MENÚ SALIR

ESTUDIOS DE LOS CAMBIOS MADURATIVOS EN EL QUESO DE CABRA DE LECHE CRUDA DE LA D.O.P. "QUESO IBORES". EFECTO DEL TRATAMIENTO DE ALTAS PRESIONES HIDROSTÁTICAS SOBRE SU MADURACIÓN

Departamento de Producción Animal y Ciencia de los Alimentos
Facultad de Veterinaria
Universidad de Extremadura

Francisco José Delgado Martínez

Mayo 2011

Edita: Universidad de Extremadura
Servicio de Publicaciones

Caldereros 2. Planta 3ª
Cáceres 10071
Correo e.: publicac@unex.es
http://www.unex.es/publicaciones

JUNTA DE EXTREMADURA
Consejería de Economía, Comercio e Innovación

Vicepresidencia Segunda y Consejería de
Economía, Comercio e Innovación

Dirección General de Innovación y
Competitividad Empresarial

Instituto Tecnológico Agroalimentario (INTAEX)
Crta. S. Vicente, s/n
Aptdo. 20107
06071 BADAJOZ
Tfno: 924 01 26 50
Fax: 924 01 26 74
intaex.juntaextremadura.net

Dña. Mª del Rosario Ramírez Bernabé, Doctora en Veterinaria y contratada-doctor INIA en el Instituto Tecnológico Agroalimentario de Extremadura (INTAEX)

INFORMA

que la Tesis Doctoral titulada "ESTUDIOS DE LOS CAMBIOS MADURATIVOS EN EL QUESO DE CABRA DE LECHE CRUDA DE LA D.O.P. "QUESO IBORES". EFECTO DEL TRATAMIENTO DE ALTAS PRESIONES HIDROSTÁTICAS SOBRE SU MADURACIÓN" presentada por el licenciado D. Francisco José Delgado Martínez, ha sido realizado bajo mi dirección en el Instituto Tecnológico Agroalimentario de Extremadura (INTAEX). Hallándose concluido y reuniendo a mi entender las condiciones necesarias, autorizo su presentación para su defensa ante el tribunal que ha de juzgarlo.

En Badajoz, a 10 de Mayo de 2011

Fdo.: Dra. Mª del Rosario Ramírez Bernabé

UNIVERSIDAD DE EXTREMADURA

FACULTAD DE VETERINARIA

Tecnología de los Alimentos

Dr. Ramón Cava López

Campus Universitario
Avda. Universidad s/n
E-10071-CÁCERES

Teléfono: 927 257169
Fax: 927 257110
e-mail: rcava@unex.es

D. Ramón Cava López, Doctor en Veterinaria y Profesor Titular del Área de Tecnología de los Alimentos de la Facultad de Veterinaria de la Universidad de Extremadura

INFORMA

que la Tesis Doctoral titulada "ESTUDIOS DE LOS CAMBIOS MADURATIVOS EN EL QUESO DE CABRA DE LECHE CRUDA DE LA D.O.P. "QUESO IBORES". EFECTO DEL TRATAMIENTO DE ALTAS PRESIONES HIDROSTÁTICAS SOBRE SU MADURACIÓN" presentada por el licenciado D. Francisco José Delgado Martínez, ha sido realizado bajo mi dirección en el Instituto Tecnológico Agroalimentario (INTAEX). Hallándose concluido y reuniendo a mi entender las condiciones necesarias, autorizo su presentación para su defensa ante el tribunal que ha de juzgarlo.

En Cáceres, a 10 de Mayo de 2011

Fdo.: Dr. Ramón Cava López

AGRADECIMIENTOS

AGRADECIMIENTOS

A mis directores de Tesis, Charo y Ramón, por su esfuerzo y dedicación. Gracias a Charo, por estar dispuesta siempre a trasladarme sus conocimientos y a atender mis necesidades, dándome también libertad para desarrollar mi trabajo. Gracias a Ramón, que, a pesar de la distancia, he tenido una sensación de cercanía y sus aportaciones han sido siempre bien recibidas.

A Pepe, mi tutor durante el período que estuve de becario en el departamento de productos lácteos del INTAEX (Instituto Tecnológico Agroalimentario de Extremadura). Gracias por la formación tecnológica que me ha transmitido durante estos años y por facilitarme siempre las cosas. Gracias por tratarme como un compañero.

A Jesús GC-MS, por enseñarme todo aquello que necesité para iniciarme en el mundo de la cromatografía y por su amistad y momentos de humor "fino". A Nandy, por ser una buena amiga y ayudarme en todo lo que he necesitado de ella. A Raquel, por estar siempre dispuesta a ayudarme ante los problemillas técnicos que surgían y su agrado al dejarme el "escaso" material que he precisado de ellos (con el consentimiento de Espe). A Juanma, por ser un gran profesional y una mejor persona.

En el departamento de lácteos, a Isidro, por sus charlas sobre el origen de la vida y el universo, y otras de temática diversa. A Joaquín, por su humor ácido. A Toni y Ana lácteos, por estar siempre dispuestas a ayudarme cuando lo necesitaba. Al departamento de carnes (Carmela, Oli, Marichu), por aguantar estoicamente el sonido celestial del destilador/valorador. A Lola y Chapi, por su contribución microbiológica. En vegetales, a Mercedes, por permitirme empezar a trastear con el cromatógrafo. A Loli, por la buena coordinación en el uso de los equipos compartidos. A Belén, por dedicarme su tiempo cuando me hacía falta. Al departamento de aceites, por sus migas comunitarias y su aceite de reparto anual. Al

personal de administración y servicios por la buena relación para con mi persona (Juan, Paulino, Mª Agustina, Andrés, Ángeles, Isabel, Ángela).

A los compañeros de desayuno que no han sido mencionados con anterioridad: Junra, Paco, Ana, Cristina, Sergio, Roberto, Carmina, Laura, Rocio...

Al panel de cata entrenado que ha aportado su granito de arena a este trabajo ("el orden de los factores no altera el producto"): Manolo Plaza, Teresa, Jose María, Tomasa, Lola, Isidro, Pepe, Rafa, Toni, Espe, Raquel, Dani, Antonio.

A Luis y Víctor, del departamento de Producción Animal de la Facultad de Veterinaria de la Universidad de Extremadura en Cáceres, por recibirme tan bien el tiempo que estuve con ellos.

A las queserías de la DOP Queso Ibores que han colaborado en esta Tesis: "Berrocales Trujillanos", "S.A.T. Quesería de las Villuercas" y "Quesería Almonte".

A los organismos que han financiado los proyectos de investigación con los que ha sido posible el desarrollado este trabajo: Junta de Extremadura y fondos FEDER (Fondo Europeo de Desarrollo Regional). A la Junta de Extremadura por la beca concedida (DOE 130, 2007), cofinanciada con fondos FEDER. Al INTAEX, como centro de investigación de acogida. Al grupo de investigación TRADINNOVAL, por el contrato realizado para la finalización de la Tesis.

Quiero dedicar esta tesis doctoral a aquellas personas que compartieron su vida conmigo y que ahora no están:

A mi tío, a mi abuela

También quiero dedicársela a las personas que se encuentran ahora a mi lado:

A mi mujer, a mis padres, a mi familia

ÍNDICE

ÍNDICE

	Pág.
1. Introducción	1
1.1. Estado actual del sector lácteo a nivel nacional y regional	3
1.1.1. La industria quesera	5
1.1.2. Las Denominaciones de Origen Protegidas de queso en España	8
1.1.3. Características generales del Queso Ibores	10
1.2. Procesos bioquímicos que tienen lugar durante la maduración de los quesos	13
1.2.1. Microorganismos implicados en la maduración del queso	15
1.2.2. Metabolismo de la lactosa, el lactato y el citrato	18
1.2.3. Lipolisis y metabolismo de ácidos grasos	20
1.2.4. Proteolisis y fenómenos relacionados	26
1.3. La Tecnología de Altas Presiones Hidrostáticas	28
1.3.1. Procesado de alimentos mediante Alta Presión Hidrostática	31
1.3.2. Aplicaciones de la APH en queso	34
1.3.2.1.Inactivación de microorganismos en quesos presurizados	35
1.3.2.2.Cambios bioquímicos inducidos por la presión en el queso	38
1.3.2.3.La textura y el análisis sensorial de quesos tratados por APH	41
2. Justificación y objetivos	53
3. Diseño experimental	57
4. Materiales y métodos	63
4.1. Materiales	65
4.1.1. Ensayo I: quesos empleados para el estudio a lo largo de la maduración	65
4.1.2. Ensayo II: quesos sin recubrimiento de pimentón en corteza tratados por APH a lo largo de la maduración	66
4.1.3. Ensayo III: quesos recubiertos con pimentón en corteza tratados con APH al final de la maduración	66
4.1.4. Tratamiento de los quesos por Alta Presión Hidrostática	67
4.2. Métodos	69
4.2.1. Análisis microbiológico	69
4.2.2. Composición físico-química	71

	Pág.
4.2.3. Color instrumental	71
4.2.4. Análisis del Perfil de Textura (APT)	72
4.2.5. Índices de proteolisis secundaria: fracciones de Nitrógeno y aminoácidos libres	72
4.2.6. Cuantificación de ácidos grasos libres (AGL) mediante cromatografía de gases-detector de ionización de llama	74
4.2.7. Determinación de la oxidación de lípidos y proteínas	75
4.2.8. Identificación de compuestos volátiles mediante micro-extracción de la fase sólida y cromatografía de gases acoplada a espectrometría de masas	76
4.2.9. Evaluación sensorial	77
4.2.10. Análisis estadístico	78
5. Resultados	83
Capítulo I. "Proteolysis, texture and colour of a raw goat milk cheese throughout the maturation"	85
Capítulo II. "Free fatty acids and oxidative changes of a raw goat milk cheese through maturation"	103
Capítulo III. "Formation of the aroma of a raw goat milk cheese along maturation analysed by SPME–GC–MS"	121
Capítulo IV. "Changes in microbiology, proteolysis, texture and sensorial characteristics in raw goat milk cheeses treated by high-pressure during maturation"	147
Capítulo V. "Influence of high pressure treatment on free fatty acids and oxidation processes throughout maturation of a raw goat milk cheese"	177
Capítulo VI. "Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation"	197
Capítulo VII. "Microbiology, proteolysis, texture and sensorial evaluation of a raw goat milk cheese with paprika on rind high pressure treated at the end of ripening"	219
Capítulo VIII. "Effect of high-pressure treatment on the volatile profile of a mature raw goat milk cheese with paprika on rind"	243
6. Discusión general	265
6.1. Evaluación de los principales procesos de maduración relacionados con la formación de los parámetros de calidad del Queso Ibores	267
6.1.1. Parámetros físico-químicos y color y textura instrumental	267
6.1.2. Principales rutas metabólicas del proceso de maduración	268
6.1.3. Perfil de compuestos volátiles en el espacio de cabeza del queso	271

	Pág.
6.2. Efectos del tratamiento de Alta Presión Hidrostática (APH) sobre el Queso Ibores	275
6.2.1. Quesos sin recubrimiento en corteza tratados por APH a diferentes estados de maduración	275
6.2.2. Quesos con recubrimiento en corteza (pimentón) tratados por APH al final de la maduración. Evolución de su almacenamiento posterior en refrigeración	285
7. Conclusiones	297

1. INTRODUCCIÓN

1. INTRODUCCIÓN

1.1. Estado actual del sector lácteo a nivel nacional y regional

Desde el punto de vista social y económico, la producción de leche sigue teniendo una gran importancia en España, si bien el sector ha ido perdiendo peso en los últimos años. En el año 2009, la producción de leche de vaca, oveja y cabra supuso el 18% del valor generado por todo el sector ganadero y el 6,3% del valor total del sector agrario, por encima de casi todas las producciones ganaderas y de muchas de las producciones agrícolas. De la producción nacional de leche, el 75% corresponde a la producción de leche de vaca, el 13% a la producción de leche de oveja y el 12% a la producción de leche de cabra. La producción de leche de vaca está limitada por unas cuotas nacionales de producción. España tiene una cuota de producción de 6,23 millones de toneladas de leche, pero el sistema de cuotas está previsto que desaparezca en el año 2015. España es el séptimo país productor de leche de la Unión Europea, tras Alemania, Francia, Reino Unido, Países Bajos, Italia y Polonia.

Lo que diferencia a España de otros países dentro de la UE es nuestro importante déficit de cuota en relación con el consumo. Así, nuestro consumo anual de leche se sitúa en 9,5 millones de toneladas de leche y ello obliga a importar grandes cantidades todos los años, fundamentalmente de Francia y Portugal. Sin embargo, pese a este desfase entre oferta y demanda, el mercado de la leche arrastra desde hace años una situación crítica. Los precios que se pagan al ganadero llegan a situarse por debajo de los costes de producción y además hay explotaciones con problemas en la recogida de la leche.

Anualmente, la producción de leche supone en la Unión Europea en torno al 14% de la producción agraria y más del 30% de la producción ganadera, porcentajes en ambos casos muy superiores a los españoles. Según los datos de Eurostat, la producción de leche (todas las cabañas) en el año 2009 sufrió un descenso en volumen de un 0,5%,

mientras que los precios experimentaron un crecimiento del 20,5%. Esta caída espectacular es un claro signo de las dificultades que el sector lácteo atravesó en el año 2009, no sólo en España sino en toda la UE.

El sector lácteo español presenta una estructura empresarial orientada esencialmente hacia la producción de leche líquida envasada, ya que alrededor del 60% de toda la leche recogida en nuestro país se destina a ese mercado. Esta estructura es diferente de la de los grandes productores lácteos de Europa, donde el queso, la mantequilla o la leche en polvo tienen mucha más importancia.

En la base del sector se encuentran en actividad 26.320 explotaciones ganaderas dedicadas preferentemente a la producción de leche. La producción promedio por explotación se sitúa en unos 232.800 kilos anuales, mucho más cerca del promedio europeo que hace unos pocos años. De todas formas, la atomización en esa base productiva es todavía muy acusada.

