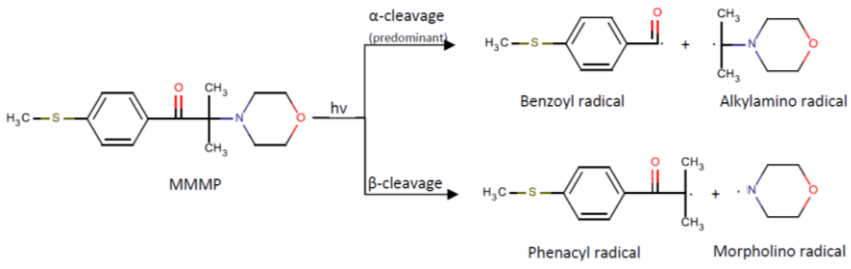

Figure 5.2: Photocleavage of MMMP.

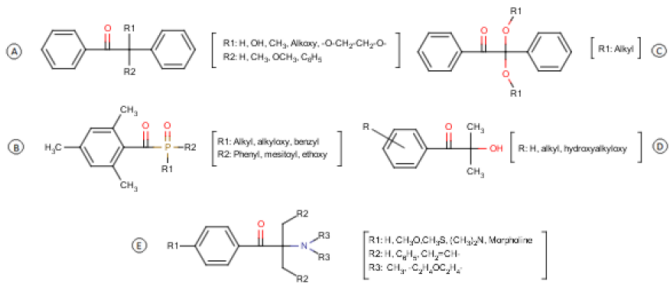

Figure 5.3: Main families of type I photoinitiators. A: benzoin derivatives, B: phospine oxides, C: benzyl ketals, D: hydroxyacetophenones (HAP's) and E: α-aminoalkylacetophenones (AAAP's).

- Hydroxyacetophenones (HAP's), used since the 70's. These compounds have a strong light absorption between 230-270 nm and weaker up to 360 nm. The triplet's lifetimes of these substances are longer than for other type I photoinitiators, which is the reason why these compounds can be quenched and consequently suffer minor cleavage rate (Allen *et al.*, 1999b; Green, 2010).

- Benzylketals, versatile family of substances that absorbs between 230-270 nm, with very short triplet lifetime and a high rate of photocleavage. The principal drawback is the coating surface yellowing and, in some cases, as for example in the case of 2,2-dimethoxy-2-phenylacetophenone (BDK), the generation of by-products like methyl benzoate which makes it not suitable for food packaging applications. Nevertheless, this substance is listed in the EuPIA (European Printing Inks Association) *Suitability List of Photoinitiators for Low Migration UV Printing Inks and Varnishes* of February 2013 (Decker, 1996; Segurola *et al.*, 1999a; Allen *et al.*, 1999b; Green, 2010; EuPIA, 2013a).

- Benzoin derivatives, represent the more heterogeneous family and the most used of type I photoinitiators. They have a strong absorption between 300-400 nm and a relatively short-lived triplet state. Among them, benzoin ethers are included, which were extensively used compounds in the past, and absorb between 230-270 nm. Benzoin ethers have been gradually replaced by the hydroxyacetophenones, mainly due to their poor shelf life caused by thermal instability (Arsu *et al.*, 2009; Green, 2010).

- α- Aminoalkylacetophenones (AAAP's). Their N substitutions make them highly reactive compounds, with strong absorption in the mid UV range at 280-350 nm, being useful in applications different from those of the above mentioned families. Some AAAP's , such as MMMP, produce yellowing and odor but other components like 2-benzyl-2-dimethylamino-1-(4-morpholinophenyl)-butan-1-one (BDMB) does not pose this problem. Moreover, some substances of this family, like BDMB, can also suffer β-cleavage (Rist *et al.*, 1992; Segurola *et al.*, 1999b; Allen *et al.*, 1999b; Arsu, 2006; Arsu et al, 2009; Green, 2010).

- Phospine oxides, with excellent properties for depth cures, as a result of their small absorption near the visible light (350-420 nm), and for curing processes in presence of Ti_2O. Although phosphine oxides are yellow colored products, the resulting coating turns white due to the subsequent photobleaching by UV or daylight radiations on the phosphine oxides previously unreacted (Segurola *et al.*, 1999b; Corrales *et al.*, 2003; Arsu, 2006, 2009; Green, 2010).

Other type I photoinitiators, but less used in FCM, include: o-acyl-α-oximino ketones, HABI's, acyloximino esters, α-haloacetophenones, trichloromethyl-S-triazine and photobase/acid generators.

5.3.1.1.2. Type II

The absorption of UV light by these photoinitiators leads to an excited state and, in this state, these photoinitiators, in contrast to type I photoinitiators, cannot spontaneously generate radicals. This is because CO–aryl bond energies are too high to be broken by UV energy, so the presence of a coinitiator is necessary. The problem with these systems lies on the long triplet lifetime that makes them more sensitive to quenching.

The coinitiator may function in two different ways: hydrogen abstraction (from an H donor compound, or the environment), and through electron transfer (from an electron donor and subsequent proton transfer step). The most widely used coinitiators are tertiary amines, which also act as oxygen

scavengers; other alternatives include alcohols, ethers and thiols, but with less efficiency (Fouassier & Rabek, 1993a; Fouassier, 1995, 1998; Allen, 1996; Allen *et al.*, 1999a; Arsu & Aidin, 1999; Andrzejewska *et al.*, 2006; Yagci *et al.*, 2006; Arsu *et al.*, 2009; Green, 2010; Fouassier & Lalevèe, 2012).

The efficiency of tertiary amines is inversely proportional to the ionization potential of the amine, so the most active radicals are the ones generated from the weakest amines. The reactivity and efficiency of these molecules are increased with the incorporation of hydroxyl groups in their chains (Green, 2010).

But, the amines have a big drawback, because the photoyellowing effect appears in these formulations due to post-cure oxidative processes. For this reason, in high-quality works, the type II photoinitiators are not so commonly selected as the first choice and, type I photoinitiators are preferred (Green, 2010; Fouassier & Lalavée, 2012a).

In the figure 5.4 the two major families of type II photoinitiators and its photoinitiation mechanism are represented:

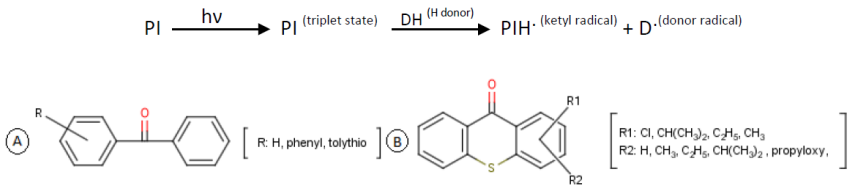

Figure 5.4: General steps of a type II photoinitiator leading to radical generation and the main families: A: benzophenone and B: thioxantone.

- Benzophenones, one of the most worldwide photoinitiators used due to its characteristics and low cost, with strong absorption between 230-260 nm and weak around 330 nm. Their properties make them an excellent choice alone or in combination with other photoinitiators. There are also many derivatives with strong absorption in the 280-330 nm range and similar reactivity to type I photoinitiators (Arsu *et al.*, 2009; Green, 2010).

- Thioxanthones, with maximum absorption at 380-420 nm. They are extensively used alone or with other photoinitiators, having the advantage of

does not produce photoyellowing, and also their excellent behavior as sensitizers. Polymeric thioxanthones are designed with very low migration rate for food packaging use (Arsu *et al.*, 2006b, 2009; Green, 2010).

Less important are the anthraquinones, increasingly used as sensitizers (Crivello & Jang, 2003), camphorquinone (mainly used in dentistry) (Andrzejewska *et al.*, 2006) and benzylformate esters which suffer Norrish type II reactions through intramolecular hydrogen abstraction (Hu *et al.*, 2000).

5.3.1.2. Cationic photoinitiators

In the last two decades, this type of photoinitiators have experimented a boost in the industry due to their advantages over the free-radical photoinitiators. Features like insensitivity to oxygen quenching, very low shrinkage, excellent adhesion and resistance allow to producing more uniform and stable polymers (Crivello & Sangermano, 2001; Decker *et al.*, 2001; Crivello *et al.*, 2003; Yagci, 2004; Aydogan *et al.*, 2006; Yagci *et al.*, 2006; Green, 2010).

Since Crivello & Lam described the photochemical mechanism of iodonium salts (1977, 1979), other onium salts have been described and used. An overview of iodonium salts' photochemical mechanism is shown in Figure 5.5 with the general structure of the major chemical families of cationic photoinitiators. In this figure, the final H+ belongs to a Brønsted acid, responsible for the polymerization initiation (Abdul-Rasoul *et al.*, 1978; Yagci, 1989, 1999; Decker, 1996; Yagci *et al.*, 2006; Green, 2010).

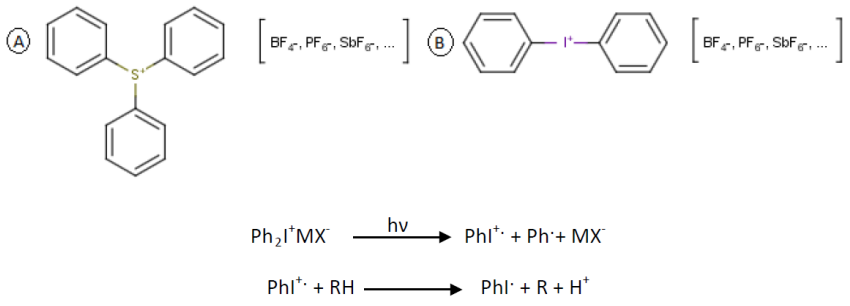

Figure 5.5: Main families of cationic photoinitiators: A: triarylsulphonium salts and B: diaryliodonium salts and the general mechanism of cationic fotoinitiation for diaryiodonium salts.

Also, it is frequent the use of a radical photoinitiator to sensitize cationic photoinitiators in order to increase the range of absorption from 220-310 nm to 300-400 nm (Yagci, 1989, 2004; De Voe *et al.*, 1990; Aydogan *et al.*, 2006; Green, 2010). Nowadays, this type of photoinitiators includes the following families:

5.3.1.2.1. Sulphonium salts

Usually triarylsulphonium salts. They have strong absorption at 220-230 nm and weak around 300-325 nm. These salts have a good thermal stability and reactivity that result in fast surface curing. To increase the reactivity, it is usual the thiophenyl substitution which changes the absorption pattern to 300-400 nm (Green, 2010).

5.3.1.2.2. Iodonium salts

Usually diaryliodonium salts. They have a similar thermal stability to sulphonic salts (Dektar & Hacker, 1990a), but lower reactivity because their absorption pattern is at 220-270nm. To achieve the reactivity of sulphonic salts it is necessary to sensitize them with other radical photoinitiators, such as isopropylthioxanthone (ITX) (Green, 2010).

But the photolysis of the first generation of iodonium and sulphonium salts has a serious drawback, because these photoinitiators yields benzene or toluene. For this reason the following families were designed to avoid this weak point (Fouassier & Lalavée, 2012b; Fouassier, 2013). Also, in the case of sulphonium salts this kind of cationic photoinitiators leads to a residual odor that decreases on light exposure.

Related with the toxicity, it is important to take into account the anion because e.g. the use of antimony is not suitable in printing inks for FCM due to their demonstrated toxicity (EPA, 2000; Green, 2010). Despite this, different photoinitiators included in the Suitability List of Photoinitiators for Low Migration UV Printing Inks and Varnishes provided by the EuPIA have the anion SbF_6^-.

Other cationic photoinitiators with minor impact are the ferrocenium salts. These substances produce excellent cures in whole depth, being the main disadvantage their poor solubility (Green, 2010).

5.3.1.3. Novel Options and the future

In the recent years the photoinitiators' chemistry followed different routes to obtain more reactive, greener and cheaper photoinitiation systems. Nevertheless, its introduction in FCM is slow and this fact is confirmed by the suitability list proposed by the printing industry (EuPIA, 2013a).

In pursuing a greener chemistry, the industry diminished the energy sources, the lamps and other parts of the photopolymerization process, developing new light sources, using natural light or even searching for natural products or renewable monomers that could be biodegradable (Tefhe, 2013). But, the attempts to decrease the migration were in other directions.

A few years ago, the concept of "Low Migration Inks" was introduced by the industry. These inks are specially designed for food packaging and must fulfill the requirements set out in legislations (de Mondt, 2012). Most of current photoinitiators have a molecular weight under 500 Da (the vast majority is around 250 Da or less) and the scission products are smaller; all these molecules, in appropriate circumstances, are likely to migrate to foodstuffs and could be a risk for the human health. For this reason, it is necessary to minimize the migration of these compounds (Dietliker, 2007).

The more easy way to avoid the migration is the development of compounds with higher molecular weight. Examples of these inks are polymeric or oligomeric photoinitiators or macrophotoinitiators (Fouassier & Lalavée, 2012b; Bauer, 2014). This kind of photoinitiators usually has a molecular weight > 1,000 Da and for this reason are without toxicological concern. These new molecules need to have: high reactivity and solubility, low volatility, not release odorous compounds; and without photoyellowing effect (Fouassier & Lalavée, 2012b).

There are many different proposed macrophotoinitiators which combines different kinds of photoinitiators with amine synergists, sensitizers, chromophores and even other photoinitiators (of the same or different type). There are also innovative ideas with the structure of the polymer photoinitiators, being proposed linear structures, pendant initiator species, initiator species within the chain itself, or dendritic structures (Green, 2010; de Mondt, 2012). Other recent option is the possibility to anchor the photoinitiator to a nanoparticle to increase the size and thus minimize the migration ratios (Bauer, 2014).

The use of Multicomponent Photoinitiating Systems is another novel alternative. The use of various components leads to an increase in the efficiency of the photoinitiating system being very useful in high speed cures. The Generally Encountered Mechanism is the most used system, being based in the addition of a quencher to promote the efficiency of the initiator radical by the extinction of the concomitant radical that could scavenge the growing polymer chain (Green 2010; Fouassier & Lalavée 2012b).

There are more Multicomponent Photoinitiating Systems, with three components or even with four components but the photopolymerization process is beyond this review. Examples of molecules that can participate in these systems are dyes and other photoinitiators that have been already discussed in the previous parts of the review.

Other alternative is the use of UV-inks without photoinitiators, self-initiating resins, but these systems have produced historically low-efficiency processes. Nowadays, new approaches based on charge-transfer systems are developed and have increased the photopolymerization rate (Dietliker, 2007; Fouassier & Lalavée, 2012b). Related to the non-use of photoinitiators, fillers as magnetite nanoparticles have exceptional photoinitiator activity and could be a good alternative too (Bauer, 2014).

There is another technology that can be part of the future of the ink industry in the following years: the electron beam curing, process that provides

some advantages over the UV-curing as: instantaneous complete curing, no requirement of photoinitiators, and the possibility of curing thick and pigmented coatings (Kumar, 2013; Bauer, 2014).

Other future options have been discussed by different authors as the use of other bonds instead the typical C-C bond (e.g: C-Ge, C-Si, Si-Si, etc.) (Fouassier & Lalavée, 2012b); however, these options are currently on their early stages, and probably will not achieve relevance in the following years in FCM

5.4. MIGRATION PROCESSES

Prior to begin with the presentation and discussion of the previous and current legislations, the possible migration ways followed by photoinitiators to reach the foodstuffs must be described for a better understanding of the problem.

Since in 2005 the RASFF was informed by the Italian authorities of the presence of ITX, a photoinitiator used in inks and varnishes, in baby milk packaged in beverage cartons (RASFF, 2005), many other alerts related to substances used in printing inks have been notified, being more than a hundred cases since then and eighteen notifications since 2010 (RASFF, 2014; Aparicio & Elizalde, 2015).

A review of RASFF alerts and literature in more detail allows drawing the following conclusion: the photoinitiators can reach the food from different kinds of packages (cartonboards, paperboards, different plastics and even multilayer packages) by two different ways: indirect and direct transfer (Choi *et al.*, 2002; Bradley *et al.*, 2005):

- Indirect transfer: the photoinitiator or by-product could reach the foodstuff through the vapor. This is the case of semi-volatile photoinitiators as benzophenone and derivatives (Rothenbacher *et al.*, 2007; Pastorelli *et al.*, 2008; Sanches-Silva *et al.*, 2008a; Koivikko *et al.*, 2010).

- Direct transfer: the photoinitiator goes from the printed surface to the food by diffusion or by *set-off* effect. The low molecular weight molecules can diffuse through the different layer/s of the package but this phenomenon is slower for components of high molecular weight (photoinitiators or pigments). The *set-off* effect could be defined as the transfer of printing inks components from the external layer to the inner one when they are in contact during storage of packages in reels or stacks until final assembling. The *set-off* should be the main source of contamination of foodstuffs with photoinitiators due to the step of passing through the polymeric matrix of the package is avoided (Bradley *et al.*, 2005; Jickells *et al.*, 2005; Rothenbacher *et al.*, 2007).

There are three different *set-off* mechanisms: blocking, rubbing and peeling. In the first one, the ink in the external layer is detached when the stock is separated for its use when all the packages are stored in reels, stacks or nests. The rubbing happens when the printed layer suffers abrasive damage during the storing, and finally, the peeling occurs when the combination ink-substrate is not adequate and the ink is separated from the surface. To avoid the *set-off* effect and to ensure the quality of the coating, different strategies are followed like the use of lacquers or varnishes or the selection of the correct match of inks with the substrate to achieve a good adhesion between them.

These are the main proposed mechanisms that could explain the presence of these components in the foodstuffs. Other authors proposed that the wetting contact could be another possible way of migration, but its impact is smaller and less studied in photoinitiators (Jung *et al.*, 2013).

In addition it is also necessary to introduce the significance of to study their kinetics to assess the hazard. To study the migration kinetics it is usual to carry out the migration experiments, but this fact is expensive, time-consuming and, in some cases, complicated due to the complexity of food matrixes, or the low concentration of the migrants (Lau *et al.*, 2000; Brandsch *et al.*, 2002; Helmroth *et al.*, 2002a, 2002b; Petersen *et al.*, 2005; Sanches Silva *et al.*, 2006, 2007, 2009, 2010). To avoid these experiments, the current legislation allows the performance of theoretical predictions based on scientific evidences (EC, 2011).

The mass transfer is a predictable physical event. The process of mass transfer from plastic materials into foodstuffs obeys to a differential equation: the Fick's Second Law. Crank (1975) developed analytical solutions to this equation that today are used to predict the two capital parameters that allow the prediction of the migration phenomenon: the partition coefficient ($K_{P/F}$) of the migrant between the polymer and the food and the diffusion coefficient (D_P) of the migrant in the polymer. With these two parameters, applying the Arrhenius equation, the speed and magnitude of the migration can be predicted (Piringer, 1991; Brandsh *et al.*, 2002; Sanches-Silva *et al.*, 2008, 2010; Simoneau, 2010).

5.5. ANALYTICAL METHODS TO DETERMINE MIGRATION OF PHOTOINITIATORS

After describing the different migration processes followed by photoinitiators (and its by-products) to reach the food, it is necessary to describe how it could be performed its determination in food and FCM. Nevertheless, this is a hard task due to three main reasons: a) only photoinitiators are expected to be in UV inks formulations, there is no statement of the presence of by-products, so their presence is not expected b) except for photoinitiators, there are no information about them or it is scarce, and c) all these compounds take part of a very heterogeneous group of molecules and so their physico-chemical properties are clearly different. To make this evident in the table 5.1 are shown the range of some physic-chemical properties values between the different photoinitiators that take part in the EuPIA suitability list (EuPIA, 2013a).

Physico-chemical properties	Ranges	
	Smaller value	Higher value
Molecular weight* (D)	MBF (164.16)	RHO 2074 (1,016.25)
T/V† (Topological Polar Surface Area/ Van der Waals Surface Area)	DBA (0.03)	2-DMEB (0.9)
Vapor Pressure‡ (mmHg) (25 ºC)	o-cl-HABI (2.63E-2)	MBF (2.58E-26)
Melting point (ºC)	2-MBP (-18.00)	EHA (243.00)
Boiling point‡ (ºC)	CPQ (226.50)	o-cl-HABI (810.30)
Log P‡ (o/w)	OPAc (0.63)	o-cl-HABI (11.15)
Water Solubility‡ (mg L^{-1}) (25 ºC) (pH=7)	o-cl-HABI (4.40E-5)	2-DMEB (1.40E+5)

Table 5.1: Ranges of photoinitiators physic-chemical properties obtained from Scifinder web version. *: The polymeric photoinitiators without defined molecular weight are not considered. ‡: estimated data. †: obtained with Jchem® software (version 6.2) by ChemAxon®.

For these reasons, in the reviewed literature only methods for the determination of the photoinitiators and related starting substances are developed. The main techniques used were Liquid Chromatography (LC) and Gas Chromatography (GC). Lately, another useful technique developed was Direct Analysis in Real Time coupled to a mass detector (DART-MS).

In the tables 5.2 to 5.5, important data about the methodology to extract the photoinitiators from the food/FCM and the analysis of the samples are described. The tables 5.2 and 5.3 described the most followed technique of determination and/or quantification of photoinitiators in foodstuffs and FCM, which is the LC. This is the most common analytical technique because offers to the analyst the possibility to select between different methodologies in function of requirements as: cost, time, determination or quantification of one or several components and detectors (diode array (DAD), fluorescence (FLD), mass (MS) or mass/mass (MS/MS), so nowadays is probably the most used as routine in food safety laboratories.

The HPLC-DAD is the option selected for current methods for its combination of low cost and low time analysis. Most of the analyzed photoinitiators could be determined by this technique with acceptable limits of detection in the range of µg of photoinitiator/kg of foodstuff (Sanches-Silva *et*

al., 2008c). The lower LOD values were founded for ITX in foodstuffs by Jung *et al.* (2010) with a LOD = 0.4 μg kg^{-1} and for various photoinitiators in package by Lago *et al.* (2014) with LODs = 0.31 μg dm^{-2}, However, in the case of polymeric photoinitiators, as benzophenone acrylate the LOD is 1.22 mg dm^{-2}, this increase is due to the chromatographic resolution, yielding a sum of peaks rather than a single peak in the chromatogram depending on the monomer units of the polymer (Lago *et al.*, 2014).

If more accuracy is needed, the LC-MS/MS methodologies are the chosen option. This technique reaches a LOD = 2 ng kg^{-1} foodstuff by Gallart-Ayala *et al.* (2014). The gas chromatography (GC) is also a technique very common for the determination of benzophenone related compounds as can be seen in tables 5.4 and 5.5. As LC, GC offers the opportunity to perform different methods depending on its objectives and requirements, using a non-specific detector as flame ionization (FID) or specific as mass (MS) or mass-mass detector (MS/MS). The obtained LODs by GC reach values of 0.1 μg kg^{-1} (Allegrone *et al.,* 2008; Gil-Vergara *et al.,* 2008). GC-MS technique reaches LODs almost comparable to LC-MS/MS.

Nowadays these two techniques are the methods of reference, but in the recent years the DART-MS gain ground to LC and GC because its big advantage: there are no sample treatment (extraction and concentration steps) with the consequent saving of time. But DART-MS also has drawbacks, the price of the equipment, and the lack of sensitivity due to the absence of extraction or concentration steps. Furthermore is not highly quantitative, because the ion intensities are variable. Bentayeb *et al.* (2012, 2013) have reported LODs between 0.20 mg dm^{-2} and 20 mg dm^{-2}.

Method	Photoinitiators	Detector parameters	Analytical column	Mobile phase	Type of Sample	Extraction procedure	Reference
HPLC-DAD/FLD	II: 2-ITX	DAD: 260nm FLD exc: 272nm FLD em: 440nm	LC-PAH Supelcosil (250mm x 4.6mm i.d.; 5µm particle size)	A: H2O B: AcN Isocratic	Food FCM	LLE (QuEChERS)	Rothenbacher *et al.*, 2007
	I: MMMP II: ITX Amine syn: EDB	DAD: 260nm FLD exc: 264nm FLD em: 440nm	Luna C18 (2) (100mm x 2mm i.d.; 3µm particle size)	A: H2O B: AcN Gradient	Food	LLE (QuEChERS)	Jung *et al.*, 2010
HPLC-DAD	I: HCPK, BDK, MMMP II: BP, ITX Amine syn: EHA	HCPK: 246nm BDK, BP: 256nm MMMP: 306nm EHA: 310nm ITX: 386nm	Kromasil 100 C18 (150mm x 4mm i.d; 5µm particle size)	A: H2O B: AcN Gradient	Food FCM	LLE	Sanches-Silva *et al.*, 2008a
	I: HCPK, BDK, MMMP II: BP, ITX Amine syn: EHA	HCPK: 246nm BDK, BP: 256nm MMMP: 306nm EHA: 310nm ITX: 386nm	Kromasil 100 C18 (150mm x 4mm i.d; 5µm particle size)	A: H2O B: AcN Gradient	Food Simulants	Direct Injection	Sanches-Silva *et al.*, 2009
	II: BP	256nm	Kromasil 100 C18 (150mm x 4mm i.d; 5µm particle size)	A: H2O B: AcN Gradient	Food FCM	LLE	Pastorelli *et al.*, 2008
	I: HCPK, BDK, MMMP II: BP, ITX Amine syn: EHA Cat: BIS, THIO	---	CN Nucleosil 100-5 (250mm x 4.6mm)	A: H2O (TFA/TBAP) B: MeOH C: AcN Gradient	Food Simulants	SPE	Papilloud & Baudraz 2002a, 2002b
	I: HCPK II: BP, ITX Amine syn: EDB, EHA	HCPK: 245nm BP, ITX: 254nm EDB, EHA: 310nm	Luna C18 (250mm x 4.6mm i.d.; 5µm particle size)	A: H2O B: MeOH Gradient	Food	1st LLE 2nd SPE	Sagratinit *et al.*, 2008
	II: BP	BP: 256nm	Diamonsil C18 (250mm x 4.6mm i.d. x 5µm particle size)	A: H2O B: AcN Gradient	Food Simulant	LLE	Li *et al.*, 2012

I: HCPK, BDK, MMMP II: BP, ITX Amine syn: EHA	HCPK: 246nm BDK, BP: 256nm MMMP: 306nm EHA: 310nm ITX: 386nm	Kromasil 100 C18 (150mm x 4mm i.d; 5µm particle size)	A: H2O B: AcN Gradient	Food FCM	LLE	Sanches-Silva *et al.*, 2008b, 2008c
II: 2-HBP, 4-HBP, 4-MBP, MBB, DEAB, PBZ, BP	2-HBP, 4-HBP, 4-MBP, MBB, DEAB, BP: 254nm PBZ: 290nm	Kromasil 100 C18 (250mm x 4mm i.d; 5µm particle size)	A: H2O B: AcN Gradient	Food FCM	LLE	Rodríguez-Bernaldo de Quirós *et al.*, 2009
II: MK, DEAB, DMAB	350nm	Zorbax SC85 (250mm x 4.6mm i.d; 5µm particle size)	MeOH:H2O (4:1) Isocratic	FCM	LLE	Castle *et al.*, 1997
II: 2-HBP, 4-HBP, 4-MBP, MBB, DEAB, PBZ, BP, BPAcr Amine syn: EHA	BP, BPAcr: 256nm 4-MBP: 260nm PBZ: 290nm	Eclipse XDB-C18 (250mm x 4.6mm i.d. x 5µm particle size)	A: H2O B: AcN Gradient	FCM	LLE	Koivikko *et al.*, 2010
I: HCPK II: 4-MBP, PBZ, MBB, 4-HBP, DEAB, MK, DMAB Amine syn: EDB, EHA	HCPK, MB: 245nm BP, 4-MBP: 255nm 4-HBP, PBZ: 290nm EHA, EDB: 310nm MK, DEAB, DMAB: 365nm	Star RP-18e (250mm x 4mm i.d. x 5µm particle size)	A: H2O B: AcN Gradient	FCM	LLE	Jung *et al.*, 2013

Table 5.2: HPLC-DAD/FLD methods for photoinitiator analysis.

Method	Photoinitiators	Analytical Conditions		Analytical column	Mobile phase	Type of Sample	Extraction procedure	Reference
		Source / Detector	m/z					
LC-MS	I: HCPK, DMPA, MMMP II: BP, ITX Amine syn: EHA	ESI (+) TOF	HCPK: 227 DMPA: 279, 225 MMMP: 280 BP: 183, 205 ITX: 277, 255 EHA: 278	Zorbax Eclipse XDB C18 (150 mm x 4.6 mm i.d.; 5 µm particle size)	A: AcN (0.1 % FA) B: H_2O (0.2 % FA) Gradient	Food FCM	LLE	Sanches-Silva *et al.*, 2008a
	II: ITX	ESI (+) Q	255 → 213	Gemini C18 (100 mm x 2 mm i.d.; 5 µm particle size)	A:MeOH B: AF 20 mM Gradient	Food	LLE	Benetti *et al.*, 2008
	I: HCPK II: BP, ITX Amine syn: EHA, EDB	ESI (+) Q	HCPK: 227 BP: 205 ITX: 303 EHA: 277 EDB: 216	Luna C18 (150 mm x 4.6 mm i.d.; 5µm particle size)	A: H_2O B: MeOH Gradient	Food	1st LLE 2nd SPE	Sagratini *et al.*, 2008
	II: ITX	ESI (+) / Q ESI (+) / QIT	277 255 → 213	Luna C18 (150 mm x 4.6 mm i.d.; 5µm particle size)	A: H2O B: MeOH Isocratic	Food	PLE	Sagratini *et al.*, 2006
	II: 4-MBP, PBZ, MBB, 4-HBP, DEAB, MK, DMAB Amine syn: EDB, EHA	ESI (+) / Q	4-HBP: 199 → 121, 105 MBB: 241 → 209,152,105 BP: 183 → 105 EDB: 194 → 179, 166, 134 DMAB: 226 → 148, 120 , 105 4-MBP: 197 → 105 MK: 269 → 148, 120, 105 PBZ: 259 →181, 152, 105 DEAB: 325 → 281, 176, 133 EHA: 278 → 166, 151, 134	Hypersil GOLD (100 mm x 2.1 mm i.d. x 3 µm particle size)	A: MeOH (AF 5mM/FA 0.1 %) B: H_2O (AF 5 mM/FA 0.1 %) Gradient	Food	LLE	Jung *et al.*, 2013
LC-MS/MS	II: 2-ITX	ESI (+) QIT	255 → 213,184	BDS Hypersil C18 (100 mm x 3 mm i.d.; 5 µm particle size).	A: H_2O (0.1 % FA) B: MeOH Gradient	Food FCM	1st LLE 2nd SPE LLE	Sun *et al.*, 2007

I: HCPK, DMPA, HMPP II: BP, PBZ, DEAB, 2-ITX, 4-ITX, DETX Amine syn: EDB, EHA	ESI (+) QqQ	HCPK: 205 → 165,187 DMPA: 225 → 197,105 HMPP: 165 → 91,119 BP: 183 →105,77 PBZ: 259 → 105,181 DEAB: 325 → 176, 281 2-,4-ITX: 255 → 213,184 DETX: 269 → 241,213 EDB: 194 → 151,134 EHA: 278 → 151,134	Discovery HS F5 (150 mm x 2.1 mm i.d.; 3µm particle size)	A: AcN B: H_2O (AF/FA 25 mM) Gradient	Food FCM	1st LLE 2nd SPE (QuEChERS) LLE	Gallart-Ayala *et al.*, 2011
I: HCPK II BP, ITX Amine syn: EHA, EDB	ESI (+) QIT APPI (+) QIT	HCPK: 227 BP: 183 → 105 ITX: 255 → 213 EDB: 194 → 166 EHA: 278 → 166	Luna C18 (250 mm x 4.6 mm i.d.; 5 µm particle size)	A: H_2O (5 mM AF + 0.1 % FA) B: MeOH Gradient A: H_2O B: MeOH Isocratic	Food FCM	1st LLE 2nd SPE	Sagratini *et al.*, 2008
II: 2-ITX Amine syn: EHA	ESI (+) QqQ	2-ITX: 255 → 184,213 EHA: 278 → 166,151	SunFire C18 (50 mm x 2.1 mm i.d.; 3.5 µm particle size)	MeOH: H_2O (AF 10 mM) (80:20) Isocratic	Food	A: PLE B: LLE	Gil-Vergara *et al.*, 2007
II: ITX	ESI (+) QqQ	255 → 213 255 → 184	Luna C18 (250 mm x 4.6 mm i.d.; 5 µm particle size)	A: H_2O B: MeOH Isocratic	Food	PLE	Sagratini *et al.*, 2006
II: 2-ITX, 4-ITX	Turboions pray source (+) QqQ	255 → 213,184	Luna C8 (2) (50 mm x 2 mm i.d.; 5 µm particle size) Discovery ZR-PS (150 mm x 2.1 mm i.d.; 3 µm particle size)	A: H_2O (0.05 % AcA) B: AcN Gradient	Food	LLE	Bagnati *et al.*, 2007
I: HCPK, DMPA II: BP, 4-MBP, 2-ITX, MBB, PBZ Amine syn: EDB, EHA	--	--	--	--	Food FCM	1st LLE 2nd SPE (QuEChERS) LLE	Biederman *et al.*, 2013

II: 2-ITX, 4-ITX	ESI (+) QqQ	255 → 213,184	Discovery HS F5 (150 mm x 2.1 mm i.d.; 3 µm particle size) SunFire C18 (150 mm x 2.1 mm i.d.; 3.5 µm particle size)	A: H_2O (AF/FA 25 mM) B: AcN Gradient	Food	1st LLE 2nd SPE	Gallart-Ayala *et al.*, 2008
I: HCPK, BDMB, MMMP, TPO II:BP, ITX Amine syn: EHA	ESI/APCI (+) QqQ	HCPK: 205 → 77,187 BDMB: 367 → 176,190 MMMP: 280 → 165,117 TPO: 349 → 147,119 BP: 183 → 105,77 ITX: 255 → 184,213 EHA: 278 → 151,166	Gemini C18 (150 mm x 2 mm i.d.; 3 µm particle size)	A: MeOH B: H_2O (5 mM AA + 0.1 % FA) Gradient	Food FCM	1st LLE 2nd SPE LLE	Shen *et al.*, 2009
I: HCPK II: 4-MBP, PBZ, MBB, 4-HBP, DEAB, MK, DMAB Amine syn: EDB, EHA	ESI (+) QqQ	4-HBP: 199 → 121, 105 MBB: 241 → 209,152 BP: 183 → 105, 77 EDB: 194 → 151, 134 DMAB: 226 → 105, 77 4-MBP: 197 → 105, 77 MK: 269 → 148, 77 PBZ: 259 → 105, 77 DEAB: 325 → 176, 133 EHA: 278 → 151, 166 HCPK: 205 → 105, 77	Synergi MAX -RP 80 (150 mm x 2 mm i.d.; 4 µm particle size)	A: MeOH (AF 2 mM/FA 0.05 %) B: H_2O (AF 2 mM/FA 0.05 %) Gradient	Food	LLE	Jung *et al.*, 2013

Table 5.3: LC-MS and LC-MS/MS methods for photoinitiator analysis.

Method	Photoinitiators	Analytical Conditions		Analytical column	Oven program	Type of Sample	Extraction procedure	Reference
		Mode	Detector					
GC-FID	I: HCPK, DMPA	Splitless	250 °C	HP-5 (30 m x 0.32 mm i.d.; 0.25 µm film thickness)	100 → 300 °C	Food Simulants	Aq. Simulants: LLE Org. Simulant: Concentration	Wang *et al.*, 2009; Huang *et al.*, 2012

Table 5.4: GC-FID methods for photoinitiator analysis.

Method	Photoinitiators	Analytical Conditions		Analytical column	Oven program	Type of Sample	Extraction procedure	Reference
		Source / Mode	m/z					
GC-MS	II: BP	EI Split (1:20)	---	Rtx-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness)	180 → 250 ºC	Food FCM	LLE	Pastorelli *et al.*, 2013
	II: BP, 4-MBP	EI Splitless QIT	BP: 182 → 153, 181 4-MBP: 196 → 119, 181	SGE BPX-5 (25 m x 0.22 mm i.d.; 0.25 µm film thickness)	50 → 280 ºC	Food	1st LLE 2nd SPE	Van Hoeck *et al.*, 2010
	II: BP	EI Splitless	BP: 182,105	Rtx-1 (60 m x 0.25 mm i.d.; 0.25 µm film thickness)	50 → 280 ºC	Food FCM	LLE	Anderson *et al.*, 2003
	I: HCPK II BP, ITX Amine syn: EHA, EDB	EI Split (1:50)	HCPK, EDB: 81, 99, 148, 164, 193 BP: 105, 182 ITX: 239, 254 EHA: 148, 165, 277	HP 5 MSI (30 m x 0.25 mm i.d.; 0.25 µm film thickness)	80 → 300 ºC	Food FCM	1st LLE 2nd SPE	Sagratini *et al.*, 2008
	II: 2-ITX Amine syn: EHA	EI Split (---)	2-ITX: 254, 239, 184 EHA: 277, 165, 148	DB 5 MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness)	50 → 270 ºC	Food	A: PLE B: LLE	Gil-Vergara *et al.*, 2007
	I: HCPK, DMPA, MMMP II: BP, ITX Amine syn: EHA	EI Splitless	HCPK: 151, 105, 57 DMPA:98, 81, 77 MMMP: 128 BP: 105, 77, 182 ITX: 239, 254 EHA: 165, 148, 277	Rtx-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness)	120 → 300 ºC	FCM	LLE	Sanches-Silva *et al.*, 2008b
	II: MK, DEAB, DMAB	EI Splitless	MK: 224, 265/148, 268 DEAB: 309, 324	CP-Sil 5CB (17 m x 0.25 mm i.d.; 0.12 µm film thickness)	100 → 300 ºC 60 → 300 ºC	Food FCM	LLE	Castle *et al.*, 1997
	I: HCPK, DMPA, MMMP II: BP, ITX Amine syn: EHA	IT	---	Optima Delta 6 (30 m x 0.25 mm i.d.)	50 → 250 ºC	Food Simulants	SPE	Papilloud & Baudraz 2002a, 2002b

	II: 2-HBP, 4-HBP, 4-MBP, MBB, DEAB, PBZ, BP, BPAcr Amine syn: EHA	EI Split (1:40)	---	DB 5-HT (30 m x 0.25 mm i.d.; 0.1 µm film thickness)	100 → 250 ºC	FCM	LLE	Koivikko *et al.*, 2010
	I: HCPK, BDMB, DMMB, MMMP, OMN 910, TPO II: BP, PBZ, ITX, SP 7005, SP 7010 Amine syn: EHA	EI Splitless	---	HP 5 MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness)	50 → 295 ºC	FCM	LLE	Bentayeb *et al.*, 2013
	I: HCPK, DMPA II: BP, 4-MBP, ITX Amine syn: EHA, EDB	EI Splitless EI Splitless QIT	HCPK: 99, 81 DMPA:151, 105 BP: 105, 77, 182 4-MBP:119, 196 ITX: 254, 239 EHA: 277, 165 EDB: 148, 193, 164	HP 5 MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) DB 5-HT (30 m x 0.25 mm i.d.; 0.1 µm film thickness)	70 → 280 ºC	Food FCM	SPME LLE	Negreira *et al.*, 2010
	I: HCPK, DMPA, MMMP II: BP, 2-MBP, 3-MBP, 4-MBP, PBZ, 2-HBP, 4-HBP, MBB, 2-ITX, 4-ITX, DETX Amine syn: EHA, EDB	EI Splitless	BP: 77, 105, 182 2-MBP: 91, 119, 195 3-MBP: 105, 119, 196 4-MBP: 105, 119, 196 PBZ:152, 181, 258 4-HBP: 105, 121, 198 2-HBP: 77, 121, 198 MBB: 105, 163, 240 HCPK: 55, 81, 99 2-ITX: 184, 239, 254 4-ITX: 184, 239, 254 DMPA: 77, 105, 151 DETX: 239, 254, 268 MMMP: 84, 128, 151 EHA: 184, 227, 304 EDB: 148, 165, 277 MPBP: 184, 227, 304	ZB-5ms (30 m x 0.25 mm i.d.; 0.25 µm film thickness)	100 → 320 ºC	Food FCM	LLE	Bradley *et al.*, 2012
GC-MS/MS	II: ITX	ESI (+) QIT Splitless	ITX: 254 → 239, 184	Restek RTX-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness)	100 → 280 ºC	Food	1st: LLE 2nd: SPE 1st: Alkaly Hydrolisis 2nd: SPE	Allegrone *et al.*, 2009

Table 5.5: GC-MS and GC-MS/MS methods for photoinitiator analysis.

5.6. WHAT IF PHOTOPRODUCTS ARE MORE DANGEROUS SUBSTANCES THAN PHOTOINITIATORS THEMSELVES?

Ideally a future specific legislation on printing inks should take into account the photoinitiators mechanism during the photopolymerization. Three kinds of compounds should be considered: photoinitiators, their photoproducts and the radicals created in the photocuring process.

The EFSA ESCO WG on non-plastic food contact materials report (EFSA, 2011a) remarks the absence of a specific legislation for different materials like UV inks, and the Swiss legislation is proposed as a base to a future legislation (FDHA, 2005), but in this ordinance there is not any information about the possible photoproducts. These compounds, photoproducts and radicals formed in the printing process, are included in the definition of Non-Added Intentionally Substances (NIAS), i.e. "impurity in the substances used or a reaction intermediate formed during the production process or a decomposition or reaction product", given in the Commission Regulation (EU) No 10/2011 (EC, 2011). The EFSA-ESCO WG on non-plastic food contact materials in its report (EFSA, 2011a) addresses the issue concerning the need of toxicity evaluation of NIAS.

In order to consider the potential migration of NIAS into foodstuffs, derived from the use of printing inks in packaging materials, the exact routes of photocuring mechanisms should be known in detail. Many of these NIAS can be harmless molecules, but if for instance, the already known photocleavage processes of three different photoinitiators used in FCM (and included in EUPIA's list (2013a)), reported by various authors, are observed in this work, it can be determined that there are many substances involved for which toxicity cannot be excluded.

To illustrate the just above mentioned, the hazards of several photoproducts were tentatively evaluated by categorization according to Cramer classification, using the Toxtree open-source software from EURL-

ECVAM (European Union Reference Laboratory for alternatives to animal testing). The Cramer classification is considered as "the best known approach for structuring chemicals in order to make a Toxicological Threshold Concern (TCC) estimation" by the EURL-ECVAM. Also the IARC (International Agency for Research on Cancer) databases were used to determine the carcinogenic potential of these compounds.

Green (2010) shows the photoproducts of one of the most used HAP's, 2-hydroxy-2-methylpropiophenone (HMPP), a molecule classified as class II in Cramer toxicological classification (EFSA, 2011b). From HMPP, three radicals could be formed being able to interact with any molecule present in the formulation, besides with other photoproducts.