Un 80% de la producción lechera se concentra en las comunidades autónomas de Galicia, Castilla y León, Asturias, Cataluña y Cantabria, mientras que el 86% de las explotaciones está radicado en Galicia, Castilla y León, Asturias y Cantabria. Extremadura tiene cierta relevancia a nivel nacional en la producción de leche de cabra (Tabla 1). Las explotaciones con menores dimensiones se encuentran en las provincias de A Coruña, Ourense y Pontevedra, mientras que las mayores, con más de un millón de kilos de producción anual como promedio, están radicadas en Albacete, Comunidad Valenciana o Murcia.

En la actualidad se encuentran en actividad unas 1.500 industrias, de las cuales menos de 200 empresas y/o cooperativas industrializadoras tienen una actividad significativa como elaboradores y/o comercializadores de leche líquida envasada. Únicamente ocho empresas están envasando más de 200 millones de litros al año. Se detecta una cada vez mayor concentración en la cúspide de la pirámide empresarial, ya que los 10

grupos lácteos más importantes manejan el 80% de toda la leche que se recoge en España.

Tabla 1. Producciones españolas de leche de cabra por comunidades autónomas en miles de toneladas

Comunidad Autónoma	2007	2008
ANDALUCÍA	234	228,7
ARAGÓN	1,4	1,5
CANARIAS	90	91,2
CASTILLA LA MANCHA	59	62,4
CASTILLA LEÓN	27	29
CATALUÑA	6,7	7,6
COMUNIDAD VALENCIANA	11	11,7
EXTREMADURA	26	27
MADRID	4	4,4
MURCIA	26	23,7
OTRAS COMUNIDADES	2,9	3,8
TOTAL ESPAÑA	488	491

Fuente: Ministerio de Medio Ambiente, y Medio Rural y Marino.

1.1.1. La industria quesera

El mercado español de quesos ha experimentado durante el último año un notable crecimiento del 6,5% en volumen. De esa manera, se han superado las 300.000 toneladas comercializadas. Ese dinamismo del sector se ha producido por un abaratamiento generalizado de los precios, por una fuerte competencia entre las marcas blancas y los productos marquistas y por un repunte de las importaciones.

Atendiendo a los volúmenes vendidos, la principal oferta dentro del sector es la de los quesos manchegos y regionales, con el 31,6% del total vendido. A continuación aparecen los quesos frescos, los fundidos, las especialidades de importación, los quesos rallados, los blancos para untar, los de pasta blanda, otros quesos frescos, los Emmental y Gruyere, los quesos de pasta veteada y los surtidos de quesos. Los porcentajes del mercado en valor de los mismos aparecen en la Figura 1. Entre los

quesos regionales, los de mezcla acaparan el 76,7% de todas las ventas en volumen y el 73,3% en valor, seguidos a mucha distancia por el de Mahón, el manchego y el gallego. Dentro de las especialidades de importación destacan los edam/maasdam, con cuotas del 30,8% en volumen y del 27,3% en valor, seguidos por el gouda (25,8% y 21,5% respectivamente), el queso italiano, el queso inglés y los quesos de bola.

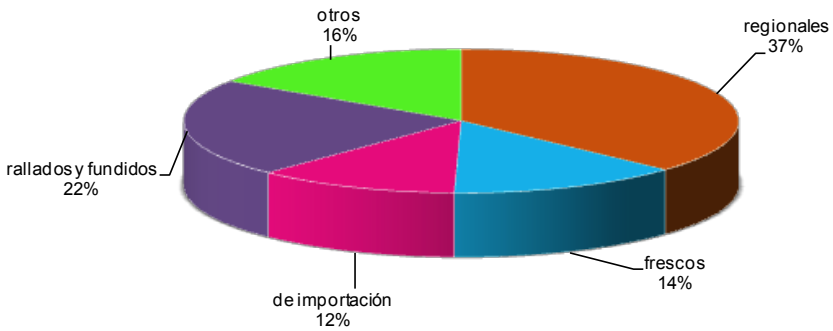

Figura 1. Porcentajes del mercado en valor de los distintos tipos de queso

La atomización y el carácter semiartesanal de buena parte de sus operadores constituían los dos rasgos más característicos del sector quesero español, pero esa situación está variando durante los últimos tiempos. Se han registrado algunos movimientos de concentración empresarial, los principales operadores han aumentado su capacidad productiva y diversificando sus ofertas e, incluso, se percibe una cierta penetración de capitales internacionales en un sector que presenta buenas perspectivas de crecimiento.

El principal grupo dentro de los fabricantes e importadores de quesos en España presenta un volumen de producción de 95.000 toneladas, mientras que los dos siguientes operadores rondan las 30.000 toneladas en cada caso. Entre las 20.000 y las 28.000 toneladas aparecen otras siete empresas.

Las marcas de distribución empiezan a ocupar un lugar importante en este sector y durante el último ejercicio han registrado incrementos del 35% en el caso de los

quesos regionales y del 14% en las especialidades de importación. En algunas categorías el predominio de las marcas blancas es ya absoluto. Así, en el queso en lonchas, las marcas de distribución acaparan el 65,3% del mercado frente al 15,2% de la primera oferta marquista. En los quesos tipo Burgos, las marcas blancas presentan porcentajes del 54,9% en volumen y del 42,7% en valor, mientras que el primer fabricante con marca propia se queda en el 19,3% y el 27,7%. La firma de acuerdos entre los fabricantes y las grandes cadenas de distribución está siendo un factor clave para el crecimiento de las empresas del sector.

El comercio exterior de quesos presenta una situación estable en la que las importaciones resultan mucho más importantes que las exportaciones y donde sólo se perciben cambios anuales en algunas categorías concretas. Durante el último ejercicio computado se importaron algo más de 219.500 toneladas, lo que supuso un descenso interanual del 7,6%. Esta reducción se concentró en el segmento de los quesos rallados, que pasaron a algo menos de 13.000 toneladas, cuando en el año anterior superaron las 34.600 toneladas. Otras partidas significativas son las de los quesos frescos (48.500 toneladas), los quesos fundidos (24.650 toneladas) y los quesos azules (8.110 toneladas). Los quesos importados provienen preferentemente de otros países de la Unión Europea, entre los que destacan Francia, Holanda, Alemania, Dinamarca e Italia. También resultan importantes las importaciones de quesos industriales, utilizados en los platos preparados. Éstas llegaron hasta las 24.300 toneladas, provenientes de Alemania, Francia e Italia.

Las exportaciones son mucho menos importantes y apenas superaron las 43.300 toneladas, sin apenas variaciones con respecto a años precedentes. Estas exportaciones se dirigen fundamentalmente a Francia, Alemania, Holanda, Estados Unidos y Dinamarca. Las partidas indican la importancia de los quesos frescos (6.000 toneladas), los fundidos (5.100 toneladas) y los rallados (3.350 toneladas), mientras que una partida sin especificar reúne a las restantes 28.900 toneladas. Las

exportaciones de quesos industriales rondaron las 5.000 toneladas y sus principales mercados de destino fueron Portugal, Francia, Alemania y Reino Unido.

1.1.2. Las Denominaciones de Origen Protegidas de queso en España

El Reglamento (CE) 510/2006 del Consejo, de 20 de marzo de 2006, sobre protección de las indicaciones geográficas y de las denominaciones de origen de los productos agrícolas y alimenticios, establece las definiciones de Denominación de Origen Protegida (DOP) y de Indicación Geográfica Protegida (IGP). Estas son las dos figuras de protección que se aplican a los productos agrícolas y alimenticios diferentes del vino y de las bebidas espirituosas.

Así, en dicho Reglamento se define una DOP como "El nombre de una región, de un lugar determinado o, en casos excepcionales, de un país, que sirve para designar un producto agrícola o un producto alimenticio originario de dicha región, de dicho lugar determinado o de dicho país, cuya calidad o características se deben fundamental o exclusivamente al medio geográfico con sus factores naturales y humanos, y cuya producción, transformación y elaboración se realicen en la zona geográfica delimitada".

En España existen un total de 27 DOP en queso, de las cuales 3 pertenecen a la comunidad autónoma de Extremadura: "Queso Ibores", "Queso de La Serena" y "Torta del Casar". Las tres DOP extremeñas emplean leche cruda para la elaboración de sus quesos, en el primer caso de cabra y las otras dos de oveja.

En la DOP "Queso de La Serena" la zona de elaboración y producción de queso abarca 21 municipios de la comarca de La Serena, en la provincia extremeña de Badajoz. Para la elaboración del queso se utiliza exclusivamente leche cruda de oveja de raza Merina y cuajo vegetal procedente del cardo *Cynara cardunculus*. El período de maduración mínimo es de 60 días y el peso oscila entre 700 gramos y 2 kilos. Su cremosidad depende del grado de maduración, denominándose "Tortas" a los que presentan una pasta blanda, fluida y mantecosa, perceptible al paladar. Cuando es añejo presenta una

pasta y corteza duras y un ligero aunque exquisito sabor picante. 126.000 ovejas y 115 explotaciones ganaderas producen más de 1,1 millones de litros de leche al año. Las 13 queserías registradas producen una media anual de más de 200.000 kilos de queso; el 60% se comercializa en el mercado regional, el 35% en el nacional y el 5% restante se destina a la exportación.

La Torta del Casar se elabora en 36 municipios de las comarcas de Llanos de Cáceres, Sierra de Fuentes y Montánchez en la provincia de Cáceres. El queso se elabora con leche cruda de ovejas pertenecientes a los troncos merino y entrefino, cuajo vegetal procedente de la flor del cardo *Cynara cardunculus* y sal, con una maduración mínima de 60 días. Presenta una corteza semidura de color entre amarillo y ocre, pasta untable de color blanco a marfil y textura blanda y untuosa, fundente al paladar. Olor intenso y sabor desarrollado, apenas salado y ligeramente amargo, característica esta debida al uso del cuajo vegetal. El Consejo Regulador tiene inscritas 50 explotaciones ganaderas con 27.650 ovejas que producen 3,6 millones de litros de leche al año. Las 13 queserías inscritas certifican unos 351.702 kilos de Torta del Casar, que se comercializan en formatos de entre 0,5 y 1 kilos, dedicándose más del 94% al mercado nacional.

Figura 2. Quesos de La Serena (izquierda) y Torta del Casar (derecha)

1.1.3. Características generales del Queso Ibores

El queso de Ibores es un queso graso de pasta semidura con DOP elaborado en Extremadura siguiendo un proceso tradicional y cuya producción está regulada por el "Reglamento de la Denominación de Origen Protegida Queso Ibores" publicado en el DOE nº61/2003 (Decreto 69/2003, de 20 de mayo). Este queso se elabora exclusivamente a partir de leche cruda de cabras de las razas Serrana, Verata, Retinta y de los cruces entre ellas. Al término de su proceso de maduración, el queso presenta un color amarillo céreo a ocre oscuro, siendo tradicional la presentación pimentonada, así como untados en aceite o sin pimentonar (Figura 3). El empleo de leche cruda de cabra implica el mantenimiento de unos controles rigurosos de calidad en ganaderías y queserías inscritas en el Consejo Regulador, precisando además un período mínimo de 60 días de maduración para garantizar su seguridad alimentaria.

Figura 3. Quesos pertenecientes a la DOP "Queso Ibores", pimentonado (izquierda) y sin pimentonar (derecha)

Existen unas 20.632 cabras censadas en 84 explotaciones ganaderas extensivas y familiares que producen 2,3 millones de litros de leche de calidad, que elaboran las 9 queserías inscritas productoras de Queso Ibores. Durante la campaña se comercializan en torno a 150.000 kilos, de los que cerca de un 80% se destinan al mercado nacional.

La zona geográfica tanto de producción de la leche como de elaboración del queso abarca los municipios comprendidos en las comarcas naturales de Ibores, Villuercas, la Jara y Trujillo en el sureste de la provincia de Cáceres (Figura 4). Las características orográficas de la zona de producción de la D.O. y su flora se traducen en adecuadas producciones lácteas caracterizadas por la calidad. Las encinas, alcornoques y sotobosque de jaras, tomillo y brezo, característicos de este ecosistema, confieren a la alimentación del ganado unas características específicas que, junto con su elaboración a base de leche cruda, proporcionan al queso su singularidad.

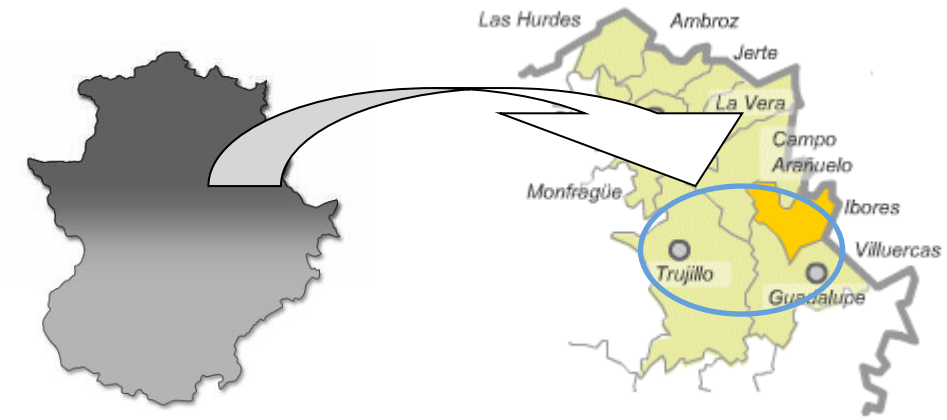

Figura 4. Zona geográfica que engloba a los productores de Queso Ibores

Según la D.O.P. Queso Ibores, una vez ordeñadas las cabras, la leche se conservará a una temperatura inferior a los 6 ºC para limitar el desarrollo microbiano, durante un

período máximo de 48 horas. La leche debe estar limpia y sin impurezas, libre de calostros, conservantes etc., que puedan influir negativamente en la maduración y conservación del queso, así como en las condiciones higiénicas y sanitarias del mismo. Las características fisicoquímicas que debe tener la leche son las siguientes:

- Proteína: 3% mínimo.
- Materia grasa: 4% mínimo.
- Extracto seco (ES): 13% mínimo.
- Acidez máxima: 15º Dornic.
- pH: 6,5 mínimo.

La coagulación de la leche se realiza mediante cuajo animal u otras enzimas coagulantes que estén expresamente autorizadas por el Consejo Regulador. El proceso de cuajado se lleva a cabo a una temperatura de 28–32 ºC, durante un período de 60–90 min. Una vez obtenida la cuajada, ésta se somete a cortes sucesivos hasta conseguir granos de tamaño de 5 a 10 mm de diámetro. Posteriormente se introduce la cuajada en moldes cilíndricos autorizados (moldes de paño y microperforados) con el tamaño adecuado para que los quesos, una vez madurados, presenten la forma y dimensiones peculiares de los mismos. El siguiente paso es el prensado mediante los equipos adecuados para tal fin, durante un tiempo de 3 a 8 horas a presiones de 1 a 2 Kg/cm^2. Una vez terminado el prensado, la salazón será húmeda o seca, utilizándose exclusivamente cloruro sódico. En caso de salazón húmeda, el tiempo máximo de permanencia será de 24 h, en una solución salina de concentración salina máxima de 20 ºBeaumé. La maduración mínima del Queso Ibores es de 60 días y bajo unos parámetros de temperatura de 4–15 ºC y una humedad relativa superior al 75%. El Queso Ibores debe presentar al final de la maduración las características reflejadas en la Tabla 2.

Con objeto de cumplir el reglamento, velar por la calidad de la materia prima y del producto y proteger los intereses de la Denominación de Origen “Queso Ibores” se

constituye el Consejo Regulador de la Denominación de Origen. El Consejo Regulador es una entidad pública sin ánimo de lucro que goza de entidad jurídica propia y plena capacidad de obrar en el desempeño de las funciones que le atribuye expresamente el ordenamiento jurídico y el reglamento de la DOP.

1.2. Procesos bioquímicos que tienen lugar durante la maduración de los quesos

El queso es un alimento bioquímicamente dinámico que sufre cambios significativos durante su maduración. Las principales vías para la formación de compuestos de aroma y sabor en queso son i) el metabolismo de lactosa, lactato y citrato (denominado con frecuencia "glucolisis"), ii) la lipolisis y iii) la proteolisis. En la glucolisis, dependiendo del tipo de queso, los microorganismos y las condiciones de maduración, el lactato puede ser metabolizado por diferentes vías formando compuestos que contribuyen al aroma y sabor del queso, junto con los compuestos derivados de la grasa formados por lipolisis y por reacciones de oxidación de lípidos, tales como ácidos grasos libres (AGL), ésteres, lactonas y cetonas, que generalmente tienen bajos umbrales de percepción (Kinsella, 1975; Rothe y cols., 1972; Siek y cols., 1971). Sin embargo, la proteolisis de las caseínas proporciona péptidos de pequeño a mediano tamaño y aminoácidos libres, que probablemente contribuyen en menor medida al aroma de la mayoría de variedades de queso, pero sí tienen una influencia relevante en el sabor. En los procesos metabólicos descritos con anterioridad, los microorganismos participan en mayor o menor medida y, por lo tanto, se incluye un primer apartado sobre los microorganismos que guardan relación con la maduración de los quesos.

Los compuestos volátiles generados a lo largo de la maduración tienen su origen en los componentes presentes en los alimentos. Estos compuestos pueden originarse durante el proceso de maduración de forma intencionada o no y también se forman durante el almacenamiento de los alimentos (Plutowska y Wardencki, 2007). Los perfiles aromáticos que presentan los alimentos son el resultado de un número elevado de

reacciones que tienen lugar entre sus componentes. Las características del aroma resultante dependen de distintos factores: disponibilidad y estructura de los reactivos, participación de la grasa, aminoácidos y sacáridos y condiciones de reacción (temperatura, duración, actividad del agua, pH, nivel de oxígeno, etc.).

Tabla 2. Características del Queso Ibores al final de su maduración

Físicas	Fisicoquímicas	Organolépticas	Microbiológicas
FORMA cilíndrica, con caras sensiblemente planas ALTURA de 5 a 9 cm DIÁMETRO de 11 a 15 cm PESO de 650–1200 g CORTEZA semidura, de color amarillo céreo a ocre oscuro, pimentonada o untada de aceite PASTA semidura, de color blanco marfil, presentando ojos pequeños, poco abundantes y desigualmente distribuidos	GRASA mínima del 45% sobre ES ES mínimo 50% pH de 5,0 a 5,5 PROTEINA mínima del 30% sobre ES NaCl máximo 4%	AROMA de suave a moderado, a queso de cabra de leche cruda SABOR franco característico, ligeramente ácido, moderadamente picante, algo salado, suavemente caprino en el retrogusto y muy agradable al paladar	Según la normativa higiénico-sanitaria vigente: *Salmonella*, *Listeria monocytogenes* y enterotoxinas estafilocócicas: n=5, c=0 (ausencia en 25 g) Estafilococos coagulasa positivos: n=5, c=2, m= 10^4 ufc/g, M=10^5 ufc/g

Se han identificado más de 600 compuestos volátiles en queso, sin embargo, todos los compuestos tienen una importancia relativa dependiente del umbral de percepción, por lo tanto, no todos participan en la misma medida en el aroma final del producto. Por este motivo, es imposible reproducir el aroma de ninguna variedad de queso

mezclando únicamente compuestos puros (McSweeney y Sousa, 2000). Entre los compuestos odor-activos identificados en queso se encuentran alcoholes, aldehídos, cetonas, ésteres, lactonas, furanos, compuestos nitrogenados, pirazinas, compuestos azufrados, terpenos, hidrocarburos aromáticos y ácidos grasos libres (revisado por Curioni y Bosset, 2002).

El aroma del queso es uno de los criterios más importantes que determinan su elección y aceptación por parte del consumidor. Generalmente, la fracción volátil refleja una imagen aproximada de ese aroma. El flavor típico de cada variedad de queso, entendido éste como la combinación compleja de sensaciones olfativas y gustativas percibidas durante la degustación, es el resultado de un complejo balance entre compuestos químicos volátiles y no volátiles originados durante el proceso de maduración a partir de la grasa, proteínas y carbohidratos de la leche (Fox y Wallace, 1997); y como consecuencia de esto, cada producto tiene un perfil único y característico de compuestos volátiles (Plutowska y Wardencki, 2007).

Debido al interés creciente en la caracterización de los productos tradicionales con DOP se ha estudiado el perfil volátil de algunos quesos españoles en los últimos años. Entre ellos, quesos de oveja: Manchego (Martínez-Castro y cols., 1991; Villaseñor y cols., 2000), Roncal (Izco y Torre, 2000), Idiazábal (Larráyoz y cols., 2001), La Serena (Carbonell y cols., 2002), Zamorano (Fernández-García y cols., 2004) y Torta del Casar (Delgado y cols., 2010); quesos de cabra: Palmero (Guillén y cols., 2004) y Majorero (Castillo y cols., 2007); quesos de vaca: Mahón (Mulet y cols., 1999). En estos estudios se identificaron un gran rango de compuestos volátiles que contribuyen al aroma de esos quesos. Los cambios en su perfil volátil y en su abundancia pueden ser considerables lo que proporciona notas aromáticas diferentes a cada tipo de queso.

1.2.1. Microorganismos implicados en la maduración del queso

Las enzimas de los microorganismos relacionados con el queso y más particularmente las bacterias del ácido láctico (BAL), contribuyen notablemente al desarrollo de su

aroma y sabor. Estos organismos pueden proceder de la leche o ser intencionadamente añadidos durante el proceso de fabricación del queso.

Los microorganismos implicados en la elaboración y maduración del queso se pueden dividir en dos grandes grupos (Figura 5):

1) Microorganismos que se añaden a la leche empleada para la elaboración del queso después de ser cuidadosamente seleccionados por empresas fabricantes de bacterias iniciadoras o fabricantes de quesos. También se incluyen los microorganismos iniciadores presentes en la leche cruda empleada para la elaboración de ciertos tipos de quesos, como el Queso Ibores.

2) Bacterias del ácido láctico no iniciadoras (BALNS).

El grupo 1 puede ser subdividido a su vez en dos grupos: los microorganismos iniciadores primarios y los iniciadores secundarios. El papel de los cultivos iniciadores primarios es asegurar la producción de ácido durante la elaboración del queso. Este grupo también está involucrado en la degradación de proteínas y grasa durante la maduración. Por último, juegan un importante papel en la protección biológica del producto (por ejemplo, disminuyendo el pH y produciendo bacteriocinas). Dentro de este grupo los más frecuentes son *Lactococcus lactis* y *Streptococcus termophilus*, que son BAL. Los iniciadores secundarios son cultivos que se añaden a un número limitado de tipos de quesos para mejorar funciones bien definidas; por ejemplo, la producción de gas en quesos tipo suizo se debe a *Propionibacterium shermanii ssp. freudenreichii*, mientras que *Brevibacterium linens* es el principal contribuidor a la coloración superficial de quesos madurados con microorganismos de superficie. *Penicillium roqueforti* y *Penicillium camemberti* también pueden incluirse en este grupo. Además, los iniciadores secundarios, mediante sus sistemas enzimáticos diversos, pueden estar también implicados en el proceso de maduración. De hecho, sus contribuciones son indispensables para el

desarrollo del aroma y sabor típicos de muchos quesos, como son el Roquefort, el Emmental, el Camembert y el Limburger (Johnson, 1998).

El grupo 2, las bacterias del ácido láctico no iniciadoras, contribuyen al desarrollo del aroma y sabor de algunas variedades de quesos y podrían considerarse por lo tanto como contaminantes deseables de la leche o del queso con posterioridad. Cepas de *Lactobacillus* son las más comunes y se pueden encontrar en un número relativamente alto: *L. casei*, *L. paracasei*, *L. plantarum* y *L. curvatus* son las especies predominantes. Los pediococos y enterococos también son miembros del grupo pero están presentes normalmente en menor número (Fox y cols., 1998). Los micrococos, que no son BAL, también pueden jugar un papel significativo en la formación del aroma de ciertos tipos de quesos.

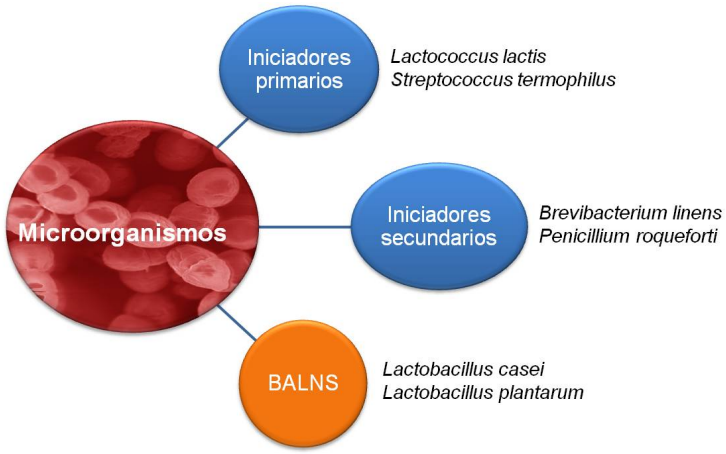

Figura 5. Microorganismos implicados en la maduración del queso. BALNS (bacterias del ácido láctico no iniciadoras)

Por otra parte, en los últimos años ha aparecido una nueva tecnología en la industria quesera conocida como "cultivos adjuntos" que ofrece a los consumidores por un lado seguridad y por otro lado quesos con notables propiedades organolépticas en un período de maduración razonable. Los cultivos adjuntos pueden ser definidos como

cepas seleccionadas a partir de microorganismos relacionados con quesos que son añadidos a la leche para mejorar la calidad sensorial del queso. A diferencia de los BALNS, los adjuntos son específicamente seleccionados e intencionadamente añadidos para suplementar los microorganismos de la leche y mejorar la calidad final del queso.

Se ha realizado un estudio previo sobre la evolución de microorganismos presentes a lo largo de la maduración del Queso Ibores, en el cual se analizaron mesófilos totales, lactobacilos, enterobacterias, coliformes fecales, enterococos, estafilococos, micrococos, levaduras y mohos (Mas y cols., 1991). En la Tabla 3 aparece un resumen de los resultados obtenidos en dicho trabajo. Enterobacterias y coliformes fecales tuvieron un escaso desarrollo debido a que el extracto seco y la sal fueron elevados y el pH bajo. Por su parte, los estafilococos y micrococos fueron abundantes.

Tabla 3. Microorganismos (log ufc/g queso) presentes en el interior del Queso Ibores durante su maduración (n=6). (Fuente: Mas y cols., 1991)

Días	Mesófilos totales	Lactobacilos	Enterobacterias	Coliformes fecales	Enterococos	Estafilococos y micrococos	Mohos y levaduras
3	8,69	6,72	4,09	2,60	5,77	5,37	3,69
30	8,53	7,60	2,35	1,05	6,04	3,83	4,92
60	7,82	7,71	1,83	1,38	5,57	4,67	2,98

1.2.2. Metabolismo de la lactosa, el lactato y el citrato

El metabolismo de la lactosa a lactato es esencial para la maduración de todas las variedades de quesos. Dependiendo del tipo de cultivo iniciador, la lactosa es metabolizada por la vía glucolítica (la mayoría de bacterias iniciadoras) o por la vía de la fosfocetolasa (*Leuconostoc spp*) (Cogan y Hill, 1993). Los principales productos resultantes del metabolismo de la lactosa son el L- o D-lactato o una mezcla de ambos a iguales proporciones, aunque algunas cepas producen otros compuestos, como por ejemplo, *Leuconostoc* que produce etanol. El lactato contribuye al aroma típico de los quesos de cuajada ácida y probablemente también al de las variedades de quesos

maduros, particularmente al inicio de la maduración (McSweeney y Sousa, 2000). La acidificación del queso tiene un efecto indirecto en el flavor, al determinar la capacidad tampón del queso y en consecuencia el crecimiento de distintos tipos de microorganismos durante la maduración y la actividad de enzimas involucradas en la misma. Dependiendo del tipo de queso, el lactato puede ser metabolizado mediante varias vías a otros compuestos que contribuyen al aroma y sabor del queso (Figura 6).

El acetato, un importante compuesto implicado en el aroma de muchos quesos con un típico olor a vinagre, es muy abundante en los quesos Cheddar, Gruyère, Roncal y Emmental (revisado por Curioni y Bosset, 2002). Este compuesto, además de originarse a partir de la lactosa por la acción de las BAL, se puede también formar como resultado del metabolismo de citrato y lactato, o como un producto del catabolismo de aminoácidos y, en menor medida, mediante la lipolisis de la grasa de la leche.