Apart from the generated radicals, two volatile products like acetone and isopropanol are formed from the alkyl radical. These two compounds are Class I molecules in Cramer classification. Isopropanol has an ADI (Acceptable Daily Intake) of 2.4 mg kg bw^{-1} day^{-1} and acetone is a molecule that is correctly metabolized by the human body (EFSA, 2005, 2011b). Tetramethylethylene glycol, a molecule classified as class III, could also arise from the alkyl radical dimerization.

From the benzyl radical could be generated benzaldehyde, which would be oxidized to benzoic acid; both compounds with low toxic hazard (Class I) and with an ADI of 0-5 mg kg bw^{-1} for benzaldehyde due to "lack of teratogenic, reproductive or carcinogenic potential" (JECFA, 2002; EFSA, 2010).

The last described route from HMPP could produce two benzyl radicals which might react to form three possible Class III compounds, diphenylethanedione, a photoinitiator not used in FCM, a semi-quinoid product and 1,2-ethanedione, 1-(4-benzoylphenyl)-2-phenyl.

In summary, from a typical type I photoinitiator like HMPP, categorized in Class II in Cramer's classification, new 4 Class I compounds, 4 Class III and 3 radicals are formed. Then, the potential migration of these eleven molecules

should be also considered in the migration assays of HMPP from the FCM into the food.

The proposed first photocleavage stages of the type II photoinitiator ITX (class III) in presence of an amine was described by Green (2010), in this case the aminobenzoate EDB (Benzoic acid, 4-(dimethylamino)-, ethyl ester) (class III). When UV light reaches the photoinitiator molecule, two radicals are formed: a ketyl radical, with poor reactivity, and the alkylamine, the true initiator of the photopolymerization process.

This ketyl radical could follow three different routes: dimerization, disproportionation to return to ITX and its hydrolyzed product, and a termination product after the polymer chain growth. All of them are Class II photoproducts. The alkylamine radical could react with the monomer/oligomers or generate peroxi radicals due to oxygen scavenging to obtain hydroperoxide that can follows different routes where there are at least 4 compounds of class III, and 4 different radicals, which could interact with any other compounds. Dektar & Hacker (1990b) proposed the photoproducts of a cationic photoinitiator model, triphenyl sulphonium hexafluorophosphate. The photocleavage of this photoinitiator can proceed by heterolytic and homolytic cleavage of the singlet state of the sulphonium salt (Knapzyck & McEwen, 1969, 1970; Crivello & Lam, 1979).

The heterolytic cleavage route leads to phenyl cation and diphenylsulphide, which could react and generate six different compounds. The three (phenylthio)biphenyl isomers, and diphenylsulphide are classified by Cramer as class III. The products of reaction of the phenyl cation and the H donor would depend on the H donor.

From the homolytic cleavage route, first a phenyl radical and a diphenylsulphinyl radical cation pair can be formed. From these two radicals the reaction products that involve an H donor, all 5 generated molecules, are classified as high hazard (class III). Benzene deserves special attention due to its demonstrated carcinogenicity, being classified by IARC (International Agency for

Research on Cancer) as a Group I compound (carcinogenic to humans) (IARC, 2012).

These three examples mentioned above show that from a simple photoinitiator (of any type), various molecules could be generated, and they could be of more toxicological importance than the initial photoinitiator for the consumers' health.

5.7. LEGISLATION ON PHOTOINITIATORS USED IN FCM

The printing inks legislations are different all over the world, for that reason this review refers to the 3 main cases: USA, Japan and Europe.

a) United States of America, the migrants from FCM are considered as potential Indirect Food Additives, because they are no added intentionally; however, it is reasonable that they could migrate to foodstuffs, becoming a component of it. Exempted from this safety assessment are substances known to be Generally Recognized As Safe (GRAS) and all compounds used before 1958 (Muncke, 2012).

Printing inks and photoinitiators are Indirect Food Additives; however, they are not regulated by the 21 Code of Food Regulations (CFR) parts 174-177, which regulates food packaging substrates as well other materials as adhesives and it provides positives lists of authorized substances (FDA, 2014). There are no specific guidelines for printing inks or coatings applied on the non-food contact surface of food packaging neither.

Nevertheless the photoinitiators must be complying with the indirect food additive guidelines (21 CFR 170-190) (FDA, 2014). The manufacturer is responsible for ensuring that a barrier ensures that there is no migration. It is considered negligible migration if after carry out all the migration and diffusion tests, the migration is minor than 50 μg kg^{-1} foodstuff, the Threshold of Regulation (TOR) (FDA, 2012). These data is related on the dietary exposure.

In the case of migration between 50 µg kg^{-1} and 1 mg kg^{-1} the indirect food additive enter in the Food Contact Substance Notification (FCSN) system (FDA, 2013). The company needs to provide in vitro genotoxicity and /or sub chronic toxicity studies (other studies are recommended also). With this system the Food and Drug Administration (FDA) could provide a Letter of No Objections that allows its use.

The last case is for migration levels above 1 mg kg^{-1}. In this case the company needs a dedicated petition, where is going to need different toxicological studies regulated by the FDA's Redbook (FDA, 2007). The selection of these studies is decided in agreement with the FDA.

b) Japan, the Food Sanitation Law sets the legal framework (JETRO, 2006). This law says that the inertness of food packaging must be ensured and the packaging which contains or bears harmful substances is banned. The Japanese FCM Industry is self-regulated, being associated to adopt similar approaches to EU legislation, with positive lists for additives and starting substances (Mori, 2010, Muncke, 2014). The overall migration limit is set in 30 mg kg^{-1} foodstuff.

c) European Union. Currently, there is no specific or harmonized legislation in the European Union on printing inks regarding FCM. Some photoinitiators have also other uses such as, for example, benzophenone which also acts as UV blocker and is used in plastic materials. For benzophenone, and its derivatives, 2-, 3- and 4-methyl benzophenone, a specific migration limit (SML) has been set in the European legislation on plastic materials and articles intended to come into contact with foodstuffs (Regulation (EU) No 10/2011) (EC, 2011). But, this is an exceptional case due to those other uses of benzophenone.

Printing inks are one of the 17 groups of materials included in the framework regulation on the materials and articles intended to come into contact with food (Regulation (EC) No 1935/2004) (EC, 2004), and therefore these materials must fulfill with the requirements there established, being of special relevance the general safety requirements set in Article 3, such as not

endanger human health, no unacceptable changes in the composition and no deterioration of the organoleptic characteristics.

Moreover, in the European Union, the manufacturing of these FCM must be in agreement with the Commission Regulation (EC) No 2023/2006 on good manufacturing practice for materials and articles intended to come into contact with food (EC, 2006).

The implementation of a specific legislation on printing inks used in food contact materials stand to become ever more necessary. But this is a hard task and, for this reason, some countries developed a legislation to regulate the use of printing inks in food packages in its own territory instead of waiting for a harmonized European Union legislation.

The first country was Switzerland with the Ordinance of the FDHA on Materials and Articles (817.023.21) issued in 2005. This ordinance was drafted on the basis of the Inventory List of substances given by EuPIA and the cursory risk assessment conducted by Swiss authorities; it presents two different parts: part A, that contains evaluated substances intended to be used in the manufacture of FCM (these substances need to comply with the restrictions), and part B, where the substances listed are those that have not been evaluated by any officially recognized scientific test. In the part A there are set SML for 18 photoinitiators and amine synergists more than in the Regulation (EU) No 10/2011. The substances of the part B may be used if no transfer of these substances to foodstuffs/food simulants can be detected with a target migration limit of 10 μg kg^{-1} (FDHA, 2005; Pedersen, 2012).

Based on the Swiss legislation, it is expected that the Federal Ministry of Food, Agriculture and Consumers Protection of Germany presents shortly a draft ordinance amending the German Consumer Goods Ordinance ("German Ink Ordinance"). The draft of this legislation has a positive list of substances similar to that proposed by Swiss Ordinance and the substances not regulated might be used if their migration is negligible with an LOD of 10 μg kg^{-1} and they

are not classified as carcinogenic, mutagenic nor reproductive toxicity (Pedersen, 2012; EuPIA, 2013b).

To address the potential problem of substances used in non-plastic food contact materials, and no covered by specific legislation, the European Food Safety Authority Scientific Cooperation Working Group (ESCO-WG) published an external scientific report (EFSA, 2011a), where food industries and European food safety agencies took part. The report collects the information available in Member States concerning the evaluation of substances, identifying the needs and then proposing strategies for prioritizing the evaluation of substances. One of the strategies suggested by the ESCO-WG to set priorities in the evaluation of substances, when limited or no information on toxicity is available, is based on the human exposure threshold approach (Pinalli *et al.*, 2011). This approach uses structural information based on Cramer classification (Cramer *et al.*, 1978), which categorizes substances according to their potential toxicity in 3 types: class I for simple chemical structures and with efficient modes of metabolism, suggesting a low order of oral toxicity. Class III are substances with reactive functional groups and that may suggest significant toxicity. Class II substances are in the middle. The annex of the report shows the classification of some photoinitiators used in printing inks for food contact materials. This report should be the basis of the first steps in the elaboration of specific legislations for non-plastic materials, including printing inks.

5.8. CONCLUSIONS

The photoinitiators' issue is hard to address mainly due to three reasons: a) not all the photoinitiators migrate from the packaging into the food by the same mechanism because of the unique properties of each compound; b) not all the photoinitiators have the same action mechanism; and, c) several photoproducts can be formed in the photopolymerization process (nowadays considered as NIAS), and moreover some of them could represent a health hazard (e.g. benzene).In this scenario is not an easy task to elaborate legislation for this

subject. All the above mentioned could be the reason why there is no specific UV inks legislation and the regulations where this group of substances are included are very heterogeneous over the world; nevertheless, a specific UV inks' legislation in Europe is more necessary over time. The Swiss and future German legislation represent a good starting point for this future legislation. Focus in this goal, this review tries to address all the aspects related to photoinitiators that should be considered prior its specific regulation. However, deeper and broader knowledge is necessary to draft this legislation in order to protect the consumers' health.

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6. ANALYTICAL METHODS FOR DETERMINING PHOTOINITIATORS IN FOOD-CONTACT MATERIALS

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6.1. INTRODUCTION

In the past, the main function of food packaging was as a container. Nowadays, the main function of packaging is to protect food and preserve its quality (De Kruijf *et al.*, 2002; Risch, 2009).

In this context, food packaging must form an inert barrier to prevent transfer of mass to the foodstuff from outside. However, mass transfer may also occur from the food-contact material (FCM) to the foodstuff, representing a possible hazard to human health (Widén *et al.*, 2004; Poças & Hogg, 2007; Wang *et al.*, 2009).

The packaging sector represents about 2% of the gross national product (GNP) in developed countries, and half of the packaging produced is used for foodstuffs (Robertson, 2013). Any problems associated with the FCM could therefore have serious effects on public health and national economies. The use of printing inks on the external face of food packaging is of particular concern, as many of these are UV-curable inks that contain photoinitiators.

The first reported case of migration of photoinitiators to foodstuffs occurred in September 2005 (RASFF, 2005). The Italian authorities detected ITX in baby milk from Spain. Since then, the European Union's Rapid Alert System for Food and Feed (RASFF), (a network involving the European Commission's Directorate-General for Health and Consumer Protection, the European Food Safety Authority (EFSA), the EFTA Surveillance Authority (ESA) and the members states) has reported another 144 notifications (44 alerts and 100 informations) until the end of 2013 (RASFF, 2013).

A brief glance at these 144 notifications shows the following: 12 different photoinitiators and one amine synergist were detected in foodstuffs; the notifications originated in 16 different countries; the migration occurred from/through different types of packaging to reach many different kinds of foodstuffs; and finally, not all the products were seized or withdrawn from the

markets. The data gathered by the RASFF indicate that there are many different factors involved in the problem of migration of photoinitiators from FCM to foods, making this problem difficult to address.

The European Food Safety Agency (EFSA) considered the problem of photoinitiators in an external scientific report (ESCO) compiled jointly by the food industry and food safety agencies (EFSA, 2011). This report formed the basis of future legislation, and the potential toxicity of some photoinitiators was determined by the Cramer classification, whereby the photoinitiators and other molecules are classified as of potentially high, medium or low toxicity on the basis of their molecular structure (Cramer *et al.*, 1978).

Some researchers have studied how photoinitiators can reach the foodstuffs and have identified two main routes (Choi *et al.*, 2002; Jung *et al.*, 2010): (a) direct transfer by permeation from the outer printed surface through different layer(s) of packaging, or by *set-off* (transfer of the photoinitiators during storage of the printed material in reels or stacks) (Morlock & Schwack, 2006; Rothenbacher *et al.*,2007), and (b) indirect transfer by gas phase, for volatile photoinitiators such as benzophenone and derivatives, see Figure 6.1

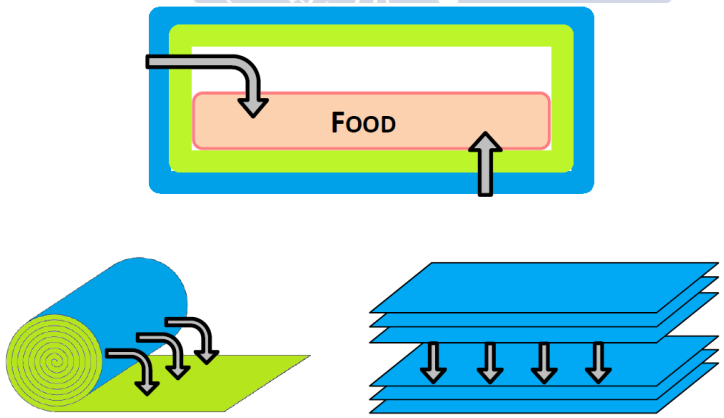


Figure 6.1: The upper scheme illustrates the indirect transfer and direct transfer of photoinitiators from the external layer of the packaging to the food. In the lower scheme, the *set-off* effect in reels and stacks is represented.

However, it is not only the starting substances that can migrate to the foodstuffs and contaminate them. Depending on the initial photoinitiator(s)

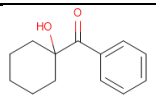
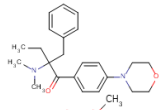
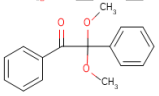
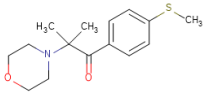
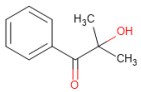
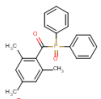
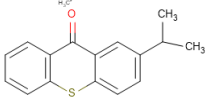
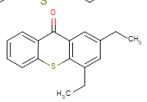
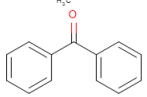
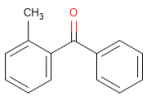
involved, different byproducts (photolysis products and radicals) can be generated by photopolymerization and photocuring reactions. Foodstuffs can also be contaminated with these products, in a complex process about which very little is known, which makes it very difficult to evaluate the hazards associated with these unknown substances.

The above-mentioned byproducts are included in the so-called non-intentionally added substances (NIAS), which are defined as ‘‘impurities in the substances used or reaction intermediates formed during the production process or decomposition or reaction products which can occur in the final product’’ by European Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food (EC, 2011).

Although there is no standard procedure for the determination of photoinitiators and NIAS in FCM or foodstuffs, some authors have developed methods for the detection and/or quantification of one or more photoinitiators. To date, methods have been developed for determining 28 photoinitiators, of which only 19 have been declared as suitable for use in coatings, inks and varnishes for the noncontact side of food packaging by the European Printing Ink Association (EuPIA) (EuPIA, 2013). This EuPIA list, see Table 6.1, contains more than 100 photoinitiators declared as appropriated for the mentioned uses.

Determination of NIAS is a difficult task, mainly because these products are unknown. The reactions that occur in the process of photopolymerization in FCM have not been widely studied, and it is not generally know which molecules are involved. Thus, the first step required is to identify and quantify the photolysis products generated by each photoinitiator or combination of photoinitiators/synergists (Lord *et al.*, 2013).

In accordance with this scenario, the different techniques for determining photoinitiators and their derivatives in FCM and foodstuffs are discussed in this chapter.

Structure	Name	PI type	Mw	CAS No.	Mp (°C)	Bp (°C)	Log P (o/w)	Water Solubility (mg/L) (25°C) (pH=7)	Vapor Pressure (mmHg)	SML (mg/kg)
	HCPK	I	204.26	947-19-3	48.00	339.00*	2.18*	1400.00*	3.75 x 10-5*	--
	BDMB	I	366.50	119313-12-1	N/A	528.80*	3.38*	730.00*	2.87 x 10-11*	0.15+
	DMPA	I	256.30	24650-42-8	64.00	361.10*	3.62*	150.00*	1.06 x 10-05*	--
	MMMP	I	279.40	71868-10-5	N/A	420.10*	2.44*	500.00*	2.89 x 10-07*	0.05+
	HMPP	I	164.20	7473-98-5	164.00	260.80*	1.49*	4400.00*	6.08 x 10-03*	--
	TPO®	I	348.37	75980-60-8	N/A	519.00*	4.62*	11.00	6.71 x 10-11*	0.05+
	ITX	II	254.35	5495-84-1	76.75	398.20*	5.11*	28.00*	1.43 x 10-06*	0.05
	DETX	II	268.37	82799-44-8	N/A	427.90*	5.67*	13.00*	1.58 x 10-07*	--
	BP	II	182.22	119-61-9	47.80	305.40	3.18	137.00	8.23 x 10-04*	0.6**
	2-MBP	II	196.24	131-58-8	-18.00	308.00	3.69*	71.00*	6.17 x 10-04*	0.05

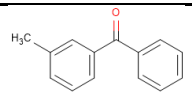
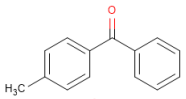
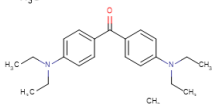
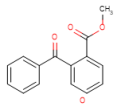
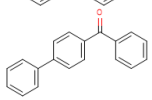
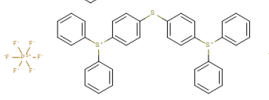
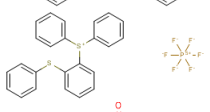
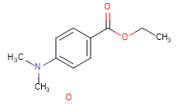
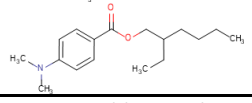
	3-MBP	II	196.24	643-65-2	159.50	317.00	3.69*	65.00*	3.92 x 10-04*	0.05
	4-MBP	II	196.24	134-84-9	59.50	328.10*	3.69*	65.00*	1.94 x 10-04*	0.05
	DEAB	II	324.46	90-93-7	96.00	475.70*	5.91*	0.39*	3.25 x 10-09*	--
	MBB	II	240.25	606-28-0	52.00	351.00	2.70	80.00	1.53 x 10-05*	0.05
	PBZ	II	258.31	2128-93-0	102.00	419.50	5.14*	3.90*	3.11 x 10-07*	--
	BIS	Cationic	876.43	74227-35-3	235.00	N/A	N/A	N/A	N/A	--
	THIO	Cationic	516.51	8156-13-8	N/A	N/A	N/A	N/A	N/A	--
	EDB	Amine synergist	193.24	10287-53-3	63.50	296.50*	2.51*	410.00*	1.43 x 10-03*	0.05+
	EHA	Amine synergist	277.40	21245-02-3	243.00	382.90*	5.41*	4.70*	4.57 x 10-06*	2.4

Table 6.1: Photoinitiators included in the EuPIA list and the analytical methods developed for their determination and quantification. *:experimental data. Obtained from SciFinder® version web. N/A: Not available.

6.2. PHOTOINITIATORS

Several studies have considered the identification and/or quantification of one or more photoinitiators. The technique most commonly used is chromatography, in its different variants: liquid (LC), gas (GC) or high-performance thin-layer (HPTLC), coupled to different detectors: diode array (DAD), fluorescence (FLD), flame ionization (FID), mass (MS), or some combination of these. Other techniques such as voltammetric methods or, the most novel, direct analysis in real time (DART) are also used.

The different analytical methods developed for the determination of photoinitiators in food and FCM are described below.

6.2.1. CHROMATOGRAPHY

Selection of liquid or gas chromatography should be based on the nature and the properties of the target photoinitiators, i.e. liquid chromatography is usually most suitable for photoinitiators of low volatility.

6.2.1.1. Liquid Chromatography (LC)

This is the most commonly used technique for the determination of photoinitiators in foodstuffs and FCM (Castle *et al.*, 1997; Papilloud & Baudraz, 2002a, 2002b; Sagratini *et al.*, 2006, 2008; Bagnati *et al.*, 2007; Gil-Vergara *et al.*, 2007; Rothenbacher *et al.*, 2007; Sun *et al.*, 2007; Benetti *et al.*, 2008; Gallart-Ayala *et al.*, 2008, 2011; Pastorelli *et al.*, 2008; Sanches-Silva *et al.*, 2008a, 2008b; 2009a, 2009b; Rodríguez-Bernaldo de Quirós *et al.*, 2009; Shen *et al.*, 2009; Jung *et al.*, 2010, 2013; Koivikko *et al.*, 2010; Biedermann *et al.*, 2013). The sum of these methods determines a total of 20 different photoinitiators of different kinds (cationic, type I, type II and even amine synergists).

The analytical methods can be classified into two main groups depending on the detector used: diode array or fluorescence detectors, and mass

spectrometry. The techniques in the first group are those most extensively used for the identification and quantification of photoinitiators and those in the second group are very useful for confirmation purposes.

6.2.1.1.1. HPLC-DAD/FLD

Before the scandal involving the presence of ITX in baby's milk, very few studies involving the determination of photoinitiators had been reported (Castle *et al.*, 1997 Papilloud & Baudraz, 2002a, 2002b; RASFF, 2005). Castle *et al.* (1997) determined 3 different photoinitiators (MK, DEAB and DMAB) in different paper and cardboard FCM, using ethanol as solvent in the extraction. The chromatographic separation was achieved with a Zorbax SC85 (Hichrom®) (i.d., 250 mm x 4.6 mm and particle size, 5 µm) reversed-phase column with low carbon loading (6 %), in isocratic mode (methanol/water 4:1). Detection was performed with DAD at 350 nm.

MK, DEAB and DMAB were detected in 10-26 % of the samples at concentrations of respectively 0.05, 0.1 and 0.1 mg kg^{-1}, which are above the limits of detection (LOD), but unlikely to pose a risk to human health (Castle *et al.*, 1997).

In a later study, Papilloud and Baudraz (2002a, 2002b) used a method involving solid-phase extraction (SPE) to determine the following compounds in food simulants: ITX, BP, BIS, THIO, HCPK, DMPA, MMMP and EHA. Chromatographic separation was performed with a reversed-phase column with cyano groups (CN Nucleosil 100-5; i.d., 250 mm x 4.6 mm and particle size, 5 µm), in gradient mode and with a mobile phase composed of buffered water, methanol and acetonitrile. The results obtained indicated good sensitivity, with LOD and LOQ of µg kg^{-1} order of magnitude (Papilloud & Baudraz, 2002a, 2002b).

After the above-mentioned scandal, many researchers focused on the health problem of photoinitiators in foodstuffs, and many analytical methods have since been published. Sanches-Silva *et al.* (2008a, 2008b, 2008c, 2009),

Pastorelli *et al.* (2008), Rodríguez-Bernaldo de Quirós *et al.* (2009) and Koivikko *et al.* (2010) developed some methods of identifying and quantifying the following photoinitiators in different FCM, foodstuffs and food simulants: (BP, ITX, EHA, DEAB, HCPK, MMMP, BDMB, 4-MBP, 2-HBP, 4-HBP, MBB, PBZ and BPAcr.

These authors used similar schemes to develop their methods. All achieved the chromatographic separation with a C18 column: Kromasil 100 (Teknocroma®) or Eclipse XDB (Agilent Technologies®) of length 15 cm, i.d. 4.6 mm and particle size 5 µm, except Rodríguez-Bernaldo de Quirós *et al.* (2009) and Pastorelli *et al.* (2008) who used columns of length 25 cm. The mobile phase used was a gradient of acetonitrile and water.

The photoinitiators and amine synergist were detected with DAD at different wavelengths: 246 nm for HCPK, 254 or 256 nm for BP, 256 nm for BDMB, MBB, DEAB, BPAcr, 2-HBP and 4-HBP, 256 or 260 nm for 4-MBP, 290 nm for PBZ, 306 nm for MMMP, 310 nm for EHA and 386 or 256 nm for ITX.

Regarding the extraction procedure, single-solvent extraction with acetonitrile was used for FCM, while for the foodstuffs; the procedure depended on the nature of the samples. Thus, the single-solvent extraction procedure was used for samples such as powdered milk and cakes; for beverages, the samples were simply diluted with acetonitrile and for milk (because of its acidic character), the extraction was carried out with ammoniac and two successive extractions with hexane. Samples of food simulants were injected directly into the HPLC system.

These methods showed low LODs of between 17-30 µg L^{-1} for HCPK, BDMB, MMMP, ITX, BP and EHA and slightly higher values (46 µg L^{-1}) for 4-MBP and PBZ. For all samples, the LOQ was around 0.14 mg L^{-1}. Because of the nature of BPAcr, a polymeric photoinitiator, the chromatographic behavior was different; this compound yielded a sum of peaks rather than a single peak in the chromatograms, and the LOD and LOQ were therefore higher than those obtained for the other photoinitiators (LOD = 1.22 mg L^{-1}, LOQ = 3.67 mg L^{-1}).

Benzophenone was common in the FCM analyzed, and some other photoinitiators were also detected. Photoinitiators were detected in foodstuffs but to a lesser extent than in FCM.

More recently, Rothenbacher *et al.* (2007) developed an HPLC-DAD/FLD method for the determination of 2-ITX in different types of packaging and foodstuffs purchased in German markets. The FCM extraction was carried out with hexafluoro-2-propanol and ethanol. However, for foodstuffs, the extraction was based on the ''quick, easy, cheap, effective, rugged, and safe'' (QuEChERS) approach (Anastassiades *et al.*, 2003). The QuEChERS method is based on 4 steps (with shaking of the mixture between each): extraction with acetonitrile; addition of magnesium sulfate and sodium chloride; addition of the internal standard; and, finally, mixing of the supernatant with magnesium sulfate (to remove the residual water) and primary secondary amine (PSA) (to remove sugars and fatty acids).

The chromatographic method was carried out with a Supelcosil® LC-PAH (Agilent Technologies®) reversed-phase C18 column (i.d., 250 mm x 4.6 mm and particle size, 5 µm) and acetonitrile and water as mobile phase in isocratic mode. 2-ITX was detected with DAD, at 260 nm, and with FLD, at 273 nm (excitation) and 440 nm (emission). In this study, ITX was detected in 26 % of the packages at concentrations higher than the LOD and LOQ (2 and 5 µg L^{-1}), but lower than those obtained by Sanches-Silva *et al.* (2008a, 2008b, 2009a, 2009b) in the previously mentioned studies. Regarding the food, the concentrations of 2-ITX varied up to 357 mg kg^{-1} in orange juice.

Jung *et al.* (2010) first developed an HPLC-DAD-FLD method and more recently another HPLC-DAD method for determining different photoinitiators (Jung *et al.*, 2013). They used the first method to determine MMMP, ITX and EDB in yoghurt samples. The extraction procedure was the same as described by Rothenbacher *et al.*, (2007) with slight differences to improve extraction. The analysis was performed with a gradient of acetonitrile and water as mobile phase, and a Luna C18 column (i.d., 100 mm x 2 mm and particle size, 3 µm) was used as the stationary phase. Quantification was carried on with DAD, at 260

nm, and with FLD, at 264 and 440 nm as excitation and emission wavelengths. The limits of detection and quantification of the compounds in yoghurt were as follows: 0.4 and 2.5 μg kg^{-1} for ITX; 3.5 and 10.7 μg kg^{-1} for EDB, and 3.8 and 11.9 μg kg^{-1} for MMMP. These data confirm better results for ITX than in the previous studies and are similar to those obtained for MMMP.

In a recent study, Jung *et al.* (2013) analyzed eleven photoinitiators (BP, 4-MBP, PBZ, HCPK, 4-HBP, DEAB, MK, EDB, EHA, DMAB and MBB) in cartonboard packaging, confirming the tendency of the development of multimethods for ever greater numbers of photoinitiators. The method used to extract these photoinitiators from the FCM was that proposed by Pastorelli *et al.* (2008).

Chromatographic separation was achieved with a reversed-phase C18 column (Purospher® Star RP 18e: i.d., 250 mm x 4 mm and particle size, 5 μm) and a mobile phase composed by water and acetonitrile, in gradient mode. This method yielded good resolution, but the time required for the analysis was 70 min. The photoinitiators were detected at 245 nm (HCPK and MBB), 255 nm (BP and 4-MBP), 290 nm (4-HBP and PBZ), 310 nm (EHA and EDB) and finally at 365 nm (DEAB, MK and DMABP).

The results showed good sensitivity, with LODs and LOQs in the different cartonboards ranging from 2.8 μg dm^{-2} (for BP) to 29.0 μg dm^{-2} (for PBZ). The LOQs ranged from 9.4 μg dm^{-2} (for BP) to 95.0 μg dm^{-2} (for PBZ). BP was detected and quantified in 49 % of the 310 samples analyzed, and the other photoinitiators, except 4-HBP, were detected in at least one of the samples. This data complemented and enhanced the survey of these photoinitiators initiated by Koivikko *et al.* (2010) in which only BP was detected in paper and cartonboard.

Only one other HPLC-DAD method for the determination of photoinitiators in beverages has been reported. Sagratini *et al.* (2008) determined 5 different photoinitiators (ITX, BP, EHA, EDB and HCPK) in beverages and packaging. To determine these photoinitiators, the beverages were centrifuged and the

resulting supernatant was extracted with n-hexane in the presence of Na2SO4 and then subjected to SPE.

The results, obtained with a Luna C18 reversed phase column (i.d., 250 mm x 4.6 mm and particle size, 5 µm) and a mobile phase composed by methanol and water in gradient mode, showed LODs ranging between 20 and 50 µg L^{-1} and LOQs between 100 and 300 µg L^{-1}, i.e. similar values to those obtained by other authors for the same matrices (Sanches-Silva *et al.*, 2008b).

In summary, on the basis of the methods reported in the literature reviewed, the following is a suitable and standardized HPLC-DAD method for the determination of photoinitiators in FCM and food: chromatographic separation with a general reversed-phase C18 column and a mobile phase composed by water and acetonitrile/methanol. By way of example, an HPLC-DAD chromatogram of fourteen photoinitiators is illustrated in Figure 6.2.

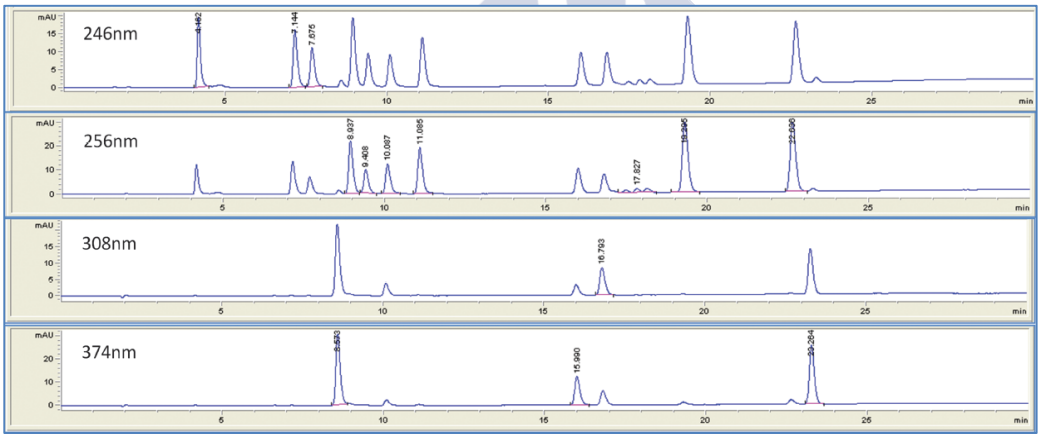


Figure 6.2: HPLC chromatograms of fourteen photoinitiators and an amine synergist (EHA): HMPP (4.16 min), HCPK (7.14 min), MBB (7.67 min), EDB (8.57 min), BP (8.94min), 2-HBP (9.41 min), DMPA (10.09 min), 4-MBP (11.09 min), PBZ (15.99 min), DEBP (16.79 min), BPAcr (17.83 min), ITX (19.29 min), DETX (22.69 min) and EHA (23.26 min).

Regarding the extraction procedure for FCM, a single-step extraction with acetonitrile 24 h at 70 ºC is appropriate. However, the procedure is more complex for foodstuffs, and several methods have been developed. The current tendency is to follow the QuEChERS approach, see Figure 6.3, with some variations depending on the complexity of the food matrix.

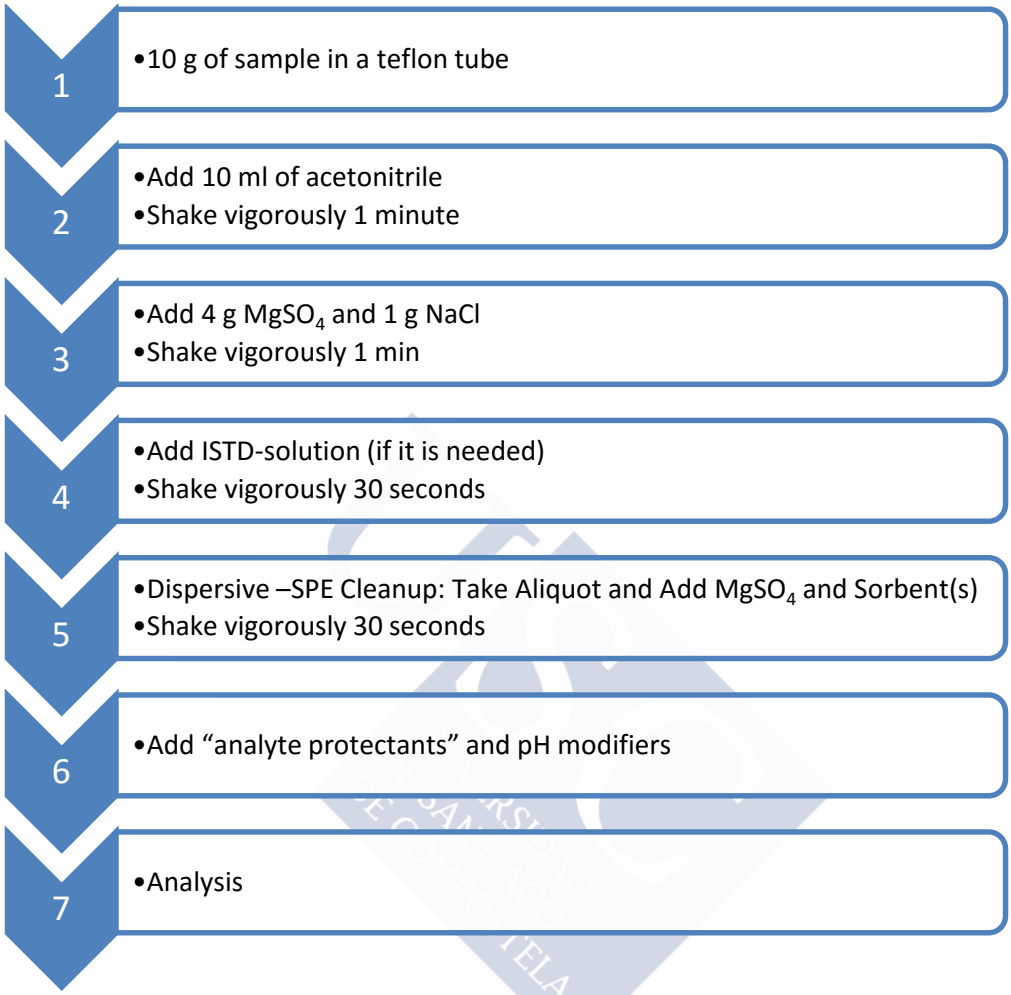


Figure 6.3: Steps included in the ''quick, easy, cheap, effective, rugged, and safe'' (QuEChERS) approach (Anastassiades *et al.*, 2003).

6.2.1.1.2. HPLC-MS and HPLC-MS/MS

These techniques can be used for confirmatory purposes or for both identification and confirmation. Previously, HPLC-MS was only used to confirm the results obtained by other methods because of the low availability of mass detectors; however, HPLC-MS and MS/MS are now commonly used to detect various substances.

Sanches-Silva *et al.* (2008a) used HPLC-MS-TOF with electrospray ion source in positive mode (ESI +) as a confirmatory technique under the same chromatographic conditions as in the HPLC-DAD method, although acidification of the mobile phases was required, in this case with formic acid (0.1 % and 0.2 %, respectively).

Confirmation was carried out by comparison of mass spectra of samples and standards. The most-abundant fragments were protonated molecules $[M+H]^+$ in the case of MMMP and EHA; a protonated molecule and a sodium adduct $[M+Na]^+$ for BP and ITX, only a sodium adduct for HCPK; and, finally, a sodium adduct and a molecule without a methoxy group $[M-OMe]^+$ for DMPA.

Benetti *et al.* (2008) used HPLC-MS (ESI +) to determine ITX in dairy products. The extraction procedure was single-solvent extraction with acetonitrile, and the chromatographic separation was achieved with a Gemini reversed-phase C18 column (i.d., 100 mm x 2.0 mm and particle size, 5 µm) (Phenomenex®) and a gradient of methanol and 20 mM ammonium formate.

This method showed a good detection capability (7.2 µg kg^{-1}). The protonated molecule $[M+H]^+$ m/z = 255 was selected as identification and quantification ion, and m/z = 213 was chosen as the confirmation ion. This method was tested with 50 samples of milk and yoghurt, and ITX was detected in 20 % of the samples.

HPLC-MS and HPLC-MS/MS methods were developed and compared in three different studies (Sagratini *et al.*, 2006, 2008; Jung *et al.*, 2013). Jung *et al.* (2013) first developed a HPLC-MS method with a single quadrupole and an ESI source in positive mode to determine photoinitiators in foodstuffs after extraction with acetonitrile. For separation, these authors used a chromatographic Hypersil® GOLD reversed phase column C18 (i.d., 100 mm x 2.1 mm and particle size, 3 µm) (Thermo® Scientifics) and a gradient of methanol and water, both modified with 5 mM ammonium formate and 0.1 % formic acid.

In this study, the protonated molecules $[M+H]^+$ were selected as quantifier ions and the qualifier ions were selected on the basis of the most abundant

fragments. The LODs ranged between 2.3 and 14 μg kg^{-1}, and LOQs ranged between 8.1 and 47 μg kg^{-1}. The BP limits were higher than for the other photoinitiators: 195 and 625 μg kg^{-1}. In the case of HPLC-MS/MS, the LODs and LOQs were lower: 2.5 and 7.5 μg kg^{-1} for all the photoinitiators except BP, for which the values were again higher (38 and 113 μg kg^{-1}).

The HPLC-MS/MS method was performed with a triple quadrupole mass detector and the same source. The photoinitiators were separated on a Synergi® MAX-RP 80A reversed-phase C12 column (i.d., 150 mm x 2.0 mm and particle size, 4 μm) (Thermo® Scientifics) and a gradient of methanol and water, both modified with 2 mM ammonium formate and 0.05 % formic acid as mobile phase, in gradient mode. Most of the precursor and product ions were the same as the qualifier and quantifier ions in the HPLC-MS method based on the most abundant fragments. Both of these methods yielded positive results in 33 % of the foodstuffs analyzed, and the legal limits were exceeded in five samples (FDHA, 2005; Council of Europe, 2009; EC, 2009, 2011).

Sagratini *et al.* (2006) developed three methods for determining ITX in juice samples, one with a single quadrupole mass detector, other with an ion trap and another one with a triple quadrupole detector. For all methods, they used a Luna reversed-phase C18 column (i.d., 150 mm x 4.6 mm and particle size, 5 μm) and methanol and water as mobile phase, in isocratic mode. The ITX was extracted from fruit-juice samples with acetone/hexane (50:50) and pressurized-liquid extraction.

For HPLC-MS (single quadrupole), the selected ion monitored was the sodium adduct of ITX. In the ion trap method, a transition was chosen from the protonated molecular ion. The same transition was selected for the HPLC-MS/MS method and another product ion was also chosen. The triple quadrupole detector exhibited very low LODs (0.01 μg L^{-1}) and LOQs (0.05 μg L^{-1}), and for single quadrupole and ion-trap detectors the corresponding values were 3 and 10 μg L^{-1}, respectively. With these methods, ITX was detected and quantified in eleven of the thirty samples analyzed.

Sagratini *et al.* (2008) reported some improvements to the above-mentioned methods. In this study, an HPLC-MS method was developed for the determination of five photoinitiators in beverages, and HPLC-MS/MS was used as a confirmatory technique. The HPLC-MS method was basically the same as in the previous study, except that the mobile phase was operated in gradient mode (Sagratini *et al.*, 2006). The ions selected for determination were the sodium adducts formed with the different photoinitiators.

The results were confirmed by HPLC-MS/MS, and two different sources were tested: ESI and ambient-pressure photoionization (APPI). Chromatographic separation was achieved with the same parameters as in the HPLC-MS method, except for the mobile phases. For the ESI source, the mobile phase comprised methanol and water modified with 0.1 % formic acid and 5 mM ammonium acetate in gradient mode, and for APPI, the mobile phase was methanol-water, although in isocratic mode.

The results obtained showed that the sensitivity of the technique depended on the photoinitiator used, but in general the LODs were lower (2-20 µg L^{-1}) with the HPLC-MS method. Better results were obtained with the APPI source than with ESI source.

Sun et al. (2007) developed an HPLC-MS/MS method for the determination of 2-ITX in beverages and packaging. The equipment used was provided with a turbo ion-spray source operated in positive mode and a triple quadrupole/linear ion-trap mass detector. Chromatographic separation was achieved with two different reversed-phase C18 columns: BDS Hypersil (Thermo® Scientifics) (i.d., 100 mm x 3 mm and particle size, 5 µm) and Inertsil ODS-3 (G.L. Sciences®) (i.d., 100 mm x 3 mm and particle size, 3 µm), and methanol and 0.1 % formic acid were used as mobile phase, in gradient mode.

The extraction procedure was carried out in FCM with acetonitrile; for the foodstuffs, extraction with acetonitrile and Carrez clarification were performed, followed by SPE. The MS parameters were the same as those reported by Sagratini *et al.* (2006). This method showed good sensitivity, with LOD =

0.15 µg kg^{-1} and LOQ = 0.50 µg kg^{-1}. Detectable amounts of ITX were found in all packaging, and in 7 of the 39 beverages analyzed.

Bagnati *et al.* (2007) developed another HPLC-MS/MS method for determining both isomers of ITX (2- and 4-ITX) in milk. The extraction was carried out with acetonitrile. The chromatographic method was performed with a triple quadrupole mass detector, with hyperbolic rods, equipped with a turbo ionspray source operated in positive mode. Two columns were tested: Luna C8 (i.d., 50 mm x 2 mm and particle size, 5 µm) (Phenomenex®), and a Discovery® ZR-PS (i.d., 150 mm x 2.1 mm and particle size, 3 µm) (Sigma-Aldrich®), with zirconium-polystyrene as the bonded phase. The mobile phase was acetonitrile and 0.05 % acetic acid, and gradient mode was used.