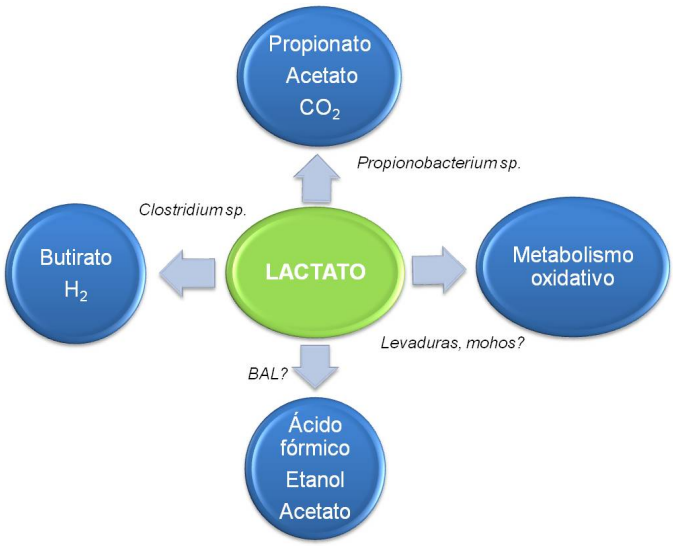

Figura 6. Vías generales del metabolismo de lactato en queso. BALNS: bacterias ácido lácticas no iniciadoras. BAL: bacterias ácido lácticas (Adaptado de McSweeney y Sousa, 2000)

Propioniobacterium spp crece en quesos tipo suizos cuando durante la maduración pasan a cámaras a temperatura templada (15–20 ºC) después del salado; y metaboliza preferentemente L-lactato formándose propionato, acetato y CO_2 (Steffen y cols., 1987). El dióxido de carbono es esencial para el desarrollo de ojos en el interior de los quesos; y el propionato y el acetato, este último en menor medida, contribuyen al flavor de algunos quesos, como Cheddar, Gruyère, y Emmental (revisado por Curioni y Bosset, 2002).

La producción de gas y aromas desagradables en ciertos quesos duros se debe al metabolismo del lactato por *Clostridium spp*, que da lugar a la formación de ácido butírico y H_2 (Fox y cols., 1995). Estos defectos se pueden evitar mediante una correcta higiene o la eliminación de esporas mediante microfiltración, entre otras medidas.

El metabolismo del citrato, a pesar de su baja concentración en la cuajada de la leche al perderse en gran parte en el suero durante la elaboración, es de gran importancia ya que puede metabolizarse produciendo un número elevado de compuestos responsables de flavor. Los microorganismos que metabolizan citrato (algunos lactococos y lactobacilos mesófilos, *Leuconostoc spp.*) no lo emplean como fuente de energía, sino mas bien son co-metabolizados con lactosa u otros azúcares. Los principales compuestos de aroma producidos a partir del citrato son el acetato, el diacetilo, la acetoina y el 2,3-butanodiol. El diacetilo es un compuesto del aroma importante en ciertos tipos de quesos, incluidos los quesos tipo holandés y el queso fresco de leche de vaca Cottage (revisado por McSweeney y Sousa, 2000). El metabolismo del citrato tiene una importancia particular en los quesos tipo holandés donde el CO_2 producido es responsable de la formación de ojos, aunque también puede causar agrietamientos indeseables y el defecto conocido como “cuajada flotante” en los quesos Cheddar y Cottage.

1.2.3. Lipolisis y metabolismo de ácidos grasos

Los principales lípidos presentes en la leche son los triglicéridos, que pueden

representar hasta el 98% del total de los lípidos (Christie, 1983; Gunstone y cols., 1994; Jensen y cols., 1991). Los triglicéridos son ésteres de glicerol compuestos por una molécula de glicerol unido a tres ácidos grasos. La lipolisis consiste en la hidrólisis enzimática de los triglicéridos dando lugar a ácidos grasos libres (AGL) y glicerol, mono- o diglicéridos. Esta vía metabólica es esencial para el desarrollo del aroma y sabor en muchos tipos de queso, debido a que la grasa de la leche contiene altas concentraciones de ácidos grasos de cadena corta e intermedia (C4:0–C8:0 y C10:0–C14:0, respectivamente) que, cuando son liberados mediante lipolisis, contribuyen directamente al flavor del queso (McSweeney y Sousa, 2000).

La lipolisis en el queso se debe a la presencia de enzimas lipolíticas (lipasas y esterasas) que surgen de diferentes fuentes: la leche, la preparación del cuajo animal (y sobre todo la pasta de cuajo), las bacterias iniciadoras, las bacterias iniciadoras adjuntas, las no iniciadoras y, posiblemente, lipasas exógenas añadidas (Deeth y Fitz-Gerald, 1995; Fox y Wallace, 1997; McSweeney y Sousa, 2000):

a) *Lipasa de la leche*. La leche contiene una lipoproteína lipasa (LPL) endógena muy potente, que normalmente nunca alcanza su máxima actividad en la leche (Fox y cols., 1993; Fox y Stepaniak, 1993). La LPL muestra una preferencia por la hidrólisis de triglicéridos de cadena media, debido fundamentalmente a la mayor solubilidad y movilidad de estos triglicéridos en sistemas emulsionados, lo que permite una hidrólisis más rápida comparada con los triglicéridos de cadena larga. La LPL es relativamente poco específica para el tipo de ácido graso a liberar pero sí es específica con respecto al lugar de corte, liberando ácidos grasos a partir de las posiciones *sn-1* y *sn-3* de mono- di- o triglicéridos (Olivecrona y cols., 1992). Por lo tanto, los ácidos grasos de cadena corta y media son liberados preferentemente por la LPL, debido a que son los que suelen ocupar esas posiciones. La LPL es más importante en quesos elaborados a partir de leche cruda, como el Queso Ibores, ya que su actividad se reduce por pasteurización, aunque probablemente también tenga alguna contribución

en la lipolisis de quesos elaborados a partir de leche pasteurizada debido a que se requieren al menos 78ºC durante 10 segundos para su completa inactivación (McSweeney y Sousa, 2000).

b) ***Pasta de cuajo***. La pasta de cuajo se elabora a partir del abomaso de terneros, cabritos o corderos sacrificados después de la lactancia y por lo tanto es un cuajo de origen animal. Esta fuente de lipolisis carece de interés en el caso del Queso Ibores debido al empleo de cuajo animal comercial durante su elaboración, que no contiene lipasas. La pasta de cuajo se emplea en la elaboración de algunas variedades de quesos italianos de pasta dura como son el Provolone o el Romano. La pasta de cuajo contiene una lipasa conocida como esterasa pregástrica (PGE, en inglés) que es altamente específica para ácidos grasos de cadena corta esterificados en la posición *sn-3* (Collins y cols., 2003).

c) *Enzimas lipolíticas microbianas*. Las lipasas y esterasas de las BAL parecen ser los principales agentes lipolíticos en el queso Cheddar y en los quesos holandeses elaborados a partir de leche pasteurizada (Fox y cols., 2000). Para hidrolizar la grasa de la leche las BAL poseen enzimas capaces de hidrolizar esteres de AGL, tri-, di- y monoacilglicéridos (Fox y Wallace, 1997). A pesar de la presencia de esas enzimas, las BAL, especialmente *Lactococcus* y *Lactobacillus spp*, son por lo general débilmente lipolíticas comparadas con otras especies. Sin embargo, debido a su abundante presencia en quesos con un largo período de la maduración, las BAL son consideradas responsables de la liberación de niveles significativos de AGL (Collins y cols., 2003). La localización de la mayor parte de la actividad esterasa y lipasa parece ser intracelular. Por lo tanto, puede ser necesaria la liberación hacia la matriz del queso mediante la autolisis de las bacterias o, simplemente, la existencia en las BAL de algún sistema de transporte activo transmembrana de glicéridos o ácidos grasos implicados en la formación de AGL y otros productos lipolíticos. A

día de hoy, son necesarios más estudios que establezcan la contribución a la lipolisis de estos mecanismos propuestos.

El desarrollo de las reacciones lipolíticas tiene gran importancia durante maduración del queso ya que se liberan AGL implicados directa o indirectamente en el aroma del queso. La lipolisis ha sido bastante estudiada quesos azules y quesos de pasta dura italianos donde la lipolisis alcanza elevados niveles y es la mayor vía de generación de aroma (revisado por Collins y cols., 2003). Existen escasos estudios sobre el perfil de AGL de quesos de cabra. Buffa y cols. (2001) analizaron el contenido de AGL de quesos elaborados a partir de leche cruda, pasteurizada y presurizada. Franco y cols. (2003) estudiaron la evolución del patrón lipolítico del queso de cabra español de cuajada ácida Babia-Laciana. También se ha descrito la lipolisis durante la maduración del queso Urfa producido en Turquía, elaborado a partir de leche cruda y pasteurizada de cabra y vaca (Atasoy y Türkoglu, 2009). Por otro lado, se ha estudiado la lipolisis de algunos quesos españoles elaborados a partir de leche pasteurizada de cabra (Fontecha y cols., 2006; Poveda y Cabezas, 2006).

Los AGL liberados durante la lipolisis, especialmente AGL de cadena corta y media, contribuyen, junto con los compuestos volátiles y los productos de proteolisis, directamente al aroma del queso (McSweeney y Sousa, 2000; Urbach, 1993). Los AGL de cadena larga (más de 14 átomos de carbono) juegan un papel menor en el flavor de los quesos debido a sus altos umbrales de percepción (Molimard y Spinnler, 1996). Los niveles de lipolisis varían considerablemente entre los diferentes tipos de quesos. Además, el análisis del perfil de los AGL de cadena corta y media ha sido sugerido como un índice para la caracterización del periodo de maduración de los quesos (Woo y cols., 1984). Los AGL no son solamente compuestos aromáticos por sí mismos, sino que también actúan como moléculas precursoras de una serie de reacciones catabólicas que llevan a la producción de compuestos del aroma, tales como metilcetonas, ésteres, alcoholes secundarios, lactonas y aldehídos (Figura 7).

Las metilcetonas o 2-alcanonas son importantes catabolitos de los ácidos grasos, particularmente en quesos azules, donde constituyen los compuestos más importantes relacionados con el flavor (revisado por Collins y cols., 2003). La vía por la cual las metil cetonas son producidas involucra la liberación de ácidos grasos por lipolisis, su oxidación a β-cetoácidos y la descarboxilación a 2-alcanonas con la pérdida de un átomo de carbono (Figura 7). Las 2-alcanonas se pueden reducir a sus alcoholes secundarios correspondientes (2-alcanoles), un paso que es reversible bajo condiciones aeróbicas. Las metil cetonas se forman en queso gracias a la acción de las lipasas de los mohos, como por ejemplo, *Penicillium roqueforti* (Urbach, 1997).

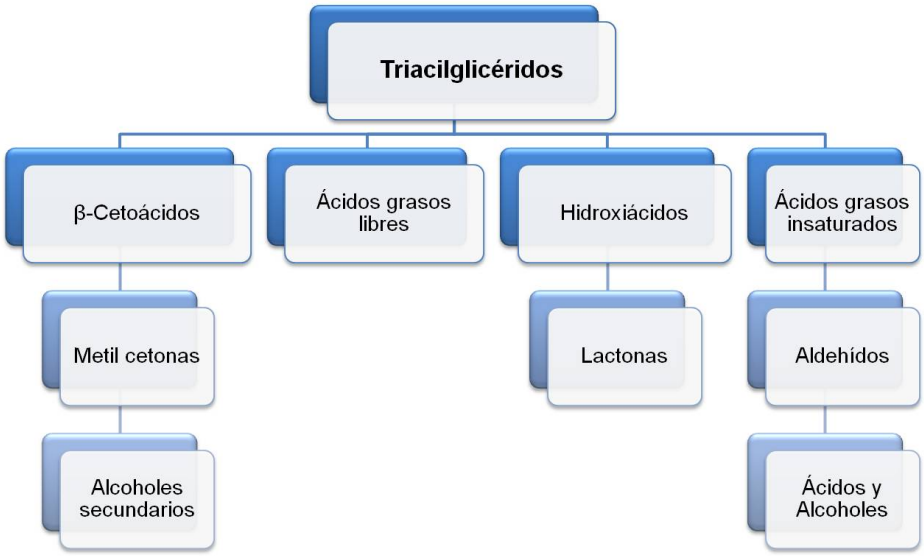

Figura 7. Catabolismo de ácidos grasos libres (Adaptado de Molimard y Spinnler, 1996)

Los ésteres son otros compuestos derivados del catabolismo de ácidos grasos y son comunes en la fracción volátil de los quesos (Urbach, 1997). Hay una gran diversidad de ésteres presentes en el queso (Molimard y Spinnler, 1996). Estos compuestos influyen de forma importante en el aroma y sabor de los quesos y se forman cuando

los AGL reaccionan con alcoholes. La mayoría de los ésteres encontrados proporcionan notas aromáticas a “fresco”, “afrutado” y “floral”, y su importancia radica en el bajo umbral de percepción de alguno de ellos.

Los alcoholes secundarios pueden formarse en queso por reducción enzimática de metil cetonas. El 2-propanol, 2-butanol, 2-octanol y 2-nonanol se encuentran en la mayoría de quesos de pasta blanda y son compuestos típicos del aroma de los quesos azules (Engels y cols., 1997).

Las lactonas son compuestos cíclicos formados por la esterificación intramolecular de hidroxiácidos, mediante la pérdida de agua y la formación de una estructura con forma de anillo. Las principales lactonas presentes en queso son la γ- y δ-lactonas, que son estables y con un fuerte flavor (McSweeney y Sousa, 2000).

Los aldehídos se suelen formar a partir de aminoácidos por transaminación, resultando en la formación de una imida que puede ser descarboxilada (Collins y cols., 2003). Sin embargo, algunos aldehídos de cadena lineal (butanal, heptanal, nonanal) se pueden formar como resultado de la β-oxidación de ácidos grasos insaturados. Los aldehídos de cadena lineal se caracterizan por sus aromas a hierba verde (Moio y cols., 1993). De todas formas, los aldehídos son compuestos transitorios en queso porque son rápidamente reducidos a alcoholes primarios o incluso oxidados a sus ácidos correspondientes.

Por su parte, los ácidos grasos lineales son compuestos importantes, o incluso predominantes, para el aroma de muchos tipos de quesos. Entre ellos, el ácido butanoico o butírico tiene un olor a “queso rancio” y juega un importante papel en tipos de quesos como el Camembert, Cheddar, Grana Padano, Gruyère, Pecorino, Ragusano y Roncal (revisado por Curioni y Bosset, 2002). Sin embargo, cantidades elevadas de este ácido pueden resultar desagradables. En la Tabla 4 aparecen los ácidos grasos de cadena lineal identificados en distintos tipos de queso y sus descriptores de olor más característicos.

1.2.4. Proteolisis y fenómenos relacionados

La proteolisis es el más complejo y, en la mayoría de variedades, el más importante de los eventos primarios que ocurren durante la maduración del queso (McSweeney y Sousa, 2000). La proteolisis juega un papel fundamental en el desarrollo de:

i) Cambios texturales en la cuajada del queso, debido a la ruptura de la red proteica.

ii) Contribución directa al sabor del queso mediante la formación de péptidos y aminoácidos libres.

iii) Liberación de sustratos (aminoácidos) para reacciones catabólicas secundarias (deaminación, descarboxilación, etc.).

iv) Cambios en la matriz del queso que facilitan la liberación de compuestos con sabor durante la masticación.

Según Sousa y cols. (2001), durante la maduración, la proteolisis del queso está catalizada por enzimas procedentes del coagulante o cuajo (quimosina, pepsina, proteinasas ácidas de plantas o fúngicas), la leche (plasmina, catepsina D), las bacterias iniciadoras, las no iniciadoras o los iniciadores secundarios (por ejemplo, *Penicillium camemberti*) y proteinasas/peptidasas exógenas empleadas para acelerar la maduración.

En la mayoría de variedades de quesos, la hidrólisis inicial de las caseínas se debe al coagulante y en menor extensión a la plasmina y quizás a proteinasas de células somáticas (catepsina D), que dan lugar a la formación de péptidos de gran tamaño (insolubles en agua) y péptidos de tamaño intermedio (solubles en agua) que son degradados por el coagulante y enzimas procedentes de los microorganismos iniciadores y no iniciadores del queso. La producción de pequeños péptidos y aminoácidos libres se debe a la acción de proteinasas y peptidasas microbianas (McSweeney y Sousa, 2000).

Tabla 4. Ácidos grasos de cadena lineal identificados como compuestos odor-activos en diferentes tipos de quesos (Adaptado de Curioni y Bosset, 2002)

Ácido graso	Descriptor/es de olor	Tipo de queso
Ácido etanoico	Vinagre, acético	Camembert, Cheddar, Emmental, Gruyère, Roncal
Ácido propanoico	Gas, acre	Cheddar, Emmental, Gruyère
Ácido butanoico	Rancio, queso maduro	Camembert, Cheddar, Emmental, Gruyère, Roncal
Ácido pentanoico	Madera, nueces	Camembert, Cheddar, Grana Padano
Ácido hexanoico	Cabra, queso sudado	Camembert, Cheddar, Gruyère, Roncal
Ácido heptanoico	Rancio	Grana Padano
Ácido octanoico	Rancio, sudado	Camembert, Cheddar, Grana Padano, Roncal
Ácido nonanoico	Cabra	Bouton de Culotte
Ácido decanoico	Podrido, añejo	Camembert, Cheddar, Grana Padano, Roncal
Ácido dodecanoico	Jabón, leche caliente	Camembert, Cheddar, Roncal
Ácido tetradecanoico	Sudado	Roncal

Las proteasas procedentes de las flores secas de *Cynara cardunculus* presentan la capacidad de coagular la leche y se han empleado con éxito durante mucho tiempo en la elaboración de quesos tradicionales de la Península Ibérica, como son los quesos portugués Serra da Estrela o Serpa y los quesos extremeños de La Serena y la Torta del Casar. Los extractos de *C. cardunculus* contienen dos proteínas, la cardosina A y la B (Sousa, 1993). Estas proteinasas producen mayores niveles de proteolisis que los cuajos animales comerciales. Relacionado con este aspecto, Galán y cols. (2008) encontraron que la actividad de estas enzimas da lugar a quesos de oveja completamente maduros (con todas sus características organolépticas) tres meses antes que con el empleo de coagulante de ternero, acelerando por tanto la maduración del queso.

La proteolisis contribuye fundamentalmente al sabor del queso mediante la producción de péptidos y aminoácidos libres. Los péptidos de gran tamaño no contribuyen directamente al sabor del queso, pero son importantes para conseguir la textura idónea del mismo. El papel exacto de los péptidos de mediano y pequeño tamaño en el aroma y sabor del queso no está claro, aunque parece que contribuyen al sabor de fondo de algunos quesos como el Cheddar (revisado por McSweeney, 1997).

Los productos finales de la proteolisis son los aminoácidos libres que se encuentran a distintas concentraciones dependiendo del tipo de queso y han sido empleados como índices de maduración (McSweeney y Fox, 1997). La proporción relativa de aminoácidos individuales parece ser similar en muchos tipos de queso e incrementos en la concentración de aminoácidos libres no necesariamente acelera la maduración ni tampoco la intensidad del sabor. Fox y Wallace (1997) sugirieron que el flavor del queso y la concentración de aminoácidos no están correlacionados debido a que distintos quesos tenían aromas muy diferentes, aunque la concentración y proporción relativa de aminoácidos libres eran generalmente similares. Esos resultados muestran que la producción de aminoácidos no es un paso limitante en la maduración del queso y quizás sea la modificación enzimática o química de los aminoácidos el factor crítico. De hecho, el catabolismo de aminoácidos libres puede dar lugar a un número de compuestos que podrían contribuir al aroma y sabor del queso: amoniaco, aminas, aldehídos, fenoles, indol y alcoholes (Sousa y cols., 2001).

1.3. La Tecnología de Altas Presiones Hidrostáticas

La tecnología de procesado de alimentos mediante altas presiones hidrostáticas (APH) ha emergido fundamentalmente por el interés creciente por parte de los consumidores de nuevos métodos para la preservación de alimentos que garanticen su seguridad microbiológica, alta calidad, mínimo procesado y ausencia de aditivos.

El procesado por APH implica la exposición de un producto a presiones extremadamente elevadas que van desde 100 a 1000 MPa (1000-10000 bar), aunque

las presiones que se suelen utilizar en sistemas comerciales se encuentran entre los 400 y 600 MPa. Debido a que la presión es aplicada isostáticamente (igual en todas direcciones) y de forma instantánea, en general, los alimentos sólidos mantienen su forma original.

La mayoría de los sistemas de APH consisten en una vasija de presión con una tapa, un sistema de presurización y un mecanismo de manejo. La alta presión dentro de la vasija se genera mediante compresión directa, en la cual el volumen de la cámara de tratamiento se reduce, por la acción de la presión hidráulica sobre un pistón, o compresión indirecta, donde una bomba de alta presión o un intensificador bombea el medio presurizado hacia la cámara de tratamiento. Este último es el método más frecuente a nivel comercial. Si la máquina está equipada con sistemas de calentamiento/enfriamiento, la temperatura de tratamiento puede ser controlada desde –20 ºC a más de 100 ºC. En la Figura 8 se representa de forma esquemática el funcionamiento básico de un equipo de APH. Una vez envasado el producto en un embalaje flexible que actué de barrera (como por ejemplo plástico) éste se coloca en un depósito plástico que se introduce en la vasija; ésta es sellada y se llena con un fluido transmisor de presión (generalmente agua) gracias al empleo de una bomba de baja presión, presurizándose posteriormente la vasija mediante el uso de una bomba de alta presión que inyecta cantidades adicionales de fluido. Los alimentos envasados son sometidos a la misma presión que existe en la propia vasija gracias al fluido transmisor de presión y el embalaje flexible. Después de mantener el producto durante el tiempo deseado a una presión determinada la vasija sufre la descompresión mediante la liberación del fluido trasmisor de presión y tiene lugar la descarga del producto procesado. Para la mayoría de aplicaciones, los productos se mantienen durante 3–5 min a 600 MPa. Aproximadamente de 5 a 6 ciclos/h son posibles, teniendo en cuenta el tiempo de compresión, tiempo de tratamiento, descompresión, carga y descarga.

Otro aspecto que debe ser conocido es el incremento de temperatura que sufre el producto como consecuencia del incremento de presión. El incremento de temperatura depende de factores como la presión final, la composición del producto y la temperatura inicial. La temperatura del agua incrementa 3 ºC por cada 100 MPa a temperatura ambiente. Por otra parte, las grasas y aceites tienen un valor de calentamiento por compresión de 8–9 ºC/100 MPa y las proteínas y carbohidratos presentan valores intermedios (Rasanayagam y cols., 2003; Patazca y cols., 2007). Por lo tanto, y de forma aproximada, para un Queso Ibores tratado al final de su maduración (~40% humedad, 35% grasa y 25% proteína y carbohidratos) el incrementó de temperatura en función de la presión se situaría en torno a los 5–5,5 ºC/100 MPa.

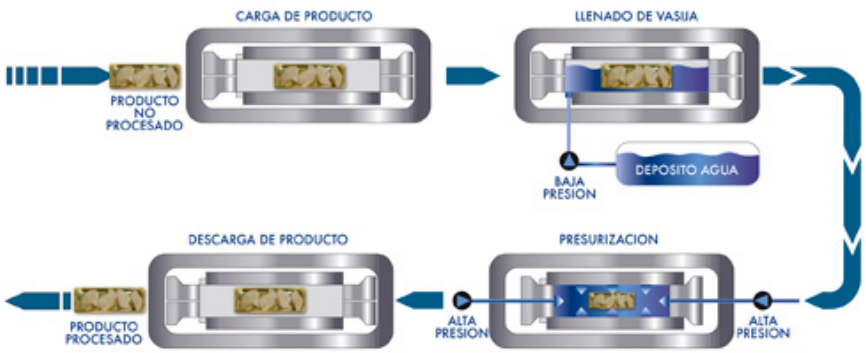

Figura 8. Esquema del funcionamiento de una unidad de APH. Fuente: NC-Hyperbaric

En la actualidad, configuraciones verticales y horizontales están comercialmente disponibles en función del producto a procesar y de la disponibilidad de espacio. Los volúmenes de la cámara de tratamiento van desde los 35 a los 600 L con un máximo de presión de ~700 MPa. Existen dos principales fabricantes de equipos de APH, Avure Technologies (Kent, WA, USA) y NC-Hyperbaric (Burgos, España).

Recientemente se ha desarrollado un proceso continuo, un tipo diferente de sistema que también opera bajo alta presión, que es referenciado en la literatura como Alta Presión Dinámica (APD) u Homogenización por Alta Presión. Los homogeneizadores se han empleado en la industria láctea durante muchos años, aunque operan a presiones más bajas que las alcanzadas en sistemas isostáticos. En los nuevos homogeneizadores, se aplican presiones de hasta 400 MPa a un líquido que es forzado a pasar a través de una válvula ajustable, generalmente de cerámica, causando un incremento en la velocidad de flujo, cavitación, colisión en la superficie estacionaria, etc. Los sistemas de APD también disminuyen las poblaciones microbianas en leche cruda inoculada, aunque los mecanismos de inactivación probablemente son diferentes de los de los sistemas hidrostáticos.

1.3.1. Procesado de alimentos mediante Alta Presión Hidrostática

El procesado por alta presión hidrostática (APH) es una técnica de preservación de alimentos que inactiva microorganismos patógenos y microorganismos vegetativos causantes del deterioro. A nivel industrial la aplicación de APH se realiza a presiones intensas y temperaturas moderadas (<45 ºC), lo que permite la conservación de la mayoría de alimentos con un mínimo efecto sobre el sabor, la textura, la apariencia o el valor nutricional. El tratamiento por presión puede ser empleado para el procesado de alimentos líquidos o sólidos con un alto contenido en humedad. De esta manera, la APH es una tecnología que mantiene la calidad alimentaria evitando la necesidad de un tratamiento térmico excesivo o conservantes químicos.

A diferencia del tradicional procesado térmico, la aplicación de APH sobre alimentos afecta en menor medida a su valor nutricional, sabor, color, flavor o contenido en vitaminas (Hayashi, 1989). Esto se debe a que sólo las uniones no covalentes que se establecen dentro de la materia biológica son alteradas mediante APH. De esta manera, pequeñas moléculas como aminoácidos o vitaminas, se ven afectadas en menor medida por la APH, mientras que la estructura de moléculas de gran tamaño

(proteínas, ácidos nucleicos) puede ser alterada (Balci y Wilbey, 1999). Por ello, la APH se está convirtiendo en una tecnología potencial en la industria láctea para la elaboración de yogurt, algunas variedades de queso y productos lácteos con texturas novedosas.

La tecnología de APH es considerada como la mayor innovación en el procesado de alimentos en los últimos 50 años (Dunne, 2005). El uso comercial del procesado por alta presión está cada vez más expandido, ya que proporciona a los productores de alimentos la oportunidad de preservar los alimentos con una etiqueta de ingredientes "limpia" y además es el procesado elegido para aplicaciones en la que la pasteurización térmica podría afectar negativamente a la calidad del producto. Por ejemplo, para asegurar la seguridad alimentaria de un producto como el salami, la pasteurización del producto envasado mediante calentamiento da lugar a la pérdida de líquido y migración de la grasa.

La APH permite a productos como la charcutería loncheada ser "pasteurizados" después del loncheado y envasado, reduciendo así el riesgo de contaminación durante la manufactura. El Servicio de Inspección y Seguridad Alimentaria del Departamento de Agricultura de los Estados Unidos reconoce que el procesado mediante alta presión es un mecanismo de seguridad alimentaria aceptable para la eliminación de *Listeria monocytogenes* en productos cárnicos procesados. El tratamiento de presión es también efectivo en la inactivación de otros microorganismos peligrosos como *Escherichia coli*, *Salmonella* y *Vibrio*, así como muchas levaduras, mohos y bacterias responsables del deterioro de los alimentos.