The mass parameters were the same as in the last two methods for the determination of ITX. Under those conditions, the method yielded a lower LOQ with the Luna C8 column (2.5 µg L^{-1}) but only Discovery® ZR-PS yielded acceptable separation of the two isomers. The results showed 43 % of positive cases in the milk samples tested.

Gallart-Ayala *et al.* (2008) also studied both of the isomers of ITX by using highly selective reaction monitoring (H-SRM). Two columns were tested for the chromatographic separation: SunFire® C18 (i.d., 150 mm x 2.1 mm and particle size, 3.5 µm) (Waters®) and a Discovery® HS F5 (i.d., 150mm x 2.1mm and particle size, 3 µm) (Supelco®). The liquid chromatograph was equipped with a triple quadrupole mass detector and an ESI source operated in positive mode.

The extraction procedure and mass parameters were the same as reported by Sun *et al.* (2007). For analysis of foodstuffs, the column selected was the pentafluorophenylpropyl Discovery® HS F5, because separation of both isomers was not achieved with the Sunfire® column. The method yielded excellent LODs of 12.0 (for 2-ITX) and 13.0 ng kg^{-1} (for 4-ITX) in milk samples and LODs of 2.0 (for 2-ITX) and 3.6 ng kg^{-1} (for 4-ITX) in multifruit purée samples; 2-ITX was detected in 4 of the 18 analyzed samples and 4-ITX in only one sample.

Gil-Vergara *et al.* (2007) used HPLC-MS/MS to detect 2-ITX and the amine synergist EHA in milk samples. In this study, two different extraction procedures were tested: pressurized-liquid extraction and liquid–liquid extraction (successive extractions with citrate buffer, acetonitrile and a mixture of tert-butyl methyl ether and hexane). Both extraction procedures yielded the same recoveries.

Chromatographic separation was achieved with a SunFire® reversed phase C18 column (i.d., 50 mm x 2.1 mm and particle size, 3.5 µm), in isocratic mode, and with methanol and water and 10 mM ammonium formate. The analysis was performed in a liquid chromatograph with triple quadrupole mass detector and an ESI source (+). The 2-ITX mass transitions were the same as in the previously mentioned studies; for EHA, the transitions were selected from the protonated molecule. For EHA and 2-ITX, the LODs obtained with this method were 0.01 and 0.03 µg L^{-1} and the LOQs were 0.03 and 0.1 µg L^{-1}, respectively. 2-ITX and EHA were detected in 15 % of the samples.

Shen *et al.* (2009) developed another multimethod for the determination of seven photoinitiators (BP, ITX, EHA, HCPK, BDMB and TPO) in milk samples and packaging. Extraction was performed with acetonitrile for the packaging and with acetonitrile followed by SPE for the foodstuffs.

This method was carried out with a triple quadrupole mass detector and two different sources were tested: ESI and APCI. The best results were obtained with the ESI source operated in positive mode. Chromatographic separation was achieved with a Gemini® reversed-phase C18 column (i.d., 150 mm x 2.0 mm and particle size, 3 µm) (Phenomenex®) and a gradient of methanol and 20 mM ammonium formate with 0.1 % formic acid. The precursor ions selected were the protonated molecules of each photoinitiator, and the product ions were chosen on the basis of the most abundant fragments originated from each precursor ion. The LODs of the method ranged from 0.05 µg kg^{-1} for TPO and BDMB to 2.5 µg kg^{-1} for HCPK, and the LOQs ranged between 0.1 and 5 µg kg^{-1}. In this study, it was concluded that ITX and BP were the photoinitiators most commonly used in milk packaging.

In 2011, Gallart-Ayala *et al.* (2011) improved a method that they had developed three years before for the determination of eleven photoinitiators (BP, 2-ITX, 4-ITX, PBZ, DEAB, EDB, EHA, HCPK, DMPA, HMPP and DETX) in different foodstuffs and packaging. The equipment and chromatographic parameters were the same in both methods and the precursor ions of the photoinitiators were the protonated molecules $[M+H]^+$, except in the case of DMPA, and the precursor ion was the molecular ion without a methoxy group $[M-CH_3O]^+$. The product ions were selected on the basis of the most-abundant fragments generated from the precursor ions (Gallart-Ayala *et al.*, 2008, 2011).

The extraction solvent used for the packages was dichloromethane. Two different methods were tested for the foodstuffs: the QuEChERS method and another based on a method used in a previous study, but no differences were found between the results obtained (Sagratini *et al.*, 2008). The LOQ ranged between 0.2 and 2.3 µg kg^{-1}, except for HCPK and HMPP, for which the LOQ ranged between 500 to 710 µg kg^{-1} depending on the extraction method. Positive results were obtained for the foodstuffs and packaging.

The most recent HPLC-MS/MS method reviewed was that used by Biedermann *et al.* (2013) to determine EHA, EDB, BP, 4-MBP, MBB, 2-ITX, PBZ, HCPK and DMPA in different foodstuffs and their respective recycled paperboard packages . The QuEChERS method was used to extract the photoinitiators from foodstuffs, and the packages were extracted with acetonitrile. The method yielded a LOD = 0.15 mg kg^{-1}.

From the studies reviewed, it can be concluded that HPLC-MS or HPLC-MS/MS are suitable techniques for the analysis and/or confirmation of the presence of photoinitiators in foodstuffs and/or FCM. A standard method could achieve the chromatographic separation with a C18 column. In the case of ITX isomers, columns containing pentafluorophenylpropyl and zirconium-polystyrene, as active compounds of the matrix, proved to be good options.

Use of an ESI source in positive mode and a triple quadrupole mass detector seem to be the best options, with LODs and LOQs as low as the ng kg^{-1} order of magnitude for both FCM and foodstuffs.

A chromatogram obtained from the extraction of a beer can, under the suggested conditions is represented in Figure 6.4. In the same sample analyzed by HPLC-DAD, only BP and EDB were identified, whereas ITX, DEAB, MBB, HCPK and EHA were also detected by the LC-MS/MS method.

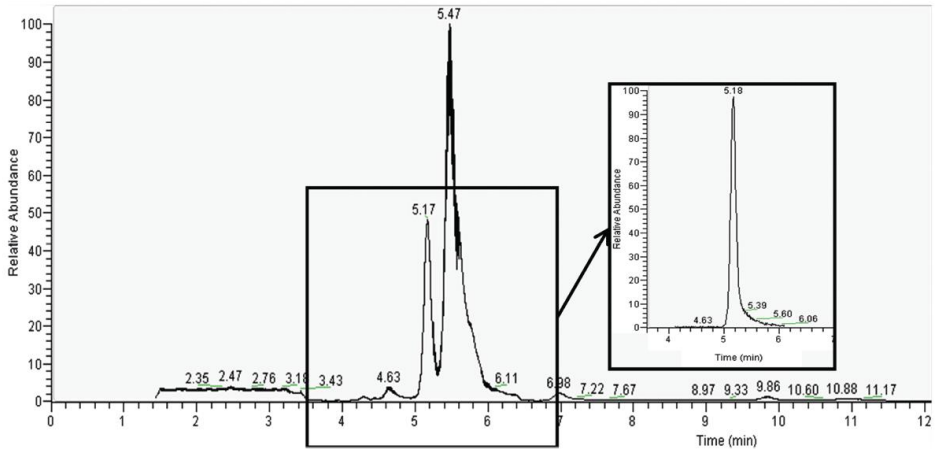


Figure 6.4: HPLC-MS/MS chromatogram in SRM (single reaction monitoring) mode of a beer can extract in which the following photoinitiators and amine synergist (EHA) were detected: MBB (4.29 min), HCPK (4.64 min), EDB (5.17 min), BP (5.48 min), MBB (9.84 min), ITX (13.52 min) and EHA (17.62 min).

6.2.1.2. Gas Chromatography (GC)

This technique is very common for determining semivolatile photoinitiators such as benzophenone and derivatives. As in liquid chromatography, different detectors can be used with this technique, and the mass detector is the most commonly used (GC-MS), although in this case the use of mass-mass tandem is not very common (Castle *et al.*, 1997; Anderson & Castle, 2003; Gil-Vergara *et al.*, 2007; Allegrone, 2008; Pastorelli *et al.*, 2008; Sanches-Silva *et al.*, 2008b; Koivikko *et al.*, 2010;. Negreira *et al.*, 2010; Van Hoeck *et al.*, 2010; FSA, 2011; Bentayeb *et al.*, 2013; Bradley *et al.*, 2013). A flame ionization detector (FID) was also used (Wang *et al.*, 2009; Huang *et al.*, 2012).

6.2.1.2.1. GC-FID

Wang *et al.* (2009) and Huang *et al.* (2012) developed a GC-FID method to study the stability of HCPK and DMPA in food simulants. Dichloromethane was used for sample extraction.

The chromatographic separation was achieved with a low polar capillary column: HP-5 (Agilent®) ((5 %-phenyl)-methylpolysiloxane) (i.d., 30 m x 0.32 mm and film thickness, 0.25 µm). The system was operated in splitless mode, with the oven temperature programmed to increase from 100 to 300 ^{o}C, and the detector temperature was set at 250 ^{o}C. The photoinitiators were identified by comparison of the retention time with the corresponding peak in a standard solution.

This methodology may be a good option for daily analysis because of the simplicity of sample processing and the low cost of the equipment.

6.2.1.2.2. GC-MS and GC-MS/MS

Three GC-MS methods were developed before the first scandal involving ITX was reported (Castle *et al.*, 1997; Papilloud & Baudraz, 2002a, 2002b; Anderson & Castle, 2003). In the first method, developed by Castle *et al.* (1997), three different photoinitiators (DMAB, MK and DEAB) were confirmed in food packages. The extraction procedure was the same as in the HPLC-DAD method described in a previous section. The source used was electron impact (EI) and the injection was in splitless mode. The oven temperature was programmed to increase from 60 ^{o}C to 300 ^{o}C. The chromatographic separation was achieved with a dimethylpolysiloxane column: CP-Sil 5CB (Chrompack®) (i.d., 17 m x 0.25 mm and film thickness, 0.12 µm). The LODs, which ranged from 0.14 to 0.6 mg kg^{-1}, indicated a lower sensitivity for MK determination than with the HPLC-DAD method developed in the same study.

Another method for the determination and quantification of the same three photoinitiators in food samples is also reported in the same study (Castle *et al.*, 1997). In this case, the same equipment was used, but the oven

temperature was increased from 100 ºC to 300 ºC. Sample (food) extraction was carried out (twice) with ethanol and triethylamine. The LOD was 2 µg kg^{-1}. The results revealed quantities of photoinitiators that are unlikely to pose a risk to human health.

Papilloud and Baudraz (2002a, 2002b) developed a method for determining BP, ITX, EHA, HCPK, DMPA and MMMP in food simulants extracted by SPE. The system was a gas chromatograph coupled to an ion-trap mass spectrometric detector and equipped with a Optima Delta-6 (Mackerey-Nagel®) medium polar column of i.d. 30 m x 0.25 mm. The oven temperature was programmed to increase from 50 ºC to 250 ºC. The method showed good linearity and reproducibility.

Anderson and Castle (2003) developed a GC-MS method for the determination of residues of BP in food and paperboard packaging. The chromatographic method used a nonpolar Rtx-1 column (i.d., 60 m x 0.25 mm and film thickness, 0.25 mm) (Restek®). The oven temperature was programmed to increase from 50 to 280 ºC and the injection was in splitless mode.

The results showed a good sensitivity in food, with a LOD = 0.01 mg kg^{-1} and a LOQ = 0.05 mg kg^{-1}. BP was found in 41 % of the packaging and in the foodstuff contained in the contaminated packaging, 71 % of the samples contained appreciable amounts of this photoinitiator.

Since the first case of milk contaminated with ITX was reported in Italy, many other GC-MS methods have been developed. Sanches-Silva *et al.* (2008b) determined the same photoinitiators as Papilloud and Baudraz (2002a, 2002b) in beverage packaging. Chromatographic separation was achieved with a low polarity column Rtx®-5MS (i.d., 30 m x 0.25 mm and film thickness, 0.25 µm) (Restek®) operated in splitless mode. The oven temperature was programmed to increase from 120 to 300 ºC.

Extraction was performed with acetonitrile. The results showed that this method was suitable for the determination of the six above-mentioned photoinitiators in FCM.

Pastorelli *et al.* (2008) used GC-MS as confirmatory technique for the determination of BP in food and FCM. The equipment was the same as in Sanches-Silva *et al.* (2008b) but with the split mode of operation (1:20), and the extraction was achieved with acetonitrile. The method showed good results with an analytical run time of only eleven minutes.

Sagratini *et al.* (2008) developed a GC-MS method combined with HPLC-DAD, HPLC-MS and HPLC-MS/MS methods discussed in a previous section. In this method BP, ITX, EHA, EDB and HCPK were determined in beverages and packaging. The FCM was first extracted with dichloromethane and then by SPE; the foodstuffs were extracted three times with n-hexane, sodium sulfate was added and extracted by solid-phase extraction.

Chromatographic separation was achieved with an HP-5 MSI column (i.d., 30 m x 0.25 mm and film thickness, 0.25 µm) (Agilent Technologies®) operated in split mode (1:40). The oven temperature was programmed to increase from 80 ^{o}C to 300 ^{o}C. The lowest LODs and LOQs were obtained using GC/MS (0.2-1 and 1-5 µg L^{-1}).

Koivikko *et al.* (2010) developed another GC-MS method to confirm the presence of BP, 4-MBP and various derivatives in FCM. A low-polar column DB-5-HT (Agilent®) (i.d., 30 m x 0.25 mm and film thickness, 0.1 µm) was used to achieve for chromatographic separation of all photoinitiators. Injection was in split mode (1:40). The oven temperature was programmed to increase from 100 ^{o}C to 250 ^{o}C. The results confirmed the data obtained with the HPLC-DAD method discussed in a previous section.

Gil-Vergara *et al.* (2007) compared LC-MS/MS and a GC-MS method for the determination of EHA and ITX in milk and milk-based beverages. For this purpose, the gas chromatograph was equipped with a nonpolar column DB 5 MS (i.d., 30 m x 0.25 mm and film thickness, 0.25 µm) (J&W Scientific®). The

oven temperature was programmed to increase from 50 to 270 ^{o}C and injection was in split mode.

The results showed LODs of 0.1 for ITX and 0.3 µg L^{-1} for EHA and LOQs of 0.5 and 1.0 µg L^{-1}, respectively. These values were higher than those reported for HPLC-MS/MS, but the values were lower than other HPLC-MS/MS methods, and this method proved to be a good alternative to HPLC-MS methods (Sun *et al.*, 2007).

To determine 7 different photoinitiators (HCPK, DMPA, BP, 4-MBP, ITX, EHA and EDB) in packaged milk, Negreira *et al.* (2010) developed two GC-MS methods, the first for determination and the second to confirm the results. In the first method, chromatographic separation was achieved with a low polar column HP-5 MS (i.d., 30 m x 0.25 mm and film thickness, 0.25 µm) (Agilent Technologies®). The oven temperature was programmed to range from 70 ^{o}C to 280 ^{o}C, and the injection mode was splitless. In the confirmatory method, an ion trap was used, and the column was a low-polar column DB-5-HT (Agilent Technologies®) (i.d., 30 m x 0.25 mm and film thickness, 0.1 µm).

SPME was used for the milk samples and acetonitrile was used for the packaging. The LOQs ranged from 0.6 and 3.5 µg L^{-1}. Only benzophenone was detected in the milk samples.

Bentayeb *et al.* (2013) developed a GC-MS method to confirm the results of a DART/TOF-MS method (discussed in the DART section) in FCM. For confirmation, the packages were extracted with methylene chloride and injected into the gas chromatograph in splitless mode. The chromatographic conditions (source and column) were the same as those described by Negreira *et al.* (2010), but the oven temperature was programmed to range from 50 to 295 ^{o}C. The results obtained suggest that GC-MS is a good technique for confirmation of detection of photoinitiators in FCM.

Van Hoeck *et al.* (2010) determined BP and 4-MBP in breakfast cereals. For this purpose, the cereals were extracted using a mixture of dichloromethane and acetonitrile, followed by SPE. The chromatographic method used nonpolar

5% phenyl-polysilphenylene-siloxane SGE BPX-5 column (i.d., 25 m x 0.22 mm and film thickness, 0.25 µm) (SGE®). The injection mode was splitless, and the oven temperature was programmed to increase from 50 to 280 ºC. The detector was an ion trap. This method was able to detect low concentrations of BP and 4-MBP (LODs = 2 µg $kg^{-1}$and LOQs = 6-8 µg kg^{-1}).

Bradley *et al.* (2013) developed a multimethod for analyzing 17 photoinitiators (BP, PBZ, 2-HBP, 4-HBP, MBB, HCPK, DMPA, MMMP, 2-ITX, 4-ITX, DETX, EHA, EDB, 4-MBP, 3-MBP), 2-MBP and BMS) in 350 different foodstuffs and for determining whether the food packaging was the origin of contamination (Bradley *et al.*, 2013; FSA, 2011). In this study, the food was extracted twice with acetonitrile/dichloromethane (1:1), followed by two more extractions with hexane and acetonitrile. The paper/cardboard samples were extracted with acetonitrile.

Chromatographic separation was performed with a low-polarity column: Phenomenex® ZB-5ms (i.d., 30 m x 0.25 mm and film thickness, 0.25 µm), with injection in splitless mode. The oven temperature was programmed to range from 100 ºC to 320 ºC. The results showed positive results for nine different photoinitiators in foodstuffs. The LODs ranged between 0.44 and 17 µg kg^{-1} and the LOQs between 1.5 and 57 µg kg^{-1} for the different food samples.

Finally, only one reference to a gas chromatography method with a MS/MS detector was found. Allegrone *et al.* (2008) developed a method for determining ITX in milk. For the extraction procedure, two alternative methods were assayed. In the first method, the milk was mixed with ethanol and water and extracted with SPE; in the second option, an alkaline hydrolysis was carried out previous to the SPE. Chromatographic separation was achieved with a low-polarity column Rtx®-5MS (i.d., 30 m x 0.25 mm and film thickness, 0.25 µm) (Restek®) operated in splitless mode. The chromatograph was equipped with an ESI source operated in positive mode and an ion-trap mass spectrometer.

With this method, the LOD was 0.1 µg L^{-1} and the LOQ, 0.5 µg L^{-1}, and appreciable amounts of ITX were detected in all of the milk samples.

Gas chromatography can be recommended for the analysis and confirmation of several photoinitiators, e.g. Bradley *et al.* (2013) analyzed 20 different photoinitiators. However, the method is not suitable for some photoinitiators because of their physicochemical properties, e.g. Benzophenone acrylate or BDMB. Generally, for analysis of these compounds, a low polar column, an EI source and a MS or MS/MS are recommended, yielding LODs in the µg kg^{-1} order in most cases. By way of example, Figure 6.5 shows a chromatogram for sixteen photoinitiators obtained with the proposal suggestions.

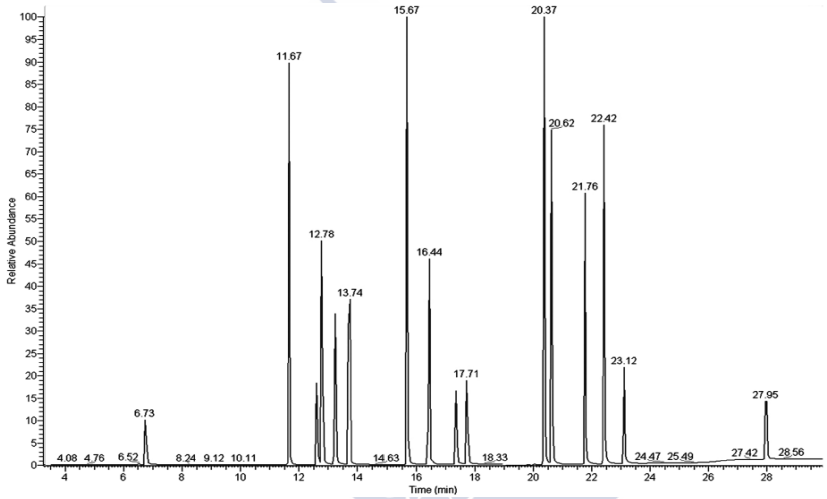


Figure 6.5: GC-MS chromatogram of sixteen photoinitiators and an amine synergist (EHA): HMPP (6.73 min), BP (11.67 min), HCPK (12.65 min), EDB (12.81 min), 2-HBP (13.19 min), 4-MBP (13.75 min), DMPA (15.67 min), MBB (16.45 min), 4-HBP (17.29 min), HMB (17.75 min), EHA (20.39 min), MMMP (20.64 min), ITX (21.78 min), PBZ (22.40 min), DETX (23.10 min) and DEAB (27.95 min).

6.2.1.3. High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC was one of the first techniques developed for the identification and quantification of photoinitiators. Morlock & Schwack (2006) developed two methods for quantifying ITX in milk, yoghurt, margarine and soybean oil. The extraction procedure was conducted with various solvents, and the best results were obtained with hexane/ethyl acetate. Two stationary phases were tested: silica gel 60 and RP18 HPTLC. For quantification, FLD was used at 254 / >400 nm.

To confirm the results, two different approaches were tested: ESI/MS and DART/MS, and good robust results were obtained with LOD = 64 pg and a LOQ = 128 pg in ESI/MS mode.

6.2.2. VOLTAMMETRY

Ranganathan *et al.* (2011) developed cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods for the determination of ITX in wine. For this purpose, they used an electrochemical workstation with three-electrode cell: a glassy-carbon solid electrode as working electrode, the counter electrode was a Pt wire and as reference electrode Ag/AgCl. Lithium perchlorate was used as supporting electrolyte. A small amount of LiClO4 was added to the samples.

Haidong *et al.* (2012) developed an amperometric sensor for the determination of benzophenone in FCM. The sensor was developed by electropolymerization of o-phenylenediamine on a glassy-carbon electrode in the presence of BP. As BP is nonelectroactive, potassium ferricyanide was used. The BP molecules enter the holes of the imprinted film, blocking electron transfer and the concentrations of BP are indicated by the resulting decrease in the current.

The equipment used to test this sensor was the same as that used by Ranganathan *et al.* (2011). The extraction procedure was carried out with ethanol. The results were compared with an HPLC-DAD method developed in the same study with a Diamonsil C18 column (i.d., 250 mm x 4.6 mm and particle size, 5 µm), with acetonitrile and water as mobile phase, in gradient mode. The data obtained revealed similar LODs as the HPLC-DAD method and proved to be a good alternative to HPLC-DAD and GC-MS methods.

6.2.3. DART

This new technique is presented as the most suitable for screening photoinitiators in FCM by offset transfer because of the huge advantage that no sample preparation is required, and the analytical run time is therefore minimal.

Bentayeb *et al.* (2013) developed a DART/TOF-MS method to determine photoinitiators in FCM. The DART parameters were 500 V of exit grid voltage and the helium current flow was carried out at a temperature of 530 ^{o}C in positive-ion mode. However, this methodology has the drawback that it is not expected to be highly quantitative: ion intensities were quite variable, and the LODs were therefore presented as a range: 0.20 μg dm^{-2}<LOD<20 μg dm^{-2}.

6.3. NON-INTENTIONALLY ADDED SUBSTANCES (NIAS)

Two main approaches can be used for the determination of compounds related with the use of photoinitiators can be followed: study of possible byproducts or performance of a non-targeted screening analysis of the printed materials.

6.3.1. STUDY OF BYPRODUCTS

Photochemistry studies can demonstrate the cleavage routes that each photoinitiator can follow and the intermediate and final molecules generated during the process. Although some of these routes have been described, this information is not available for all photoinitiators. Nonetheless, many molecules can be generated from a single photoinitiator (Dektar & Hacker, 1978; Green, 2010)

As these products can also react with the environment, the number of byproducts increases exponentially (see Figure 6.6).

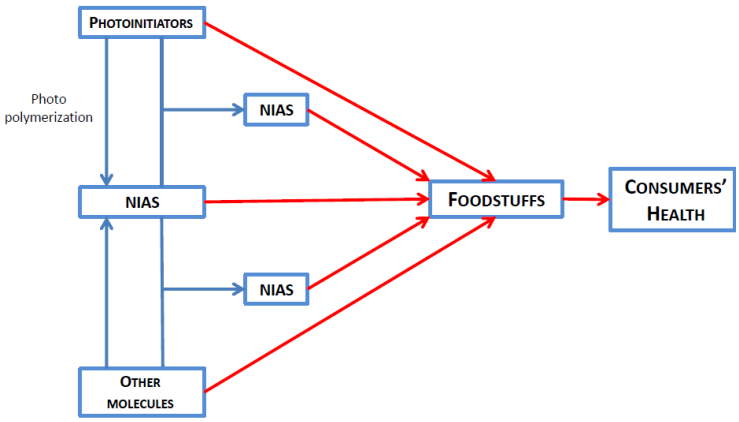


Figure 6.6: Scheme of the different molecules (and possible byproducts/NIAS) that can reach foodstuffs and pose a risk to consumer health.

As observed in these studies, some hazardous products can be generated, e.g. benzene is a photolysis product of the cationic photoinitiator triphenylsulfonium hexafluorophosphate (Dektar & Hacker, 1978). The International Agency for Research on Cancer (IARC) has classified this molecule as Group I (carcinogenic to humans) on the basis of the results of scientific studies (IARC, 2012)

If all byproducts, impurities and other photoproducts were known, analytical methods could be developed for determination of these substances in FCM and foodstuffs. However, as there is a huge number of such compounds, this task is almost impossible. Nevertheless, some potentially hazardous molecules should be identified (e.g. benzene) and monitored to prevent risks to consumer health.

6.3.2. NON-TARGETED SCREENING ANALYSIS

The number of possible byproducts is enormous because the byproducts of the photoinitiators are added to others derived from adhesives, plasticizers, and also from many other components of the FCM, foodstuffs and even the environment.

To analyze such molecules, some authors have proposed some strategies for overcoming the above-mentioned problem (Feigenbaum *et al.*, 2002; Coulier *et al.*, 2008). These can be summarized in 4 different steps: sample work-up, selection of the analytical technique, determination and/or quantification of the compounds, and interpretation of the results.

First, the food or FCM should be extracted using any of the techniques previously discussed in this chapter. For this purpose, it is necessary to consider the compounds of interest, because volatile compounds may readily disappear, and many transformations may occur, such as oxidation processes or other reactions with the environment. The extraction procedure should therefore differ in relation to the sample and the compounds of interest. According to Coulier *et al.* (2008), the best option is to avoid this step, although this was not possible until the appearance of the DART technique, which enables direct analysis of samples (Coulier *et al.*, 2008; Hajslova *et al.*, 2009).

Selection of the analytical technique is critical because non-targeted analysis does not distinguish compounds, which is a problem for the chromatographic separation. GC and LC are therefore the most commonly used techniques in this field, even in two-dimensional separations (GC x GC or LC x LC). Use of MS or TOF detectors is the most convenient option with these techniques (Coulier *et al.*, 2008).

A pure standard should be used for the quantitative determination of the possible migrating molecules; however, in many cases such standards are not available and comparison of the response of the unknown with another molecule of similar structure is the best solution.

The final step in this strategy is to analyze the data obtained. Whether the quantity of migrant is relevant or not will depend on the results and on the LODs and LOQs of the method. However, if a component does not appear in the chromatograms, this does not mean that it does not represent a risk to consumer health, because the LODs may be higher than the toxicological thresholds of this unknown compound.

In the absence of toxicological data on the migrant, it is imperative to follow some criteria to decide whether the quantities of the molecules represent potential risks to consumer health. Under this premise, the threshold of toxicological concern (TTC) can be applied to the evaluation of these substances in FCM based on the Cramer classification of the structure of each of the molecules (Cramer *et al.* 1978; Munro *et al.*, 1996). With this methodology, molecules are classified as of low toxicity (Class I) or significant toxicity (Class III), with class II being of intermediate toxicity. This strategy has been recommended since the last decade and followed by most researchers and authorities see Figure 6.7 (Kroes *et al.*, 2000, 2004; Pinalli *et al.*, 2011).

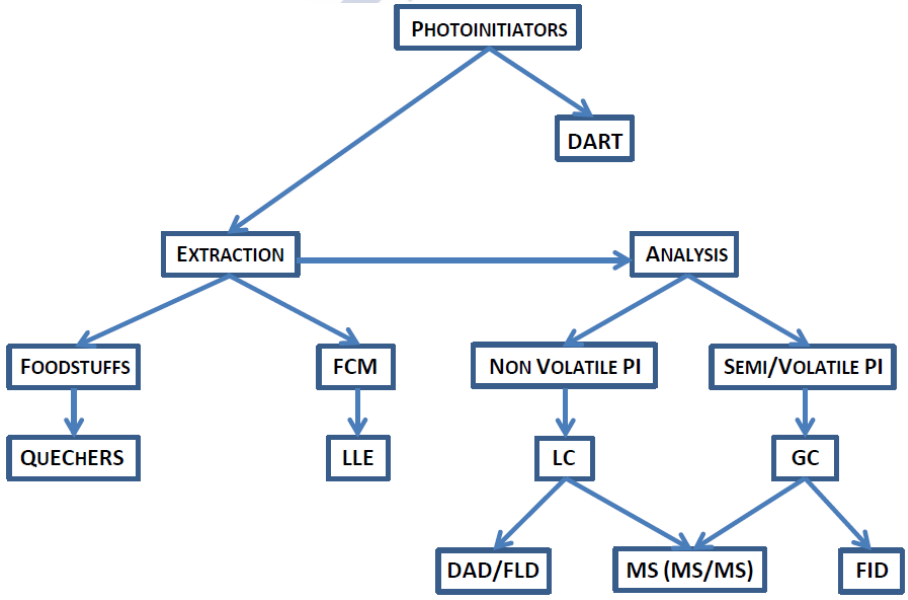


Figure 6.7: Proposed method for determining and/or quantifying photoinitiators in FCM/foodstuffs based on the predominant extractions and analytical techniques in use at present.

6.4. CONCLUSIONS AND FUTURE OVERVIEW

The lack of specific European legislation for the use of printing inks in food packaging potentially creates a risk to consumer health. General standardized protocols must be developed to harmonize procedures for the determination of

photoinitiators and their byproducts. The methods proposed by different authors are shown in the tables 5.2, 5.3, 5.4 and 5.5, and the figure 6.7, which can be used as a starting point for standardizing the procedure.

From the literature reviewed, HPLC-MS/MS-MS appears to be the analytical tool of choice because the limits of detection are the lowest obtained and this may be the most versatile of all of the methods tested. However, the most difficult challenge is to use analytical methods to determine NIAS. It is therefore imperative to carry out more detailed studies of the photoproducts derived from each photoinitiator used in FCM to identify as yet unknown products.

Moreover, legislation must also be developed in relation to NIAS. Although efforts are being made in this field, they are not yet sufficient and the following years will be decisive as regards protection of consumer health.

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7. DEVELOPMENT OF A CHROMATOGRAPHIC METHOD FOR THE SIMULTANEOUS DETERMINATION OF PHOTOINITIATORS AND AMINE SYNERGISTS IN FCM

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7.1. ABSTRACT

Photoinitiators (PIs) are components of UV-cured inks widely used in printing of food packaging. These substances can be transferred into food and may present a hazard for the human health. In the present work, a high-performance liquid chromatography method with diode-array detection (HPLC-DAD) to determine PIs and amine synergists in food packaging samples is reported. The analysis was performed with a Kromasil C18 (250 × 3.2 mm i. d., 5 µm particle size) column using a binary solvent gradient consisting of acetonitrile (A) and Milli-Q water (B) at a flow rate of 0.5 mL min^{-1} as mobile phase. The proposed method allows the separation of fourteen PIs and amine synergists in a single run. The method was validated with respect to linearity, repeatability and limits of detection and quantification. Excellent sensitivity (LODs ≤ 1.56 µg dm^{-2}) and appropriate repeatability (RSD (n = 10) < 0.9 %) were achieved.

Different types of food packaging materials including, plastic films, cardboard and cans were analyzed and PIs were detected in 47 % of the samples tested (n=17). Positive samples were confirmed by LC-MS/MS using electrospray ionization (ESI) in positive mode.

Keywords: photoinitiators, HPLC-DAD, food packaging, LC-MS/MS.

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7.2. INTRODUCTION

Since September of 2005, when the Rapid Alert System for Food and Feed (RASFF) published an alert of contamination with 2-isopropylthioxanthone (ITX) in baby milk notified from the Italian authorities, other alerts have appeared related to contaminations with photoinitiators (PIs) in foods (RASFF, 2005). A brief glance at the RASFF database shows 16 notifications of photoinitiators since 2010 (15 informations and 1 alert), this fact shows the relevance of the problem generated by the migration of photoinitiators from the Food Contact Material (FCM) into foodstuffs (RASFF, 2014).

The migration/transfer of photoinitiators from the FCM into the food can occurs by three different mechanisms (Choi *et al.*, 2002; Jung *et al.*, 2010):

- permeation of analytes from the outer printed surface through the packaging material to the food contact surface, if a barrier is not used;
- by *set-off*, the transfer of substances to the food contact surface occurs during the storage, when the printed material is stored in reels or stacks (Morlock & Schwack, 2006; Rothenbacher *et al.*, 2007); and
- indirect mass transfer by gas phase, in case of volatile compounds like benzophenone and its derivatives (Pastorelli *et al.*, 2008; Sanches-Silva *et al.*, 2008a; EFSA, 2009).

The use of photoinitiators in UV inks is considered environmentally friendly because no organic solvents are incorporated in their formulation. However, risk cannot be excluded from the use of these inks as a result of the process of photopolymerization many PIs residues are formed from the photoinitiator and monomer/oligomers are still present due to incomplete polymerization. Is possible that these substances can migrate into the foodstuffs, representing a food safety issue (Papilloud & Baudraz, 2002a; Rothenbacher *et al.*, 2007; Pastorelli *et al.*, 2008; Sanches-Silva *et al.*, 2008a; EFSA, 2009).

Most of the photoinitiators have not been fully evaluated, and until now there is not a specific European legislation about their use in FCM, except for the family of benzophenones, which is subjected to restrictions. The Regulation EU No 10/2011 on plastic materials and articles intended to come into contact with food, establishes a specific migration limit of 0.6 mg kg^{-1} for benzophenone and derivatives (EC, 2011).

Some European countries, like Switzerland, have a more detailed regulation on printing inks. The Annex 6 of the Swiss Ordinance 817.023.21 sets migration restrictions for photoinitiators, based on the existing toxicological data (FDHA, 2005).

The use of photoinitiators in FCM have to be in compliance with the framework legislation (1935/2004/EC) on the materials and articles intended to come into contact with food and with the Good Manufacturing Practices (GMP) relating to groups of materials and articles intended to come into contact with food appeared at Regulation 2023/2006 (EC, 2004, 2006).

Based on all these facts, many methods have been developed for the identification and/or quantification of photoinitiators in order to guarantee the safety in the use of printed FCM (Castle *et al.*, 1997; Papilloud & Baudraz, 2002a, 2002b; Anderson & Castle, 2003; Quinto-Fernández *et al.*, 2003; Morlock *et al.*, 2006; Sagratini *et al.*, 2006, 2008; Bagnati *et al.*, 2007; Rothenbacher, *et al.*, 2007; Sun *et al.*, 2007; Allegrone *et al.*, 2008; Benetti *et al.*, 2008; Gallart-Ayala *et al.*, 2008, 2011; Pastorelli *et al.*, 2008; Sanches-Silva *et al.*, 2008a, 2008b, 2008c, 2009; Rodríguez-Bernaldo de Quirós *et al.* 2009; Wang *et al.*, 2009; Koivikko *et al.* 2010; Van Hoeck *et al.*, 2010; Huang *et al.*, 2012). For that purpose, analytical methodologies such as HPTLC (High Performance Thin Layer Chromatography), or mainly HPLC with Diode Array-Fluorescence Detectors (DAD-FLD) have been widely used. When positive confirmation of the identity is required, mass spectrometry coupled to liquid or gas chromatography (especially for low molecular weight and high/semi-volatile molecules like benzophenone and derivatives (e.g.: 4-MBP) are the techniques of choice (Simal-Gándara *et al.*, 2003; Rial-Otero *et al.*, 2009).

Recently, a new technique, Direct Real Time Analysis (DART) coupled to time of flight - mass spectrometry (DART/TOF-MS), has proved to be particularly useful and it has been successfully applied in the determination of these compounds in kitchenware and other FCM by Bentayeb *et al.* (2012, 2013).

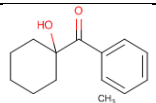
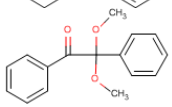
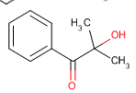
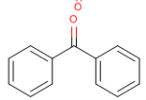
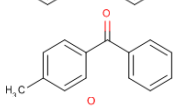
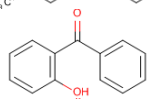
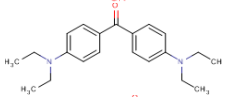
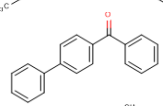
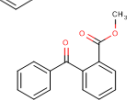
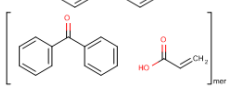
The aim of this paper was to develop a multi-analyte method for the determination of type I and type II photoinitiators and synergist amines notified in the last RASFF alerts/notifications. Performance characteristics of the method were studied. The method was further applied to determine PIs in different types of food packaging samples. LC-MS/MS operating in positive electrospray ionization (ESI) mode was used as a confirmatory technique

7.3. MATERIALS AND METHODS

7.3.1. REAGENTS AND CHEMICALS

Analytical standards of methyl-2-benzoylbenzoate (MBB) 97%, 2-hydroxybenzophenone (2-HBP) 99%, 4-methyl benzophenone (4-MBP) 99%, 4-benzoylbiphenyl (PBZ) 99%, 4,4'-bis(diethylamino) benzophenone (DEAB) 99%, 2-ethylhexyl-4-(dimethylamino)benzoate (EHA) 98%, ethyl-4-(dimethylamino) benzoate (EDB) >99%, 2-isopropyl-9H-thioxanthen-9-one (ITX) 97%, 2,4-diethyl-9H-thioxanthen-9-one (DETX) 98%, methyl-1-(4-methylthio)phenyl-2-morpholinopropan-1-one, (HCPK) 99%, 2,2-dimethoxy-2-phenyl acetophenone (DMPA) 99%, 2-hydroxy-2-methyl propiophenone (HMPP) 97%, were obtained from Aldrich. Benzophenone (BP) 99% was supplied by Fluka®. Benzophenone acrylate (BPAcr) was obtained directly from a supplier. The chemical structures and the main physico-chemical properties of the photoinitiators are summarized in Table 7.1.

Acetonitrile (AcN) HPLC grade and LC-MS grade were purchased from Merck® (Darmstadt, Germany). Formic acid was mass spectrometry grade (Sigma-Aldrich®, St.Louis, Mo, USA). Ultrapure water was obtained with a Milli-Q system (Millipore®, Bedford, MA, USA).

Structure	Formula	Name	CAS No.	Molecular Weight	Melting Point (°C)	Boiling Point (°C)	Log P (o/w)	Vapor Pressure (mm Hg) 25 °C
	C_{13} H_{16} O_2	HCPK	947-19-3	204.26	48.00	339.00*	2.18*	3.65e-05*
	C_{16} H_{16} O_3	DMPA	24650-42-8	256.30	64.00	371.10*	3.62*	1.06E-05*
	C_{10} H_{12} O_2	HMPP	7473-98-5	164.20	184.00	260.80*	1.49*	6.08E-03*
	C_{13} H_{10} O	BP	119-61-9	182.22	47.80	305.40	3.18	8.23E-04
	C_{14} H_{12} O	4-MBP	134-84-9	196.24	59.50	328.10*	3.69	1.94E-04*
	C_{13} H_{10} O_2	2-HBP	117-99-7	198.22	38.00	308.00*	3.53*	4.39E-04*
	C_{21} H_{28} N O_2	DEAB	90-93-7	324.46	96.00	475.70*	5.91*	3.25E-09*
	C_{19} H_{14} O	PBZ	2128-93-0	258.31	102.00	419.50	5.14*	3.11E-07*
	C_{15} H_{12} O_9	MBB	606-28-0	240.25	52.00	351.00	2.70	1.53E-05
	NA	BPAcr	59626-79-8	N/A	N/A	N/A	N/A	N/A

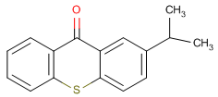
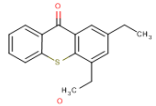
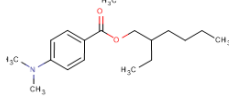
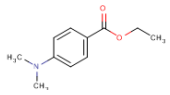
	$C_{16}H_{14}OS$	ITX	5495-84-1	254.35	76.75	398.20*	5.11*	1.43E-06
	$C_{17}H_{16}OS$	DETX	82799-44-8	268.37	N/A	427.90*	5.67*	1.58E-07
	$C_{17}H_{27}NO_2$	EHA	21245-02-3	277.40	243.00	382.90*	5.41*	4.57E-06*
	$C_{11}H_{15}NO_2$	EDB	10287-53-3	193.24	63.50	296.50*	2.51*	1.43E-03*

Table 7.1: Structures and physicochemical properties of the photoinitiators and amine synergists. * Estimated. N/A: Not available.

A primary stock solution of each photoinitiator (PI) was prepared in AcN (1,000 µg mL^{-1}) and stored at 4 ºC in darkness. Working solutions were prepared by dilution with AcN.

7.3.2. SAMPLE TREATMENT

A total of 17 food packaging samples including different kinds of cans (3), paper (1) and cardboards (8) and plastic materials (6) were analysed (Simal-Gándara *et al.*, 1999). All the packed foods were purchased in local supermarkets.

Photoinitiators were extracted from the packaging materials as follows: a known surface (0.8 dm^2) of the material was immersed into 25-50 ml of AcN and then maintained in an oven at 70 °C for 24 hours. Then an aliquot was removed from the extraction solution and filtered with 0.45 µm PTFE membrane filter (Advanted, Toyo Roshi Kaisha, Ltd., Japan).

In order to prevent a potential degradation or alteration in the composition of the extracts, the preparation was conducted under low-light conditions and stored in brown glass bottles in darkness at 4 ºC.

7.3.3. HPLC-DAD ANALYSIS

The HPLC system (Hewlett-Packard®, Waldbronn, Germany) was fitted with a HP 1100 quaternary pump, a degassing device, an autosampler, a column thermostatting system, and a diode array detector (DAD). Scan mode was used for acquisition, in the range of 190-400 nm. Chromatographic separation was achieved with a Kromasil C18 column (250 × 3.2 mm internal diameter, 5 µm particle size) (Phenomenex®, Barcelona, Spain), thermostated at 30 ºC.

A binary mobile phase with AcN and water was selected. A gradient elution method was applied, in the first two minutes the mobile phase was composed by 40 % of water and 60 % of AcN, from this minute the percentage of AcN gradually increased achieving 100 % in minute 23, then the mobile phase remaining constant until the minute 30 (in order to allow the elution of the most

retaining compounds). The flow rate was 0.5 mL min^{-1}, and the injection volume 20 µL. The wavelengths used for each photoinitiator were selected on the basis of their higher absorption peaks in UV spectra. HCPK, HMPP and MBB were monitored at 246 nm; BP, DMPA, 2-HBP, 4-MBP, BPAcr, ITX and DETX determined at 256 nm; EDB, PBZ and EHA checked at 308 nm; and DEAB observed at 374 nm.

Photoinitiators were identified by comparison of their retention times and UV spectra with those of a pure standard injected using the same HPLC conditions. In addition, a confirmation was carried out by LC-MS/MS.

Quantification was performed on the basis of linear calibration plots of peak area against concentration. Calibration lines were prepared by diluting the stock solution with AcN-water (50:50 v/v) into solutions with a concentration ranging from 0.01 mg L^{-1} to 10.0 mg L^{-1}). Each level of calibration curve is the average of three peak-area measurements.

7.3.4. HPLC-MS/MS Analysis

The HPLC-MS/MS system was comprised by an Accela autosampler, an Accela 1250 pump with a degasser, a column thermostatting system and a PDA detector, coupled to a triple quadrupole mass spectrometer TSQ Quantum Access max, controlled by Xcalibur software (Thermo Fisher Scientific, San José, CA, USA).

The chromatographic separation was performed using a reversed-phase column Kromasil C18 (150 × 3.2 mm internal diameter, 5 µm particle size) (Phenomenex®, Torrance, CA, USA), thermostatted at 30 ^{o}C, with a mobile-phase composed by AcN with 0.1 % (v/v) formic acid and Milli-Q water with 0.1 % (v/v) formic acid . The gradient elution program was the same as in the HPLC system, at a flow rate of 0.5 ml min^{-1}, and the injection volume was 20 µl. The total run time was 30 min.

PI	Retention time (min)	Precursor ion	Product ions	Collision Energy (V)	Tube Lens
HMPP	2.44	165.1 $[M+H]^+$	91.2 119.1	24 9	64
2-HBP	2.46	199.1 $[M+H]^+$	105.1 121.1	16 16	78
MBB	4.25	241.0 $[M+H]^+$	152.0 209.0	35 12	69
HCPK	4.60	205.1 $[M+H]^+$	77.2 105.1	30 12	73
EDB	5.11	194.1 $[M+H]^+$	151.1 166.1	22 13	56
BP	5.43	183.0 $[M+H]^+$	77.2 105.1	30 14	79
DMPA	5.73	225.0 $[M+CH_3O]^+$	77.2 197.0	33 12	79
4-MBP	6.92	197.0 $[M+H]^+$	105.1 119.1	14 5	77
DEAB	9.76	325.2 $[M+H]^+$	148.1 281.1	42 27	92
PBZ	10.78	259.0 $[M+H]^+$	77.2 105.1	32 17	82
BPAcr	13.1	657.1 $[X]^+$	242.8 396.6	39 32	110
	12.8	701.1 $[X]^+$	242.7 440.7	47 36	124
	12.5	745.1 $[X]^+$	242.7 484.7	46 42	132
	12.1	789.1 $[X]^+$	242.8 528.7	50 44	136
	11.8	833.2 $[X]^+$	312.7 572.8	45 40	134
ITX	13.44	255.0 $[M+H]^+$	184.0 213.0	37 21	82
DETX	16.51	269.0 $[M+H]^+$	212.9 241.0	29 22	92
EHA	17.57	278.1 $[M+H]^+$	151.1 166.1	28 20	90

Table 7.2: MS–MS conditions and retention time of each substance.

The mass spectrometer was operated in positive ESI mode. Nitrogen was used as the sheath gas at a pressure of 35 psi, and as auxiliary gas (pressure 10 arbitrary units) and argon was used as the collision gas at a pressure of 1.5

mTorr. The spray voltage was 3000 V. The vaporizer and capillary temperatures were 340 and 350 ^{o}C, respectively. Other conditions are given in Table 7.2.

7.4. RESULTS AND DISCUSSION

7.4.1. CHROMATOGRAPHIC METHOD OPTIMIZATION

The aim of this work was to develop a simple and reliable HPLC-DAD method for the identification and quantification in packaging materials of fourteen of the most commonly photoinitiators and amine synergists used nowadays by the food packaging industry.

In the development of a chromatographic method the selection of the stationary phase is a crucial step in order to achieve an appropriate separation and a good resolution of the chromatographic peaks. Three columns, Kromasil C18 (150 x 3.2 mm i.d., 5 µm); Kromasil C18 (250 x 3.2 mm i.d., 5 µm) and Kinetex Phenomenex® 100 A (150 x 4.6 mm i.d., 2.6 µm) were tested. The analysis time with, Kromasil C18 (150 x 3.2 mm i.d., 5 µm) was shorter, 19 min compared to 23 min with Kromasil C18 (250 x 3.2 mm i.d., 5 µm), nevertheless HCPK and MBB were not completely separated. On the other hand, with Kinetex Phenomenex® 100 A (150 x 4.6 mm i.d., 2.6 µm) a poor resolution was obtained for these two PIs. Therefore Kromasil C18 (250 x 3.2 mm i.d., 5 µm) was selected to carry out further analysis.

In addition, several elution gradients consisting of AcN and water in different proportions were tried. A gradient starting from AcN-water (60:40 v/v) to 100 % AcN in 23 min was selected as the best compromise between analysis time and resolution of the target analytes.

Several flow-rates, 0.4; 0.5; and 0.7 mL min^{-1} were assayed; the best separation was achieved with a 0.5 mL min^{-1} flow rate.

Under the optimized conditions a suitable separation was obtained. Chromatograms of a standard solution of PIs (A) and a food packaging sample (film 2) (B) are presented in Figure 7.1. As can be observed, BPAcr occurs as a

group of peaks, and for its quantification peaks at 17.3, 17.8 and 18.1 minutes were selected.

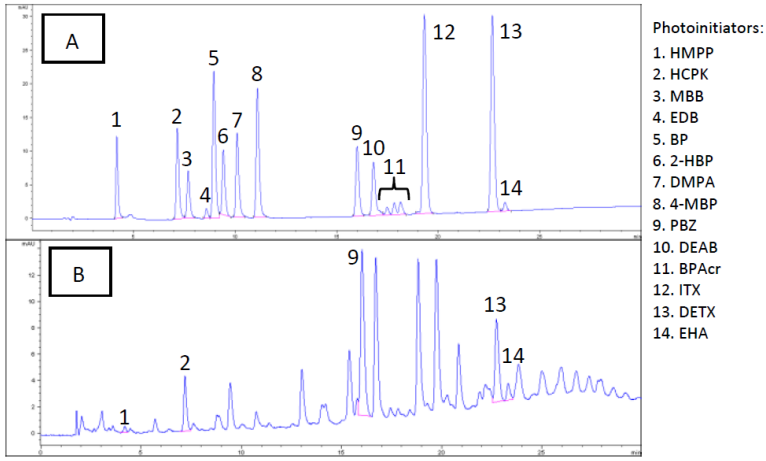


Figure 7.1: UV chromatograms at 256 nm of: (a) 10 mg L^{-1} standard solution in AcN–water (50:50) and (b) film 2 extract in acetonitrile, subsequently diluted 1:1 with water

7.4.2. IN HOUSE METHOD VALIDATION

The developed method was validated in terms of linearity, limits of detection and quantification and repeatability. The linearity of the method was tested by using a series of photoinitiator standards solutions of known concentration. The calibration curves were constructed using ten concentration levels and they were fitted to a linear regression equation. Table 7.3 shows the linear equations and the coefficients of determination (R^2). All compounds presented good linearity in the studied concentration range, the coefficients of determination were in all cases greater than 0.9998. The limits of detection and quantification (LOD and LOQ) (defined as the signal three and ten times the high of the noise level, respectively) calculated according the American Chemical Society ACS guidelines (1980) are presented in table 7.3. Due to the different volumes of FCM, each sample had a specific contact-surface/extraction-solvent-volume ratio, therefore a range of LOD and LOQ is presented. The proposed method showed a good sensitivity with detection limits in the range of 0.31-

1.56 µg dm^{-2} of printed surface, except for BPAcr (7.81-15.6 µg dm^{-2}). Our results were slightly lower than those reported by Sagratini *et al.* (2008).

Repeatability were determined by analyzing ten replicates of the standards at a concentration of 10 mg L^{-1} and expressed as the percentage of RSD (% RSD (n=10)). All analytes presented repeatabilities lower than 1 % except for BPAcr (1.3 %) (Table 7.3).

Photoinitiator	Calibration equation	r^2	LOD (µg dm^{-2})	LOQ (µg dm^{-2})	RSD (%)
HMPP	y = 138.741x - 2.661	0.9999	0.31-0.63	0.78-1.56	0.2
HCPK	y = 150.652x - 0.777	1.0000	0.78-1.56	1.56-3.13	0.4
MBB	y = 110.486x - 4.432	0.9999	0.78-1.56	1.56-3.13	0.6
EDB	y = 303.705x - 5.207	0.9999	0.31-0.63	0.78-1.56	0.4
BP	y = 219.619x - 1.244	0.9999	0.31-0.63	0.78-1.56	0.2
2-HBP	y = 109.916x - 2.597	0.9998	0.31-0.63	0.78-1.56	0.6
DMPA	y = 125.631x - 0.406	0.9999	0.78-1.56	1.56-3.13	0.5
4-MBP	y = 194.825x + 0.446	1.0000	0.31-0.63	0.78-1.56	0.9
PBZ	y = 138.204x + 0.918	0.9999	0.78-1.56	1.56-3.13	0.4
DEAB	y = 291.069 + 2.008	1.0000	0.78-1.56	1.56-3.13	0.1
BPAcr	y = 53.779x - 1.379	0.9999	7.81-15.6	15.6-31.2	1.3
ITX	y = 373.101x + 0.939	0.9999	0.31-0.63	0.78-1.56	0.1
DETX	y = 346.828x + 0.692	1.0000	0.78-1.56	1.56-3.13	0.1
EHA	y = 246.254x + 1.125	1.0000	0.31-0.63	0.78-1.56	0.1

Table 7.3: HPLC–DAD method validation data.

7.4.3. ANALYSIS OF SAMPLES

Once the chromatographic conditions were established, the proposed method was applied to the determination of PIs in real samples. A total of 17 food packaging samples including plastic films, cardboard and cans were analyzed. PIs were detected in 47 % of the samples tested. MBB, 2-HBP, 4-MBP, ITX, DMPA, HCPK and BPAcr were not found in any samples analyzed. The PIs concentrations found in the food packaging samples analyzed are summarized in Table 7.4. Values ranged from 2.23 to 102.93 µg dm^{-2}.

FCM	BP	EHA	HMPP	HCPK	DETX	PBZ	EDB
Film 1	5.92	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Film 2	n.d.	71.72	2.91	15.48	11.54	72.26	n.d.
Cardboard 1	15.26	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cardboard 3	17.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cardboard 6	14.33	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cardboard 7	13.89	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Can 1	n.d.	102.93	n.d.	n.d.	n.d.	n.d.	n.d.
Can 2	19.08	n.d.	n.d.	n.d.	n.d.	n.d.	2.23
Can 3	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Table 7.4: Detected PI's and amine synergists in FCM samples analyzed (µg dm^{-2} FCM material). n.d:Not detectable.

EHA was detected in two types of samples, a plastic film and a can; the concentrations detected were 102.93 and 71.72 µg dm^{-2}, respectively. Sagratini *et al.* (2008) reported much lower levels of this PI in the packaging materials tested.

BP was found in all the types of the packaging samples analysed, the highest values corresponded to cardboard and can samples. With respect to cardboard group samples only BP was detected in four out of the eight samples.

Seven of the fourteen photoinitiators were detected in any of the analysed FCM samples. Given the relatively low number of samples analysed (n=17), it can be concluded that the selected substances are currently amongst the most commonly used.

7.4.4. HPLC-MS/MS METHOD

To confirm the results obtained by the HPLC-DAD, an LC-MS/MS method was designed. The analysis was performed under analytical conditions described previously. ESI was used as ionization source because of its better suitability for these compounds, as it has been reported in previous works (Gallart-Ayala *et al.*, 2013).

Mass spectrometry parameters were optimized by directly infusing standard solutions in AcN-water (50:50 v/v), using a built-in syringe pump, and mixed with the mobile phase, AcN 0.1 % (v/v) formic acid-Milli-Q water 0.1 % (v/v) formic acid (60:40 v/v). Selected multiple reaction monitoring transitions (MRM), as well as retention times and adjusted voltage settings are shown in table 7.2.

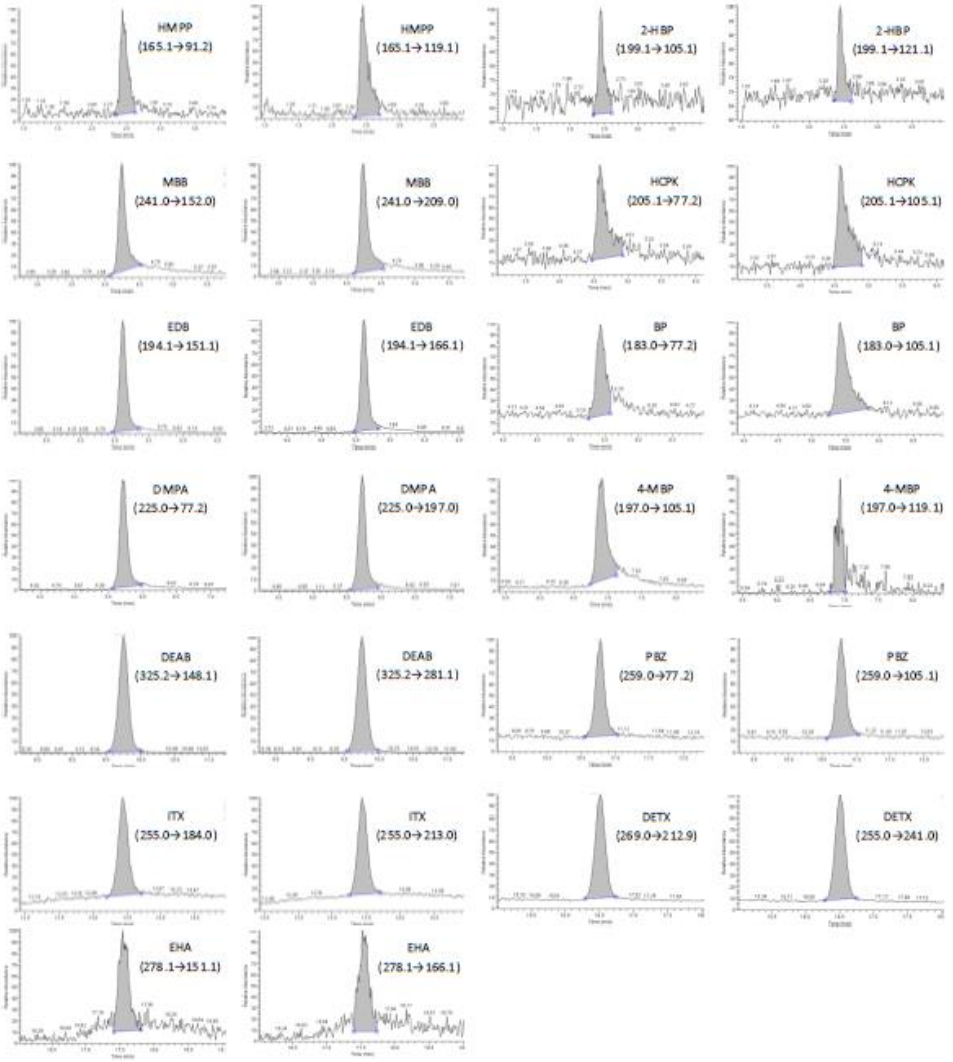


Figure 7.2: HPLC-MS/MS chromatograms of thirteen photoinitiators and amine synergists (BPAcr not included) at concentrations equal to the LOD of the HPLC-DAD method.

Spectra were acquired in the positive ion mode, and twelve of the analyzed photoinitiators showed that the most intense precursor ion was the protonated molecular ion $[M+H]^+$. In the case of DMPA, the most intense precursor ion, was the ion $[M-CH_3O]^+$ after the loss of a methoxy group. This fact is in agreement with the work of Gallart-Ayala *et al.* (2011). The two main products of each precursor ion are shown in figure 7.2.

BPAcr was a particular case. This compound occurs as a mixture of oligomers and, for this reason, several peaks between 11.8 and 13.1 min were detected at the selected wavelength (256 nm) (figure 7.1). In order to confirm the presence of this photoinitiator by HPLC-MS/MS, five precursor ions were selected, each one corresponding to the main mass of each peak appearing in the HPLC-PDA chromatogram.

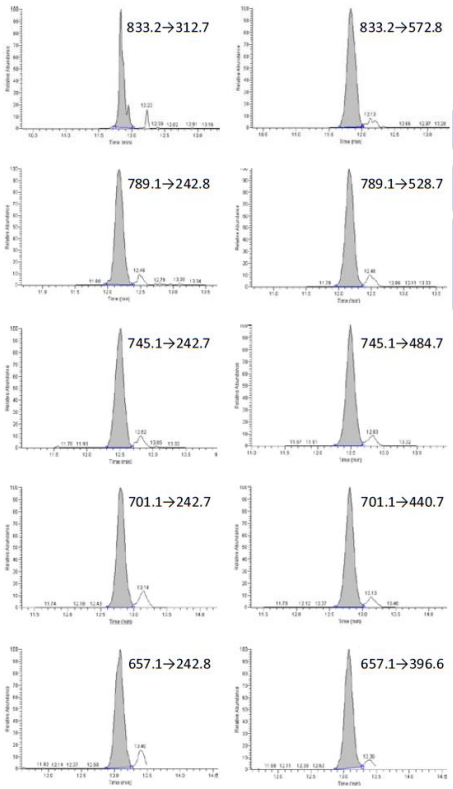


Figure 7.3: HPLC-MS/MS chromatograms of five selected BPAcr oligomers at 0.25 mg L^{-1}.

For each BPAcr precursor ion, two product ions were selected and are represented in figure 7.3. As can be observed, the peak intensity of each transition was high and well resolved signals were obtained at a concentration equal to the LOD of the HPLC-DAD method. Signal intensities were higher than 1.3×10^3, so even lower quantities can be detected using the LC-MS/MS method.

7.5. CONCLUSIONS

The proposed HPLC/DAD method is simple, reliable and could be useful as a screening tool for the routine determination of fourteen currently used photoinitiators and amine synergists, in packaging materials. It could be used as a first tool in the identification of PIs present in FCM, before proceeding to a further investigation of the possible migration into food. The method exhibited and excellent sensitivity and linearity within the range of concentration studied. Furthermore, the LC-MS/MS method allows the confirmation of the identity of substances in positive samples.

Different types of printed packaging including plastic, cardboard and cans were surveyed for the occurrence of PIs. Almost 50 % of samples presented quantifiable values of the selected substances, being benzophenone the most frequently detected substance. These PIs and amine synergists could migrate from the packaging into the food, and a potential health risk for the consumers cannot be disregarded. Further studies are required to evaluate the migration kinetics of these compounds from the food contact materials into foods and food simulants.

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8. IDENTIFICATION OF PRINT RELATED CONTAMINANTS IN FOOD PACKAGING

USC
UNIVERSIDADE
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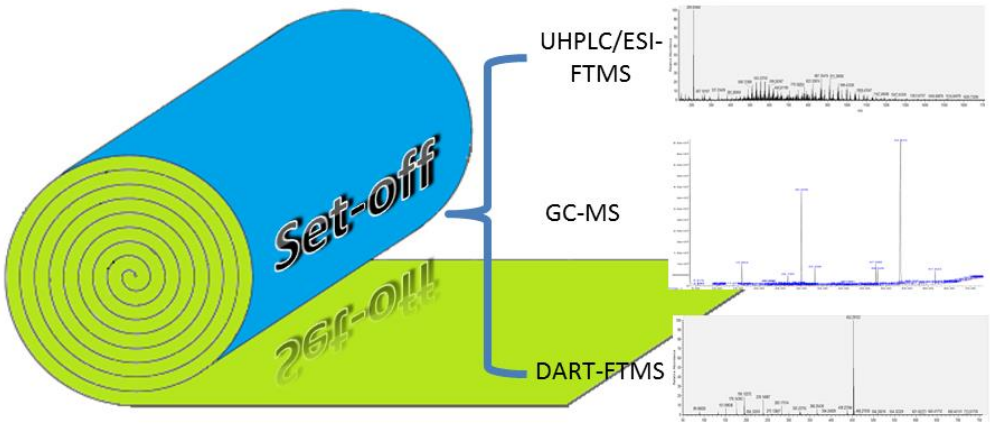
DE COMPOSTELA

8.1. ABSTRACT



Since the UV ink photoinitiator (PI) isopropylthioxanthone (ITX) was discovered in packaged milk, studies of print contamination have focused primarily on PIs but have also included amine synergists. Many other substances are used or formed during the print process, yet their identity and *set-off* properties have yet to be catalogued in food packaging. Three different techniques: direct analysis in real-time high-resolution mass spectrometry (DART-HRMS), gas chromatography-mass spectrometry (GC-MS) and ultra-high-pressure liquid chromatography electrospray ionisation/HRMS (UHPLC/ESI-HRMS) were used to detect and identify print-related molecules from the food-contact and print surfaces of three different packages with under-cured prints.

This approach tentatively identified or confirmed 110 compounds, including 35 print-related molecules. The majority of compounds identified on food-contact surfaces were packaging monomers/byproducts, solvents/plasticisers, antioxidants/degradants or slip agents/lubricants. Of these, 28 showed evidence of *set-off*. The identities of 16 PIs, seven known scission products and five probable PI degradants were confirmed, most showing signs of *set-off*. Of the print-related molecules, at least five are novel

print contaminants such as 4-morpholin-4-yl-benzaldehyde or 3-phenyl-2-benzofuran-1(3H)-one.

Keywords: Food packaging, photoinitiators, *set-off*, photo-scission, print-contaminant, DART-MS.

8.2. INTRODUCTION

UV-cured inks have become a popular printing choice for food packages in order to avoid solvents. However, print-related compounds may reach the foodstuffs in measurable quantities mainly by the so-called *set-off* effect (Rothenbacher *et al.*, 2007; Lago *et al.*, 2015). *Set-off* is an unintentional contamination of the food-contact surface of food packaging materials that can occur when package materials are stored in reels or stacks so the inner part of the package is in contact with the outer surface (printed surface) of adjacent packages. To determine the *set-off* effect in packaging, most of the printing industry carries out visual quality checks, but many of the print components are not always visible (Bradley *et al.*, 2005; Bentayeb *et al.*, 2012).

To avoid the *set-off* effect there are various strategies, such as the use of varnishes or lacquers, the minimisation of solvents, faster or more efficient photoinitiators (PIs) (including multifunctional PIs), or using powders/particles to reduce the friction and contact in stacks and rolls (Bentayeb *et al.*, 2012). Some strategies involve minimising known 'migrants' through the use of >1000 Da oligomeric or polymeric PIs, or formulations of multiple PIs. However, the polymeric PIs often lack characterising information which complicates their detection or assessment in packages/foods. Recently, the first analytical methods for the determination of a limited number of polymeric PIs were reported (Bentayeb *et al.*, 2013; Jung *et al.*, 2014; Jung & Simat 2014; Lago *et al.*, 2014). It is not clear if these approaches truly minimise *set-off* or shift which molecules might undergo *set-off*.

Ten years after the first food safety alert related to a food contamination with UV-cured inks (RASFF, 2005), more than 100 notifications related to the presence of PIs in foodstuffs have been reported by the Rapid Alert System for Food and Feed (RASFF). This fact indicates that the food contamination continues (RASFF, 2015). Since 2005, most print contamination studies have focused on methods to determine/quantify PIs in packaging, food and food simulants, (Castle *et al.*, 1997; Papilloud & Baudraz, 2002a, 2002b; Anderson &

Castle, 2003; Sagratini *et al.*, 2006, 2008; Sanches-Silva *et al.*, 2006, 2008a, 2008b, 2008c; Bagnati *et al.*, 2007; Gil-Vergara *et al.*, 2007; Rothenbacher *et al.*, 2007; Sun *et al.*, 2007; Allegrone *et al.*, 2008; Benetti *et al.*, 2008; Pastorelli *et al.* 2008; Shen *et al.*, 2009; Wang *et al.*, 2009; Koivikko *et al.*, 2010; Negreira *et al.*, 2010; Van Hoeck *et al.*, 2010; Gallart-Ayala *et al.*, 2011; Bentayeb et al,. 2012, 2013; Li *et al.*, 2012; Bradley *et al.*, 2013; Jung *et al.*, 2013, 2014; Jung & Simat, 2014; Lago *et al.*, 2014) or investigated the migration kinetics/mechanisms of PIs from packaging to foods or food simulants (Anderson & Castle, 2003; Sanches-Silva *et al.*, 2008a, 2009; Pastorelli *et al.*, 2008; Rodríguez-Bernaldo de Quirós *et al.*, 2009; Jung *et al.*, 2010, 2013; Huang *et al.*, 2012; Biedermann *et al.*, 2013). The few studies of *set-off* in retail food packaging generally conclude that the migration of PIs and synergists occurs mainly by *set-off*, instead of by permeation (Johns *et al.*, 1996; Aurela *et al.*, 2001; Jung *et al.*, 2010). Many methods have been developed to determine the *set-off* effect of PIs, but very few have attempted to identify all print-related compounds, let alone those that undergo *set-off* (Papilloud & Baudraz, 2002a; Dupáková *et al.*, 2010; Bentayeb *et al.*, 2012).

To our knowledge, no published study has attempted to identify comprehensively print transformation products and their *set-off* capabilities. The generation of these print transformation products is unavoidable due to the nature of the photo-curing process. Type I PIs generate their own scission products; and type II PIs can generate other products with their co-initiators (Lago *et al.*, 2015). As a result, many different print-related compounds can undergo *set-off* and be transferred to the foodstuffs and because many were never expected or intended to contact foods they may lack authorisation. Ambient ionisation mass spectrometry (AMI-MS) is useful in identifying *set-off* compounds and packaging additives (Ackerman *et al.*, 2009; Bentayeb *et al.*, 2012, 2013). The elimination of an extraction step can avoid narrowing the window of potential unknowns with solvent compatibility, and speeds analysis. Bentayeb *et al.* (2012, 2013) used DART/TOF-MS to detect and identify a broader range of *set-off* unknowns than was previously achieved with GC-MS

methods. However, it has also been reported that false-positives by DART/TOF-MS are possible, and that volatility can limit DART-MS sensitivity. Because GC-MS adds identifying power (chromatography and a large spectral database) and LC-MS broadens the analyte window to non-volatile substances, a combined analytical approach is likely needed to identify as many print-related contaminants as possible. The promising results in previous works suggested that a more comprehensive and aggressive approach might identify novel print transformation products capable of undergoing *set-off*.

The aim of this paper was to determine the print-related components that have been transferred from the printed surfaces to the food-contact surfaces of three different packages. The samples studied were intentionally under-cured in order to increase *set-off* and thus represented a better opportunity to identify print-related substances capable of *set-off*.

8.3. MATERIALS AND METHODS

8.3.1. CHEMICALS

Methanol (Optima® LC/MS, CAS number 67-56-1), acetonitrile (Optima® LC/MS, 75-05-8) and formic acid (Optima® LC/MS, 64-18-6) were acquired from Fisher Scientific Co. (Pittsburgh, PA, USA). Hexane (pesticide residue analysis grade, 110-54-3) was obtained from Fluka (Milwaukee, WI, USA). Dichloromethane (GC, 75-09-2) was purchased from Burdick & Jackson (Muskegon, MI, USA). Compressed nitrogen and compressed helium (99.999 %) were acquired from Airgas (Hyattsville, MD, USA). Chemical standards (unless otherwise specified) were obtained from Sigma-Aldrich (St. Louis MO, USA) at a purity of greater than 97 %. Reactive monomers (acrylates) contained inhibitors and were 90 % pure or greater. Select PIs (Table 8.1) were obtained either from Smithers-PIRA (Leatherhead, UK) or TCI-America (Philadelphia, PA, USA). Select polymeric PIs (Table 8.1) were obtained from Lamberti S.p.A. (Gallarate, Italy); Insight High Technology (Jiangsu, China); Lambson Ltd (Wetherby, UK); Rahn USA (Aurora, IL, USA); or Cytec Industries Inc. (Woodland Park, NJ, USA).

Identifying information for the chemicals used in this study are presented as follows: PIs (Table 8.1), GC confirmation stadards (Table 8.2) and print-related components/degradants (Table 8.3). All stock solutions were prepared in hexane and/or methanol in concentrations between 100 and 400 mg L^{-1}. These solutions were directly used for DART-HRMS analysis. The standard solutions for UHPLC/ESI-HRMS analysis were obtained from the stock solutions and diluted with AcN-water (50:50) with formic acid 0.1 %. The GC-MS standard solutions were also prepared from the stock solutions by dilution in dichloromethane.

CAS No.	Common name	Scientific name	Molecular formula	Monoisot. Mw	Source
86-39-5	CTX	2-chloro-thioxanthen-9-one	$C_{13}H_7ClOS$	245.99061	Sigma-Aldrich
119-61-9	BP	Benzophenone	$C_{13}H_{10}O$	182.07316	Sigma-Aldrich
131-58-8	2-MBP	2-methyl-benzophenone	$C_{14}H_{12}O$	196.08882	Sigma-Aldrich
134-84-9	4-MBP	4-methyl-benzophenone	$C_{14}H_{12}O$	196.08882	Sigma-Aldrich
530-44-9	DMAB	Methanone, [4-(dimethylamino)phenyl]phenyl-	$C_{15}H_{15}NO$	225.11536	Sigma-Aldrich
606-28-0	MBB	Methyl-2-benzoylbenzoate	$C_{15}H_{12}O_9$	240.07864	Sigma-Aldrich
947-19-3	HCPK	1-Hydroxycyclohexyl-1-phenylketone	$C_{13}H_{16}O_2$	204.11503	PIRA
1843-05-6	HOBP	12-Hydroxy-4-n-octoxybenzophenone	$C_{21}H_{26}O_3$	326.18819	Sigma-Aldrich
2128-93-0	PBZ	4-phenylbenzophenone	$C_{19}H_{14}O$	258.10447	PIRA
5495-84-1	ITX	2-isopropyl-9-thioxanthenone)	$C_{16}H_{14}OS$	254.07654	Sigma-Aldrich
7189-82-4	o-cl-HABI	2,2'-Bis(2-chlorophenyl)-4,4',5,5'-tetraphenyl-1,2'-biimidazole	$C_{42}H_{28}Cl_2N_4$	658.16910	TCI America
10287-53-3	EDB	Ethyl-4-(dimethylamino) benzoate	$C_{11}H_{15}NO_2$	193.11028	Sigma-Aldrich
21245-02-3	EHA/EHDAB	Benzoic acid, 4-(dimethylamino),2-ethylhexyl ester	$C_{17}H_{27}NO_2$	277.20418	Sigma-Aldrich
71868-10-5	MMMP	2-methyl-1-[4-(methylthio)phenyl]-2-(4-morpholinyl)-1-propanone	$C_{15}H_{21}NO_2S$	279.12930	Sigma-Aldrich
75980-60-8	TPO	2,4,6-trimethylbenzoyldiphenyl phosphine oxide	$C_{22}H_{21}O_2P$	348.12792	PIRA
106797-53-9	HPHP	1-[4-(2-Hydroxyethoxy)phenyl]-2-hydroxy-2-methyl-1-propanone	$C_{12}H_{16}O_4$	224.10486	Sigma-Aldrich
115055-18-0	Esacure® ONE	N/A	$C_{26}H_{32}O_4$	408.23006	Lamberti
119313-12-1	BDMB	2-benzyl-2-(dimethylamino)-1-(4-morpholinophenyl)-1-butanone	$C_{23}H_{30}N_2O_2$	366.23073	PIRA
119344-86-4	DMMB	2-dimethylamino-2-(4-methyl-benzyl)-1-(4-morpholin-4-yl-phenyl)-1-butanone	$C_{24}H_{32}N_2O_2$	380.24638	PIRA
142770-42-1	CPTX	1-Chloro-4-propoxythioxanthone	$C_{16}H_{13}ClO_2S$	304.03248	Sigma-Aldrich

162881-26-7	BAPO	Phenyl bis (2,4,6-trimethylbenzoyl) phosphine oxide	$C_{26}H_{27}O_3P$	418.16978	Sigma-Aldrich
272460-97-6	Esacure® 1001M	N/A	$C_{30}H_{26}O_4S_2$	514.12725	Lamberti
886463-10-1	Omnipol® 910	N/A	N/A	N/A	Insight H. T.
1182755-49-2	Speedcure® 7010	N/A	N/A	N/A	Lambson
1182753-56-5	Speedcure 7005®	N/A	N/A	N/A	Lambson
N/A	Esacure® A 198	N/A	N/A	N/A	Lamberti
N/A	Genopol® BP-2	N/A	N/A	N/A	Rahn
N/A	Genopol® TX-1	N/A	N/A	N/A	Rahn
N/A	Genopol® AB-2	N/A	N/A	N/A	Rahn
N/A	Ebecryl® P-39	N/A	N/A	N/A	Cytec Ind.

Table 8.1: Reference photoinitiators and amine synergists. (N/A: Not available). Molecular Weight is mono-isotopic.

CAS No.	Common name	Scientific name	Monoisotopic Mw	Molecular formula
57-10-3	Palmitic acid	Hexadecanoic acid	256.24023	$C_{16}H_{32}O_2$
91-01-0	Diphenyl-methanol	Benzenemethanol, α-phenyl-	184.08882	$C_{13}H_{12}O$
92-52-4	N/A	Biphenyl	154.07825	$C_{12}H_{10}$
96-76-4	N/A	2,4-Di-tert-butyl-phenol	206.16707	$C_{14}H_{22}O$
98-86-2	Acetophenone	Ethanone, 1-phenyl-	120.05751	C_8H_8O
102-76-1	Triacetin	1,2,3-Propanetriol, 1,2,3-triacetate	218.07904	$C_9H_{14}O_6$
102-82-9	Tributylamine	1-Butanamine, N,N-dibutyl-	185.21435	$C_{12}H_{27}N$
103-11-7	EHA	2-Ethylhexyl acrylate	184.14633	$C_{11}H_{20}O_2$
111-11-5	Methyl caprylate	Methyl octanoate	158.13068	$C_9H_{18}O_2$
111-82-0	Lauric methyl ester	Methyl dodecanoate	214.19328	$C_{13}H_{26}O_2$
112-27-6	Triethylene glycol (TEG)	Ethanol, 2,2'-[1,2-ethanediylbis(oxy)]bis-	150.08921	$C_6H_{14}O_4$
112-34-5	Butyl diglycol (BDG)	Diethylene glycol monobutyl ether	162.12559	$C_8H_{18}O_3$
112-60-7	Tetraethylene glycol (TEG)	Ethanol, 2,2'-[oxybis(2,1-ethanediyloxy)]bis-	194.11542	$C_8H_{18}O_5$
112-84-5	Erucamide	cis-13-Docosenamide	337.33447	$C_{22}H_{43}NO$
124-17-4	N/A	2-(2-Butoxyethoxy)ethyl acetate	204.13616	$C_{10}H_{20}O_4$
124-19-6	Nonanal	Nonylaldehyde	142.13577	$C_9H_{18}O$
128-37-0	BHT	2,6-di-tert-butyl, 4-methyl-phenol	220.18272	$C_{15}H_{24}O$
143-07-7	Lauric acid	Dodecanoic acid	200.17763	$C_{12}H_{24}O_2$
150-76-5	Mequinol	Phenol, 4-methoxy-	124.05243	$C_7H_8O_2$

502-44-3	ε-Caprolactone	Hexanoic acid, 6-hydroxy-, lactone	114.06808	$C_6H_{10}O_2$
699-61-6	N/A	1-Oxaspiro(4,5)decan-2-one	154.09938	$C_9H_{14}O_2$
719-22-2	2,6-di-tert-butylphenol	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	220.14633	$C_{14}H_{20}O_2$
761-65-9	N/A	N,N- Dibutylformamide	157.14666	$C_9H_{19}NO$
1007-32-5	N/A	1-Phenyl-2-butanone	148.08882	$C_{10}H_{12}O$
2082-79-3	Irganox® 1076	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	530.46990	$C_{35}H_{62}O_3$
3446-89-7	N/A	4-(Methylthio)benzaldehyde	152.02959	C_8H_8OS
3891-07-4	N-(2-Hydroxyethyl) phthalimide	1H-Isoindole-1,3(2H)-dione, 2-(2-hydroxyethyl)-	191.05824	$C_{10}H_9NO_3$
4098-71-9	Isophorone diisocyanate	Cyclohexane, 5-isocyanato-1-(isocyanatomethyl)-1,3,3-trimethyl-	222.13683	$C_{12}H_{18}N_2O_2$
5416-93-3	N/A	4-Methoxy phenyl isocyanate	149.04768	$C_8H_7NO_2$
7473-98-5	HMPP	2-Hydroxy-2-methylpropiophenone	164.08373	$C_{10}H_{12}O_2$
13048-33-4	HDDA	1,6-Hexanediol diacrylate	226.12051	$C_{12}H_{18}O_4$
14002-80-3	N/A	Methyl, 2,2-dimethyl-3-hydroxypropionate	132.07864	$C_6H_{12}O_3$
20071-09-4	N/A	trans-1,2-Diphenylcyclobutane	208.12520	$C_{16}H_{16}$
26530-20-1	Octhilinone®	2-octyl-4-isothiazolinone	213.11873	$C_{11}H_{19}NOS$

Table 8.2: GC confirmation standards. (N/A: Not available). All standards obtained from Sigma Aldrich, St. Louis, MO. Molecular Weight is mono-isotopic.

CAS No.	Common name	Scientific name	Molecular formula
65-85-0	N/A	Benzoic acid	$C_7H_6O_2$
68-12-2	DMFA	Formamide, N,N-dimethyl-	C_3H_7NO
94-09-7	Benzocaine	Benzoic acid, 4-amino-, ethyl ester	$C_9H_{11}NO_2$
99-77-4	N/A	Benzoic acid, 4-nitro-, ethyl ester	$C_9H_9NO_4$
99-96-7	N/A	Benzoic acid, 4-hydroxy-	$C_7H_6O_3$
100-51-6	N/A	Benzenemethanol	C_7H_8O
100-52-7	N/A	Benzaldehyde	$C_6H_6O_2$
103-30-0	Trans-Stilbene	Benzene, 1, 1'-(1E)-1, 2-ethenediylbis-	$C_{14}H_{12}$
123-31-9	Hydroquinone	1,4-Benzenediol	$C_6H_6O_2$
464-72-2	Benzopinacol	1,1,2,2-tetraphenyl-1,2-Ethanediol	$C_{26}H_{22}O_2$
487-68-3	Mesitaldehyde	Benzaldehyde, 2,4,6-trimethyl-	$C_{10}H_{12}O$
530-48-3	Phenyl-Styrene	Benzene, 1, 1'-ethenylidenebis-	$C_{14}H_{12}$
2039-49-8	N/A	Isoxazole, 3,5-diphenyl-	$C_{15}H_{11}NO$

Table 8.3: Reference (targeted) print related compounds. (N/A: Not available). All standards obtained from Sigma Aldrich, St. Louis, MO. Molecular Weight is mono-isotopic.

8.3.2. PACKAGING SAMPLES AND EXTRACTION PROCESS

The packaging samples were from the same stack of approximately 30 x 30 cm multilayer printed films as those used by Bentayeb *et al.* (2013) (Lord *et al.*, 2012). Briefly, three different package sets were studied: "Packaging A" – Ink Series X, "Packaging B" – Polymeric PI Ink Series Z and "Packaging C". Each of the package sets was under-cured and piled in stacks to increase the likelihood of *set-off*. All contained an aluminium barrier layer and polyethylene (PE) food contact layer.

The extraction procedure for the GC-MS and UHPLC/ESI-HRMS analysis was as follows: two large inner faces of a 127 x 250 x 6.5 mm cell (sandwich shape, steel with Teflon gasket) were covered with two sample films of each package set (food contact surface facing in). The cell was filled with a total of 200 ml of dichloromethane for about 30 s at room temperature. The total surface of package extracted was 629 cm^2. The 200 ml extract were evaporated (rotary evaporator) finally to obtain 2 ml (following transfer/rinses). The process was repeated for the print surface of each package. Method blanks (solvent and process) were also obtained.

8.3.3. DART-HRMS TEST

The DART-HRMS experiments were performed using a Q-Exactive HRMS (Thermo Fisher Scientific, San Jose, CA, USA) using a DART-SVP (Standardized voltage and pressure) source with Vapur™ membrane pumped interface from IonSense (Saugus, MA, USA). The source conditions were as follows: the DART to Vapur™ ceramic transfer tube (sampling gap) was set at approximately 1.5 cm; the DART to sample distance was 2-5 mm, and the Vapur™ transfer tube to MS inlet gap was about 0.5 mm. The helium flow was fixed at SVP installation (about 1 L min^{-1}), and balanced by the Vapur™ membrane pump (restrictor opened until sufficient fore-vacuum permitted MS operation). While DART temperatures tested included 350 and 500 ^oC, the 500 ^oC set point was selected for the broader range of analytes and ions generated. DART-HRMS analyses were carried out in positive and negative modes and performed across two

different mass ranges due to the 15-fold mass range limit of the HRMS (50-750 Da and 133-2,000 Da). The resolution used was 140,000 (FWHM) and the system was calibrated to < 0.2/0.3 ppm mass error daily.

The samples used were strips (15 x 2-3 cm) of the three different packages. Two different composite spectra were obtained from the length of each side of each strip: inner and outer surface, at high and low m/z scan ranges (four composite spectra per sample). To support targeted analyses in the samples, 29 PIs and 13 print-related compounds' (hexane/methanol solutions, 100 mg L^{-1}) DART spectra were collected and the top three to six unique ions tabulated.