La vida útil y la calidad de los alimentos pueden ser prolongados substancialmente mediante el uso de APH (He y cols., 2002; Hayman y cols., 2004). Adicionalmente, la calidad de los productos presurizados durante su vida útil está influenciada en mayor medida por las posteriores temperaturas de distribución y almacenamiento y por las propiedades barrera del envase, que por el tratamiento de presión.

En la actualidad, la APH se está empleando en combinación con temperatura para eliminar esporas patógenas de *Clostridium botulinum* y esporas de variedades de *Bacillus* y *Clostridia*. Este tratamiento sinérgico permite la esterilización de los alimentos con una menor exposición térmica (Matser y cols., 2004; Margosch y cols., 2006; Ahn y cols., 2007).

El procesado mediante alta presión proporciona a los productores el desarrollo de una nueva generación de productos alimenticios con un valor añadido y con una calidad superior a los producidos convencionalmente. Los primeros productos presurizados fueron mermeladas de fresa, manzana y kiwi fueron los primeros productos presurizados comercializados en el mercado japonés en 1990. Los productos basados en aguacate, especialmente guacamole, fueron posteriormente comercializados en los Estados Unidos. En 2007, unas 120 instalaciones de APH industriales en el mundo entero se dedicaban a la producción a escala industrial de alimentos tratados por alta presión (Sáiz y cols., 2008). Más del 80% del equipamiento ha sido instalado a partir del año 2000, lo que indica una tendencia acelerada en el uso de la APH. En la Figura 9 muestra algunos ejemplos de productos tratados por APH en la actualidad.

Figura 9. Productos tratados por alta presión hidrostática. Fuente: NC-Hyperbaric

La distribución de productos tratados por alta presión en función de los segmentos productores de alimentos se refleja en la Figura 10. La mayor parte de productos tratados por APH son vegetales y carnes (Samson, 2008). La posibilidad de incrementar la seguridad alimentaria de los productos presurizados y aumentar su vida útil en refrigeración ha abierto nuevas oportunidades de mercado especialmente en productos cárnicos "naturales" libres de conservantes. Varios productores de marisco han empleado también la APH para mejorara la seguridad alimentaria y la vida útil de los mismos con un beneficio añadido debido a que facilita la separación de la "carne" del caparazón o concha. Otros segmentos del mercado que emplean tratamientos de alta presión son los zumos, bebidas y productos vegetales.

1.3.2. Aplicaciones de la APH en queso

Existen numerosas áreas de interés concernientes al procesado de quesos mediante APH. Entre las más importantes se encuentran la elaboración de quesos a partir de leche tratada por APH, aceleración de la maduración del queso y la inactivación o reducción de microorganismos patógenos o causantes de deterioro en queso, para incrementar su seguridad y vida útil.

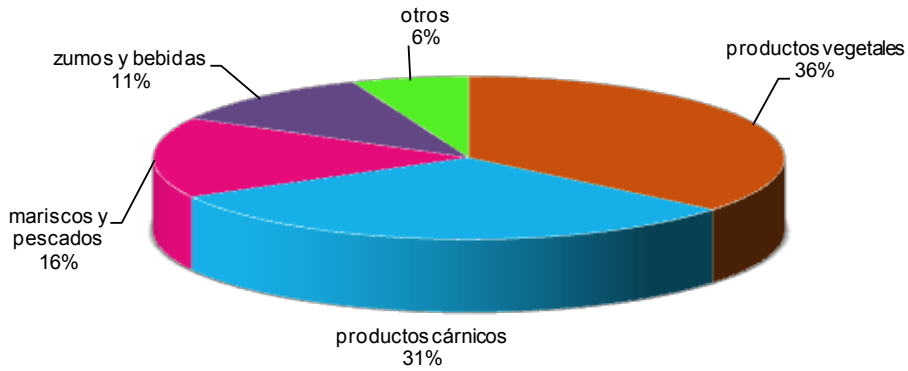

Figura 10. Porcentaje de los productos tratados por alta presión en la industria alimentaria

1.3.2.1. Inactivación de microorganismos en quesos presurizados

Existen una multitud de factores que influyen en el resultado de la inactivación de microorganismos mediante alta presión. La resistencia de los microorganismos en forma vegetativa a la presión a menudo alcanza un máximo a temperatura ambiente, por lo que la temperatura inicial del alimento previa al tratamiento de APH puede ser elevada o reducida para mejorar la inactivación producida por la presión. La inactivación también depende del tipo de microorganismos, composición del alimento, el pH y actividad de agua. Por ejemplo, las bacterias Gram-positivas son más resistentes que las Gram-negativas y, generalmente, cuanto más grande y complejo es el organismo más fácil es su inactivación. También existen variaciones significativas en cuanto a la resistencia a la alta presión entre cepas de la misma especie. Por su parte, un pH reducido tiene un efecto sinérgico con la presión en la eliminación de microorganismos. Sin embargo, una actividad de agua reducida tiende a inhibir el efecto de la presión (Balasubramaniam y cols., 2008), debido a que la presión se transmite a través del agua incluida en el alimento.

En un estudio realizado para extender la vida útil de quesos de leche de cabra, Capellas y cols. (1996) evaluaron la idoneidad del tratamiento de APH sobre quesos elaborados a partir de leche inoculada con *Escherichia coli* (10^8 ufc/g queso). Una vez elaborados, los quesos fueron tratados a 400–500 MPa de presión, con una combinación de diferentes temperaturas (2, 10 ó 25 ºC) y tiempos (5–15 min), almacenándolos posteriormente a 2–4 ºC. En los análisis realizados tras 15, 30 y 60 días después del tratamiento, no se identificaron colonias de *E. coli* en los quesos presurizados. En otros estudios, quesos de leche de cabra también han sido presurizados (50 MPa durante 72 h o 400 MPa durante 5 min) y analizados a diferentes momentos de la maduración para determinar los efectos letales del tramiento (Saldo y cols., 2000a). El tratamiento a 400 MPa/5 min redujo los recuentos de bacterias lácticas en ~3 unidades log, pero las poblaciones se recuperaron durante la maduración y, tras 3 semanas, fueron casi idénticas a la de los quesos no tratados.

También se han realizado estudios de inactivación inducida por la presión en los quesos Gouda, Camembert and Kurpiowski (tipo suizo) a diferentes estados de maduración (Kolakowski y cols., 1998; Reps y cols., 1998). Estos estudios demostraron que el tipo de queso y el grado de maduración de los mismos afectaban al nivel de inactivación microbiana.

Por otro lado, O'Reilly y cols. (2000) analizaron la capacidad de la APH para la inactivación de microorganismos patógenos y causantes de deterioro en el queso Cheddar (*Staphylococcus aureus, E. coli y Penicililum roqueforti*), mediante el tratamiento de APH realizado en un rango de intensidades de presión de 50–800 MPa, a temperaturas de 10–30 ºC, durante 20 min. De los resultados, destacar que *E. coli* fue especialmente sensible a presiones inferiores a los 200 MPa, probablemente debido al efecto sinérgico de la APH y la acidez producida durante la fermentación del queso. En otro estudio, el tratamiento de APH (400–700 MPa; 1–15 min) fue efectivo en la reducción de *L. monocytogenes* en la corteza de queso Gorgonzola, sin cambios significativos en sus propiedades sensoriales (Carminati y cols., 2004). También se ha estudiado el efecto de la APH sobre la morfología de *L. monocytogenes* tratada a 400 MPa durante 10 min en tampón citrato (Ritz y col., 2001). La presurización no afectó significativamente al volumen celular, pero sí se produjo algún daño físico como refleja la aparición de desgarros en la superficie celular y la pérdida de la integridad de la membrana en la mayor parte de la población celular.

La inactivación mediante APH de patógenos que pueden encontrarse con mayor frecuencia en queso, como *L. monocytogenes*, ha sido extensamente estudiada en la literatura (Tabla 5). López-Pedemonte y cols. (2007) obtuvieron un incremento significativo en la reducción de los recuentos de *L. monocytogenes* en un queso modelo en función de la presión aplicada (300, 400 y 500 MPa, 10 min). Estos resultados son similares a los obtenidos por Szczawinski y cols. (1997) con una mezcla de tres cepas de *L. monocytogenes* inoculadas a lonchas de quesos Gouda, Edanski y Podalaski maduros. Carminati y cols. (2004) estudiaron la inactivación de siete cepas de *L. monocytogenes*

en la corteza del queso Gouda y encontraron que fueron necesarios tratamientos de 600 MPa para obtener una reducción superior a 2,5 unidades logarítmicas de ufc/g.

Tabla 5. Investigaciones destacadas sobre la inactivación de microorganismos en quesos mediante Alta Presión Hidrostática.

Autores	Tipo de queso	Tratamiento	Resultado
Capellas y cols. (1996)	Leche pasteurizada de cabra inoculada con *E. coli* (10^8 ufc/g)	400–500 MPa 2, 10 ó 25 ºC	No se aisló *E. coli* en quesos tratados, tras 15, 30 y 60 días de almacenamiento
Saldo y cols. (2000a)	Leche de cabra	50 MPa/72 h 400 MPa/5 min	El tratamiento de 400 MPa/5 min disminuyó los recuentos de bacterias lácticas ~ 3 log ufc/g
O'Reilly y cols. (2000)	Cheddar	50–800 MPa/20 min 10–30 ºC	*E. coli* mostró mayor sensibilidad a presiones inferiores a 200 MPa
Carminati y cols. (2004)	Gorgonzola inoculado con *L. monocytogenes* (~ 7 log ufc/g)	400–700 MPa 1–15 min	Presiones superiores a los 600 MPa/10 min ó tratamientos de 700 MPa/5 min reducían más del 99% de *L. monocytogenes*
Arqués y cols. (2005)	Leche cruda de vaca inoculada con *L. monocytogenes* (~ 10^5 ufc/g)	300 y 500 MPa/5 min	Tratamiento de 500 MPa/5 min a día 2 redujo 5 log ufc/g
López-Pedemonte y cols. (2007)	Queso modelo inoculado con *L. monocytogenes* (~ 7,5 log ufc/g)	300, 400 y 500 MPa/10 min	Incremento significativo en la reducción de *Listeria monocytogenes*

Arqués y cols. (2005) encontraron que tratamientos de APH a 500 MPa durante 5 min producían una reducción de 5 log ufc/g en quesos de leche cruda de vaca inoculados con *L. monocytogenes* tratados a día 2 y analizados al día siguiente. Además, tras 20 y 30 días de maduración no se detectó su presencia. Si el mismo tratamiento se realizaba a día 50, en los recuentos realizados a 51 y 60 días tampoco se detectaba su presencia (control 6,34–5,66 log ufc/g), incluso si el tratamiento se realizaba a 300 MPa. Del mismo modo, Gallot-Lavallé (1998) presurizó quesos de cabra elaborados a partir de leche cruda y encontró reducciones de más de 5 unidades logarítmicas de una cepa *L. monocytogenes*, incluso a tratamientos de 350 MPa.

O'Reilly y cols., (2000) investigaron con mayor profundidad la posibilidad de acelerar la maduración del queso Cheddar comercial mediante su exposición a un tratamiento de 50 MPa durante 3 días a 25 ºC. Estos investigadores encontraron que el efecto sobre la proteolisis del tratamiento de APH en quesos de 2 días se redujo a lo largo del proceso de maduración. Además el incremento en los índices de proteolisis obtenido tras el tratamiento del queso no fue tan significativo como el sugerido por Yokoyama y cols. (1992) en quesos experimentales. Por lo tanto, las condiciones de presurización citadas no garantizarían el empleo industrial de la APH como tecnología para la aceleración de la maduración del queso Cheddar.

1.3.2.2. Cambios bioquímicos inducidos por la presión en el queso

Diversos estudios han examinado los cambios inducidos por la presión en aspectos bioquímicos de la propia maduración del queso (metabolismo de la lactosa y el lactato, proteolisis y lipolisis). El uso potencial de la APH para acelerar la maduración del queso se describió por primera vez en la patente de Yokama y cols. (1992). De este modo, el tratamiento de quesos Cheddar y Parmesano por APH (de 5 a 300 MPa durante 3 días a 25 ºC) mostró la posibilidad de reducir considerablemente los tiempos de maduración de esos quesos mediante la aplicación de la APH (50 MPa, 3 días, 25 ºC). Los índices que se evaluaron fueron la proteolisis y el perfil aromático del queso.

En otras ocasiones, es la leche la que es tratada mediante APH previamente a su transformación en queso. Trujillo y cols. (1999) trataron leche de cabra a 500 MPa durante 15 min a 20 ºC y los quesos obtenidos a partir de esa leche mostraron un elevado pH y contenido en sal, madurando más rápidamente y desarrollando fuertes aromas. La presencia de pequeños péptidos y aminoácidos libres indicaron una mayor proteolisis en dichos quesos. Posteriormente, Buffa y cols. (2001) encontraron niveles similares de lipolisis en quesos elaborados a partir de leche cruda y presurizada (500 MPa, 15 min, 20 ºC), mientras que los niveles de lipolisis en quesos de leche pasteurizada (72 ºC, 15 s) fueron inferiores.

El estudio del perfil volátil de los quesos tratados por alta presión es una herramienta empleada con frecuencia para valorar el efecto que dicho tratamiento tiene sobre la formación de compuestos volátiles. En este sentido, algunos investigadores analizaron los cambios producidos en el perfil volátil de quesos elaborados a partir de leche cruda de oveja. Así, Arqués y cols. (2007) evaluaron la variación en los compuestos volátiles del queso La Serena en quesos tratados a 300 y 400 MPa durante 10 min a día 2 y 50 de maduración, obteniendo una menor influencia sobre el perfil volátil y propiedades sensoriales en los quesos tratados a día 50. Por otra parte, Juan y cols. (2007a) investigaron el efecto de la APH (a 200, 300, 400 y 500 MPa, 10 min) sobre la composición volátil de quesos de leche pasteurizada de oveja tratados en dos estadios de maduración (1 y 15 días) y concluyeron que los quesos tratados a día 15 poseían un perfil volátil más similar a los quesos control que los tratados a día 1. Por tanto, estos estudios indicarían que las modificaciones de las características originales de los quesos procesados por APH fueron menores cuanto más avanzado es el estado de maduración del queso. Según nuestro conocimiento, sólo hay un estudio en quesos de cabra de leche pasteurizada que analiza el efecto de la APH (400 MPa durante 5 min a día 21 y 60 de maduración) sobre los compuestos volátiles. El estudio revela la reducción del contenido en AGL volátiles en los quesos tratados (Saldo y cols., 2003).

El efecto de la APH sobre la lipolisis de los quesos también ha sido objeto de estudio en aras a determinar la influencia del proceso de presurización en la liberación de AGL, con un papel relevante sobre el flavor de los quesos. Como vimos con anterioridad, Buffa y cols. (2001) analizaron los perfiles de AGL de quesos elaborados a partir de leche cruda, pasteurizada y presurizada. Otros investigadores evaluaron el efecto de la APH (200, 300, 400 o 500 MPa, 10 min) a día 1 y 15 de maduración, sobre quesos de leche pasteurizada de oveja (Juan y cols., 2007b), encontrando una disminución de AGL en quesos presurizados a intensidades de 400 MPa o superiores. Estos resultados les llevaron a sugerir el uso de dichos tratamientos para detener la lipolisis en aquellas variedades de quesos en las que un exceso de lipolisis no es deseable por la aparición de sabores a rancio. Recientemente Voigt y cols. (2010) también consideraron la posibilidad de detener, al menos parcialmente, la maduración del queso azul veteado tratado a 600 MPa, debido principalmente a la reducción de la liberación excesiva de AGL, impidiendo así el posible desarrollo de aromas indeseables.

La proteolisis es otra vía metabólica extensamente analizada en lo referente a la influencia del tratamiento de APH sobre el proceso de maduración de quesos. Al comienzo de este apartado se ha comentado que la proteolisis constituyó el objeto de estudio en las primeras investigaciones sobre los cambios bioquímicos inducidos en los quesos procesados por APH (Yokama y cols., 1992).

Los quesos de leche pasteurizada de cabra tratados por APH a 50 MPa durante 3 días ó 400 MPa 5min seguido de 50 MPa, 72 h, a 14 ºC, mostraron una proteolisis más rápida y unos mayores valores de pH que los quesos sin tratar, posiblemente debido a un efecto primario de la presión sobre las enzimas o un efecto secundario debido a cambios de pH (Saldo y cols., 2000b). Saldo y cols. (2002) encontraron que el tratamiento mediante APH produjo un aumento de los niveles de aminoácidos libres, aunque los quesos tratados a 400 MPa (5 min) presentaron perfiles peptídicos y caseínicos similares a los quesos no tratados con un menor tiempo de maduración y a los quesos tratados a 50 MPa (72 h). La actividad plasmina no se vio afectada por la

presurización, mientras que la actividad coagulante disminuyó con el tratamiento a 400 MPa.

El queso Cheddar tratado a presiones superiores a los 400 MPa tuvo una tasa de liberación de aminoácidos libres significativamente inferior a la de los quesos control (Wick y cols., 2004). Por su parte, Juan y cols. (2004) consiguieron aumentar la proteolisis en quesos de leche de oveja tras la aplicación de tratamientos de 300 MPa; estos cambios fueron atribuidos a la lisis celular inducida por la presión. Además, propusieron que los tratamientos a intensidades de 400 MPa o superiores podían ser un método útil para disminuir o detener la proteolisis del queso. En relación con estos resultados, en quesos de oveja tratados a 500 MPa (10 min, 12 ºC) los contenidos de aminoácidos libres fueron menores que en quesos control; sin embargo, se observó un aumento de la proteolisis en quesos tratados a 300 MPa, que fue atribuido igualmente a la lisis celular, aunque también podrían influir los cambios conformacionales producidos en la matriz del queso por la APH.

Por lo tanto, el efecto del tratamiento mediante APH depende de varios factores, como son el tiempo de tratamiento, intensidades de presión, momento del tratamiento y/o tipo de quesos, entre otros.

1.3.2.3. La textura y el análisis sensorial de quesos tratados por APH

Las propiedades viscoelásticas del queso Gouda tratado a 225 y 400 MPa, inmediatamente post-presurización, difirieron significativamente a las de los quesos no tratados (Messens y cols., 2000). Después de 42 días de maduración, las propiedades reológicas del queso Gouda tratado por APH fueron iguales que en los quesos control, por lo que dichas propiedades se restablecieron durante la maduración. En este mismo queso, Kolakowski y cols. (1998) vieron que los quesos presurizado entre 0 y 500 MPa durante 4 h tuvieron mayor elasticidad y mejores propiedades organolépticas que los quesos control, evaluado por un panel de catadores expertos.

La microscopía de escaneo confocal es una técnica útil para observar cambios microestructurales después del tratamiento mediante APH de los quesos. Con el empleo de esta técnica, O'Reilly y cols. (2000) no observaron ningún cambio estructural notable en queso Cheddar comercial presurizado a 50 MPa durante 3 días a 25 ºC. Por otro lado, los quesos de leche de cabra tratados a 450 MPa a 10 ºC durante 5–30 min, mostraron una matriz proteica más homogénea que la de quesos sin presurizar (Capellas y cols., 1996). De esta manera, existe la posibilidad de obtener nuevas texturas en quesos reduciendo la variabilidad.

En un estudio realizado sobre quesos elaborados a partir de leche cruda, pasteurizada (72 ºC, 15 s) y presurizada (500 MPa, 15 min, 20 ºC) de cabra (Buffa y cols., 2001), los quesos elaborados con leche presurizada recibieron las mayores puntuaciones en la evaluación sensorial de un panel entrenado; sin embargo, no hubo diferencias significativas entre los distintos quesos. Juan y cols. (2008) encontraron que el tratamiento de APH (300 MPa durante 10 min) aplicado a quesos de oveja de 1 día de maduración modificó las propiedades reológicas y sensoriales de los quesos. Los quesos presurizados fueron más blandos y elásticos y menos friables, y los valores para el sabor, olor y calidad del aroma descendieron con respecto a los quesos control. Por otro lado, los quesos tratados a día 15 fueron muy similares a los quesos control, pero con una red proteica más homogénea y una textura menos friable. En este sentido, Saldo y cols. (2000b) concluyeron que los quesos presurizados (400 MPa, 5 min, seguido de 50 MPa, 72 h, a 14 ºC) fueron menos friables y más elásticos que los control. En cuanto a los atributos sensoriales se detectaron notas amargas en alguno de los quesos presurizados.

Referencias

Ahn, J., Balasubramaniam, V.M., & Yousef, A.E. (2007). Inactivation kinetics of selected aerobic and anaerobic bacteria spores by pressure-assisted thermal processing. International Journal of Food Microbiology, 113, 321–329.

Arqués, J.L., Garde, S., Fernández-García, E., Gaya, P., & Núñez, M. (2007). Volatile Compounds, Odor, and Aroma of La Serena Cheese High-Pressure Treated at Two Different Stages of Ripening. Journal of Dairy Science, 90, 3627–3639.

Arqués, J.L., Rodríguez, E., Gaya, P., Medina, M., & Nuñez, M. (2005). Effect of combinations of high-pressure treatment and bacteriocin-producing lactic acid bacteria on the survival of *Listeria monocytogenes* in raw milk cheese. International Dairy Journal, 15, 893–900.

Atasoy, A.F., & Türkoglu, H. (2009). Lipolysis in Urfa cheese produced from raw and pasteurized goats' and cows'milk with mesophilic or thermophilic cultures during ripening. Food Chemistry, 115, 71–78.

Balasubramaniam, V.M., Farkas, D. & Turek, E.J. (2008). By destroying pathogenic and spoilage organisms while keeping food chemistry basically intact, high-pressure technology enables pasteurization of foods with minimal effects on taste, texture, appearance, or nutritional value. Food Technology, 11, 32–38.

Balci, A.T., & Wilbey, R.A. (1999). High Pressure Processing of Milk–the First 100 Years in the Development of a New Technology. International Dairy Technology, 52, 149–155.

Buffa, M.N., Guamis, B., Pavia, M., & Trujillo, A.J. (2001). Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 175–179.

Capellas, M., Mor-Mur, M., Sendra, E., Pla, R., & Guamis, B. (1996). Populations of Aerobic Mesophiles and Inoculated E. coli During Storage of Fresh Goat's Milk' Cheese Treated with High Pressure. Journal of Food Protection, 59, 582–587.

Carbonell, M., Núñez, M., & Fernández-García, E. (2002). Evolution of the volatile components of ewe raw milk La Serena cheese during ripening. Correlation with flavour characteristics. Le Lait, 82, 683–698.

Carminati, D., Gatti, M., Bonvini, B., Neviani, E., & Mucchetti, G. (2004). High pressure processing of Gorgonzola cheese: influence on *Listeria monocytogenes* inactivation and on sensory characteristics. Journal of Food Protection, 67, 1671–1675.

Castillo, I., Calvo, M.V., Alonso, L., Juárez, M., & Fontecha, J. (2007). Changes in lipolysis and volatile fraction of a goat cheese manufactured employing a hygienized rennet paste and a defined strain starter. Food Chemistry, 100, 590–598.

Christie, W.W. (1983). The composition and structure of milk lipids. In Fox P.F. (Ed.), Developments in dairy chemistry–2 (pp. 1–35). Applied Science Publishers, London.

Cogan, T.M., & Hill, C. (1993). Cheese starter cultures. In Fox P.F. (Ed.), Cheese: Chemistry, Physics and Microbiology (2nd ed.) (pp. 193–255). Chapman & Hall, London.

Collins, Y.F., McSweeney, P.L.H., & Wilkinson, M.G. (2003). Lipolysis and free fatty acid catyabolism in cheese: A review of current knowledge. International Dairy Journal, 13, 841–866.

Curioni, P.M.G., & Bosset, J.O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

Deeth, H.C., & Fitz-Gerald, C.H. (1995). Lipolyticenzymes and hydrolytic rancidity in milk and milk products. In Fox P.F. (Ed.), Advanced dairy chemistry–2–Lipids (pp. 247–308). Chapman & Hall, London.

Delgado, F.J., González-Crespo, J., Cava, R., García-Parra, J., & Ramírez, R. (2010). Characterization of the volatile profile of a Spanish ewe raw milk soft cheese P.D.O. Torta del Casar during ripening by SPME-GC-MS. Food Chemistry, 118, 182–189.

Dunne, C.P. (2005). High pressure keeps food fresher. Available at http://www.natick.army.mil/about/pao/05/05-22.htm. Accessed October 8, 2008.

Engels, W.J.M., Dekker, R., de Jong, C., Neeter, R., & Visser, S. (1997). A comparative study of volatile compounds in the water soluble fraction of various types of ripened cheese. International Dairy Journal, 7, 255–263.

Fernández-García, E., Gaya, P., Medina, M., & Núñez, M. (2004). Evolution of the volatile components of ewes' raw milk Zamorano cheese: seasonal variation. International Dairy Journal, 14, 701–711.

Fontecha, J., Castillo, I., Blasco, L., Alonso, L., & Juárez, M. (2006). Effect of artisanal kid rennet paste on lipolysis in semi-hard goat cheese. Food Chemistry, 98, 253–259.

Fox, P.F., & Stepaniak, L. (1993). Enzymes in cheese technology. International Dairy Journal, 3, 509–530.

Fox, P.F., Law, J., McSweeney, P.L.H., & Wallace, J. (1993). Biochemistry of cheese ripening. In Fox P.F. (Ed.), Cheese: Chemistry, physics and microbiology (2nd ed.), Vol. 1 (pp. 389–438). Chapman & Hall, London.

Fox, P.F., Singh, T.K., & McSweeney, P.L.H. (1995). Biogenesis of flavour compounds in cheese. In Malin E.L., Tunick M.H. (Eds.), Chemistry of Structure/Function Relationships in Cheese (pp. 59–98). Plenum Press, New York.

Fox, P.F., McSweeney, P.L.H., & Lynch, C.M. (1998). Significance on nonstarter lactic acid bacteria in Cheddar cheese**.** Australian Journal of Dairy Technology, 53, 83–89.

Fox, P.F., & Wallace, J.M. (1997). Formation of flavour compounds in cheese. Advances in Applied Microbiology, 45, 17–85.

Fox, P.F., Guinee, T.P., Cogan, T.M., & McSweeney, P.L.H. (2000). Fundamentals of cheese science. Gaithersburg, MD: Aspen Publishers Inc.

Franco, I., Prieto, B., Bernardo, A., González-Prieto, J., & Carballo, J. (2003). Biochemical changes throughout the ripening of a traditional Spanish goat cheese variety (Babia-Laciana). International Dairy Journal, 13, 221–230.

Galán, E., Prados, F., Pino, A., Tejada, L., & Fernández-Salguero, J. (2008). Influence of different amounts of vegetable coagulant from cardoon Cynara cardunculus and calf rennet on the proteolysis and sensory characteristics of cheeses made with sheep milk. International Dairy Journal, 18, 93–98.

Gallot-Lavallé, T. (1998). Efficacité du traitement par les hautes pressions sur la destruction de Listeria monocytogenes dans les fromages de chèvre au lait cru. Science of Aliments, 18, 647–655.

Guillén, M.D., Ibargoitia, M.L., Sopelana, P., Palencia, G., & Fresno, N. (2004). Components Detected by Means of Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry in the Headspace of Artisan Fresh Goat Cheese Smoked by Traditional Methods. Journal of Dairy Science, 87, 284–299.

Gunstone, F.D., Harwood, J.L., & Padley, F.B. (1994). Milk lipids. In The lipid handbook (2nd ed.) (pp. 195–197). Chapman & Hall, London.

Hayashi, R. (1989). Application of High Pressure to Food Processing and Preservation: Philosophy and Development. In Spiess, W.E.L. and Schubert, H. (Eds.), Engineering and Food (pp. 815–826). Elsevier Applied Science, London.

Hayman, M., Baxter, I., Oriordan, P.J., & Stewart, C.M. (2004). Effects of high-pressure processing on the safety, quality, and shelf life of ready-to-eat meats. Journal of Food Protection 67, 1709–1718.