To confirm the presence of the target compounds in the samples, the molecular formula for the main adducts and fragments of each analyte were determined from standard spectra and listed. ToxID™ software (Thermo Fisher Scientific, San Jose, CA) was used to check for targeted formula in the spectra obtained from the samples. The typical ion forms in the targeted list were $[M+H]^+$, $[M-H_2O+H]^+$ and $[M+NH_4]^+$, as well as $[M-H]^+$, $[M+Cl]^-$ and $[M+O_2]^-$. Also, the molecular formulas of the two main fragments were determined for each compound and searched for as separate molecules (Table 8.4). Ions' molecular formulae were verified by mass accuracy and corresponding isotopic ions' (A+1, A+2) relative abundance and mass. A targeted analyte was considered DART-MS confirmed in a sample by detecting at least three of the searched adducts or fragments produced by its standard.

PI/Photoscission product	Positive Fragments	Negative Fragments
CTX	$C_{13}H_7ClO^{34}S+H^+$: 248.99368 $C_{12}{}^{13}CH_7ClOS+H^+$: 248.00124	$C_{13}H_7ClSO_2-H^+$: 260.97825 $C_{20}H_{10}ClSO_2-H^+$: 348.00173
BP	$C_{12}{}^{13}CH_{10}O+H^+$: 184.08380	$C_{21}H_{20}O_6-H^+$: 367.11871 $C^{20}H^{18}O^5-H^+$: 337.10815
2/4-MBP	$C_{13}{}^{13}CH_{12}O+H^+$: 198.09945 $C_8H_7O\cdot$: 119.04914	-
DMAB	$C_{14}{}^{13}CH_{15}NO+H^+$: 227.12599 $C_{13}{}^{13}C_2H_{15}NO+H^+$: 228.12935	$C_{13}H_{11}NO-H^+$: 196.07679 $C_{12}{}^{13}CH_{11}NO-H^+$: 197.08014
MBB	$C_{14}H_8O_2+H^+$: 209.05971 $C_{13}{}^{13}CH_8O_2+H^+$: 210.06306	$C_{14}H_{10}O_3-H^+$: 225.05572 $C_{13}{}^{13}CH_{10}O_3-H^+$: 226.05907
HCPK	$C_7{}^{13}CH_5O\cdot$: 106.03739 $C_{13}H_{15}O+H+$: 188.11510	$C_8H_9NO_2-H^+$:150.05605 $C_6H_{10}O_4-H+$:145.05063
HOBP	$C_{20}{}^{13}CH_{26}O_3+H^+$: 328.19883 $C_{19}{}^{13}C_2H_{26}O_3+H^+$: 329.20218	$C_{20}{}^{13}CH_{26}O_3-H^+$: 326.18427 $C_{21}H_{25}O_3-H^+$: 340.16801
PBZ	$C_{18}{}^{13}CH_{14}O+H^+$: 260.11510 $C_{17}{}^{13}C_2H_{14}O+H^+$: 261.11845	-

ITX	$C_{15}{}^{13}CH_{14}OS+H^+$: 256.08717 $C_{16}H_{14}O^{34}S+H^+$: 257.07961	$C_{16}H_{14}O_2S-H^+$: 269.06417 $C_4H_{10}O_4-H^+$: 121.05063
o-cl-HABI	$C_{21}H_{15}ClN_2+H^+$: 331.09910 $C_{21}H_{15}{}^{37}ClN_2+H^+$: 333.09490	$C_{21}H_{15}N_2Cl-H^+$: 329.08510 $C_{20}{}^{13}CH_{15}N_2Cl-H^+$: 330.08846
EDB	$C_{11}H_{15}NO_2+H^+$: 195.12091	$C_{10}H_{11}NO_3-H^+$: 192.06662
EHA/EHDAB	$C_{16}{}^{13}CH_{27}NO_2+H^+$: 279.21481 $C_{34}H_{54}N_2O_4+H^+$: 555.41563	$C_9H_{11}NO_2-H^+$: 164.07170 $C_8{}^{13}CH_{11}NO_2-H^+$: 165.07506
MMMP	$C_{14}{}^{13}CH_{21}NO_2S+H^+$: 281.13993 $C_{15}H_{21}NO_2{}^{34}S+H^+$: 282.13237	$C_8H_8O_2S-H^+$: 167.01722 $C_{14}H_{19}NO_2S-H^+$: 264.10637
TPO	$C_{44}H_{42}O_4P_2+H^+$: 697.26311 $C_{43}{}^{13}CH_{42}O_4P_2+H^+$: 698.26647	$C_{12}H_{11}O_2P-H^+$: 217.04239 $C_{11}{}^{13}CH_{11}O_2P-H^+$: 218.04576
HPHP	$C_{11}H_{14}O_2+H^+$: 179.10666 $C_{12}{}^{13}CH_{16}O_4+H^+$: 226.11549	$C_{10}H_{12}O_3-H^+$: 179.07137 $C_9{}^{13}CH_{12}O_3-H^+$: 180.07472
Esacure® ONE	$C_{25}{}^{13}CH_{32}O_4+H^+$: 410.24069 $C_8H_{17}O_3+H^+$: 163.13287	$C_{25}{}^{13}CH_{33}O_6-H^+$: 441.22379 $C_{26}H_{33}O_6N-H^+$: 454.22351
BDMB	$C_{22}{}^{13}CH_{30}N_2O_2+H^+$: 368.24136 $C_{22}H_{28}N_2O_2+H^+$: 353.22235	$C_{11}H_{13}O_3N-H^+$: 206.08227 $C_{10}{}^{13}CH_{13}O_3N-H^+$: 207.08562
DMMB	$C_{23}{}^{13}CH_{32}N_2O_2+H^+$: 382.25701 $C_{13}H_{19}N+H^+$: 190.15903	-
CPTX	$C_{16}H_{13}ClO_2{}^{34}S+H^+$: 307.03556 $C_{15}{}^{13}CH_{13}ClO_2S+H^+$: 306.04311	$C_{13}H_7ClO_2S-H^+$: 260,97825 $C_{12}{}^{13}CH_7ClO_2S-H^+$: 261.98161
BAPO	-	-
Esacure® 1001M	$C_{29}{}^{13}CH_{26}O_4S_2+H^+$: 516.13788 $C_{19}H_{20}O_4S_4+H^+$: 441.03172	$C_7H_8O_3S-H^+$: 171.01214 $C_7H_7O_3S-H^+$: 170.00431
Omnipol® 910	$C_{27}H_{37}N_3O_3+H^+$: 452.29077 $C_{26}{}^{13}CH_{37}N_3O_3+H^+$: 453.29412 $C_{26}{}^{13}C_2H_{37}N_3O_3+H^+$: 454.29748 $C_{23}H_{31}ON_3+H^+$: 366.253989 $C_{26}H_{35}N_3O_3+H^+$: 438.27512	$C_{15}H_{20}O_4N_2-H^+$: 291.13503 $C_{12}H_{26}O_7-H^+$: 281.16058 $C_{10}H_{22}O_6-H^+$: 237.13436 $C_{12}H_{27}O_9-H^+$: 314.15823 $C_{14}H_{31}O_{10}-H^+$: 358.18444
Speedcure® 7005	$C_{14}H_8O_2+H^+$:209.05971 $C_{13}{}^{13}CH_8O_2+H^+$:210.06306 $C_{15}H_{12}O_3+H^+$: 241.08592 $C_{14}H_{10}O_2+H^+$: 211.07536 $C_{17}H_{16}O_3+H^+$: 269.11722	$C_{14}H_{10}O_3-H^+$: 225.05572 $C_{13}H_{10}O-H^+$:181.06589 $C_{13}{}^{13}CH_{10}O_3-H^+$: 226.05908 $C_{12}{}^{13}C_2H_{10}O_3-H^+$: 227.06243
Speedcure® 7010	$C_{17}H_{13}ClO_4S+H^+$:349.02958 $C_{13}H_7ClO_2S+H^+$:262.99280 $C_{17}H_{13}ClO_4{}^{34}S+H^+$: 351.02538 $C_{15}H_9ClO_4S+H^+$: 320.99828 $C_{13}H_7ClO_2{}^{34}S+H^+$: 264.98860	$C_{13}H_7ClO_2S-H^+$: 260.97825 $C_{13}H_7ClO_2{}^{34}S-H^+$: 262.97405 $C_{12}{}^{13}CH_7ClO_2S-H^+$: 261.98161 $C_{12}{}^{13}CH_7ClO_2{}^{34}S-H^+$: 263.97740 $C_{13}H_7ClO_3S-H^+$: 276.97317
Esacure® A 198	$C_{21}H_{29}N_6O_3+H^+$: 414.23739 $C_{20}{}^{13}CH_{29}N_6O_3+H^+$: 415.24075	$C_{22}H_{30}O_6N_3-H^+$: 431.20618 $C_{23}H_{32}O_6N_3-H^+$: 445.22183
Genopol® BP-2	$C_{16}H_{12}O_3+H^+$: 253.08592 $C_{20}H_{42}O_{10}+H^+$: 443.28507	$C_{14}H_{10}O_2-H^+$: 209.06080 $C_{14}H_{10}O_3-H^+$: 225.05572
Genopol® TX-1	-	-
Genopol® AB-2	-	$C_6H_5ON_2Cl-H^+$: 155.00176 $C_8H_9O_2N_2Cl-H^+$: 199.02798
Ebecryl® P-39	$C_5H_6O_2+H^+$: 99.04460	$C_3H_5O_3Cl-H^+$: 122.98545 $C_3H_3O_4Cl-H^+$: 136.96471
Benzoic acid	-	$C_6{}^{13}CH_6O_2-H^+$: 122.03286
DMFA	-	$C_9H_{16}O_4-H^+$: 187.09758 $C_4H_6O_5-H^+$: 133.01425
Benzocaine	$C_7H_7NO_2+H^+$: 138.05495 $C_8{}^{13}CH_{11}NO_2+H^+$: 167.08961	$C_8{}^{13}CH_{11}NO_2-H^+$: 165.07506 $C_9H_{10}O_3N-H^+$: 179.05879
Benzoic acid, 4-nitro-, ethyl ester	$C_7H_5NO_4+H^+$: 168.02913 $C_6{}^{13}CH_5NO_4+H^+$: 169.03249	$C_9H_{10}NO_4-H^+$: 195.05371 $C_7H_5NO_4-H^+$: 166.01458

Benzoic acid, 4-hydroxy-	$C_6{}^{13}CH_6O_3+H^+$: 140.04233 $C_6H_6O+H^+$: 95.04914	$C_7H_5O_3-H^+$: 136.01659 $C_6{}^{13}CH_6O_3-H^+$: 138.02777
Benzenemethanol	$C_8H_4O_3+H^+$: 149.02332 $C_8H_8O_3+H^+$: 153.05462	$C_7H_8O_2-H^+$: 123.04515 $C_7H_6O_2-H^+$: 121.02950
Benzaldehyde	$C_6H_{12}O_2+H^+$: 117.09101 $C_{13}H_{20}+H^+$: 177.16378	$C_6H_6O_2-H^+$: 121.029503 $C_5{}^{13}CH_6O_2-H^+$: 122.03286
Trans-Stilbene	$C_{22}H_{18}+H^+$: 283.14813 $C_{13}H_{10}+H^+$: 167.08553	-
Hydroquinone	$C_6H_6O_3+H^+$: 127.03897 $C_5{}^{13}CH_6O_2+H^+$: 111.04013	$C_6H_5O_2\cdot$: 108.02168 $C_6H_4O_3-H^+$: 123.00877
Benzopinacol	$C_{20}H_{14}O+H^+$: 271.11174 $C_{25}{}^{13}CH_{20}O+H^+$: 350.16205	$C_{13}H_{10}O_2-H^+$: 197.06080 $C_{13}H_{12}O\cdot$: 183.08154
Mesitaldehyde	$C_9H_{10}O+H^+$: 165.09101 $C_9H_{12}+H^+$: 121.10118	$C_{10}H_{12}O_2-H^+$: 163.07645 $C_9{}^{13}CH_{12}O_2-H^+$: 164.07981
Phenyl-Styrene	$C_{16}H_{14}O+H^+$: 223.11174	-
Isoxazole, 3,5-diphenyl-	$C_{14}{}^{13}CH_{11}NO+H^+$: 223.09470 $C_{13}{}^{13}C_2H_{11}NO+H^+$: 224.09805	$C_{15}H_{11}NO_2-H^+$: 236.07170

Table 8.4: List of the two (five in the case of polymeric photoinitiators) main DART-HRMS fragments of each photoinitiator or photoscission product. Common fragments are not shown in order to avoid false positives.

8.3.4. GC-MS ANALYSIS

The GC-MS analysis was performed with three objectives: (1) to confirm the targeted compounds' DART-HRMS detections; (2) to detect unknown package, print, and *set-off* compounds; and (3) to standard-confirm the identity of unknown *set-off* compounds. An Agilent 6890 gas chromatograph coupled to a 5973N mass spectrometer (Agilent®, Palo Alto, CA, USA) was used with two different columns to cover a broad polarity range: an Agilent HP-5MS (30 m x 0.25mm x 0.25 µm film) and a Restek Rtx-200 (30 m x 0.25 mm x 0.25 µm film) capillary column (Bellefonte, PA, USA).

Two different methods were used: a long method to detect unknowns in the samples, and a shorter method to confirm their identities with standards. A longer method with a slow temperature ramp was used on all blanks and samples with both columns. The longer method used a flow of 1.0 ml min^{-1} of helium with a 3 min hold at 50 °C, followed by a 4 °C min^{-1} ramp to 320 °C and a 2.5 min hold. The total run time was 73 min. A 1 µl splitless injection was used, and the solvent delay was set at 3 min. Interface, source and quadrupole temperatures were 300, 230 and 150 °C, respectively. The GC-MS scan range was m/z 42-550. To match the identified peaks from the sample extracts and

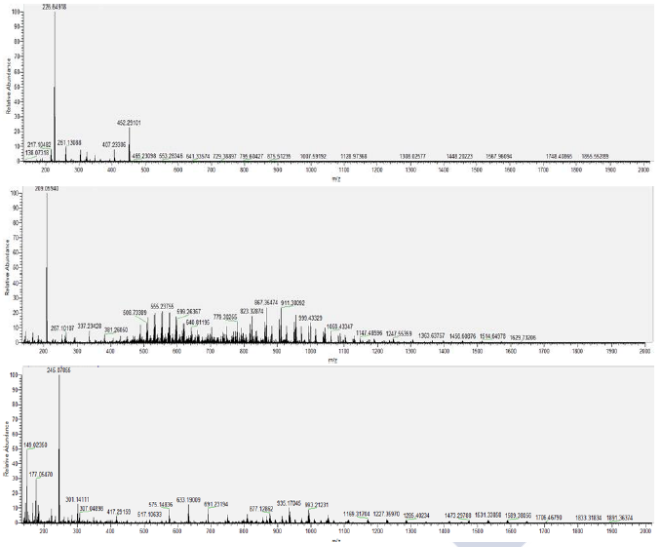
the standards, a C10-C40 solution was also injected with each sequence in order to obtain the Kovats retention index of each peak. Grob mixtures were injected to confirm the methods' chromatographic performance.

To confirm their identity, all targeted and tentatively identified compounds were compared with available standards' retention indeces and EI spectra. Standards' retention indices and EI spectra were verified using a shorter method in order to confirm approximately 100 compounds in a timely fashion. The short method used an oven ramp of 10 °C min^{-1} instead of 4 °C min^{-1}. Kovats retention indices were required to be inside the 5 % of variance of the standards. EI spectra were required to match the standard's top three ions and yield an NIST library match (> 40 % match manually, > 75 % automatically and 95 % automatch of alkane structures) for a tentative identification to be confirmed by a standard. The 17 tentative EI-MS identifications that failed standard matches are not shown. Tentative identifications without standards available for confirmation were required to yield an EI-spectra automatch of > 75 % and a retention index within about 100 of structure based estimates or reported values (for similar columns) from the NIST database.

8.3.5. UHPLC/ESI-HRMS ANALYSIS

The single-sided package extracts were also analysed by a UHPLC-HRMS system (Thermo Fisher Scientific). Chromatographic separation was performed at 0.35 mL min^{-1} with a Luna C8 reversed-phase column (150 × 2.0 mm-i.d., 3 µm particle size) (Phenomenex®, Torrance, CA, USA). The mobile phases (95:5 and 5:95 H_2O-AcN, A and B respectively) were acidified with 0.1 % (v/v) formic acid. Following a gradient from 100 % A (after 1.25 min) to 100 % B at 26.25 min, the organic phase was held for 2 min, and then a reverse gradient (6.5 min) to the starting conditions (1.25 min equilibration) for a cycle time of 36 min. The HRMS parameters were the same as in the DART-HRMS analysis, and the HESI-2 (heated electrospray ionization) probe was operated in the positive mode at 3.0 kV, sheath gas at 48 psi, auxiliary gas flow of 15, and source vaporiser and capillary transfer tube temperatures of 300 and 370 °C respectively.

The spectra of PIs and print-related compounds were obtained by infusion (5-15 µL min^{-1}) and their main adducts and fragments were tabulated (as in DART-HRMS analysis) to be used by ToxID™ for targeted analysis. The selected adducts typically chosen for ESI spectra were $[M+H]^+$, $[M+Na]^+$ and $[M+K]^+$, as well as several fragments (Tables 8.5 and Figure 8.1). As with DART-HRMS, when at least any three adducts or fragments (from standard spectra) were detected as a peak in a sample chromatogram, the compound was then considered a UHPLC/ESI-HRMS tentative identification. Ions' molecular formulae were verified by mass accuracy and corresponding isotopic ions' (A+1, A+2) relative abundance and mass.



Omnipol® 910:
$[C_{27}H_{37}O_4N_3+2H^+]$: 226.64902
$[C_{10}H_{18}O_2N_4+H^+]$: 262.99280
$[C_{27}H_{37}O_4N_3+H^+]$: 452.29077
$[C_{32}H_{32}\ O_3N_4+2H^+]$: 261.13100
$[C_{25}H_{30}\ O_3N_2+H^+]$: 407.23292

Speedcure® 7005:
$[C_{14}H_9O_2]$: 209.05971
$[C_{31}H_{67}O_{27}+H^+]$: 867.35512
$[C_{33}H_{71}O_{28}+H^+]$: 911.38134
$[C_{34}H_{34}O_7+2H^+]$: 555.23773
$[C_{37}H_{34}O+K^+]$: 533.22412

Speedcure® 7010:
$[C_8H_{17}O_6Cl+H^+]$: 245.07864
$[C_8H_4O_3+H^+]$: 149.02332
$[C_{10}H_8O_3+H^+]$: 177.05462
$[C_{12}H_{25}O_6Cl+H^+]$: 301.14124
$[C_7{}^{13}CH_{17}O_6Cl+H^+]$: 246.08200

Figure 8.1: Polymeric Photoinitiators ESI-HRMS spectra in positive mode with the obtained main ions. The spectra correspond to: Omnipol® 910 (top), Speedcure® 7005 (middle) and Speedcure® 7010 (bottom).

PI/Photosc. product	Positive Fragments (m/z)	PI/Photosc. product	Positive Fragments (m/z)
CTX	$C_{13}H_7{}^{37}ClOS+H^+$: 248.99494 $C_{12}{}^{13}CH_7ClOS+H^+$: 248.00124	**Omnipol® 910**	$C_{27}H_{37}O_3N_3+2H^+$: 226.64902 $C_{10}H_{18}O_2N_4+H^+$: 227.15025 $C_{27}H_{37}O_3N_3+H^+$: 452.29077 $C_{32}H_{32}N_4O_3+2H^+$: 261.13100 $C_{25}H_{30}N_2O_3+H^+$: 407.23292
BP	$C_{12}{}^{13}CH_{10}O+H^+$: 184.08380 C_7H_4O+H+: 105.03349	**Speedcure® 7005**	$C_{14}H_8O_2+H^+$: 209.05971 $C_{31}H_{62}O_{27}+H^+$: 867.35512 $C_{33}H_{66}O_{28}+H^+$: 911.38134 $C_{34}H_{34}O_7+H^+$: 555.23773 $C_{37}H_{34}O+K^+$: 533.22412
2/4-MBP	$C_{13}{}^{13}CH_{12}O+H^+$:198.09945	**Speedcure® 7010**	$C_8H_{17}O_6Cl+H^+$: 245.07864 $C_8H_4O_3+H^+$: 149.02332 $C_{10}H_8O_3+H^+$: 177.05462 $C_{12}H_{25}O_6Cl+H^+$: 301.14124 $C_7{}^{13}CH_{17}O_6Cl+H^+$: 246.08200
DMAB	$C_{14}{}^{13}CH_{15}NO+H^+$: 227.12600 $C_{13}{}^{13}C_2H_{15}NO+H^+$: 228.12935	**Esacure® A 198**	$C_{23}H_{31}O_4N_3+H+$: 414.23873 $C_{22}{}^{13}CH_{31}O_4N_3+H^+$: 415.24209
MBB	$C_{14}H_8O_2+H^+$: 209.05971 $C_{14}{}^{13}CH_{12}O_3+H^+$: 264.07122	**Genopol® BP-2**	$C_{14}H_8O_2+H^+$: 209.05971 $C_{46}H_{54}O_{13}+Na^+$: 837.34566
HCPK	$C_{13}H_{14}O+H^+$:188.11510 $C_7H_4O+H^+$: 105.03349	**Genopol® TX-1**	$C_9H_9ON+H^+$: 148.07569 $C_9H_{11}O_2N+H^+$: 166.08626
HOBP	$C_{20}{}^{13}CH_{26}O_3+H^+$: 328.19883 $C_{19}{}^{13}C_2H_{26}O_3+H^+$: 329.20218	**Genopol® AB-2**	$C_{38}H_{36}O_7N_7+H^+$: 703.27490 $C_{36}H_{32}O_6N_7+H^+$: 659.24868
PBZ	$C_{18}{}^{13}CH_{14}O+H^+$: 260.11510 $C_{17}{}^{13}C_2H_{14}O+H^+$: 261.11845	**Ebecryl® P-39**	$C_{23}H_{36}O_{10}+Na^+$: 495,22007 $C_{25}H_{40}O_{11}+Na^+$: 539.24628
ITX	$C_{16}{}^{13}CH_{14}OS+H^+$: 256.08717 $C_{17}H_{14}O^{34}S+H^+$: 257.07961	**Benzoic acid**	-
o-cl-HABI	$C_{42}H_{28}N_4Cl^{37}Cl+H^+$: 661,17343 $C_{41}{}^{13}CH_{28}N_4Cl_2+H_+$: 660.17973	**DMFA**	-
EDB	$C_{10}{}^{13}CH_{15}NO_2+H^+$: 195.12091 $C_{10}H_{12}NO_2+H^+$: 180.10191	**Benzocaine**	$C_8{}^{13}CH_{11}NO_2+H^+$: 167.08961 $C_7H_7NO_2+H^+$: 138.05495
EHA/EHDAB	$C_{17}H_{27}NO_2+H^+$: 279.21481 $C_9H_{11}NO_2+H^+$: 166.08626	**Benzoic acid, 4-nitro-, ethyl ester**	$C_9H_{11}NO_2+H^+$: 166.08604
MMMP	$C_{14}{}^{13}CH_{21}NO_2S+H^+$: 281.13993 $C_{15}H_{21}NO_2{}^{36}S+H^+$: 282.13237	**Benzoic acid, 4-hydroxy-**	$C_{12}H_{14}O_4+Na^+$: 245.07843
TPO	$C_{10}H_{10}O_2+H^+$: 147.08044 $C_{21}{}^{13}CH_{21}O_2P+H^+$: 372.12049	**Benzenemethanol**	$C_8H_4O_3+H^+$: 149.02332 $C_{10}H_8O_3+H^+$: 177.05462
HPHP	$C_{11}H_{14}O_2+H^+$: 179.10666 $C_{10}{}^{13}CH_{16}O_4+H^+$: 226.11549	**Benzaldehyde**	$C_6{}^{13}CH_6O_2+H^+$: 123.04013
Esacure® ONE	$C_{25}{}^{13}CH_{32}O_4+H^+$: 410.24069	**Trans-Stilbene**	-
BDMB	$C_{22}{}^{13}CH_{30}N_2O_2+H^+$: 368.24136 $C_{11}H_{11}NO_2+H^+$:190.08626	**Hydroquinone**	-
DMMB	$C_{23}{}^{13}CH_{32}N_2O_2+H^+$: 382.25701 $C_{11}H_{11}NO_2+H^+$:190.08626	**Benzopinacol**	$C_{26}H_{20}O+H^+$: 349.15869 $C_{20}H_{14}O+H^+$: 271.11174
CPTX	$C_{16}H_{13}{}^{37}ClO_2S+H^+$: 307.03680 $C_{15}{}^{13}CH_{13}ClO_2S+H^+$: 306.04311	**Mesitaldehyde**	$C_9H_{12}+H^+$: 121.10118 $C_{10}H_{10}O+H^+$: 147.08044
BAPO	$C_{10}H_{10}O+H^+$: 147.08044 $C_{10}H_{12}O_2+H^+$: 165.09101	**Phenyl-Styrene**	$C_{13}H_{10}O+H^+$: 183.08044 $C_{14}H_{12}O+H^+$: 197.09609
Esacure® 1001M	$C_{30}H_{26}O_4S_2+K^+$: 553.09041 $C_{29}{}^{13}CH_{26}O_4S_2+Na^+$:538.11983	**Isoxazole, 3,5-diphenyl-**	$C_{14}{}^{13}CH_{11}NO+H^+$:223.09470 $C_{13}{}^{13}C_2H_{11}NO+H^+$: 224.09805

Table 8.5: List of the two (five in the case of polymeric photoinitiators) ESI-HRMS main fragments of each photoinitiator or photoscission product.

8.4. RESULTS

8.4.1. TARGETED ANALYSIS

Three packaging materials were tested by three techniques; the PIs and print-related components detected are listed in table 8.6. In most cases the formulated PIs were detected by multiple techniques. Also, there is evidence of *set-off* for a total of 14 PIs and seven print-related compounds. These results correlate well with the ink formulation of each package and are clearly consistent with the results previously obtained by Lord *et al.* (2013) and Bentayeb *et al.* (2013).

By DART-HRMS, the mass of the detected ions typically yielded a mass error below 2 ppm. Therefore, it was necessary to construct a somewhat restrictive identification criterion apart from mass accuracy in order to avoid possible false-positives as explained previously. The molecular ion $[M+H]^+$ was the main form observed for most print-related compounds; however, no molecular ion was observed for the polymeric PIs (> 1,000 Da) as can be seen in Figures 8.2 to 8.4. Only the intermediate mass ions of various oligomeric and PI moieties were observed in all the standard and sample DART spectra (listed in table 8.7). This is consistent with Bentayeb *et al.* (2013) suggestion of probable decomposition prior to ionisation at the temperatures used in the DART-HRMS method.

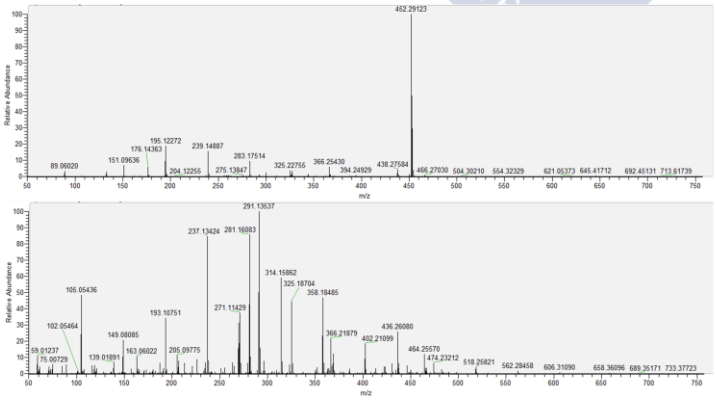


Figure 8.2: Omnipol® 910 DART-HRMS spectra with the main ions observed. Positive (top) and negative (bottom) modes are represented.

CAS No.	Name	DART-HRMS			GC-MS			UHPLC/ESI-HRMS		
		Package A	Package B	Package C	Package A	Package B	Package C	Package A	Package B	Package C
119-61-9	BP			*Set-off*			*Set-off*			*Set-off*
606-28-0	MBB				*Set-off*	PS	*Set-off*	*Set-off*	*Set-off*	*Set-off*
947-19-3	HCPK	*Set-off*	*Set-off*		*Set-off*			*Set-off*		
2128-93-0	PBZ	*Set-off*			*Set-off*	*Set-off*	*Set-off*	*Set-off*		
5495-84-1	ITX						*Set-off*			*Set-off*
10287-53-3	EDB	*Set-off*	*Set-off*	*Set-off*	PS		*Set-off*			*Set-off*
21245-02-3	EHA/EHDAB								FCS	
71868-10-5	MMMP	*Set-off*	PS	*Set-off*	*Set-off*		PS	*Set-off*		*Set-off*
75980-60-8	TPO				n.d.	n.d.	n.d.	*Set-off*		
106797-53-9	HPHP	*Set-off*	*Set-off*	*Set-off*				n.d.	n.d.	nd
119313-12-1	BDMB							*Set-off*		*Set-off*
119344-86-4	DMMB							*Set-off*		
886463-10-1	Omnipol 910®		PS						*Set-off*	
1182755-49-2	Speedcure 7010®		*Set-off*						*Set-off*	
1182753-56-5	Speedcure 7005®		*Set-off*						*Set-off*	
94-09-7	Benzocaine									*Set-off*
99-96-7	Benzoic acid, 4-hydroxy-	*Set-off*	*Set-off*	*Set-off*						
100-51-6	Benzenemethanol	*Set-off*	*Set-off*	*Set-off*						
100-52-7	Benzaldehyde	*Set-off*	*Set-off*	*Set-off*	PS					
123-31-9	Hydroquinone	*Set-off*	*Set-off*	*Set-off*						
487-68-3	Mesitaldehyde	*Set-off*	*Set-off*	*Set-off*	PS			PS	PS	PS
530-48-3	Phenyl-Styrene							*Set-off*		

Table 8.6: Photoinitiators and amine synergists (top) and print related compounds (bottom) confirmed by DART-HRMS, UHPLC/ESI-HRMS and/or GC-MS. (n.d.: not detected or below blanks; PS: Print Side; FCS: Food Contact Side; *Set-off*: detected on both sides).

Polymeric PI	DART-HRMS Fragments detected	UHPLC/ESI-HRMS Fragments detected
Omnipol® 910	$[C_{27}H_{37}N_3O_3+H^+]$:452.29077	$[C_{10}H_{18}O_2N_4+H^+]$: 227.15025
	$[C_{23}H_{31}N_3O+H^+]$: 366.25399	$[C_{27}H_{37}O_3N_3+H^+]$: 452.29077
	$[C_{12}H_{17}N+H^+]$: 176.14338	$[C_{25}H_{30}N_2O_3+H^+]$: 407.23292
	$[C_{51}H_{66}N_3O_4+H^+]$: 785.51261	$[C_{13}H_{22}O_3N_4+H^+]$: 283.17647
	$[C_{26}H_{35}N_3O_3+H^+]$: 438.27512	$[C_{12}H_{22}O_3N_6+H^+]$: 305.15896
Speedcure® 7005	$[C_{14}H_8O_2+H^+]$: 209.05971	$[C_{68}H_{68}O_{14}+2H^+]$: 555.23773
	$[C_{16}H_{12}O_3+H^+]$: 253.08592	$[C_{66}H_{64}O_{13}+2H^+]$: 533.22412
	$[C_{17}H_{14}O_3+H^+]$: 267.10157	$[C_{70}H_{72}O_{15}+2H^+]$: 577.25034
	$[C_{15}H_{12}O_3+H^+]$: 241.08592	$[C_{64}H_{60}O_{12}+2H^+]$: 511.21152
	$[C_{17}H_{16}O_3+H^+]$: 269.11722	$[C_{72}H_{76}O_{16}+2H^+]$: 599.26395
	$[C_{29}H_{40}O_{10}+H^+]$: 549.26942	-
Speedcure® 7010	$[C_{17}H_{13}ClO_4S+H^+]$:349.02958	$[C_{32}H_{37}O_9ClS+H^+]$: 633.18976
	$[C_{15}H_9ClO_4S+H^+]$: 320.99828	$[C_{32}H_{37}O_9Cl^{37}ClS+H^+]$: 635.18681*
	$[C_{18}H_{15}ClO_5S+H^+]$: 379.04015	$[C_{35}H_{43}O_{10}ClS+H^+]$: 691.23163*
	$[C_{13}H_7ClO_2S+H^+]$: 262.99280	$[C_{12}H_{14}O_4+H^+]$: 223.09649
	-	$[C_{10}H_8O_3+H^+]$: 177.05462

Table 8.7: Polymeric PIs fragments detected by DART-HRMS and UHPLC/ESI-HRMS. The molecular ions are not detected. * - very low intensities.

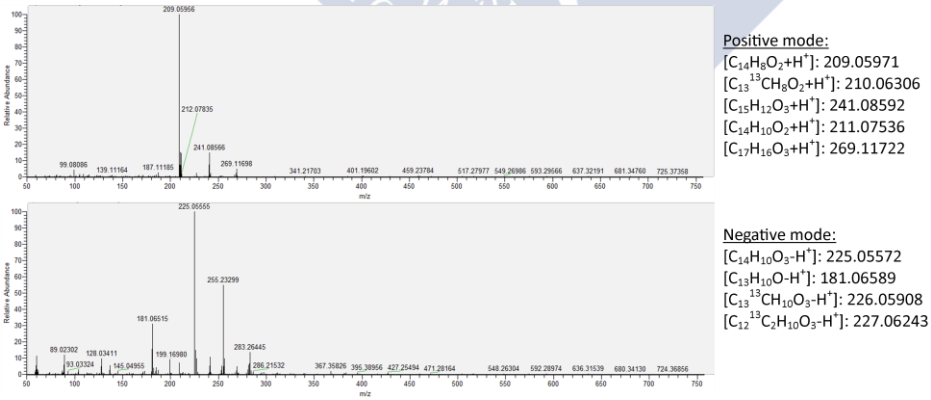


Figure 8.3: Speedcure® 7005 DART-HRMS spectra with the main ions observed. Positive (top) and negative (bottom) modes are represented.

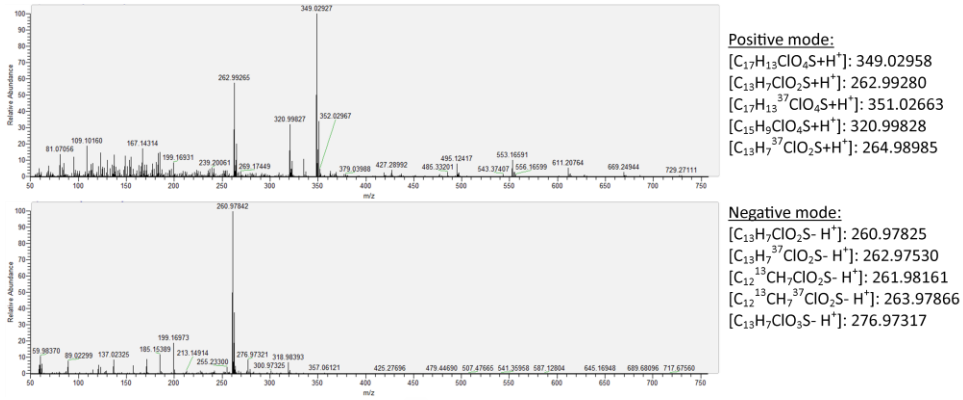


Figure 8.4: Speedcure® 7010 DART-HRMS spectra with the main ions observed. Positive (top) and negative (bottom) modes are represented.

Seven photo-scission products were detected, most frequently by DART-HRMS. The PIs and other components of the packaging share some low molecular weight structures with the print-related compounds, and as a consequence these fragments/ions are less specific and some could be false-positives or impurities/breakdown products of other PI formulations. Nevertheless, the ions used for the identification criteria were truncated to remove any common ions in order to avoid false-positives. In the case of the five targeted print-related compounds detected by DART-HRMS, benzaldehyde and mesitaldehyde were also confirmed by other techniques. Differences in ionisation sensitivities likely accounted some compounds (4-hydroxy benzoic acid) only being detected by DART-HRMS, although poor chromatography or in-extract loss could also have played a role.

Two PIs, methyl-o-benzoyl-benzoate (MBB) and ITX, were observed in contrast to Lord *et al.* (2012) and Bentayeb *et al.* (2013) who did not detect these two compounds in their samples. The PI MBB was observed by all three techniques; however, by DART-HRMS the fragments of MBB detected were common with Genopol® BP-2 and Speedcure® 7005, and its positive confirmation was impracticable with chromatography or tandem MS. ITX was also not detected by DART-HRMS, probably due to its possible presence in only one color of the print in a correspondingly small area. It was likely only detected after the extraction/concentration with a solvent by GC-MS and UHPLC/ESI-

HRMS. These cases, as with the fate of other PIs not suitable for DART-HRMS (trimethylbenzoyldiphenyl phosphine oxide (TPO) or BDMP according to Bentayeb *et al.* (2013), justify analysis by three techniques to achieve more reliable results.

8.4.2. NON-TARGETED ANALYSIS

The 34 confirmed and 54 tentatively identified compounds obtained by GC-EI/MS analysis (not including targeted PIs and photo-products) are shown in tables 8.8 and 8.9. A total of 18 confirmed and nine tentatively identified compounds show evidence of *set-off* or presence on both print and food-contact sides of the packaging. Most of the non-targeted compounds were monomers, co-monomers, monomer byproducts, solvents or plasticisers. Approximately one of seven non-targeted molecules were PIs or print-related components and almost 60 % (seven of 12) showed evidence of *set-off*.

CAS number	Name	Function*	Package A	Package B	Package C
57-10-3	Palmitic acid	Slip Agent Degradant	FCS/PS	FCS/PS	FCS/PS
91-01-0	Diphenylmethanol	PI degradant	-	-	*Set-off*
92-52-4	Biphenyl	PI degradant	*Set-off*	-	FCS
96-76-4	2,4-Di-tertbutylphenol	Antioxidant degradant	FCS/PS	FCS/PS	-
98-86-2	Acetophenone	Monomer	PS	PS	FCS
102-76-1	Triacetin	Solvent	FCS/PS	FCS/PS	FCS/PS
102-82-9	Tributylamine	Additive	-	-	PS
103-11-7	2-Ethylhexyl acrylate	Monomer	PS	PS	FCS
111-11-5	Methyl octanoate	Slip agent	FCS/PS	PS	-
111-82-0	Lauric methyl ester	Slip agent	FCS/PS	PS	-
112-27-6	Triethylene glycol	Solvent	-	-	PS
112-34-5	Diethylene glycol monobutyl ether	Solvent	FCS/PS	FCS	PS
112-60-7	Tetraethylene glycol	Solvent	-	PS	-
112-84-5	Erucamide	Slip agent	FCS/PS	FCS/PS	FCS/PS
124-17-4	2-(2-Butoxyethoxy)ethyl acetate	Solvent	PS	PS	FCS/PS
124-19-6	Nonylaldehyde	Solvent	FCS	FCS/PS	FCS
128-37-0	2,6-di-tert-butyl4-methyl-phenol	Antioxidant	-	-	FCS/PS
143-07-7	Lauric acid	Monomer	-	PS	-

150-76-5	Mequinol	Monomer	PS	PS	-
502-44-3	ε-Caprolactone	Monomer byproduct	PS	-	-
699-61-6	1-Oxaspiro(4,5)decan-2-one	Antioxidant degradant	PS	-	-
719-22-2	2,6-di tert-butyl-1,4-benzoquinone	Antioxidant degradant	PS	FCS/PS	FCS
761-65-9	N,N-Dibutylformamide	Solvent	-	-	PS
1007-32-5	1-Phenyl-2-butanone	PI degradant	*Set-off*	*Set-off*	FCS
2082-79-3	Irganox 1076®	Antioxidant	FCS/PS	FCS/PS	FCS/PS
3446-89-7	4-(Methylthio)benzaldehyde	PI degradant	*Set-off*	PS	*Set-off*
3891-07-4	N-(2-Hydroxyethyl) phthalimide	Comonomer	-	-	PS
4098-71-9	Isophorone diisocyanate	Monomer	PS	PS	-
5416-93-3	4-Methoxy phenyl isocyanate	Monomer	PS	PS	-
7473-98-5	2-Hydroxy-2-methylpropiophenone	PI	-	-	*Set-off*
13048-33-4	1,6-Hexanediol diacrylate	Monomer	*Set-off*	PS	-
14002-80-3	Methyl, 2,2-dimethyl-3-hydroxypropionate	PI degradant	PS	PS	PS
20071-09-4	trans-1,2-Diphenylcyclobutane	Monomer byproduct	*Set-off*	*Set-off*	FCS
26530-20-1	2-octyl-4-isothiazolinone	Biocide	-	-	PS

Table 8.8: 34 Non-targeted compounds confirmed by GC-MS using HP-5MS and Rtx-200 columns. * - technical functions obtained from Skjevrak *et al.* (2005), EuPIA (2013), Salamone (1996), FDA (2015), Bentayeb *et al.*(2012), and product literature. (-: not detected).

Included in the 18 confirmed *set-off* compounds were other PIs such as 2-hydroxy-2-methylpropiophenone (HMPP), photo-products such as 4-(methylthio)benzaldehyde, antioxidants such as Irganox® 1076, plasticisers (triacetin), slip agents (erucamide) and monomers (1,6-hexanediol diacrylate, HDDA). Many of these are commonly used in food-contact materials and some are included in the European Union positive list of authorised substances included in the European FCM legislation (Regulation (EU) No 10/2011) (EC, 2011). However, other compounds are "Non-Intentionally Added Substances" (NIAS). Some of these NIAS, such as 2,6-di-tert-butyl-1,4-benzoquinone or 2,4-di-tert-butylphenol are degradation products of the very common antioxidants Irgafos® 168 and Irganox® 1010 (Burman *et al.*, 2005; Alin & Hakkarainen, 2011), and as such their safety assessments were often part of the original additive's food contact assessment. Others are used in UV curing inks (1-phenyl-2-butanone) (Bradley *et al.*, 2013) in varnishes/lacquers or over prints (isocyanates /reactive linkers).

CAS number	Name	Function*	Package A	Package B	Package C
57-11-4	Octadecanoic acid	Monomer/slip agent	PS	PS	FCS
84-66-2	Diethyl Phthalate	Plasticizer	FCS	FCS	-
85-44-9	α-Phthalic anhydride	Monomer	-	-	PS
98-83-9	Methylstyrene	Monomer	-	PS	
101-81-5	Diphenylmethane	Monomer degradant	-	-	FCS
110-42-9	Decanoic acid, methyl ester	Slip agent	FCS/PS	-	FCS
112-39-0	Hexadecanoic acid, methyl ester	Monomer	FCS	-	-
112-61-8	Methyl stearate	Slip agent	FCS	-	-
115-86-6	Triphenyl phosphate	Monomer/Plasticizer	-	-	PS
119-47-1	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	Antioxidant	FCS	-	-
122-99-6	Ethanol, 2-phenoxy-	Solvent	PS	PS	-
124-10-7	Methyl tetradecanoate	Slip agent	FCS	-	-
124-26-5	Octadecanamide	Slip agent	-	FCS	-
141-02-6	2-Butanedienoic acid E, bis (2-ethylhexyl)ester	Monomer	PS	-	-
629-54-9	Hexadecanamide	Slip agent	-	PS	FCS
1115-20-4	Propanoic acid, 3-hydroxy-2,2-dimethyl-, 3-hydroxy-2,2-dimethylpropyl ester	Monomer	PS	PS	PS
1235-74-1	Methyl dehydroabietate	Monomer	-	-	FCS/PS
1520-44-1	Benzene, 1,1'-(1-methyl-1,3-propanediyl)bis-	Antioxidant degradant	FCS/PS	FCS	FCS
1589-60-2	1-Phenylcyclohexanol	PI degradant	PS	-	-
1731-92-6	Heptadecanoic acid, methyl ester	Slip agent	FCS	-	FCS
1740-19-8	Dehydroabietic acid	Monomer	-	-	PS
2050-67-1	1,1'-Biphenyl, 3,3'-dichloro-	Pigment byproduct	-	FCS	-
2478-10-6	4-Hydroxybutyl acrylate	Monomer	PS	PS	-
2682-20-4	3(2H)-Isothiazolone, 2-methyl-	Biocide	PS	PS	-
2883-02-5	n-Nonylcyclohexane	-	FCS	-	-
3018-20-0	Naphthalene, 1,2,3,4-tetrahydro-1-phenyl-	Comonomer	FCS/PS	FCS/PS	FCS
5779-72-6	Benzaldehyde, 2,4,5-trimethyl-	PI degradant	FCS	-	-
6418-44-6	Heptadecane, 3-methyl-	Lubricant	FCS	FCS	FCS
7477-77-2	2(5H)-Furanone, 5,5-diphenyl-	-	-	-	PS
7614-93-9	Benzene, 1,1'-(3-methyl-1-propene-1,3-diyl)bis-	Antioxidant degradant	PS	-	-
7694-30-6	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, cis-	Antioxidant degradant	-	-	FCS
13205-48-6	Benzoic acid, 4-(methylthio)-	PI degradant	PS	-	-
13588-28-8	1-Propanol, 2-(2-methoxypropoxy)-	Solvent	PS	PS	PS
17611-16-4	13α-δ(8)-dihydroabietic acid	Monomer degradant	-	PS	PS

17831-71-9	2-Propenoic acid, oxybis(2,1-ethanediyloxy-2,1-ethanediyl) ester	Antioxidant degradant	-	PS	-
19781-73-8	n-Heptadecylcyclohexane	Lubricant	-	-	FCS
20324-32-7	2-Propanol, 1-(2-methoxy-1-methylethoxy)-	Solvent	PS	PS	FCS/PS
42978-66-5	2-Propenoic acid, (1-methyl-1,2-ethanediyl)bis[oxy(methyl-2,1-ethanediyl)] ester	Monomer	PS	PS	-
53774-19-9	3-Pentenoic acid, 4-phenyl-	Antioxidant degradant	PS	PS	-
54446-78-5	Ethanol, 1-(2-butoxyethoxy)-	Solvent	PS	PS	FCS/PS
55956-21-3	1-Propanol, 2-(2-methoxy-1-methylethoxy)-	Solvent	-	-	PS
65745-83-7	3,6,9,12,15-Oxabicyclo(15,3)heneicosa-1(21),17,19-triene-2,16-dione	Antioxidant degradant	PS	PS	-
74367-34-3	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethyphenyl trimethypentyl ester	Ink Plasticizer	PS	PS	-
82304-66-3	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	Antioxidant degradant	FCS/PS	FCS/PS	FCS/PS
93816-21-8	3,3-Diethyltridecane	Lubricant	-	FCS	-
1000161-07-9	Cyclopropane, 1-chloro-1-methyl-2-phenyl-	-	-	FCS	-
1000293-16-6	18-Norabietane	Monomer byproduct	-	-	FCS
1000304-32-9	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	Antioxidant degradant	-	-	PS
1000317-91-6	4-Morpholin-4-yl-benzaldehyde	PI degradant	PS	-	*Set-off*
1000360-41-0	5,5-Diethylpentadecane	Lubricant	FCS	FCS	-
1000360-41-6	3,3-Diethylheptadecane	Lubricant	FCS	FCS	-
1000360-42-6	3-Ethyl-3-methylnonadecane	Lubricant	FCS	-	-
1000370-33-1	3-Phenyl-2-benzofuran-1(3H)-one	PI degradant	-	PS	*Set-off*
1000373-81-4	Dehydroabietic acid	Monomer byproduct	-	-	PS

Table 8.9: 54 tentatively identified compounds by GC-MS using HP-5MS and Rtx-200 columns. *- Technical functions obtained from Skjevrak *et al.* (2005), EuPIA (2013), Salamone (1996), FDA (2015) , Bentayeb *et al.* (2012) and product literature. (-: not detected).

From the 54 compounds tentatively identified, the 10 print-related *set-off* compounds include the photo-scission product of BDMP, 4-morpholin-4-yl-benzaldehyde. Other compounds are byproducts of the antioxidant Irganox® 1010 (such as 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (Alin & Hakkarainen, 2011) or 2-propanol,1-(2-methoxy-1-methylethoxy)- (DPGME), a product commonly used in lacquers, inks and varnishes.

8.5. CONCLUSIONS

3 different techniques (DART-HRMS, GC-MS and UHPLC/ESI-HRMS) were adapted to determine the *set-off* of print-related compounds on three intentionally under-cured food packages. The results obtained emphasise the need to carry out multiple analytical techniques when screening for process transformation products in order to avoid the limitations of each technique and manage the incidence of false-positives and -negatives. For the first time, the HRMS spectra of polymeric PIs have been characterised by two different techniques (DART-HRMS and UHPLC/ESI-HRMS), and molecular formula of the fragments observed tabulated. Despite nominal masses of > 1,000 Da, the polymeric PIs ESI and DART spectra were dominated by fragments (oligomeric or other PI substructures) below 500 Da. A total of 110 molecules were identified on the different under-cured food packages, with evidence that 30 molecules likely experienced *set-off* or were present on both sides. Most of the print related compounds detected are not included in the positive list of the European FCM legislation and several are (to the best of the authors' knowledge) novel PI transformation products. The molecules reported herein can serve as guide for any future assessments of print-related contaminants.

8.6. REFERENCES

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9. STUDY OF THE PHOTO-PRODUCTS OBTAINED FROM THE UV CURING OF BP AND EDB. SURVEY IN FOOD PACKAGING MATERIALS

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9.1. ABSTRACT

UV inks are widely used in food packaging; however, since 2005, the European food safety authorities have reported many cases of food contamination with photoinitiators and, in consequence, they have put the focus on this issue. The action mechanism of photoinitiators creates photo-products that also can migrate to foodstuffs. For this reason, this work deals to identify these molecules originated from the photoinitiator system benzophenone (BP): ethyl-4-(dimethylamino) benzoate (EDB).

For that purpose, mixtures of BP-EDB have been analyzed by HPLC-DAD and GC-MS, obtaining a total of 23 photo-products, being benzaldehyde, benzocaine, benzene, 1, 1'-diphenylethylene and benzoic acid ethyl ester confirmed after the injection of standards.

Moreover, a survey of photoinitiators has been carried out in 13 food packages in order to check the presence of any of these photo-products. 30 % of the samples have presented BP and EDB, but none of their photo-products were found in the samples.

Keywords: Print-related contamination, UV inks, photoinitiators, photo-products, benzophenone, ethyl-4-(dimethylamino) benzoate

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9.2. INTRODUCTION

The benefits of using UV printing inks instead of solvent-based inks such as the speed of the process or the relative absence of residues are well known by the printing industry, accordingly they have been replacing them gradually in food packaging (Papilloud & Baudraz, 2002; Rothenbacher *et al.*, 2007). However, despite these advantages, the use of this type of inks it is no free of drawbacks as it is reflected in the fact that the Rapid Alert System for Food and Feed (RASFF) has reported more than a hundred notifications and alerts related to their presence in foodstuffs and their packaging in the last years (Aparicio & Elizalde 2015; RASFF, 2015).

These alerts notified the presence and/or migration of two components of UV inks from the food package to the foodstuffs: the photoinitiator and the amine synergist, used as coinitiator in the UV photocuring process. These compounds could reach the foodstuffs by three different ways, however the most probable and studied it is the *set-off*: the transfer of components from the print surface of the package to the inner surface when the packaging is stored in reels or stacks (Bradley *et al.*, 2005; Jickells *et al.*,2005; Rothenbacher *et al.*, 2007). To address this problem many different methodologies to determine these molecules in Food Contact Materials (FCM) and foodstuffs have been developed and many surveys have been carried out in different markets (Lago *et al.*, 2015).

Nevertheless, a previous review points out another problem derived from the process of photocuring: the photo-products (Lago *et al.*, 2015). These compounds have been usually included in the Non-Intentionally Added Substances (NIAS) definition: “impurities in the substances used or reaction intermediates formed during the production process or decomposition or reaction products”, and no targeted determination of them has been accomplished. The overall migration tests quantify both known migrants and NIAS, with an overall limit migration legally settled to 10 mg dm^{-2} from plastics.

However, the composition of this migrants is unknown, and many different compounds could be taking part of it, from harmless to very hazardous molecules, so different approaches to risk assessment and risk management must be considered in order to protect consumers' health (EC, 2011).

Considering only the photoinitiator migration from the FCM to the foodstuff, it should be necessary carry out also the targeted analysis of the photo-products for three main reasons: a) the mechanism of photopolymerization of type I photoinitiators starts with their scission, so the possible migration compounds are going to be mainly derivatives from the radicals produced from the "raw" photoinitiator, and the "raw" photoinitiator migration only will be originated from the unreacted photoinitiator, a negligible quantity if the curing process is efficient; b) on the contrary, the type II photoinitiators do not suffer scission, but they are used in combination with coinitiators, usually amine synergists, that after the hydrogen abstraction could suffer oxygen quenching obtaining new molecules as the type I photoinitiators; and, c) the appearance of the polymeric photoinitiators, also called "low migration photoinitiators" that, if there are of type II, they will not produce photo-products by themselves (the coinitiator can do it), however, they are able to produce photo-products if they are type I, as could be the case of Esacure® KIP 150 that releases acetone on the curing process (Green, 2010).

The high number of possible photoinitiators used in UV inks is the main problem in order to determine the migration from UV inks. The EuPIA (European Printing Inks Association) has published a list of "Suitable photoinitiators of low migration for UV inks and varnishes" where there are 105 photoinitiators, and considering the possible radicals and derivatives formed from them, the number of possible migrants it is almost impossible to cope with (EuPIA, 2013). With this in mind, the objective of this work has been investigate the possible photo-products obtained during the process of photocuring from one of the most used photoinitiators: Benzophenone (BP) and one of the most common amine synergist: Ethyl-4-(dimethylamino) benzoate (EDB). With this purpose, different solutions of these two compounds have been mixed in different proportions and

then irradiated with a UV lamp to initiate the process of UV curing. The obtained solutions have been injected by HPLC-DAD and GC-MS in order to identify these photo-products. Also, a survey has been carried out in 13 different packages obtained in Spanish local markets where different photoinitiators were identified by HPLC-DAD and GC-MS, looking for the proposed photo-products in those where BP and EDB have been identified.

9.3. MATERIALS AND METHODS

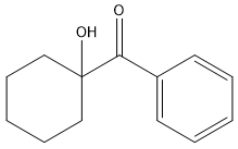
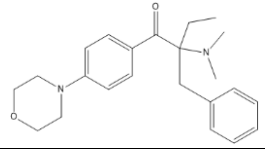
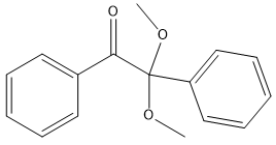
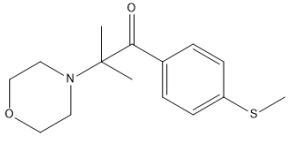
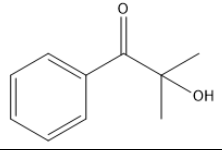
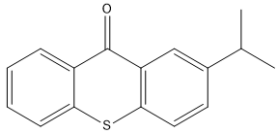
9.3.1. REAGENTS AND STANDARDS

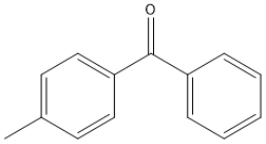
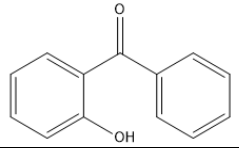
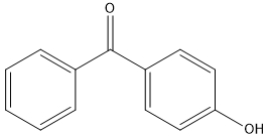
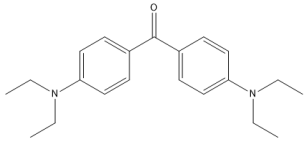
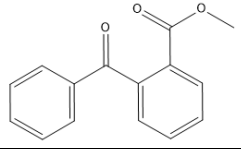
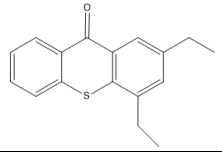
Acetonitrile (AcN) HPLC grade was acquired from Merck (Darmstadt, Germany) and ultrapure water was obtained from a Milli-Q system (Millipore, Bedford, MA, USA). The photoinitiator standards and the photo-products standards listed on table 9.1 were of reagent grade and they were obtained from Sigma-Aldrich (St. Louis, MO, USA), except for benzophenone, benzaldehyde and benzopainacol that were purchased from Fluka (Stenheim, Switzerland), dimethylformamide was of analytical grade and it was obtained from Merck (Darmstadt, Germany) and benzophenone acrylate was obtained directly from a supplier.

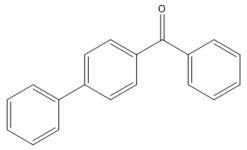
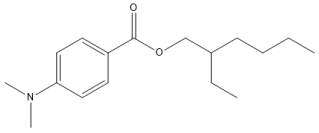
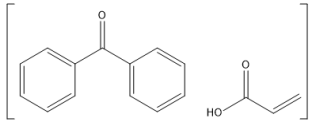
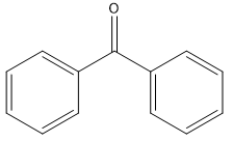
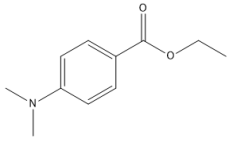
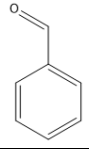
Working solutions were prepared, in AcN, in a concentration of 10,000 μg mL^{-1} for BP and EDB and 1,000 μg mL^{-1} for the rest of the molecules.

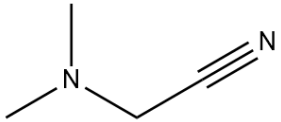
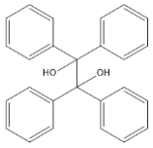
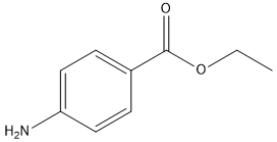
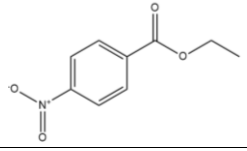
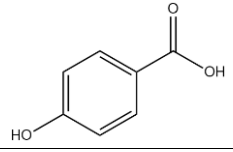
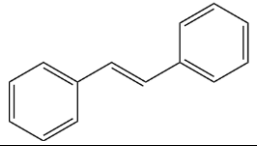
9.3.2. CURING EXPERIMENTS

The experiments were carried out with a UV lamp SwiftCure GR 250R (UV-Consulting Peschl España S.L., Castellón, Spain), using 6 different solutions (AcN, BP, EDB, BP-EDB (1:1), BP-EDB (2:1) and BP-EDB (3:1)) that were introduced in different quartz cuvettes (10 mm x 10 mm x 38 mm) and were placed at three different distances from the Hg lamp (50, 100 and 150 mm) during 0.5, 1.0, 2.0, 5.0 or 10.0 minutes. A photograph of the apparatus is given in Figure 9.1.

Structure	Common Name	Abbr.	CAS n.	Molecular formula	Melting Point (°C)	Boiling Point (°C)	Log P (octanol-water)	Vapor Pressure (mm Hg)
	1-Hydroxycyclohexyl-phenyl-ketone	HCPK	947-19-3	$C_{13}H_{16}O_2$	48.00*	339.00	2.18	3.65E-05
	2-Benzyl-2-dimethylamino-4-morpholino butyrophenone	BDMB	119313-12-1	$C_{23}H_{30}N_2O_2$	N/A	528.00	3.38	2.87E-11
	2,2-Dimethoxy-2-phenyl acetophenone	DMPA	24650-42-8	$C_{16}H_{16}O_3$	64.00*	371.10	3.62	1.06E-05
	Methyl-1-(4-methylthio)phenyl-2-morpholinopropan-1-one	MMMP	71868-10-5	$C_{15}H_{21}NO_2S$	N/A	420.10	2.44	2.89E-7
	2-Hydroxy-2-methyl propiophenone	HMPP	7473-98-5	$C_{10}H_{12}O_2$	184.00*	260.80	1.49	6.08E-03
	2-/4-Isopropyl thioxanthone	ITX	5495-84-1	$C_{16}H_{14}OS$	76.75*	398.20	5.11	1.43E-06

	4-Methyl benzophenone	4-MBP	5495-84-1	$C_{14}H_{12}O$	59.50*	328.10	3.69*	1.94E-04
	2-Hydroxy benzophenone	2-HBP	117-99-7	$C_{13}H_{10}O_2$	38.00*	308.00	3.53	4.39E-04
	4-Hydroxy benzophenone	4-HBP	1137-42-4	$C_{13}H_{10}O_2$	133.50*	322.50*	2.92	6.50E-6
	4.4'-bis(diethylamino) benzophenone	DEAB	90-93-7	$C_{21}H_{28}NO_2$	96.00*	475.70	5.91	3.25E-09
	Methyl-2-benzoylbenzoate	MBB	606-28-0	$C_{15}H_{12}O_9$	52.00*	351.00*	2.70*	1.53E-05*
	2.4-Diethyl thioxanthone	DETX	82799-44-8	$C_{17}H_{16}OS$	N/A	427.90	5.67	1.58E-07

	4-Phenyl benzophenone	PBZ	2128-93-0	$C_{19}H_{14}O$	102.00*	419.50*	5.14	3.11E-07
	2-Ethylhexyl-4-dimethylamino benzoate	EHA	21245-02-3	$C_{17}H_{27}NO_2$	243.00*	382.90	5.41	4.57E-06
	Benzophenone acrylate	BPAcr	59626-79-8	N/A	N/A	N/A	N/A	N/A
	Benzophenone	BP	119-61-9	$C_{13}H_{10}O$	47.80	305.40	3.18*	8.23E-04
	Ethyl-4-(dimethylamino) benzoate	EDB	10287-53-3	$C_{11}H_{15}NO_2$	63.50*	296.50	2.51	1.43E-03
	Benzaldehyde	Der 1	100-52-7	C_7H_6O	-26.00*	179.00*	1.45	9.74E-01

	Dimethylamino-Acetonitrile	Der2	926-64-7	$C_4H_8N_2$	N/A	137.50*	-0.19	7.03E+00
	Benzopainacol	Der 3	464-72-2	$C_{26}H_{22}O_2$	185.00*	506.90	5.04	4.26E-11
	Benzocaine	Der 4	94-09-7	$C_9H_{11}NO_2$	90.00*	310.00*	1.84	5.89E-05
	Ethyl 4-nitrobenzoate	Der 5	99-77-4	$C_9H_9NO_4$	57.00*	315.00	2.43	4.50E-04
	4-Hydroxybenzoic acid	Der 6	99-96-7	$C_7H_6O_3$	214.00*	336.20	1.40	4.48E-05
	Trans-Stilbene	Der 7	103-30-0	$C_{14}H_{12}$	124.00*	307.00*	3.89	1.35E-03

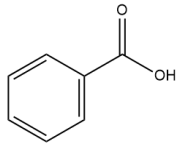
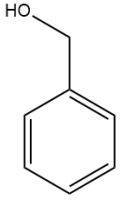
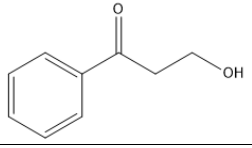
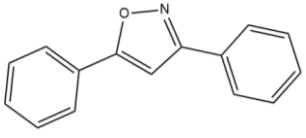
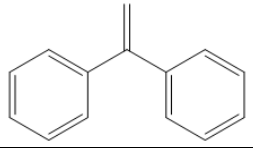
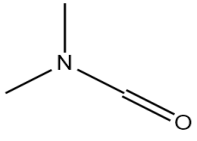
	Benzoic acid	Der 8	65-85-0	$C_7H_6O_2$	121.00*	249.20*	1.56	1.22E-02
	Benzyl alcohol	Der 9	100-51-6	C_7H_8O	-15.00*	205.00*	1.06	1.58E-01
	Ethyl Benzoate	Der 14	93-89-0	$C_9H_{10}O_2$	-34.00*	212.00*	2.63	1.80E-01
	3.5-diphenyl Isoxazole	Der 11	2039-49-8	$C_{15}H_{11}NO$	N/A	396.60	5.13	3.87E-06
	1,1-Diphenylethylene	Der 12	530-48-3	$C_{14}H_{12}$	7.50*	277.00*	3.80	7.75E-03
	Dimethyl Formamide	Der 13	68-12-2	C_3H_7NO	-60.40*	153.00*	-0.83	3.40E+00

Table 9.1: List of photoinitiators and photoproducts standards. (*: experimental data; N/A: not available).

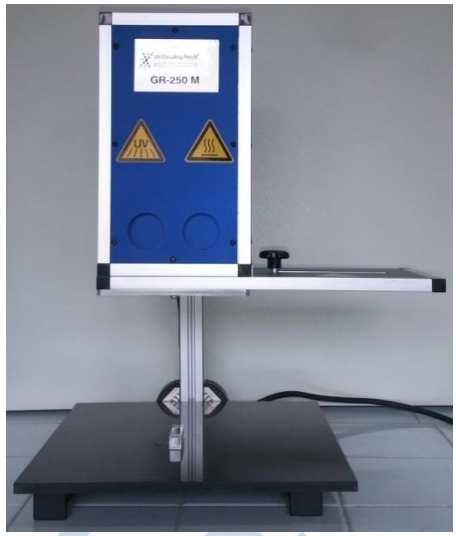


Figure 9.1: UV curing system used.

9.3.3. HPLC-DAD ANALYSIS

The HPLC system (Hewlett–Packard, Waldbronn, Germany) comprised an HP 1100 quaternary pump, a degassing device, an autosampler, a column thermostatting system, and a diode array detector (DAD). The chromatographic separation was achieved with a Kromasil C18 column (250 mm × 3.2 mm internal diameter, 5 µm particle size) (Phenomenex, Torrance, CA, USA).

The method selected was the same used by Lago *et al.* (2014) (Chapter 7).

9.3.4. GC-MS ANALYSIS

The different solutions were analyzed first in a Trace 1300 Gas Chromatographer equipped with a programmed split/splitless automatic injector AI 1300, coupled to an ISQ LT Single Quadrupole Mass Spectrometer controlled by XCalibur 2.0 software (Thermo Scientific). The mass spectra were obtained using this mass-selective detector under electron impact ionization at a voltage of 70 eV and data acquisition was done in full scan mode over an m/z range of 40-450.

A sample volume of 1 µL was injected in split mode, with a split ratio of 1:100. The separation was performed on a ZB-5MS column (30 m × 0.25 mm i.d.; 0.25 µm film thickness) using an oven temperature gradient of 50 °C for 5 min, then it was raised until 300 °C at 10 °C min^{-1} and finally it was held for 5 min. Helium was used as carrier gas with a flow rate of 1 mL min^{-1} and the temperature of injector inlet and transfer line of the detector was set at 280 and 310 °C.

Also this analysis has been carried out with a GC 6890 Gas Chromatographer equipped with a MSD 5975 Single Quadrupole Mass Spectrometer controlled by Agilent MSD Chemstation (Agilent, Waldbronn, Germany). The mass spectra were also obtained using a mass-selective detector under electron impact ionization and data acquisition was done in full scan mode (m/z range: 20-600). The sample volume injected was 1µL in splitless mode. The column used for separation was an HP-5MS column (30 m x 0.25 mm i.d.; 0.25 µm film thickness) using the following oven program: 80 °C during 1 min and then raise the temperature 15 °C min^{-1} until 300 °C and hold it for 10 min. The He rate was the same used previously and the temperatures of injector inlet and mass detector source were 280 and 230 °C.

9.3.5. SURVEY IN PACKAGES OF PHOTOINITIATORS AND PHOTO-PRODUCTS

13 different plastic packages were extracted and analyzed by HPLC-DAD and GC-MS. The extractions were carried out according the method reported by Lago *et al.* (2014) extracting a surface of 1 dm^2 with 50 mL of AcN; and they were analyzed following the method of Lago *et al.* (2014). The GC-MS analysis was carried out with the Agilent equipment described above in this section, using the same method but in SIM (Single Ion Monitoring) mode of acquisition instead of Full Scan. Benzophenone and Isopropyl thioxanthone deuterated (BP-d10 and ITX-d7) were used as internal standards. The characteristic GC-MS parameters of each compound are in table 9.2.

9.4. RESULTS AND DISCUSSION

9.4.1. UV CURING ASSAYS

To determine the photo-products obtained from the exposition to UV light of BP and EDB, 3 critical parameters were firstly evaluated: distance to the UV lamp, proportions of photoinitiator and coinitiator and time of exposition to the UV light.

Photoinitiator	Retention time (min)	Quan ion	Qual ion/s
HMPP	6.34	59.2	105.2 77.2
BP-d10	9.32	110.2	192.2 82.2
BP	9.36	105.1	182.2 77.2
HCPK	9.84	99.2	81.2
EDB	9.98	148.1	164.2
4-MBP	10.33	119.1	196.2 105.1
DMPA	11.20	151.2	105.2 77.1
MBB	11.56	163.1	105.1 240.1
4-HBP	12.02	121.1	198.2
EHA	13.59	165.1	148.2
MMMP	13.72	128.2	84.2
ITX-d7	14.32	243.1	261.2 184.9
ITX	14.36	239.1	254.2
PBZ	14.73	181.1	152.1
DETX	15.20	268.1	253.1
BDMB	18.94	176.2	275.3

Table 9.2: GC-MS parameters of each photoinitiator.

In first term, the influence of the distance to the UV lamp was studied by HPLC-DAD. Taking into account the structure of the curing system used, 3 different distances were tested (50, 100 and 150 mm) and the chromatograms obtained have been compared in order to check the possible differences between each option. The results obtained showed only slight differences

between the 3 distances for BP, EDB and the BP:EDB mixtures showing the chromatograms the same peaks with the same spectra, but with minor differences in the area obtained for each peak. Checked that there were not differences in the compounds obtained, the distance of 15 cm was selected as standard for the rest of the experiments due to allow an easier handling of samples under the UV lamp.

The assay that involved different proportions of photoinitiator-coinitiator (1:1, 2:1 and 3:1) resulted in chromatograms with identical peaks and, as expected, bigger differences in their areas than in the previous experiments were observed due to the curing solution compositions.

Finally, to study the influence of UV curing times in the formation of photo-products obtained from the photopolymerization, the solution selected was BP-EDB (1:1) due to show bigger response by the two techniques used in the previous experiment. The results obtained indicate that the time of exposition to the UV light source is a critical parameter, obtaining more curing products with increasing times of exposure to the UV light. Figure 9.2 shows the HPLC-DAD chromatogram of the mixture BP-EDB (1:1) irradiated with UV light during 0, 0.5, 1.0, 2.0, 5.0 and 10.0 minutes. It is possible to observe how the increase of time influences the system, from a time equal to zero where there is only one very broad peak (sum of EDB (8.7 min) and BP (9.0 min)) to their slight separation as two different peaks, but not completely resolved, when the time of exposition to the UV light increases. Apart from the initial compounds, different photo-products appear gradually with the passage of time and the increasing of their areas is clearly appreciated with increasing UV irradiation times.

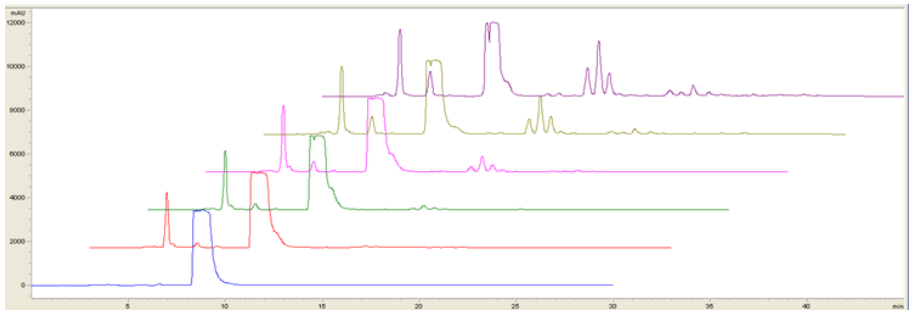


Figure 9.2: UV chromatograms at 256 nm of a 10,000 mg L^{-1} solution of BP-EDB (1:1) irradiated 0.0 (blue), 0.5 (red), 1.0 (green), 2.0 (pink), 5.0 (brown) and 10.0 min (purple). The retention times of EDB and BP are 8.7 and 9.0 min.

Based on these facts the mixture BP-EDB (1:1) were selected for the GC-MS identification experiments, in order to characterize the higher quantity of photo-products. The software peak detection (Thermo XCalibur and Agilent MSD Chemstation) and EI-spectral library matching protocol were used with the Wiley Registry™ 8th Edition and the NIST/EPA/NIH 11 Mass Spectral Libraries for detection/identification using background subtracted peak apex spectra. The first approach by GC-MS (both methods) provides a good match of a total of 23 compounds (table 9.3). In this list, there are different photo-products originated from photoscission of photoinitiator and coinitiator and their by-products. Five of these photo-products are coincident with print-related contaminants reported by Lago & Ackerman (2016) in different under-cured food packages with the same photoinitiators in the printing inks formula: benzocaine; 4-hydroxybenzoic acid; benzenemethanol; benzaldehyde and 1,1-diphenylethylene. Also, Green (2010) presented schemes of the photoscission process of different photoinitiators and tertiary amines. In these schemes, the dimerization of the BP ketyl radicals results in the obtaining of benzopainacol. Furthermore, many of the other compounds obtained by comparison with the library share structures with the coinitiator, such as benzoic acid, 4-(dimethylamino)-methyl ester obtained from the loss of a methyl group from the primary structure of EDB.

These were the tentatively identified photo-products by comparison with the mass spectral library; however, to confirm their presence, the standards of

those molecules of which an available standard could be obtained were injected by GC-MS (Table 9.1, Der 1-13). The confirmation analysis verified four of the thirteen possible photo-products: Benzaldehyde, benzocaine, benzene, 1,1'-ethenylidenebis- and benzoic acid ethyl ester. The first three photo-products confirmed agreed with the data obtained by Lago & Ackerman (2016).

CAS No.	Compound	Solution/s
65-85-0	Benzoic acid	BP+EDB
68-12-2	Dimethyl formamide	EDB
76-89-1	Benzilic acid, methyl ester	BP+EDB
93-89-0	Benzoic acid , ethyl ester	BP+EDB
94-09-7	Benzoic acid, 4-amino, ethyl ester	EDB, BP+EDB
98-86-2	Acetophenone	BP+EDB
99-77-4	Benzoic acid, 4-nitro, ethyl ester	EDB
99-96-7	4-hydroxy Benzoic acid	BP+EDB
100-51-6	Benzenemethanol	BP, BP+EDB
100-52-7	Benzaldehyde	BP+EDB
101-81-5	Diphenylmethane	BP+EDB
451-40-1	2-Phenylacetophenone	BP+EDB
464-72-2	Benzopainacol	BP+EDB
530-48-3	Benzene, 1, 1'-ethenylidenebis-	BP+EDB
768-03-6	2-Propen-1-one, 1-phenyl	BP+EDB
926-64-7	Dimethylamino acetonitrile	BP+EDB
1202-25-1	Benzoic acid, 4-(dimethylamino) methyl ester	EDB, BP+EDB
2039-49-8	Isoxazol, 3, 5-diphenyl	BP+EDB
4746-87-6	Benzilamide	BP+EDB
18358-63-9	Methyl, 4-(methylamino) benzoate	EDB, BP+EDB
28447-17-8	2-Propenoic acid, 3-(3-pyridinyl)-, ethyl ester	EDB, BP+EDB
33046-28-5	Benzenaminium, 4-carboxy,N,N,N-trimethyl- hydroxide inner salt	EDB
77629-53-9	3-Pyridinecarboxylic acid, 5, 6-dimethyl-, ethyl ester	EDB

Table 9.3: Tentatively identified products by GC-MS of BP, EDB and their mixtures dissolved in AcN after UV irradiation.

However, it is relevant the case of benzopainacol (Der 3), product from the dimerization of the ketyl radical obtained from BP, which in solution becomes BP and its confirmation cannot be performed. Also, other two molecules:

benzoic acid and 4-hydroxy benzoic acid could not be confirmed due to they are not suitable for their analysis with the GC columns used in this work.

On the other hand, only two of the confirmed photo-products are in the list of authorized compounds for plastic materials and articles intended to come into contact with food (EC, 2011), benzaldehyde and benzoic acid, ethyl ester. Nevertheless, one of the problems associated with benzaldehyde it is the possible alteration of the organoleptic properties that it can produce in the packaged food; which could lead to non-compliance with the Regulation (EC) No 1935/2004 on materials and articles intended to come into contact with food (EC, 2004, 2011). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has evaluated benzaldehyde as a flavoring agent, stablishing an ADI (Acceptable Daily Intake) of 0-5 mg kg^{-1} body weight for the group of benzaldehyde, benzoic acid, the benzoate salts (calcium, potassium and sodium), benzyl acetate and benzyl alcohol, expressed as benzoic acid equivalents. However, the benzaldehyde oxidation to benzoic acid is expected (JECFA, 1996; EFSA, 2011).

With the purpose to evaluate the toxicological profile of these four molecules the Toxtree® open-source software from EURL-ECVAM (a European Union reference laboratory for alternatives to animal testing) was applied in order to classify them according to Cramer rules (Cramer *et al.*, 1978). This classification is considered as the best known approach for structuring chemicals in order to make a Toxicological Threshold Concern (TCC) estimation' by the EURL-ECVAM (Pinalli *et al.*, 2011). It has three levels, from simple chemical structures with efficient modes of metabolism, suggesting low oral toxicity (Class I) to chemical structures with reactive functional groups that may suggest significant toxicity (Class III). In the case of the confirmed photo-products, benzaldehyde and benzoic acid, ethyl ester the software suggests low toxicity, classifying them as Class I molecules. On the contrary, the other two confirmed photo-products, benzocaine and benzene, 1,1'-ethenylidenebis-, are classified as Class III suggesting significant toxicity.

9.4.2. SURVEY IN PACKAGES OF PHOTOINITIATORS AND PHOTO-PRODUCTS

The results of the survey of photoinitiators carried out over 13 packaging samples are presented in table 9.4, with the exception of film 1 that did not present any photoinitiator. In this small survey the 92 % of the samples have presented photoinitiators after an extraction with 50 mL of AcN. This table shows the concentrations of each compound found in the samples analyzed by GC-MS, except for 2-HBP, DEAB and BPAcr, which were not included in the GC-MS method, and the HPLC-DAD data obtained for them have been included in the table.

Analyzing the data yielded by the survey, two relevant facts stand out: the diversity and the high concentrations of photoinitiators used in these package samples. In only thirteen samples, a total of 12 different photoinitiators were found, being all of them in films 4, 5 and 6, revealing the fact that nowadays the combination of different photoinitiators is a common practice in food packaging industry (Lago *et al.*, 2015). Although these packaging samples are secondary packages, some of these photoinitiators used, as BP, are volatile or semi-volatile and their migration through vapor phase to the foodstuffs is a real threat (Nerín *et al.*, 1993, 1998; López *et al.*, 2008; Pastorelli *et al.*, 2008; Sanches-Silva *et al.*, 2008, Rodríguez-Bernaldo de Quirós *et al.*, 2009; Koivikko *et al.*, 2010).

With regard to the concentrations of the photoinitiators, the sum of all of them reaches to values of 4.42 mg dm^{-2} in the film 2, being this value 40 times greater than the higher quantity found in the food package samples analyzed in our previous survey in Spanish markets (Lago *et al.*, 2014). The photoinitiators more used were HMPP, HCPK, DMPA and DETX, which are present in all the samples except for film 1. Also, the photoinitiators used in higher concentrations in the samples analyzed are the HMPP and HCPK.

PI	LOQ (mg dm^{-2})	Film											
		2	3	4	5	6	7	8	9	10	11	12	13
HMPP	0.0045	1.740	0.181	0.365	0.291	0.160	0.281	0.607	0.020	0.430	0.231	0.386	0.089
BP	0.0023	0.010	0.042	0.004*	0.010*	n.d.	0.066	n.d.	0.003*	0.007*	0.002*	0.002*	0.010*
HCPK	0.0023	0.680	1.400	1.320*	1.180*	0.670*	1.070*	0.390	0.050*	1.920*	0.159*	0.510*	0.084
EDB	0.0024	0.003*	0.030	0.004	0.003	n.d.	0.005*	n.d.	n.d.	n.d.	n.d.	0.005	n.d.
4-MBP	0.0045	n.d.	n.d.	n.d.	0.444	n.d.	0.159	n.d.	0.158*	n.d.	0.013	0.187	0.006*
DMPA	0.0023	0.229*	0.106*	0.025*	0.154*	0.100*	0.030*	0.022*	0.010*	0.030*	0.010*	0.365*	0.051*
MBB	0.0023	0.990	0.053	0.010	0.010	0.010	n.d.	0.610	0.020	n.d.	0.229	0.020	n.d.
4-HBP	0.0091	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
EHA	0.0022	0.051	0.018*	0.051	0.008*	0.010*	0.036	0.014	0.003*	n.d.	n.d.	n.d.	0.570
ITX	0.0045	n.d.	n.d.	0.012	n.d.	n.d.	0.027	0.007	0.070	n.d.	n.d.	n.d.	n.d.
PBZ	0.0023	0.243	0.008	0.043	0.003	0.011*	0.026	0.140	0.013	n.d.	0.059	n.d.	0.010*
DETX	0.0047	0.261	0.099	0.034	0.111	0.068	0.006	0.024	0.005	0.014	0.012*	0.202	0.031
MMMP	0.0046	n.d.	0.010*	0.034*	0.012*	0.009*	0.033	n.d.	0.300*	0.055*	n.d.	0.018*	n.d.
DMMB	0.0464	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.158*	n.d.
2-HBP	0.0003**	0.219**	0.081**	0.025**	0.160**	0.098**	0.025**	0.028**	n.d.**	0.048**	0.020**	0.289**	0.044**
DEAB	0.0008**	n.d.	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**
BPAcr	0.0078**	n.d.	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**	n.d.**

Table 9.4: GC-MS data of PI and amine synergists detected in the samples analyzed (µg dm^{-2} film). (n.d.*: not detected by HPLC-DAD; **: only analyzed by HPLC-DAD).

Concerning the photo-products, the samples with BP and EDB, films 2 to 5 and 7 were injected by GC-MS searching for any of the photo-products previously identified. The results of this analysis did not shown any of these photo-products in the samples, however, the low concentrations of BP and EDB detected and, also, the possibility of secondary reactions related to the UV curing process, could lead to very low concentrations of these photo-products under the detection limits of the detection method.

9.5. CONCLUSIONS

The process of UV curing of a common photoinitiator system that comprises a type II photoinitiator as BP and a coinitiator as EDB has been replicated in laboratory conditions. Different variables of the study has been tested and analyzed by HPLC-DAD and GC-MS in order to identify the possible photo-products and by-products formed during the UV curing process.

The GC-MS method has been shown as a useful tool of screening of these new compounds, helping to identify these photo-products. A total of 23 compounds have been tentatively identified, being confirmed 4 of them after the injection of the standards of 13 of them. The 9 non-confirmed compounds make evident the high difficulty that present the identification of unknowns in the screening. Also, two of the confirmed photo-products have been proposed as compounds with significant toxicity according to Cramer classification.

Also a small survey in real secondary packaging samples has been carried out detecting photoinitiators in a 92 % of the samples analyzed and 4 of them have presented BP and EDB in their UV curing formulations. However, none of the confirmed photo-products could be detected in the extractions of these packages, being the low concentrations of BP and EDB and other secondary reactions during the UV curing the probable reasons for their absence in the package extracts.

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10. MIGRATION STUDIES OF TWO COMMON COMPONENTS OF UV-CURING INKS INTO FOOD SIMULANTS

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10.1. ABSTRACT

The Rapid Alert System for Food and Feed (RASFF) has reported many cases of different UV curing inks components in foodstuffs during the last few years. These contaminants reach the foodstuffs mainly by *set-off*, their principal migration mechanism from the package. Under this premise, this work has tried to characterize the process of migration of two common UV ink components: a photoinitiator (4-Methylbenzophenone) and a coinitiator (Ethyl-4-(dimethylamino) benzoate), from the most common plastic material used in food packaging: low-density polyethylene (LDPE) into six different food simulants. The migration kinetics tests were performed at four different common storage temperatures, obtaining the key migration parameters for both molecules: the coefficients of diffusion and partition. The migration process was highly dependent on the storage conditions, the photoinitiator properties and the pH of the foodstuff.

Keywords: migration, 4-methylbenzophenone, ethyl-4-(dimethylamino) benzoate, diffusion coefficient, partition coefficient.

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10.2. INTRODUCTION

In order to improve their quality and properties, most of food packaging incorporates different additives into its structure as plasticizers, thermal and light stabilizers, slip additives or antioxidants. Consequently, the addition of these substances enables the desired properties; nonetheless, the possible interactions between the packaging and the foodstuff should be consider from a food safety concern point of view. These interactions include mass transfer processes as migration, which is defined as: "the mass transfer from an external source into food by submicroscopic processes" and it depends on several factors that can be summarized in four: food, polymer, migrant and physical conditions: time and temperature (Simoneau, 2008; Castle *et al.*, 2010).

Nevertheless, not only additives could migrate into foodstuffs, there is another wide group of compounds that could migrate from the food packaging: monomers, oligomers and their reaction products (Lau & Wong, 2000). All of these molecules could reach the foods and depending on the migrant, they could represent a serious hazard for consumers' health. To evaluate this hazard, migration experiments are usually performed, but they are expensive, time-consuming and, in some cases, complicated due to low migrant concentrations or to the complexity of food matrixes (Lau & Wong, 2000; Brandsch *et al.*, 2002; Helmroth, *et al.*, 2002a, 2002b; Petersen *et al.*, 2005; Sanches-Silva *et al.*, 2006, 2007, 2009, 2010).

To avoid these experiments, the current European legislation allows the application of theoretical prediction models based on scientific evidences (EC, 2011). These models are based on Fick's Second Law which describes the migration process in the following equation:

Eq. 1: $$\frac{\partial C_P}{\partial t} = D_P \frac{\partial^2 C_P}{\partial x_2}$$

where C_P (mg kg^{-1}) is the migrant concentration in the polymer at time t (s) and at distance x (cm), covered by the migrant since the origin; D_P (cm^2 s^{-1}) is the coefficient of diffusion in the polymer.

One group of compounds which could migrate into foodstuffs, and which has received special attention in past years, are the UV printing inks components (e.g.: photoinitiators). Printing inks are one of the seventeen groups of materials and articles included in the European Framework Regulation on materials and articles intended to come into contact with food EC 1935/2004 (EC, 2004; Bustos *et al.*, 2012). This regulation provides in its article 5 the possible adoption of "specific measures" for the different groups of materials, in order to ensure the protection of consumers' health.

Since 2005 many notifications and alerts related to photoinitiators in foodstuffs have been reported through the Rapid Alert System for Food and Feed (RASFF) (RASFF, 2015). Therefore, the study of the processes involved in the migration of photoinitiators from the packaging into foodstuffs becomes essential. Following this premise, in this work two different components of UV printing inks were studied: 4-methyl benzophenone (4-MBP), a type II photoinitiator, and ethyl-4-(dimethylamino) benzoate (EDB), an amine used as coinitiator. Low density polyethylene (LDPE) films were extruded with each substance to carry out the migration experiments at four common storage temperatures in different food simulants. The effect of the temperature on the diffusion of photoinitiators was evaluated applying the Arrhenius equation. Diffusion and partition coefficients were estimated by fitting the experimental data with a mathematical model based on Fick's Second Law (Crank, 1975).

10.3. MATERIALS AND METHODS

10.3.1. REAGENTS AND STANDARDS

The UV printing inks compounds selected in this work: 4-MBP (CAS Registry No. 134-84-9; 4-methyl benzophenone) and EDB (CAS Registry No. 10287-53-3;

Ethyl-4-(dimethylamino)benzoate), were purchased from Sigma-Aldrich® (Steinheim, Germany). Their main properties are summarized in table 10.1. Different food simulants were prepared by dilution of ethanol (absolute for analysis) and glacial acetic acid in distilled water. Acetonitrile used was HPLC grade and ultrapure water was obtained from a Milli-Q filter system (Millipore®, Bedford, MA). All the reagents were purchased from Merck® (Darmstadt, Germany).

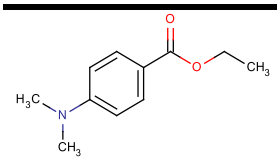
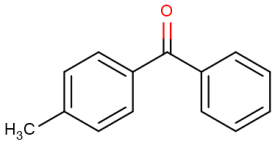
Structure	CAS No.	Common name	Mw	Log $K_{o/w}$	Mp (ºC)	Bp (ºC)	PI type
	10287-53-3	EDB	193.2	2.51[b]	63.5[a]	296.5[b]	Amine synergist
	134-84-9	4-MBP	196.2	3.69[b]	59.5[a]	328.1[b]	II

Table 10.1: Summary of the main properties of 4-MBP and EDB. Mw: molecular weight; Mp: melting point; Bp: boiling point; PI: photoinitiator; a: experimental; b: estimated. Dates extracted from SciFinder® database in 2015.

10.3.2. MIGRATION TEST

Food simulants selected in this work were those in the Commission Regulation 10/2011/EC (2011): 3 % acetic acid (w/v), 10 %, 20% and 50% ethanol (v/v). Instead of the conventional fatty simulant, vegetable oil, 95 % ethanol (v/v) was used (for throughout all this document the term simulant is used for the conventional and the substitute simulants). Tests were also performed in water, in order to compare the data obtained in the current food simulant for hydrophilic foods: 10 % ethanol (v/v) and the equivalent former food simulant, water, which is currently considered as a hydrophilic food (EC, 1985).

The temperatures selected for the migration experiments were -18 ºC (freezing temperature) only for 50 % and 95 % ethanol (other simulants are not liquid at this temperature), 4 ºC (refrigerator temperature), 20 ºC (ambient

temperature) and 40 ºC (worst case scenario for migration test of food product storage at room temperature).

To carry out the migration tests, films of low density polyethylene (LDPE) were prepared by extrusion at Gaiker (Zamudio, Spain) after mixing Alcudia® 2008F provided by Repsol-YPF® (Madrid, Spain), with EDB or 4-MBP. The obtained films were cut in 10 cm^2 sheets and accurately weighted. The film thickness ranged between 50 and 65 µm and the photoinitiators concentration measured in the LDPE films were 945.3 ± 131.0 mg kg^{-1} and 671.8 ± 195.0 mg kg^{-1}, for 4-MBP and EDB, respectively.

Migration tests were as follows: the LDPE films were introduced in a light protected tube, containing 20 ml of a food simulant at the selected temperature. At different previously pre-set times, 0.5 ml simulant were removed from the tube, filtered and injected in the HPLC-DAD system to determine the exact concentration of migrant at each time interval. The total time of migration assays were different for each temperature and photoinitiator. For 4-MBP, test times were 8 hours at 40 ºC, 2 days at 20 ºC, 7 days at 4 ºC and 210 days at -18 ºC assays. In the case of EDB: 8 hours for 40 ºC, 1 days for 20 ºC, 7 days for 4 ºC and 210 days for -18 ºC assays. At the end of each experiment, the LDPE film was removed from the tube, extracted with 20 ml of acetonitrile for 24 hours at 70 ºC, and analyzed by HPLC-DAD to determine the photoinitiator remnant in the polymer. All assays were done in duplicate.

The HPLC-DAD method was based on the method developed by Lago *et al.* (2014) with a minor modification in the mobile phase gradient: after the photoinitiator was eluted, the percentage of acetonitrile was raised to 100 % in 1 min and then held for 2 minutes.

10.4. RESULTS

10.4.1. MATHEMATICAL MODELLING

Crank (1975) proposed various solutions for Eq. 1 depending on the scenario. If a plane sheet, in our case a LDPE sheet, is suspended in a stirred solution with a finite volume, a possible solution for a Polymer-Food system could be the equation 2, which expresses the amount of migrant released from the polymer (P) into food (F) at time t (Crank, 1975; Piringer, 1994; Brandsch *et al.*, 2002; Sanches-Silva *et al.*, 2008; Simoneau, 2010):

Eq. 2: $$\frac{m_{F,t}}{A} = C_{P,0}\rho_P d_P \left(\frac{\alpha}{1+\alpha}\right) \times \left[1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-D_P t \frac{q_n^2}{d_P^2}\right)\right]$$

With: $$\alpha = \frac{1}{K_{P/F}} \frac{V_F}{V_P} \quad \text{and} \quad \alpha = \frac{1}{K_{P/F}} \frac{V_F}{V_P}$$

where $m_{F,t}$ is the mass of migrant from P into F after time t (µg); A is the area of P in contact with F (cm^2); $C_{P,0}$, $C_{P,\infty}$ and $C_{F,\infty}$ are the concentrations of migrant in the P at t = 0 , t = ∞ and the concentration of migrant in the F at t = ∞ (mg kg^{-1}); ρ_P and ρ_F are the densities of P and F (g cm^{-3}), t is the migration time (s), d_P is the thickness of P (cm), V_P and V_F are the volumes of P and F (cm^3), qn are the positive roots of the transcendent equation $\tan q_n = -\alpha q_n$, D_P is the diffusion coefficient of migrant in P (cm^2 s^{-1}), and $K_{P/F}$ is the partition coefficient of the migrant between P and F.

The experimental data obtained in the migration experiments from LDPE into the food simulants were fitted with the proposed model based on eq. 2. With this purpose, the Solver function of the software Microsoft Excel® 2010 was used. The values of diffusion (D_P) and partition coefficients ($K_{P/F}$) were determined for each migrant and food simulant at the different temperatures tested. These data are shown in table 10.2, in addition, the Root-Mean-Square Error (RMSE %). The RMSE showed values lower than 7.0 % in all cases, except for the migration of 4-MBP into 50 % ethanol (v/v) (RMSE < 9.0 %). The good

fitting of the experimental data with the model demonstrates that it can be used to predict the migration process of these migrants from LDPE to foodstuffs.

10.4.2. 4-MBP

This benzophenone derivative has a similar molecular weight than EDB; however, 4-MBP presents a higher log $K_{o/w}$ value, being more lipophilic than the amine synergist. According to this value of log $K_{o/w}$, 4-MBP, it is expected more affinity to lipophilic simulants such as 50 or 95 % ethanol (v/v) than towards hydrophilic food simulants. The $K_{P/F}$ values confirm this fact ranging from 407.0 in 3 % acetic acid (w/v) at 20 °C to values close to 1 in 95 % ethanol (v/v) at 4 °C (Table 7.2).

Food/ Food Simulant	Temperature (°C)	4-MBP			EDB		
		D_P ($cm^2\ s^{-1}$)	$K_{P/F}$ (w/v)	RMSE (%)	D_P ($cm^2\ s^{-1}$)	$K_{P/F}$ (w/v)	RMSE (%)
Water	4	1.0E-10	309.0	3.4	2.6E-10	60.3	3.9
	20	1.5E-10	365.5	3.0	8.9E-10	47.1	2.2
	40	3.2E-10	302.5	2.5	3.0E-09	119.4	3.5
3 % Acetic acid (w/v)	4	1.9E-10	279.7	3.5	4.6E-10	22.6	2.2
	20	2.4E-10	407.0	3.1	2.1E-09	22.2	4.3
	40	4.1E-10	353.3	3.9	6.8E-09	20.0	3.4
10 % Ethanol (v/v)	4	1.3E-10	257.7	6.9	3.2E-10	42.7	3.7
	20	4.9E-10	239.8	3.8	1.3E-09	38.5	5.1
	40	1.7E-09	148.6	3.9	5.1E-09	30.2	4.7
20 % Ethanol (v/v)	4	1.6E-10	148.2	5.5	3.9E-10	18.2	4.4
	20	7.8E-10	82.7	3.7	1.5E-09	23.5	4.5
	40	1.9E-09	78.9	6.3	1.2E-08	13.0	2.5
50 % Ethanol (v/v)	-18	1.5E-12	13.9	4.7	5.5E-13	25.4	2.1
	4	1.3E-09	3.3	4.3	4.7E-10	5.1	5.7
	20	5.5E-09	3.5	4.8	2.1E-09	3.9	6.0
	40	1.1E-08	4.9	8.9	8.5E-09	<2.3*	5.4
95 % Ethanol (v/v)	-18	2.8E-12	20.6	3.6	7.8E-13	37.1	3.4
	4	1.3E-09	<1.5*	3.6	4.0E-10	4.9	4.1
	20	4.5E-09	1.9	3.3	2.8E-09	<1.5*	4.0
	40	3.1E-08	2.6	3.8	6.4E-09	<1.4*	6.3

Table 10.2: Coefficients of diffusion (D_P), partition ($K_{P/F}$) and RMSE of 4-MBP and EDB. * - The method quantification limit (LOQ = 0.025 mg L^{-1}) does not allow the estimation of lower values of $K_{P/F}$.

As expected, higher temperatures lead to higher diffusion coefficients in all food simulants without exception. Even freezing temperatures allowed the

migration of both compounds, as can be observed in figure 10.1, presenting the lowest D_P values (1.5E-12) in 50 % ethanol (v/v). On the opposite, the highest value of D_P (3.1E-8) was obtained at 40 ºC, in 95 % ethanol (v/v).

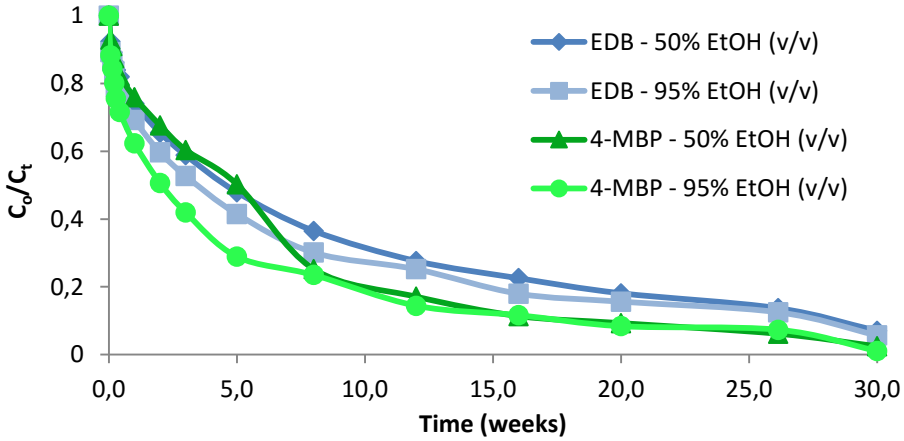


Figure 10.1: Migration curves of 4-MBP and EDB at -18 ºC. Co: Initial concentration; Ct: concentration at time t.

4-MBP is a photoinitiator derivative from the benzophenone (BP). In comparison with BP, the addition of a methyl group increases the molecular weight from 182.22 to 196.24 and the log $K_{o/w}$ from 3.18 to 3.69. These differences between both compounds affect the migration process as can be observed if these data are compared with those obtained for BP (Chapter 12). Regarding to $K_{P/F}$, from table 10.2 it can be inferred that the slightly more lipophilic character of 4-MBP leads to lower values of $K_{P/F}$, as obtained for 95 % ethanol (v/v), showing the tendency of the photoinitiator to migrate to lipophilic mediums rather than to remain in the film. On the contrary, the highest values were obtained in aqueous mediums (148-400), slightly higher than for BP (Chapter 12).

The D_P values of both photoinitiators were similar in 10, 20, 50 and 95 % ethanol (v/v); however, in water and 3 % acetic acid (w/v), 4-MBP showed lower values than BP, particularly at high temperatures (3.2E-10 and 4.1E-10 in 4-MBP and 1.0E-9 and 1.4E-9 in BP). This last fact, could be attributed to both parameters: the lower molecular weight and log $K_{o/w}$ of BP compared to 4-MBP.

Sanches-Silva *et al.* (2009) reported a correlation between the percentage of ethanol and the diffusion coefficient values for BP, HCPK and ITX (as it is reported in chapters 11 and 12). Also, in this work, 4-MBP shows a higher D_P value at higher percentage of ethanol in the food simulant. Figure 10.2 represents the influence of the percentage of ethanol in the D_P values at 4 and 40 ºC, showing a linear relation (R^2 = 0.9742) between both parameters, more evident at higher temperatures (as is the case for BP and HCPK).

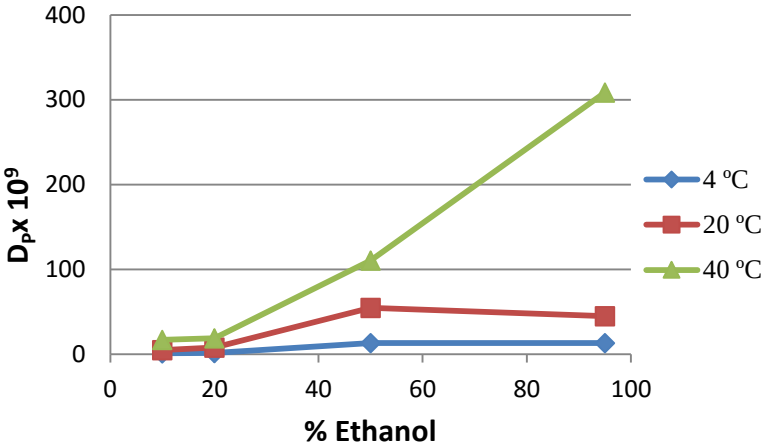


Figure 10.2: Relation between the 4-MBP diffusion coefficient (D_P) and the percentage of ethanol of the food simulant.

10.4.3. EDB

The amine synergist EDB is a tertiary amine with a molecular weight similar to 4-MBP (193.24 in front of 196.24). As for 4-MBP, the values of D_P were highly influenced by the temperature as expected, ranging from 5.5E-13 in 50 % ethanol (v/v) at -18 ºC to 1.2E-8 in 20 % ethanol (v/v) at 40 ºC. The $K_{P/F}$ values showed more affinity for fatty simulants (e.g. $K_{P/F}$ < 1.4 for 95 % ethanol (v/v)) than for the hydrophilic ones ($K_{P/F}$ values in water are over 47.1) as the log $K_{o/w}$ suggests.

Nevertheless, this coinitiator presented a peculiar behavior in 3 % acetic acid (w/v). As can be reflected in table 10.2, $K_{P/F}$ values obtained in the acidic food simulant are similar to those obtained in the more lipophilic food simulants

such as 20 % ethanol (v/v). However, the $K_{P/F}$ values in water are sensible higher, ranging from 47.1 to 119.4. Food simulant 3 % acetic acid (w/v) presents a pH close to 2.53 and, at this pH, approximately 72 % of the migrant is in the protonated form (calculated by MarvinSketch 6.2, ChemAxon® Ltd). This would explain the higher affinity for this food simulant. These $K_{P/F}$ values demonstrate that the pH of the food simulant is a key factor in the EDB migration process.

10.4.4. DIFFUSION COEFFICIENT LINEARITY

Considering that the coefficient of diffusion is dependent on the temperature (Sanches-Silva *et al.*, 2010), the Arrhenius equation (eq. 3) allows determining the relationship between both factors

Eq. 3: $$lnD = -\frac{E_A}{R}\frac{1}{T} + lnD_0$$

where D_0 is the pre-exponential factor ($cm^2 \cdot s^{-1}$), which corresponds with the theoretical values of D at a temperature equal to infinite; E_A is the activation energy (kJ mol^{-1}); R is the ideal gas constant (8.31x10-3 kJ mol^{-1} K^{-1}); and T is the temperature (K).

Arrhenius equation was applied to the diffusion coefficients obtained for EDB and 4-MBP into the different food simulants (Table 10.3). Taking into account the assumption that the coefficient of diffusion is dependent on the temperature, the linearity between ln D_P and 1/T from 4 to 40 ^oC was checked, obtaining acceptable R^2 values ($R^2 > 0.95148$ for 4-MBP and $R^2 > 0.91564$ for EDB) in all the food simulants. This fact allows the calculation of the diffusion coefficients of both molecules in this range of temperatures.

Figure 10.3 presents the application of the obtained D_P to the Arrhenius equation in the range of temperature from -18 to 40 ^oC in 50 and 95 % ethanol (v/v). As discussed above, there is a linear relation between ln D_P and 1/T from 4 to 40 ^oC; however, this linear relation is not observed between 4 and -18 ^oC. This figure shows how the experimental data obtained at -18 ^oC do not fit the linearity calculated from 4 to 40 ^oC, being the experimental D_P values more than one order of magnitude lower than the expected. These experimental results

demonstrate that the migration from LDPE of the migrants continues until freezing temperatures (-18 ºC); however, it is remarkably slower than expected and further studies should be performed in order to explain this behaviour.

Food / Food Simulant	Migrant	E_A (kJ mol^{-1})	D_0 (cm^2 s^{-1})	R^2
Water	4-MBP	22.35	1.63E-06	0.98009
	EDB	48.62	3.89E-01	0.99877
3 % Acetic Acid (w/v)	4-MBP	15.75	1.67E-07	0.95748
	EDB	53.81	6.94E+00	0.98835
10 % Ethanol (v/v)	4-MBP	52.11	8.76E-01	0.99722
	EDB	55.05	8.02E+00	0.99752
20 % Ethanol (v/v)	4-MBP	48.70	2.84E-01	0.96626
	EDB	69.20	3.91E+03	0.99036
50 % Ethanol (v/v)	4-MBP	42.57	1.59E-01	0.95148
	EDB	58.09	4.32E+01	0.99733
95 % Ethanol (v/v)	4-MBP	63.32	1.03E+03	0.99003
	EDB	60.73	1.11E+02	0.91564
Theoretical prediction (Piringer)	4-MBP	86.9	1.86E+07	-
	EDB		1.93E+07	

Table 10.3: Experimental D_0, E_A and R^2 values calculated with eq. 3 for 4-MBP and EDB.

10.4.5. WORST CASE PREDICTION

Finally, a prediction of the "worst case scenario" of the migration of EDB and 4-MBP from LDPE was carried out. For that purpose, the equational approach based on the phenomenological derivations and statistical evaluation of experimental diffusion and migration data developed by Piringer (1994) was used (Brandsch, 2002; Simoneau, 2010):

Eq. 4: $$D^*_P = 10^4 \exp(A_P - 0.1351 {M_r}^{2/3} + 0.003 M_r - \frac{10454}{T})$$

with: $$A_P = A'_P - \frac{\tau}{T} \quad \text{(Eq. 5)}$$

where D^*_P is the polymer specific upper-bound diffusion coefficient in cm^2 s^{-1}, M_r is the relative molecular mass of the migrant in D, A_P is a parameter that describes the behavior of the migrants in the diffusion, A'_P is a polymer related parameter of diffusion, independent of the temperature and τ is a

polymer specific "activation energy" parameter in K. In this case, the values for A'_P and τ are 11.5 and 0 respectively for LDPE.

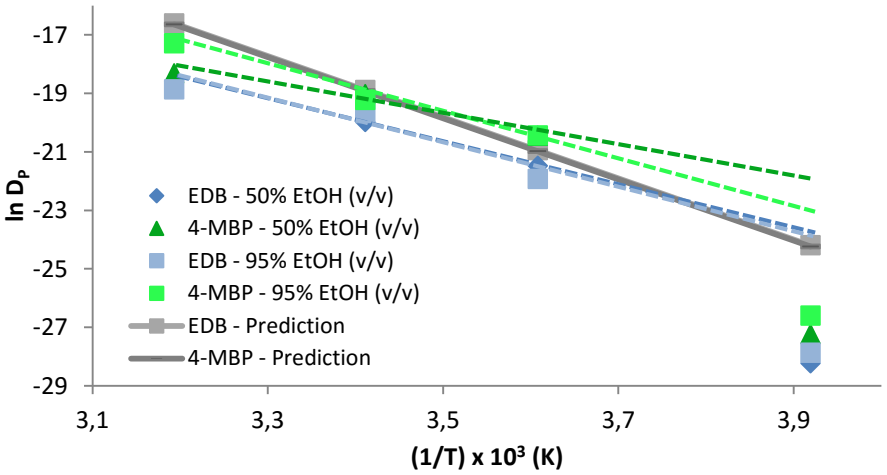


Figure 10.3: Application of the Arrhenius equation to estimate the relation between the obtained D_P for EDB and 4-MBP and the temperature. Dotted lines extrapolate the linearity estimated in the range 4-40 ºC down to -18 ºC.

For a valid estimation of the worst case scenario, the D^*_P values should be higher than the D_P obtained experimentally; however, figure 10.3 shows that $D_P > D^*_P$ for 4-MBP at 4 ºC. Taking into account the linearity of $\ln D_P$, this representation allows the calculation of the temperature at which $D_P > D^*_P$, being 16.9 ºC in 95 % ethanol (v/v), and 11.5 ºC in 50 % ethanol. Despite of these results, further studies should be accomplished in order to confirm that this equation does not overestimate the diffusion coefficients of 4-MBP in 50 % and 95 % ethanol (v/v). Different sources of error could lead to a "non-overestimation" of the diffusion coefficients: the adjustment done by the mathematical model, a non-homogenous distribution of 4-MBP in the LDPE, the polymer thickness variation or possible interactions between the film and the food simulant (Maia *et al.*, 2016).

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11. THIOXANTHONE MIGRATION INTO FOOD SIMULANTS: KINETIC STUDIES

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11.1. ABSTRACT

In the last decade the majority of the notifications reported in the Rapid Alert System for Food and Feed (RASFF) with regard to photoinitiators corresponded to thioxanthones. The objective of the present paper is to study the kinetics migration of two common thioxanthones from the food packaging into food simulants. In addition, the experimental data obtained was fit to a mathematical model, developed on the basis of the analytical solution proposed by Crank for the Fick's second law of diffusion. The good fit between the model and the experimental data allows the prediction of the key parameters of the migration of thioxanthones into foodstuffs: the partition and diffusion coefficients. Finally, the migration kinetics was also studied at -18 ^{o}C, emphasizing the fact that migration from food packaging into foodstuffs continues at freezing temperatures.

Keywords: thioxanthone, food migration, partition coefficient, diffusion coefficient, photoinitiators.

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11.2. INTRODUCTION

Thioxanthones were the first group of photoinitiators whose migration from food packaging was detected and reported by the European food control authorities (RASFF, 2005). Since that alert in 2005, eleven years have already passed; however, photoinitiators are still common food contaminants, a total of 51 notifications and 15 food alerts reported by the European authorities during this time has been assigned to the migration of thioxanthones (RASFF, 2015).

Thioxanthones are one of the two biggest groups of type II photoinitiators (the other are the benzophenones). Type II photoinitiators are the molecules responsible for the absorption of UV light to transfer its energy to the coinitiator to begin the photopolymerization process. Thioxanthones are commonly used alone or with other photoinitiators in food packaging, being extensively employed due to not produce photo-yellowing and produce very-low migration rates (Lago et al., 2015). Nowadays, different thioxanthone approaches have been designed in order to improve their quality, and to diminish their migration rates, by increasing their molecular weight, as can be the case of Speedcure® 7010 or Genopol® TX-2, polymeric photoinitiators with Mw > 1000 Da. Nonetheless, the migration of these polymeric photoinitiators and related compounds is still possible, even increasing their molecular weight, due to the set-off phenomena, which allows the transference from the outer surface to the inner one, during the storage of the food packaging in reels or stacks (Bentayeb *et al.*, 2013, Lago *et al.*, 2016).

To cope with the possible migration of photoinitiators to foodstuffs, many articles have reported different analytical methods developed to detect these food contaminants in foodstuffs and/or food packaging by different techniques (Lago *et al.*, 2015). Nevertheless, other strategy can be very useful to evaluate the migration of thioxanthones to foodstuffs: the prediction of their migration from food packaging. The development of a valid methodology to characterize the migration process could be a valuable tool to avoid carry out expensive and

time-consuming experiments and to determine if the quantity migrated is enough to pose a concern for humans' health. Also, the current European legislation, Commission Regulation (EU) No. 10/2011, on plastic materials and articles intended to come into contact with food, allows the migration prediction of food contaminants only applying generally recognised diffusion models based on scientific evidences that are constructed such as to overestimate real migration (European Commission, 2011). The migration into food and food simulants is a predictable physical event that, in absence of interactions with food and from monolayer materials, obeys to Fick's 2nd law of diffusion (Simoneau, 2010). Most of the mathematical models developed to predict the migration to foodstuffs are based on this theory. To characterize the migration process, two key parameters should be obtained: the diffusion and partition coefficient between the polymer and the foodstuff (D_P and $K_{P/F}$). However, to the best of authors' knowledge, only a few works have studied the photoinitiator migration into food or food simulants, obtaining migration kinetic parameters of only seven photoinitiators, being ITX the unique thioxanthone studied (Dole *et al.*, 2006; Pennarum *et al.*, 2004a, 2004b; Sanches-Silva *et al.*, 2008, 2009; Stoffers *et al.*, 2005; Zülch and Piringer, 2010; Maia *et al.*, 2016).

The aim of this work is to study the migration of the two main thioxanthone photoinitiators used in food packaging: 2/4-Isopropylthioxanthen-2-one (ITX) and 2,4-Diethylthioxanthen-9-one (DETX). For this purpose low density polyethylene (LDPE) films were spiked with each thioxanthone photoinitiator. The migration experiments were carried out at 4 different temperatures from -18 to 40 ^{o}C in the food simulants 50 and 95 % ethanol (v/v). The obtained experimental data were fitted to a mathematical model based on the solutions proposed by Crank (1975) for Fick's 2nd law. Finally, the effect of the temperature on the diffusion of the photoinitiators was study applying Arrhenius equation.

11.3. MATERIALS AND METHODS

11.3.1. REAGENTS AND CHEMICALS

Analytical standards of isopropyl-9H-thioxanthen-9-one (ITX) (mixture of 2 and 4 isomers) 97 % (CAS No. 5495-84-1) and 2, 4-diethyl-9H-thioxanthen-9-one (DETX) 98 % (CAS No. 82799-44-8), were obtained from Sigma-Aldrich (Schnelldorf, Germany). To prepare the different food simulants (50 and 95 % ethanol (v/v)), ethanol (absolute for analysis) was mixed with distilled water. Finally, the mobile phase, used in the HPLC analysis, was composed by acetonitrile (HPLC grade) and ultrapure water obtained with a Milli-Q filter system (Millipore, Bedford, MA). All the reagents were purchased from Merck (Darmstadt, Germany).

11.3.2. MIGRATION TESTS

11.3.2.1. Sources

Low density polyethylene (LDPE), Alcudia® 2008F, supplied by Repsol-YPF (Madrid, Spain), was used as photoinitiator source. The LDPE was extruded in presence of ITX or DETX, for spiking the films. The process was carried out in Gaiker (Zamudio, Spain). The ITX film presented a concentration of 1,160.5 ± 85.2 mg kg^{-1} and the DETX film had a concentration of 1,437.2 ± 26.4 mg kg^{-1}. The thickness of the films ranged between 56 and 68 µm.

11.3.2.2. Food simulants

After carry out solubility test, the migration assays were only performed in the food simulants where ITX and DETX did not have a limited solubility: 50 and 95 % ethanol (v/v). The assays were carried out by duplicate.

11.3.2.3. Procedure

The spiked films were cut in pieces of 10 cm^2 to obtain a total surface of contact with the food simulant of 20 cm^2. Each strip was immersed in 20 ml of food simulant in tubes protected from the light, previously placed at -18, 4, 20

or 40 ºC (in order to replicate the common conditions of food storage and a worst case scenario). At pre-set times, aliquots of 0.5 ml were removed from each tube. The migration studies lasted for 11 months at -18 ºC, 7 days at 4 ºC, 2 days at 20 ºC and 12 hours at 40 ºC. After the last aliquot was obtained, the sample was removed from the tube and the photoinitiator was extracted from the film with acetonitrile for 24 hours at 70 ºC. Each aliquot and the sample extraction were analyzed by HPLC-DAD to determine the migration kinetics with the HPLC-DAD method developed by Lago *et al.* (2014).

11.3.3. MATHEMATICAL MODELLING

The experimental data obtained from the migration tests were fitted, with the Solver function of Microsoft Excel 2010®, with the proposed model based on the analytical solution to the Fick's 2nd law of diffusion proposed by Crank (1975) for a plane sheet (LDPE in this case) into a stirred solution of limited volume (food simulant):

Eq. 1: $$\frac{m_{F,t}}{A} = C_{P,0}\rho_P d_P \left(\frac{\alpha}{1+\alpha}\right) \times \left[1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-D_P t \frac{q_n^2}{d_P^2}\right)\right]$$

With: $$\alpha = \frac{1}{K_{P/F}} \frac{V_F}{V_P}$$ and $$\alpha = \frac{1}{K_{P/F}} \frac{V_F}{V_P}$$

This solution expresses the migrant released from the polymer (P) into food (F) at time *t*, where $m_{F,t}$ is the mass of migrant from P into F after time *t* (µg); *A* is the area of P in contact with F (cm^2); $C_{(P,0)}$, $C_{(P,\infty)}$ and $C_{(F,\infty)}$ are the concentrations of migrant in the P at t = 0 , t = ∞ and the concentration of migrant in the F at t = ∞ (mg kg^{-1}); ρ_P and ρ_F are the densities of the P and the F (g cm^{-3}), *t* is the migration time (s), d_p is the thickness of P (cm), V_P and V_F are the volumes of P and F (cm^3), qn are the positive roots of the transcendent equation, D_P is the diffusion coefficient of migrant in P (cm^2 s^{-1}), and $K_{P/F}$ is the partition coefficient of the migrant between P and F (Crank, 1975).

The values of the partition and diffusion coefficients were obtained ($K_{P/F}$ and D_P). Also, the Root Mean Square Error (RMSE) was calculated to evaluate the fit of the experimental data and the proposed model.

11.4. RESULTS

11.4.1. THIOXANTHONE MIGRATION FROM LDPE INTO FOOD SIMULANTS

There was a good fit between the model and the experimental data with low RMSE values for DETX (RMSE ≤ 5.3 %) and a little higher for ITX (RMSE ≤ 6.3 %) except in 95 % ethanol (v/v) at -18 ºC (RMSE = 9.3 %). This fact indicates that the model can be a suitable tool for the thioxanthone migration estimation from LDPE into these food simulants.

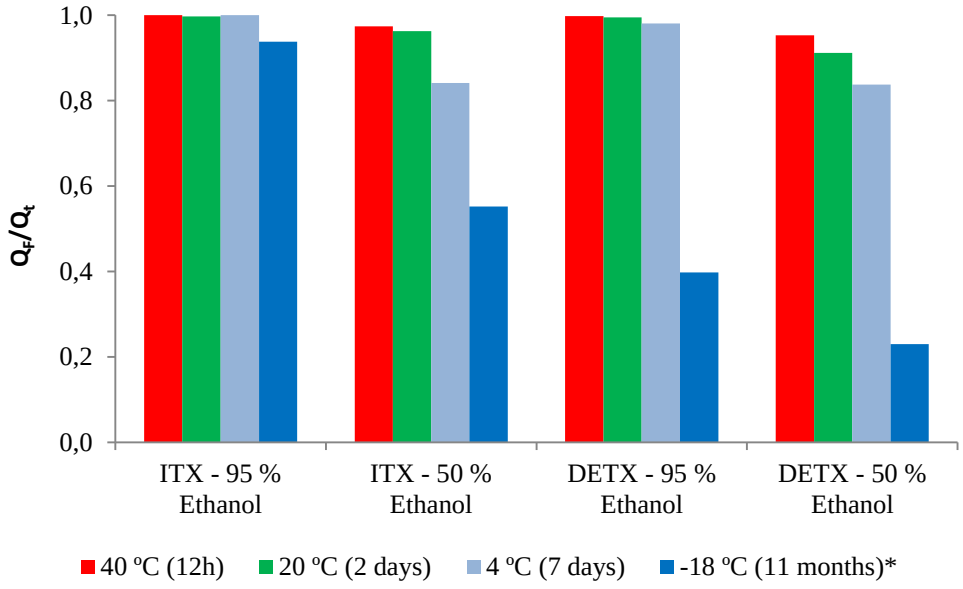

Figure 11.1: Ratio between the thioxanthone in the food when the equilibrium is reached and the initial amount in the LDPE film. * - the migration tests at -18 ºC did not reach the equilibrium after 11 months, so the migrated thioxanthone at this time is represented.

ITX has a slightly lower molecular weight (254.35) than DETX (268.37), showing both a non-hydrophilic character with log $K_{o/w}$ values of 5.11 for ITX and 5.67 for DETX, respectively. These properties could explain the data represented in figure 11.1 that shows the ratio between the thioxanthone migrated from the LDPE into the food simulants and the initial amount in the LDPE film. The results show that ITX and DETX migrate almost completely into

50 and 95 % ethanol (v/v), being enough very short times of storage to achieve migration rates of > 98 % in 95 % ethanol (v/v) and > 84 % in 50 % ethanol (v/v) at 4, 20 and 40 ºC.

However, at -18 ºC, the migration curves of ITX and DETX in both food simulants did not achieve the equilibrium after 11 months of assay. Figure 11.2 shows all the migration kinetics carried out at freezing temperatures for both molecules. As can be seen, after 11 months of assay, ITX presents faster migration and higher rates of migration, probably due to a lower molecular weight; however all the kinetics are slow and the equilibrium is not reached in the time of testing, achieving only migration rates of DETX < 40 % and for ITX 55 % at 50 % ethanol (v/v) and 94 % in 95 % ethanol (v/v).

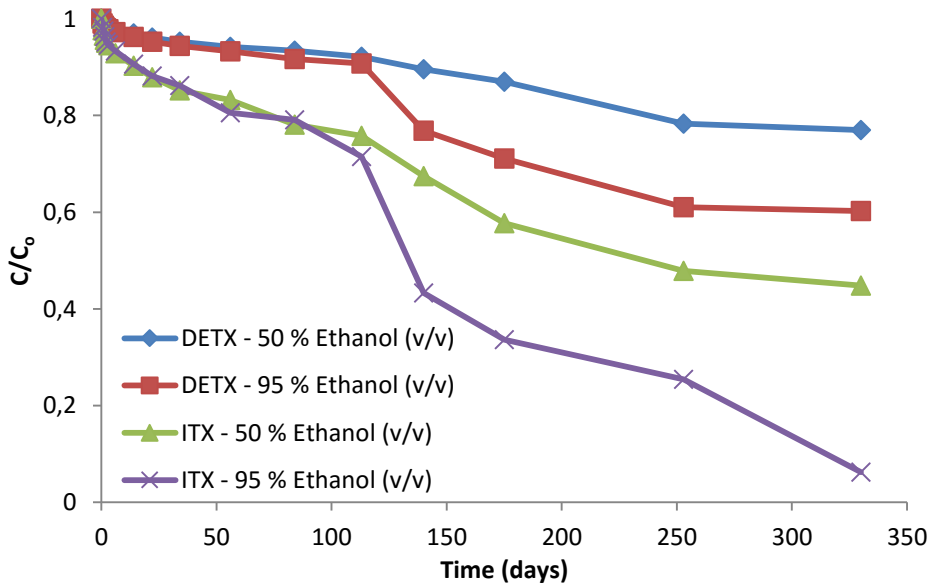

Figure 11.2: Migration kinetic curves of ITX and DETX in 50 and 95 % ethanol (v/v).

11.4.2. PARTITION COEFFICIENTS ($K_{P/F}$)

Calculated $K_{P/F}$ values and the diffusion coefficients are showed in table 11.1. The values obtained at -18 ºC are high due to the fact that the equilibrium was not reached at the end of the study time and, in consequence, the

concentrations in the food simulant are lower than the expected at the equilibrium (on the contrary that the concentration in the polymer).

Food Simulant	Temperature (ºC)	D_P (cm^2 s^{-1})		$K_{P/F}$ (w/v)		RMSE (%)	
		ITX	DETX	ITX	DETX	ITX	DETX
50 % Ethanol (v/v)	-18	1.6E-12	2.5E-13	197.3	877.8	2.8	2.0
	4	1.3E-10	4.6E-11	66.4	61.7	3.5	3.3
	20	9.7E-10	4.4E-10	12.7	29.7	6.3	2.7
	40	5.3E-09	2.0E-09	8.7	14.8	5.3	5.3
95 % Ethanol (v/v)	-18	3.6E-12	8.1E-13	18.2	359.8	9.6	4.7
	4	1.6E-10	5.5E-11	<1.0*	5.9	2.7	2.7
	20	1.5E-09	7.0E-10	<1.6*	1.5	3.1	2.9
	40	6.1E-09	4.0E-09	<0.9*	0.7	4.3	1.9

Table 11.1: D_P, $K_{P/F}$ and RMSE values obtained for the migration of both thioxanthones into 50 and 95 % ethanol (v/v). * - The limit of quantification of ITX (LOQ = 0.01mg L^{-1}) did not allow achieve lower accurate $K_{P/F}$ results.

The $K_{P/F}$ values indicate that the thioxanthones have lipophilic tendency, obtaining higher $K_{P/F}$ values in 50 % ethanol (v/v) than in 95 % ethanol (v/v). $K_{P/F}$ values are favorable to the polymer in 50 % ethanol (v/v) ($K_{P/F} \geq 8.7$ in ITX and $K_{P/F} \geq 14.8$ for DETX); however, both thioxanthones have presented more affinity for 95 % ethanol (v/v), obtaining values even favorable to the food simulant ($K_{P/F} < 1$).

In a study conducted by Sanches-Silva *et al.* (2009), the migration kinetics of ITX into the food simulant 95% ethanol (v/v) was evaluated at 5, 25 and 40 ºC; the values of $K_{P/F}$ reported at the temperatures studied (1,481; 1,932 and 1,389) were noticeably higher than those obtained in this work. Different reasons can explain the divergence of results; it could be due to the different process of incorporation of additives, which can lead to a heterogeneous distribution of the migrant in the film, the different source, differences in the thickness of the polymer or even possible interaction between the polymer and the food simulant.

11.4.3. DIFFUSION COEFFICIENT (D_P)

The values represented in table 11.1 show that the food simulant does not have influence in this parameter, due to do not exist significant differences between the values obtained at the same temperature in both food simulants for ITX and DETX. However, unlike $K_{P/F}$, D_P depends on the temperature, ranging the values for ITX from 1.6E-12 at -18 ºC in 50 % ethanol (v/v) to 6.1E-9 in 95 % ethanol (v/v) and from 2.5E-13 in 50 % ethanol (v/v) to 4.0E-9 in 95 % ethanol (v/v) for DETX.

Sanches-Silva *et al.* (2009) have also calculated the D_P values for ITX in 95 % ethanol (v/v) from LDPE. Unlike for $K_{P/F}$, the obtained D_P values in this work are clearly in agreement with those obtained by these authors who reported D_P values of 2.6E-10, 1.3E-9 and 3.0E-9 at 5, 25 and 40 ºC.

11.4.4. LINEARITY BETWEEN THE DIFFUSION COEFFICIENT AND THE TEMPERATURE

The Arrhenius equation is a useful tool to study the relation between the diffusion coefficient and the temperature (eq. 2) (Sanches-Silva *et al.*, 2010):

Eq. 2: $$D = D_0 e^{-E_A/RT}$$

Where D_0 ($cm^2\ s^{-1}$) is the pre-exponential factor that corresponds to D at T = ∞; E_A (KJ mol^{-1}) is the activation energy; R (8.31 x 10-3 KJ $mol^{-1}\ K^{-1}$) is the ideal gas constant; and T is temperature (K).

Figure 11.3 represents the logarithm form of the Arrhenius equation. A linear relation was obtained between both parameters in the range of temperatures between 4 and 40 ºC with good R^2 values ($R^2 \geq 0.9760$). However, this linear relation is not maintained between 4 and -18 ºC, as can be observed in this figure, with a gap between the dotted line and the ln D_P obtained in this study at -18 ºC. The results showed that the migration of ITX and DETX is slower than expected at freezing temperatures; nevertheless, the deviation is not big and further studies should be carried out in order to confirm the obtained results and explain this behavior.

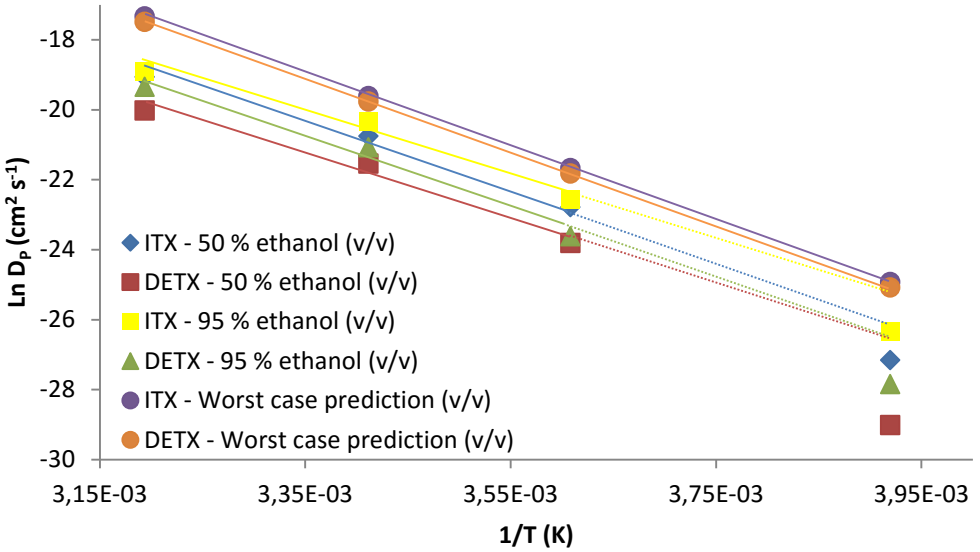

Figure 11.3: Representation of the relation between Ln D_P and 1/T for ITX and DETX in both simulants and the worst case scenario prediction obtained with equation 3. The dotted lines extend until -18 ºC the linearity between ln D_P and 1/T from 4 to 40 ºC.

11.4.5. WORST CASE PREDICTION

In order to save costs, avoiding carry out the migration experiments, different approaches have been developed for the theoretical estimation of an overestimation of D_P. The most commonly used it is the equation 3 (Brandsch *et al.*, 2002), that correlates the diffusion coefficient in the polymer (D_P) with the migrant, the polymer and the temperature without any further experimental data needed (Simoneau, 2010):

Eq. 3: $$D^*_P = 10^4 \exp(A_P - 0.1351 M_r^{2/3} + 0.003 M_r - \frac{10454}{T})$$

with: $$A_P = A'_P - \frac{\tau}{T}$$ (Eq. 4)

where D^*_P is the polymer specific upper-bound diffusion coefficient in cm^2 s^{-1}, M_r is the relative molecular mass of the migrant in D, A_P is a parameter that describes the behavior of the migrants in the diffusion, A'_P is a parameter of the diffusion related with the polymer and independent of the temperature

and τ is a polymer specific "activation energy" parameter in K. For LDPE the values for A'_P and τ are 11.5 and 0 respectively.

The values obtained, from the application of equation 3 to the system studied in this work, for D_0 and the E_A are listed on table 11.2. Also, their graphical representation was included into figure 11.3 where it can be observed that the prediction successfully overestimates the diffusion coefficients obtained experimentally.

Food/Food Simulant	Migrant	D_0 ($cm^2\ s^{-1}$)	E_A ($kJ\ mol^{-1}$)	R^2
50% Ethanol (v/v)	ITX	1.5E+04	74.4	0.9931
	DETX	1.0E+04	75.7	0.9797
95% Ethanol (v/v)	ITX	1.0E+04	72.8	0.9760
	DETX	7.8E+05	85.2	0.9810
Theoretical worse case prediction	ITX	9.3E+06	86.9	-
	DETX	8.0E+06		

Table 11.2: D_o, E_A and R^2 values obtained for ITX and DETX and the theoretical worst case prediction obtained with equation 2.

11.5. CONCLUSIONS

In the present work, the data obtained from the migration of two photoinitiator thioxanthones from LDPE into food simulants has been fitted to a mathematical model based on Fick's 2nd law of diffusion. The good fit between the predicted and experimental data shows this model as a useful tool to predict the migration of these food contaminants into foodstuffs at the common storage temperatures. These results allow to achieve a deeper knowledge about the thioxanthone migration phenomena into food simulants, in order to improve the already developed migration models, trying to avoid the experimental studies needed to check the compliance of the food packages with the current legislations.

In addition, this work provides experimental data of migration at freezing temperatures of these two common food contaminants. The data obtained has confirmed that, as expected, the migration continues at temperatures under 0 ^oC, and non-negligible quantities of thioxanthone continue reaching the food

simulants. Finally, after check the linearity between the diffusion coefficient and the temperature, this relation between these parameters detoured at freezing temperatures and this behavior should be studied deeper in the future.

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12. STUDY OF THE MIGRATION OF TWO PHOTOINITIATORS FROM LOW DENSITY POLYETHYLENE INTO FOOD SIMULANTS

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12.1. ABSTRACT

The capability of photoinitiators to reach the foodstuffs from the external layer of food packages has been demonstrated by many studies in the last decade. However, these migration studies have been carried out at temperatures above 4 ^{o}C, and there is no data in one of the most common temperatures of food storage: freezing temperature (-18 ^{o}C). In order to cover the whole range of food storage temperatures, in the present paper, the migration phenomena of two of the most common photoinitiators have been studied into water and five different food simulants, in a range of temperatures between -18 and 40 ^{o}C. The migration results showed that the process strongly depends on storage conditions and type of food, e.g.: benzophenone reaches values of D_P = 2.8E-08 at 40 ^{o}C in 95 % ethanol (v/v), being the migration slower at 4 ^{o}C in aqueous simulants: D_P = 9.3E-11 and even slower at freezing temperatures. Also, the linearity between the diffusion coefficient D_P and the temperature was checked, fitting to this linear model only between 4 and 40 ^{o}C.

Keywords: migration, photoinitiators, diffusion coefficient, partition coefficient, mathematical modelling.

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12.2. INTRODUCTION

Food packaging plays an important role to prevent food contamination and, consequently, to protect the consumers' health. For that purpose, many researchers have studied the interactions between packages and foodstuffs to evaluate their risks. The possibility of a mass transfer from package to food under normal or foreseeable conditions of use always exists, and then two different problems could occur: a) the food quality could be modified and, b) its ingestion could be dangerous. The packaging materials must not transfer their constituents to food that may affect product quality or represent a risk to consumers' health (EC, 2004).

In order to protect consumers' health a European legal framework already exists. The European Regulation 1935/2004/EC establishes the principles on the materials and articles intended to come into contact with food (EC, 2004). Based on this regulation, the Good Manufacturing Regulation 2023/2006/EC and the specific legislation for plastic materials and articles intended to come into contact with foodstuffs, Regulation 10/2011/EC complete the European framework (EC, 2006, 2011). To check compliance of the Food Contact Materials (FCM) with the European regulations, there are two options: a) carry out an analysis to perform the migration of substances from these FCM to foodstuffs, or b) calculating or modelling the migrations based on scientific evidences (EC, 2011).

The photoinitiators take part in UV curing inks formulations, which could remain in the package after the curing process, and then reach the foodstuff by direct contact, by permeation or *set-off*, or by indirect contact, through the vapor phase (Johns *et al.*, 2000; Anderson & Castle, 2003; Rothenbacher *et al.*, 2007; Rodríguez-Bernaldo de Quirós *et al.*, 2009; Sanches-Silva *et al.*, 2009; Pinalli *et al.*, 2011; Bentayeb *et al.*, 2013; Lago *et al*., 2015). The photoinitiators must be in compliance with the general framework for FCM; however, there is not a European specific legislation for them (Lago *et al.*, 2015).

In 2005, the Italian Authorities informed to the Rapid Alert System for Food and Feed (RASFF) of baby milk contaminated with the photoinitiator 2-ITX (RASFF, 2005). Since that day, many works related to photoinitiators and UV inks have been published, nevertheless most of them are studies concerned to analytical methods for their determination in food packaging and foodstuffs (Castle *et al.*, 1997; Papilloud *et al.*, 2002a, 2002b; Anderson & Castle, 2003; Morlock *et al.*, 2006; Sagratini *et al.*, 2006, 2008; Bagnati *et al.*, 2007; Gil-Vergara *et al.*, 2007; Rothenbacher *et al.*, 2007; Sun *et al.*, 2007; Allegrone *et al.*, 2008; Benetti *et al.*, 2008; Gallart-Ayala *et al.*, 2008, 2011; Sanches-Silva *et al.*, 2008a, 2008b, 2009; Koivikko *et al.*, 2009; Pastorelli *et al.*, 2009; Rodríguez-Bernaldo de Quirós *et al.*, 2009; Shen *et al.*, 2009; Wang *et al.*, 2009; Jung *et al.*, 2010; Negreira *et al.* 2010; Van Hoeck et al, 2010; Ranganathan *et al.*, 2011; Huang *et al.*, 2012; Lago *et al.*, 2014) and there are not many experimental data that allow characterize the migration process of photoinitiators to foodstuffs. More than a hundred photoinitiators, or mixtures of them, are listed in the EuPIA "Suitability list of photoinitiators for low migration UV printing inks and varnishes" (2013), and to the best of authors' knowledge, there are only available migration data of seven [Benzophenone, Chimassorb® 81, ITX, EHA, HCPK, DMPA and MMMP] in a few works (Pennarum *et al.,* 2004a, 2004b; Stoffers *et al.*, 2005; Dole *et al.*, 2006; Sanches-Silva *et al.*, 2008c; Zülch & Piringer, 2010; Alves-Feiteira-Maia, 2013).

The aim of this study is the characterization of the photoinitiators' migration process from packages to foodstuffs, in order to obtain a better understanding of this mass transfer process. With this in mind, two photoinitiators commonly used in FCM were selected: benzophenone (BP) and 1-Hydroxycyclohexyl-phenyl-ketone (HCPK). The assays were carried out in water and the simulants included in Commission Regulation (EU) No 10/2011: 3 % acetic acid (w/v), 10 %, 20 % and 50 % ethanol (v/v). Instead of vegetable oil, 95 % ethanol (v/v) was used as a substitute.

12.3. METHODS

12.3.1. REAGENTS AND STANDARDS

To prepare the different food simulants, ethanol (absolute for analysis) and glacial acetic acid (anhydrous for analysis) were diluted in distilled water in different percentages. The acetonitrile used was HPLC grade and ultrapure water was prepared with a Milli-Q filter system (Millipore, Bedford, MA). All the reagents were purchased from Merck (Darmstadt, Germany).

The photoinitiators selected were: HCPK [CAS Registry No. 947-19-3; 1-Hydroxycyclohexyl-phenyl-ketone; 99 %] and benzophenone (BP) [CAS Registry No. 119-61-9; Methanone diphenyl; 99 %]; supplied by Sigma-Aldrich (Steinheim, Germany). The main properties of these photoinitiators are summarized in table 12.1.

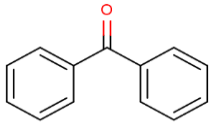
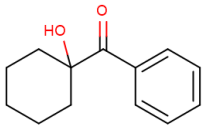
Structure	CAS nr.	Common name	Mw	Log $K_{o/w}$	Mp (ºC)	Bp (ºC)	PI type
	119-61-9	BP	182.22	3.18[a]	47.80[a]	305.40[a]	II
	947-19-3	HCPK	204.26	2.18[b]	48.00[a]	339.00[b]	I

Table 12.1: Photoinitiators selected for the study. Mw: Molecular weight; Mp: Melting point; Bp: Boiling point; PI: Photoinitiator. a: experimental; b: estimated. Data obtained from Sci-Finder® database in 2015.

12.3.2. HPLC METHOD

An Agilent 1200 HPLC system (Agilent Technologies, Waldbronn, Germany) comprising a quaternary pump, a degassing device, an autosampler, a column thermostatting system, and a diode array UV detector was used. The detector was continuously performing a scan in the range of 190 - 400 nm. The bandwidth used was 4 nm. Chromatographic separation was achieved with a

Kromasil C18 column (250 × 3.2 mm internal diameter, 5 µm of particle size) (Phenomenex, Torrance, CA, USA), thermostatted at 30 ^{o}C.

The chromatographic method has been the same used by Lago *et al.* (2014) with a slight modification in the mobile phase gradient. In order to shorten the analysis time, after the analyzed photoinitiator had eluted, the percentage of acetonitrile was raised until 100 % in 1 min and the mobile phase was held for 2 min. The photoinitiators were identified by comparison of their retention times and UV spectra with those of a pure standard injected using the same HPLC conditions.

12.3.3. INCORPORATION OF THE PHOTOINITIATORS INTO THE FILM

The low density polyethylene (LDPE) used was Alcudia® 2008F supplied by Repsol-YPF® (Madrid, Spain). The films were prepared by extrusion by Gaiker (Zamudio, Spain) after mixing the LDPE with BP or HCPK. The obtained films achieve a photoinitiator concentration of 1,058.5 ± 176.7 mg BP kg^{-1} film and 382.9 ± 77.6 mg HCPK kg^{-1} film. The thickness of the films ranged between 45 and 65 µm.

12.3.4. MIGRATION TEST

To perform the migration test, the films were cut into pieces of 6 x 1.66 cm, to achieve a total surface of 20 cm^2 in contact with the food simulant. These films were placed in contact with 20 ml of food simulant in tubes protected from the light.

The food simulants 3 % acetic acid (w/v), 10 %, 20 % and 50 % ethanol (v/v) were selected from the UE Regulation No. 10/2011. Instead of vegetable oil, 95 % ethanol (v/v) was chosen as substitute based on scientific evidences. The migrant assays were also carried out in water, in order to compare the differences between the current simulant for hydrophilic foods (10 % ethanol) with the food simulant of the previous legislation (water) (CEC, 1985). Here, water and 95 % ethanol (v/v) will be named as food simulants, although in the current legislation they are not considered as such.

To construct the migration curves, 20 different samples were placed at each temperature: -18 ºC (freezing temperature) (only in 50 % and 95 % ethanol (v/v), due to the other simulants are solid at this temperature), 4 ºC (refrigeration temperature), 20 ºC (ambient temperature) and 40 ºC (worst case scenario for migration test of product storage at room temperature). The total time of analysis was different for each temperature (8 hours for 40 ºC, 2 days for 20 ºC, 4-7 days for 4 ºC and 15-35 weeks for -18 ºC). For each time point of the migration kinetics two samples were removed and analyzed as follows.

12.3.5. ANALYSIS BY HPLC-DAD

To determinate the migration from the film to the food simulants, at the end of the storage time, an aliquot of each sample was taken from the food simulant volume, filtered, and then injected by HPLC-DAD. To quantify the total amount of photoinitiator in the film before the migration analysis, various films have been completely extracted in an oven at 70 ºC for 24 hours with 20 ml of acetonitrile.

12.4. RESULTS AND DISCUSSION

The mass transfer is a predictable physical event. In the case of the mass transfer from plastic materials into foodstuffs, this process obeys to Fick's Second Law (Crank, 1975; Piringer, 1994; Brandcsh *et al.*, 2002; Sanches-Silva *et al.*, 2008c). To characterize the migration from the Polymer (P) to the Food (F), two capital parameters should be determined: the partition coefficient ($K_{P/F}$) of the migrant between the polymer and the food, and the diffusion coefficient (D_P) of the migrant in the P (Sanches-Silva *et al.*, 2008d; Simoneau, 2010).

For the determination of these parameters various assumptions should be considered: a) the migrant is homogenously distributed in the polymer, and when the migration begins, in the food; b) the process of migration does not have any boundary resistance for the mass transfer from the surface of P to F, and c) decomposition or evaporation of the migrant does not exist, and

consequently the total amount of the migrant in the system P-F remains constant (Crank, 1975; Begley *et al.*, 2005).

Based on this mathematical model, experimental data were exported to Solver function of Microsoft Excel® 2010 software. The values of parameters D_P, $K_{P/F}$ and RMSE (%) for each photoinitiator are shown in tables 12.2 for BP and 12.3 for HCPK.

12.4.1. DIFFUSION COEFFICIENTS

The obtained results show that the migration process is faster at higher temperatures in all the performed assays without any exception. This fact confirms that the coefficient of diffusion (D_P) depends on the temperature. The D_P values are higher for HCPK in water, 3 % acetic acid (w/v) and 10 and 20 % ethanol (v/v) than in the case of BP; however, in 50 and 95 % ethanol (v/v) is on the contrary. These results are due to HCPK presents higher affinity for the hydrophilic food simulants (Log $K_{o/w}$ = 2.18) than BP (Log $K_{o/w}$ = 3.18).

The D_P values obtained at -18 ^oC are sensible lower than those obtained at refrigeration temperature; however, the migration of the two photoinitiators is not negligible at temperatures under 0 ^oC (Figure 12.1). The D_P values ranged between 1.4E-12 and 2.0E-12 in the case of BP and 1.3E-13 and 1.7E-13 for HCPK; values in agreement with the diffusion coefficients of plasticizers from PVC in different meats in the unique work (as far as the authors know) that has carry out the migration experiments at this temperature (Lau & Wong, 1997). Despite these low D_P values, 15 weeks (BP) or 35 weeks (HCPK) are enough to achieve a photoinitiator migration rate > 90 % into the food simulant. This fact should be carefully considered in order to protect the consumers' health. There are different foodstuffs that can be stored in the freezer up to 9-12 months, and as can be inferred from the data obtained, this time is more than enough for the almost complete migration of the two photoinitiators.

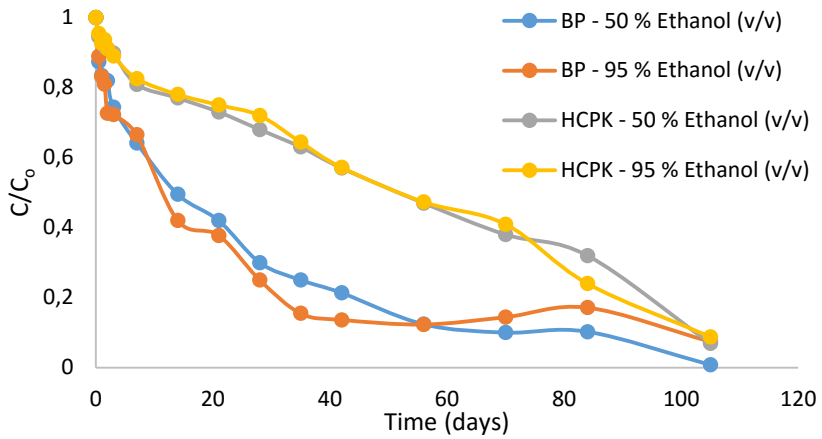


Figure 12.1: Migration kinetic curves of BP and HCPK in 50 and 95 % ethanol (v/v) at -18 ºC.

Food / Food Simulant	Temperature (ºC)	BP		HCPK	
		D_P (cm^2 s^{-1})	RMSE (%)	D_P (cm^2 s^{-1})	RMSE (%)
Water	4	3.5E-10	3.7	2.3E-10	8.3
	20	4.7E-10	2.7	1.5E-09	2.7
	40	1.0E-09	2.3	8.8E-09	8.0
3 % Acetic Acid (w/v)	4	5.3E-10	3.4	2.0E-10	6.4
	20	9.0E-10	3.5	1.1E-09	6.0
	40	1.4E-09	2.5	6.7E-09	5.3
10 % Ethanol (v/v)	4	9.3E-11	6.9	2.0E-10	4.8
	20	3.5E-10	3.0	1.1E-09	3.9
	40	5.8E-10	4.4	6.1E-09	5.5
20 % Ethanol (v/v)	4	3.5E-10	4.1	4.4E-10	2.7
	20	9.5E-10	3.9	9.3E-10	3.2
	40	3.8E-09	2.3	6.5E-09	7.5
50 % Ethanol (v/v)	-18	1.4E-12	6.8	1.2E-13	11.9
	4	9.8E-10	5.2	2.9E-10	3.6
	20	2.3E-09	2.9	1.2E-09	2.6
	40	1.4E-08	3.0	7.2E-09	6.4
95 % Ethanol (v/v)	-18	2.0E-12	4.8	1.7E-13	6.9
	4	1.1E-09	3.5	4.1E-10	6.2
	20	4.4E-09	4.9	1.3E-09	2.2
	40	2.8E-08	2.8	1.1E-08	6.3

Table 12.2: Diffusion coefficients (D_P) and RMSE obtained for BP and HCPK.

There are few articles that perform the migration kinetics of BP or HCPK (Pennarum *et al.*, 2004a, 2004b; Dole *et al.*, 2006; Sanches-Silva *et al.*, 2008c, 2009; Zülch *et al.*, 2010; Alves-Feiteira-Maia, 2013), but most of them carry out

the analysis from other FCM (food board, PET, PET/PS or HDPE), only Sanches-Silva *et al.* (2008c, 2009) and Alves-Feiteira-Maia *et al.* (2013) have performed their studies with LDPE as photoinitiator source.

Sanches-Silva *et al.* (2008c) performed the migration kinetics of BP and HCPK from LDPE to powdered milk. Also, the migration kinetics of HCPK have been performed in water, 3 % acetic acid (w/v) and 10, 20, 30, 60 and 95 % ethanol (v/v) and of BP in the last three (Sanches-Silva *et al.*, 2009). In general, the obtained D_P values are similar to those obtained in this work, being the effect of the temperature smaller in both photoinitiators in all the food simulants. As an example, BP, at 5 ^oC in 95% ethanol (v/v), has a D_P = 3.0E-9, being in the present work at 4 ^oC: 1.1E-9; however, the value obtained at 40 ^oC is lower (5.6E-9) than the obtained value in the mentioned study: 2.8E-8. The differences between the values could be due to the photoinitiator source used, although in both cases LDPE was the polymer used, the manufacture process was different. Moreover, experimental and mathematical differences in the model used have contributed to these small differences observed in the D_P values between both works.

Sanches-Silva *et al.* (2009) reported that the diffusion coefficients are smaller in 60 than in 95 % ethanol (v/v), being lower in food simulants with lower % of ethanol (for example, BP values are 6.2E-10, 7.0E-10 and 7.1E-10 at 5, 25 and 40 ^oC in 60% ethanol (v/v), and 3.0E-10, 3.3E-9 and 5.0E-9 in 95% ethanol (v/v)), following the same pattern observed in the present study for both photoinitiators: lower percentage of ethanol leads to lower D_P values. Assuming that "*the interaction between P and F (food simulant) is negligible and no swelling of P by uptake of F occurs during the migration process*"(Begley *et al.*, 2005), the diffusion coefficient should not vary with different food simulants; nevertheless, in both works different D_P were obtained.

Figure 12.2 represents the relation between the obtained diffusion coefficients and the percentage of ethanol of the food simulants for BP. As can be observed, it seems to be a linear relation between these two parameters, being the best linear fit at higher temperatures: $R^2 > 0.998$ ($R^2 > 0.974$ for HCPK).

This fact has been already reported by Sanches-Silva *et al.* (2008c) for another photoinitiator: ITX, being attributed to a possible swelling of the polymer; however, this fact needs further studies to be confirmed.

Alves-Feiteira-Maia (2013) performed the BP migration studies in 8 different foods: turkey ham, cooked ham, red wine, tomato sauce, orange juice, pate, Gouda cheese and chocolate spread. The diffusion coefficients values obtained were similar to those obtained in this work for the food simulants assigned by the European legislation for each one of these foodstuff analyzed in the cited work. In the case of wine and tomato sauce, the D_P values are higher than those obtained for their assigned food simulant (20 % ethanol (v/v)); also in the case of turkey and cooked ham with its corresponding food simulant (10 % ethanol (v/v)). However, the differences between the diffusion coefficients of the foodstuffs and the food simulants are small.

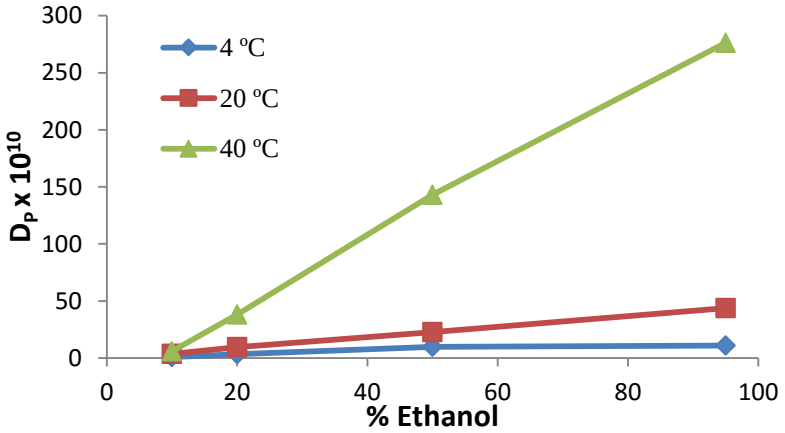


Figure 12.2: Diffusion coefficients of benzophenone vs % of ethanol of food simulants.

12.4.2. PARTITION COEFFICIENTS

The coefficients of partition of BP and HCPK obtained are always favorable to the polymer, obtaining $K_{P/F}$ values in a range from 5.5 to 168.5 for BP and from ≤ 4.3 to 56.4 for HCPK. In agreement with the log $K_{o/w}$ data, the values of $K_{P/F}$ were higher in hydrophilic food simulants for HCPK, at all the temperatures,

than for BP. On the contrary, HCPK has slightly lower $K_{P/F}$ in 95 % ethanol (v/v) than BP (Table 12.3).

The partition coefficients obtained for BP by Alves Feiteira Maia (2013) were lower than those obtained in this work, especially for foods with high fat content as Gouda cheese or chocolate spread, which have values favorable to the foodstuff (0.6 and 0.7 at 20 ºC and 0.8 and 0.4 at 40 ºC). These differences between the values in the two works, and the slight differences between the values at different temperatures, could be explained by different facts. It could be related to errors associated to the experimental method: the non-uniform distribution of the photoinitiator in the polymer matrix, the thickness variation of the polymer; or related to the differences between the experimental data and the mathematical model used (Alves Feiteira Maia, 2013). But in this work, the main reason for these variations in the values of $K_{P/F}$ could be the ratio between the weight of the polymer and the food simulant weight; little changes in the migration data can lead to significant differences in the final values of $K_{P/F}$.

Food/ Food Simulant	$K_{P/F}$							
	BP				HCPK			
	-18 ºC	4 ºC	20 ºC	40 ºC	-18 ºC	4 ºC	20 ºC	40 ºC
H_2O	-	102.2	62.8	113.4	-	≤5.4*	≤4.3*	≤12.9*
3 % Acetic Acid (w/v)	-	76.6	91.5	131.2	-	33.5	18.3	18.9
10 % Ethanol (v/v)	-	134.1	168.5	73.4	-	44.3	15.1	16.1
20 % Ethanol (v/v)	-	95.4	90.1	90.1	-	49.9	≤5.5*	≤8.2*
50 % Ethanol (v/v)	40.0	51.2	60.5	51.2	26.2	≤6.4*	≤7.1*	≤9.3*
95 % Ethanol (v/v)	48.0	29.0	33.5	5.5	48.0	23.0	56.4	21.3

Table 12.3: Partition coefficients ($K_{P/F}$) of BP and HCPK. * - The limit of quantification (LOQ) of the HPLC-DAD method used (LOQ = 0.025mg L^{-1}) does not allow to achieve values of $K_{P/F}$ under this value.

After review all the $K_{P/F}$ data, it is remarkably that the values obtained in the current simulant for hydrophilic foods (10 % ethanol) are slightly different than those obtained for the old food simulant (water) for both photoinitiators (CEC, 1985). These differences are small and could be caused by the experimental errors previously discussed; however, it is possible that not always

10 % ethanol (v/v) is the best food simulant for hydrophilic foods, as observed in the experimental data obtained for water. Nevertheless, further studies should be performed to support the data obtained in this work.

12.4.3. LINEARITY BETWEEN D_P AND THE TEMPERATURE

Under the assumption that D_P is dependent on the temperature (Simoneau, 2010) the linearity between these parameters can be checked based on Arrhenius equation (eq. 1):

Eq. 1: $$lnD = -\frac{E_A}{R}\frac{1}{T} + lnD_0$$

where D_0 is the pre-exponential factor ($cm^2\ s^{-1}$), which corresponds with the theoretical values of D at a temperature equal to infinite; E_A is the activation energy ($kJ\ mol^{-1}$); R is the ideal gas constant ($8.31x10^{-3}\ kJ\ mol^{-1}\ K^{-1}$); and T is the temperature (K).

The results obtained between 4 and 40 ^oC are shown in table 12.4:

Food / Food Simulant	Migrant	D_0 ($cm^2\ s^{-1}$)	E_A ($kJ\ mol^{-1}$)	R^2
Water	BP	3.24E-06	21.2	0.9482
	HCPK	1.17E+04	72.6	0.9983
3% Acetic Acid (w/v)	BP	2.69E-06	19.6	0.9939
	HCPK	3.27E+03	70.0	0.9996
10% Ethanol (v/v)	BP	7.21E-04	36.2	0.9219
	HCPK	1.96E+03	68.9	0.9998
20% Ethanol (v/v)	BP	3.95E-01	48.2	0.9965
	HCPK	6.14E+00	54.3	0.9526
50% Ethanol (v/v)	BP	1.31E+01	54.1	0.9678
	HCPK	3.79E+02	64.4	0.9981
95% Ethanol (v/v)	BP	1.65E+03	64.7	0.9972
	HCPK	1.42E+03	66.9	0.9789
Theoretical prediction (Piringer)	BP	2.22E+07	86.9	-
	HCPK	1.68E+07		

Table 12.4: Experimental D_0, E_A and R^2 values calculated with equations 1 and 2 between 4 and 40 ^oC.

Also, figure 12.3 shows the relation between ln D_P and 1/T for HCPK and BP in 50 and 95 % ethanol (v/v). The experimental results showed that the linear fit between ln D_P and 1/T is not good if the four temperatures tested are

considered. The D_P values obtained for both molecules at -18 ºC are more than one order of magnitude lower than required to achieve a good fit, in the whole range of temperatures tested. Nevertheless, the linearity disappears if the values obtained at -18 ºC are included. However, if it is only considered the data between 4 and 40 ºC, a good linear fit is obtained, with coefficients of correlation higher than 0.9526, except for BP that had a slightly lower value in two food simulants (R^2 = 0.9219 in 10 % ethanol (v/v) and R^2 = 0.9482 in water). These good linear fitting, allow the calculation of the parameter D_P within this range of temperatures.

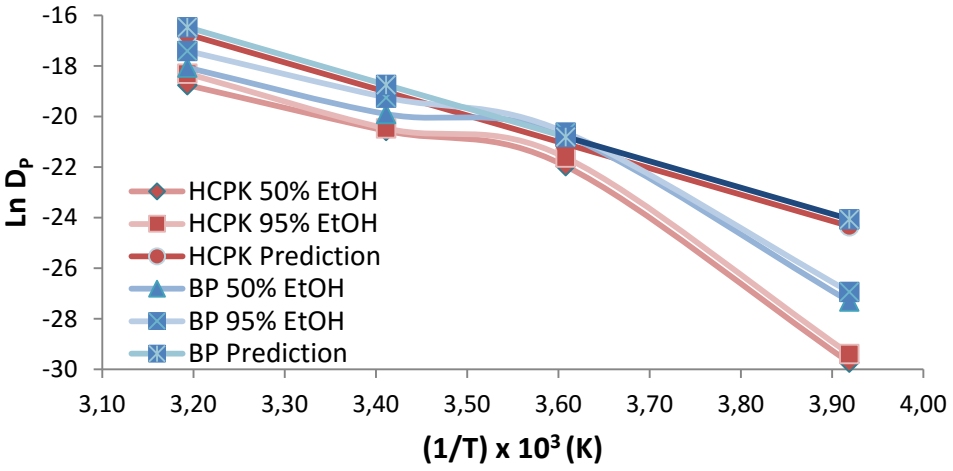


Figure 12.3: BP and HCPK diffusion coefficients linearity.

As has already been mentioned above, for the determination of migration rates, this process can be calculated by modelling. One of these models, proposed by Piringer (1994) (Eq. 2), allows the determination of the diffusion coefficients, based on the empirical relation between the molecular weight of the migrant, the temperature and the polymer used (Piringer, 1994; Brandsch *et al.*, 2000; Begley *et al.*, 2005):

Eq. 2: $$D^*_P = 10^4 \exp(A_P - 0.1351 M_r^{2/3} + 0.003 M_r - \frac{10454}{T})$$

with: $A_P = A'_P - \frac{\tau}{T}$ (Eq. 3)

where D^*_P is the polymer specific upper-bound diffusion coefficient, M_r is the molecular weight of the migrant, A_P is a parameter that describes the behavior of the migrants in the diffusion, A'_P is a parameter of the diffusion related with the polymer and independent of the temperature and τ is an specific parameter of the polymer, related to the activation energy. In this case, the values for A'_P and τ are 11.5 and 0 respectively for LDPE.

The equation 2 has been made for the overestimation of D_P values, creating a safety margin to ensure that the migration is not bigger than expected by this model. In order to confirm the utility of this equation, the experimental D_P values obtained in this work can be compared with the theoretical D^*_P obtained with equation 2. The results are represented in figure 12.3.

At the light of the obtained results, equation 2 is a useful equation for the representation of a worst case scenario for HCPK, because D^*_P is always bigger than the value obtained for D_P in the experimental studies. Nonetheless, for BP, the obtained values with equation 2 overestimate the diffusion coefficients between 4 and 40 ^oC in all the simulants except in 95 % ethanol (v/v) at 4 ^oC. In this food simulant the equation underestimates the diffusion coefficient since 7.7 ^oC to lower temperatures.

12.5. CONCLUSIONS

This work provides reliable data of the migration of two of the most used photoinitiators in UV inks for food packaging. The results showed that the process of migration is highly dependent on the storage conditions and the photoinitiator properties. The good fitting between the experimental data and the modelled values allows the use of this model to determine the migration rates of each photoinitiator from a LDPE package to most of the foodstuffs to verify the compliance with the current legislations, ensuring the food safety.

Also there are good correlation coefficients for the Arrhenius equation; this fact allows the prediction of diffusion coefficients at any temperature in the

range from 4 to 40 °C. The studies at -18 ºC demonstrates that although the process of migration is slowed down, the migration rates combined with the long term storages of frozen food, could turn the BP and HCPK migration into a food safety concern and further studies should be carried out in order to protect consumers' health.

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13. CONCLUSIONS

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The main conclusions drawn from the works that comprise this thesis are presented below:

A comprehensive review concerning the food safety aspects of the migration of photoinitiators into foodstuffs has been reported. The heterogeneity of the different groups of photoinitiators become them a food safety issue hard to address. For this reason, the main aspects that a future European UV' inks legislation should deal with are discussed.

To complete the work done in the previous review, another review concerning the multiple methods developed up-to-date, to determine and quantify photoinitiators in packaging or foodstuffs, has been done. The advantages and drawbacks of all the methods are argued and a possible decision tree to select the best analytical technique is suggested. In addition, the problem of NIAS is addressed and the possible routes for their analysis are discussed.

A multimethod for the determination of photoinitiators and amine synergists in food packaging has been developed. The described method was simple, reliable and could be useful as a screening tool for the routine determination of fourteen currently used photoinitiators and amine synergists, in packaging materials. Furthermore, a LC-MS/MS method allowed the confirmation of the identity of substances in positive samples. The results showed almost 50 % of positive samples, being benzophenone the most common photoinitiator in the analysed samples.

The photoinitiators reach the foodstuffs mainly by *set-off*. To determine the magnitude of this mechanism of migration, 3 different techniques (DART-HRMS, GC-MS and UHPLC/ESI-HRMS) have been used in under-cured packages. The results obtained underline the need of carry out different analytical techniques in order to broaden the search to transformation products, avoiding analytical limitations and the incidence of false-negatives and -positives.

Moreover, for first time, the HRMS spectra of polymeric PIs have been characterised by two different techniques (DART-HRMS and UHPLC/ESI-HRMS).

As a result, a total of 110 molecules were identified, with evidence that 30 molecules likely experienced *set-off* or were present on both sides. Most of the print related compounds detected were not included in the positive list of the European FCM legislation and several were novel PI transformation products. The molecules reported herein can serve as guide for any future assessments of print-related contaminants.

The UV photocuring process of BP and EDB has been replicated in laboratory conditions. Different variables of the study has been tested and analyzed by HPLC-DAD and GC-MS. The GC-MS method was shown as a useful tool to identify the possible photo-products and byproducts formed during the UV curing process. A total of 23 compounds were tentatively identified, being confirmed 4 of them after the injection of 13 standards. Two of these confirmed photo-products were classified as compounds with significant toxicity according to Cramer's classification.

Also a small survey in secondary packaging samples were carried out, detecting photoinitiators in a 92 % of the samples analyzed, 4 of them presented BP and EDB in their UV curing formulations, but, any of the confirmed photo-products could be detected.

Six of the most recurrent photoinitiators (BP, 4-MBP, HCPK, EDB, ITX and DETX) in the RASFF notifications list, were taken as models for the study of the migration kinetics of photoinitiators into food simulants. The studies were performed at 4 different temperatures, from -18 to 40 ^{o}C in order to study also the influence of the storage conditions. The photoinitiator source selected was LDPE due to be the most used polymer in the food packaging industry. Finally, the data has been fitted to a mathematical model based on Fick's 2nd law of diffusion

The following conclusions were extracted from these studies:

- The migration kinetic in LDPE is highly dependent on the storage conditions (time and temperature), migrant properties (Mw and log $K_{o/w}$) and type of food or food simulant (composition and pH).

- The mathematical model developed is a useful tool to predict the migration of these food contaminants into foodstuffs at the common storage temperatures. The migration key parameters: diffusion and partition coefficients (D_P and $K_{P/F}$) were obtained in all photoinitiator-food simulant-temperature systems.
- Arrhenius equation was used to study the relation between D_P and the temperature, obtaining a linear relation between log D_P and 1/T in the range of temperatures from 4 to 40 ^oC.
- Valuable migration data were obtained at -18 ^oC in 50 and 95% ethanol (v/v), temperature where there are almost no migration studies. These data showed a non-linear relation between log D_P and 1/T. For this reason, further studies should be performed in order to a better understanding of the migration processes in the range of temperatures from 4 to -18 ^oC.
- The migration studies carried out with BP, HCPK, 4-MBP, ITX and DETX showed that the D_P values obtained experiment a linear increment with the increase of the percentage of ethanol in the food simulants. This increase of D_P is clearer at higher temperatures.

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PUBLICATIONS AND COMMUNICATIONS EXTRACTED FROM THE THESIS

- **Book chapters:**
  - Lago, M.A.; Sendón, R. & Rodríguez-Bernaldo de Quirós, A. Analytical Methods for Determining Photoinitiators in Food-Contact Materials. In: Polymer Materials. Eds: Tiwari, A. & Polykarpov, A. *RSC Smart Materials*. 2015(13), 290-320.
- **Articles in international scientific journals:**
  - Lago, M.A.; Rodríguez-Bernaldo de Quirós, A.; Sendón, R.; Bustos, J.; Santillana, M.I. & Paseiro, P. Simultaneous chromatographic analysis of photoinitiators and amine synergists in food contact materials. *Anal Bioanal Chem*. 2014, 406 (16), 4251-4259.
  - Lago, M.A.; Sendón, R.; Rodríguez Bernaldo de Quirós, A.; Nieto, M. T.; Bustos, J. & Paseiro Losada, P. Photoinitiators: A Food Safety Review. *Food Addit & Contam A*. 2015. 32 (5), 779-798.
  - Lago, M. A. & Ackerman, L. K. Identification of print related contaminants in food packaging, *Food Addit & Contam A*. 2016. DOI: 10.1080/19440049.2015.1136435.
  - Lago M.A.; Rodríguez-Bernaldo de Quirós A.; Sendón R.; Bustos J.; Nieto M.T. & Paseiro P. Study of the photo-products obtained from the UV curing of benzophenone and ethyl-4-(dimethylamino) benzoate. Survey in Food Packaging Materials. (*Under preparation*).
  - Lago M.A.; Rodríguez-Bernaldo de Quirós A.; Sendón R.; Nieto M.T.; Bustos J. & Paseiro P. Migration studies of two common components of UV-curing inks into food simulants. (*Under preparation*).
  - Lago M.A.; Rodríguez-Bernaldo de Quirós A.; Sendón R.; Santillana, M. I.; Bustos J. & Paseiro P. Thioxanthone migration into food simulants: Kinetic studies. (*Under preparation*).

- Lago M.A.; Rodríguez-Bernaldo de Quirós A.; Sendón R.; Nieto M.T.; Bustos J. & Paseiro P. Migration studies of two common components of UV-curing inks into food simulants. (*Under preparation*).
- Lago M.A.; Rodríguez-Bernaldo de Quirós A.; Sendón R.; Santillana, M. I.; Bustos J. & Paseiro P. Study of the migration of two photoinitiators from low density polyethylene into food simulants. (*Under preparation*).

- **Oral communications:**
  - *XX Encontro Luso-Galego de Química* - Porto, Portugal. (2014):
    - Lago, M. A., Rodríguez-Bernaldo de Quirós, A., Sendón, R. Nieto, M. T., Bustos, J., & Paseiro, P. Benzophenone migration from LDPE to food simulants.
- **Poster communications:**
  - *Packaging Materials Symposium (ILSI)* - Berlín, Germany. (2012):
    - Lago, M.A., Rodríguez Bernaldo de Quirós, A., Sendón, R., Basadre Pampín, M.I., Nieto, M.T., Santillana, M.I., Ruíz, E., Cirugeda, M.E., Bustos, J., Sánchez, J.J. & Paseiro, P. Development of a multimethod for the determination of several photoinitiators in Food Contact Materials (FCM) by HPLC-DAD.
    - Lago, M.A., Rodríguez Bernaldo de Quirós, A., Sendón, R., Basadre Pampín, M.I., Nieto, M.T., Santillana, M.I., Ruíz, E., Cirugeda, M.E., Bustos, J., Sánchez, J.J. & Paseiro, P. Study of the photoinitiators migration from Food Contact Materials (FCM) to foodstuffs: A State-of-the-Art Review.
    - Bustos, J., Martin, P., Lago, M.A., Sendón, R., Rodríguez Bernaldo de Quirós, A., Puga, M.A., Basadre Pampín, M.I., Sanchez, J.J. GC/MS screening of ink compounds (photoinitiators) in food packaging.

- *III Encontro mocidade Investigadora* – Santiago de Compostela, Spain. (2015):
  - Lago, M. A., Rodríguez-Bernaldo de Quirós, A., Sendón, R., Bustos, J., Nieto, M. T. & Paseiro, P. Printing inks for food packaging: Migration studies of photoinitiators into food.
- *3rd International meeting on Materials/Bioproduct Interaction (MATBIM)* – Zaragoza, Spain. (2015):
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- *1st International meeting on Innovations in Food Packaging, Shelf Life and Food Safety* - Stadthalle Erding, Munich, Germany. (2015):
  - Lago, M. A., Rodríguez-Bernaldo de Quirós, A., Sendón, R., Bustos, J., Santillana, M. I. & Paseiro, P. Migration kinetics of photoinitiators and amine synergists to food simulants at freezing temperatures.
  - Lago, M. A., Rodríguez-Bernaldo de Quirós, A., Sendón, R., Bustos, J., Nieto, M. T. & Paseiro, P. Study of photoinitiators' migration kinetic in water and the aqueous food simulant 10 % ethanol.