He, H., Adams, R.M., Farkas, D.F., & Morrissey, M.T. (2002). Use of High-pressure Processing for Oyster shucking and shelf-life extension. Journal of Food Science, 67, 640–645.

Izco, J. M., & Torre, P. (2000). Characterisation of volatile flavour compounds in Roncal cheese extracted by the purge and trap method and analysed by GC-MS. Food Chemistry, 70, 409–417.

Jensen, R.G., Ferris, A.M., & Lammi-Keefe, C.J. (1991). The composition of milk fat. Journal of Dairy Science, 74, 3228–3243.

Johnson, M.E. 1998. Cheese products. In Marth E.H. and Steele J.L (Eds.), Applied Dairy Microbiology (pp.213–249). Marcel Dekker, Inc. New York.

Juan, B., Ferragut, V., Guamis, B., Buffa, M., & Trujillo, A. J. (2004). Proteolysis of a high pressure-treated ewe's milk cheese. Milchwissenschaft, 59, 616–619.

Juan, B., Barron, L.J.R., Ferragut, V., & Trujillo, A.J. (2007a). Effects of High Pressure Treatment on Volatile Profile During Ripening of Ewe Milk Cheese. Journal of Dairy Science, 90, 124–135.

Juan, B., Ferragut, V., Buffa, M., Guamis, B., & Trujillo, A.J. (2007b). Effects of high-pressure treatment on free fatty acids release during ripening of ewes' milk cheese. Journal of Dairy Research, 74, 438–445.

Juan, B., Ferragut, V., Guamis, B., & Trujillo, A.J. (2008). The effect of high-pressure treatment at 300 MPa on ripening of ewes' milk cheese. International Dairy Journal, 18, 129–138.

Kinsella, J.E. (1975). Butter flavor. Food Technology, 29, 82–98.

Kolakowski, P., Reps, A., & Babuchowski, A. (1998). Characteristics of Pressure Ripened Cheeses. Polish Journal of Food Nutritional Science, 48, 473–483.

Larrayoz, P., Addis, M., Gauch, R., & Bosset, J.O. (2001). Comparison of dynamic headspace and simultaneous distillation extraction techniques used for the

analysis of the volatile components in three European PDO ewes' milk cheeses. International Dairy Journal, 11, 911–926.

López-Pedemonte, T., Roig-Sagués, A., De Lamo, S., Hernández-Herrero, M., & Guamis, B. (2007). Reduction of counts of *Listeria monocytogenes* in cheese by means of high hydrostatic pressure. Food Microbiology, 24, 59–66.

Margosch, D., Ehrmann, M.A., Buckow, R., Heinz, V., Vogel, R.F., & Gonzalez, M.G. (2006). High-pressure-mediated survival of Clostridium botulinum and Bacillus amyloliquefaciens endospores at high temperature. Applied and Environmental Microbiology, 72, 3476–3481.

Martínez-Castro, I., Sanz, J., Amigo, L., Ramos, M., & Martínez-Alvarez, P. (1991). Volatile components of Manchego cheese. Journal of Dairy Research, 58, 239–246.

Mas, M., Timón, J., & González-Crespo, J. (1991). Queso de los Ibores: caracterización productiva, fisicoquímica y microbiológica. Archivos de Zootecnia, 40, 103–113.

Matser A.M., Krebbers B., Berg, R.W., & Bartels, P.V. (2004). Advantages of high pressure sterilisation on quality of food products. Trends in Food Science and Technology, 15, 79–85.

McSweeney, P.L.H. (1997). The flavour of milk and dairy products: III. cheese taste. International Journal of Dairy Technology, 50, 123–128.

McSweeney, P.L.H., & Fox, P.F. (1997). Indices of Cheddar cheese ripening, Proc. 5th Cheese Symp., Moorepark, Fermoy, Co. Cork, Ireland, pp. 73–89.

McSweeney, P.L.H., & Sousa, M.J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening. A review. Lait, 80, 293–324.

Messens, W., Van de Walle, D., Arevalo, J., Dewettinck, K., & Huyghebaert, A. (2000). Rheological Properties of High-pressure-treated Gouda Cheese. International Dairy Jorunal, 10, 359–367

Moio, L., Dekimpe, J., Etievant, P.X., & Addeo, F. (1993). Volatile flavour compounds of water buffalo Mozzarella cheese. Journal of Food Science, 5, 57–68.

Molimard, P., & Spinnler, H.E. (1996). Review: Compounds involved in the flavour of surface mould-ripened cheeses: Origins and properties. Journal of Dairy Science, 79, 169–184.

Mulet, A., Escriche, I., Rossello, C., & Tarrazó, J. (1999). Changes in the volatile fraction during ripening of Mahón cheese. Food Chemistry, 65, 219–225.

O'Reilly, C.E., O'Connor, P.M., Kelly, A.L., Beresford, T.P., & Murphy, P.M. (2000). Use of Hydrostatic Pressure for Inactivation of Microbial Contaminants in Cheese. Applied and Environmental Microbiology, 66, 4890–4896.

Olivecrona, T., Vilaro, S., & Bengtsson-Olivecrona, G. (1992). Indigenous enzymes in milk. II. Lipases in milk. In Fox P.F. (Ed.), Advanced dairy chemistry. 1. Proteins (pp. 292–310). Elsevier Applied Science, London.

Patazca, E., Koutchma, T., & Balasubramaniam, V.M. (2007). Quasiadiabatic temperature increase during high pressure processing of selected foods. Journal of Food Engineer, 80, 199–205.

Plutowska, B., & Wardencki, W. (2007). Aromagrams – Aromatic profiles in the appreciation of food quality. Food Chemistry, 101, 845–872.

Poveda, J.M., & Cabezas, L. (2006). Free fatty acid composition of regionally-produced Spanish goat cheese and relationship with sensory characteristics. Food Chemistry, 95, 307–311.

Rasanayagam, V., Balasubramaniam, V.M., Ting, E., Sizer, C.E., Bush, C., & Anderson, C. (2003). Compression heating of selected fatty food materials during high pressure processing. Journal of Food Science, 68, 254-259.

Reps, A., Kolakowski, P., & Dajnowiec, F. (1998). The Effect of High Pressure on Microorganisms and Enzymes of Ripening Cheeses. In Isaacs, N.S. (Ed.), High

Pressure Food Science, Bioscience and Chemistry (pp. 265–270). Royal Society of Chemistry, Cambridge, UK.

Ritz, M., Tholozan, J.L., Federighi, M., & Pilet, M.F. (2001). Morphological and physiological characterization of Listeria monocytogenes subjected to high hydrostatic pressure. Applied and Environmental Microbiology, 67, 2240–2247.

Rothe, M., Woelm, G., Tunger, L., & Siebert, H.J. (1972). Schwellenkonzentration von Aromastoffen und ihre Nutzungzur Auswertung von Aromaanalysen. Nahrung, 16, 483–495.

Sáiz, A.H., Mingo, S.T., Balda F.P., & Samson, C.T. (2008). Advances in design for successful commercial high pressure food processing. Food Australia, 60, 154–156.

Saldo, J., McSweeney, P.L.H., Sendra, E., Kelly, A.L., & Guamis, B. (2000a). Changes in Curd Acidification Caused by High Pressure Treatment. Irish Journal of Agriculture Food Research, 39, 169.

Saldo, J., Sendra, E., & Guamis, B. (2000b). High hydrostatic pressure for accelerating ripening of goat's milk cheese: Proteolysis and texture. Journal of Food Science, 65, 636–640.

Saldo, J., McSweeney, P.L.H., Sendra, E., Kelly, A.L., & Guamis, B. (2002). Proteolysis in caprine milk cheese treated by high pressure to accelerate cheese ripening. International Dairy Journal, 12, 35–44.

Saldo, J., Fernández, A., Sendra, E., Butz, P., Tauscher, B., & Guamis, B. (2003). High pressure treatment decelerates the lipolysis in a caprine cheese. Food Research International, 36, 1061–1068.

Samson, C. Tonello (2008). Personal communication. NC Hyperbaric, Spain.

Siek, T.J., Albin, I.A., Sather, L.A., & Lindsay, R.C. (1971). Comparison of flavour thresholds of aliphatic lactones with those of fatty acids, esters, aldehydes, alcohols, and ketones. Journal of Dairy Science, 54, 1–4.

Sousa, M.J.C.F. (1993). Plant Rennet Substitute from Flowers of Cynara cardunculus. M.Sc. Thesis, National University of Ireland, Cork.

Sousa, M.J., Ardö, Y., & McSweeney, P.L.H. (2001). Advances in the study of proteolysis during cheese ripening. International Dairy Journal, 11, 327–345.

Steffen, C., Flueckiger, E., Bosset, J.O., & Ruegg, M. (1987). Swisstype varieties. In Fox P.F. (Ed.), Cheese: chemistry, physics and microbiology (pp. 93–120). Elsevier Applied Science, London.

Szczawinski, J., Szczawinska, M., Stanczak, B., Fonberg-Broczek, M., & Szczepeck, J. (1997). Effects of high pressure on survival of Listeria monocytogenes in ripened, sliced cheeses at ambient temperature. In: Heremans, K. (Ed.), High Pressure Research in the Biosciences andBiotechnology. University Press, Leuven, Belgium, pp. 295–298.

Trujillo, A.J., Royo, C., Ferragut, V., & Guamis, B. (1999). Ripening profiles of goat cheese produced from milk treated with high pressure. Journal of Food Science, 64, 833–837.

Urbach, G. (1993). Relations between cheese flavour and chemical composition. International Dairy Journal, 3, 389–422.

Urbach, G. (1997). The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds. International Journal of Dairy Technology, 50, 79–89.

Villaseñor, M.J., Valero, E., Sanz, J., & Martínez- Castro, I. (2000). Analysis of volatile compounds of Manchego cheese by dynamic headspace followed by automatic thermal desorption-GCMS. Milchwissenschaft, 55, 378–382.

Voigt, D.D., Chevalier, F., Qian, M.C., & Kelly, A.L. (2010). Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innovative Food Science and Emerging Technologies, 11, 68–77.

Wick, C., Nienabert, U., Anggraeni, O., Shellhammer, T.H., & Courtney, P.D. (2004). Texture, proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure. Journal of Dairy Research, 71, 107–115.

Woo, A.H., Kollodge, S., & Lindsay, R.C. (1984). Quantification of major free fatty acids in several cheese varieties. Journal of Dairy Science, 67, 874–878.

Yokohama, H., Swamura, N., & Motobyashi, N. (1992). Method for accelerating cheese ripening. European Patent Application EP 0 469 857 A1.

2. JUSTIFICACIÓN Y OBJETIVOS

2.1. JUSTIFICACIÓN

La maduración es una de las partes del proceso de elaboración del queso en que pueden realizarse importantes acciones para reducir los costes de producción en la industria quesera. Por su naturaleza, la maduración se trata de un proceso prolongado que implica la inmovilización de producto, la inversión en cámaras de maduración de gran capacidad así como costes asociados con el mantenimiento de las condiciones termohigrométricas y de velocidad de aire necesarias para una correcta maduración. A estos factores, hay que añadir el riesgo de aparición de defectos en los quesos con la consiguiente pérdida total o parcial del valor del producto.

El empleo de leche cruda para la elaboración de quesos supone un riesgo microbiológico para los consumidores de este tipo de queso. En general, estos quesos, a pesar de cumplir los 60 días de maduración mínimos exigidos por ley (Decreto 69/2003), no están exentos de peligros microbiológicos. La tecnología de alta presión hidrostática es una de las más prometedoras de entre las llamadas tecnologías emergentes para reducir el peligro asociado con agentes patógenos en este tipo de alimento. El procesado por alta presión hidrostática (APH) podría ser una solución a este problema ya que permite la destrucción y/o inactivación de microorganismos patógenos y alterantes provocando mínimos cambios en la calidad del alimento en comparación con el efecto que tienen las tecnologías convencionales, como es el caso del tratamiento térmico. Este tratamiento permitiría aumentar la seguridad alimentaria de este tipo de queso al mismo tiempo que estabilizar el producto durante el almacenamiento posterior al reducir su carga microbiana. Sin embargo, se hace necesario evaluar si el tratamiento de APH provoca alteraciones en la maduración del queso, y por tanto, si modifica la calidad del producto final. Por ello, la aceptación de esta tecnología para su aplicación en el Queso Ibores, al igual que en otros quesos, estaría supeditada al mantenimiento de las características sensoriales y de calidad reconocida por los consumidores. No obstante, la aplicación de la tecnología de alta

presión hidrostática en los quesos podría generar la aparición de efectos secundarios de interés para su aplicación industrial.

2.2. OBJETIVOS

Los **objetivos** que se pretenden alcanzar con la realización de la tesis son:

1. Estudiar los cambios madurativos en los parámetros de calidad de quesos de cabra de la DOP Queso Ibores, así como evaluar las principales reacciones implicadas en la formación de sus características de calidad durante su maduración.

2. Analizar del efecto de los tratamientos de altas presiones hidrostáticas sobre la microbiología, las propiedades físico-químicas y sensoriales, la textura y las rutas bioquímicas de maduración implicadas en la formación del aroma y sabor de los quesos de cabra de la DOP Queso Ibores.

3. DISEÑO EXPERIMENTAL

DISEÑO EXPERIMENTAL

Para alcanzar los objetivos propuestos en el apartado anterior se diseñaron una serie de experimentos que se detallan a continuación.

Para alcanzar el **Objetivo 1** se caracterizaron los quesos en cuanto a su composición y parámetros físico-químicos y se estudiarán los cambios en los parámetros de calidad durante la maduración de los quesos tomando muestras de quesos de 3 queserías adscritas a la DOP con diferentes estados de maduración (día 1, 30, 60 y 90). Se realizaron las siguientes determinaciones (Ensayo I):

48 quesos (=lote)
(4 quesos x 3 queserías
x 4 estados de maduración)

- Composición fisicoquímica
- Color instrumental
- Análisis del perfil de textura (APT)
- Índices de proteolisis secundaria
- Cuantificación de ácidos grasos libres
- Oxidación de lípidos y proteínas
- Identificación de compuestos volátiles

Para lograr el **Objetivo 2**, se trataron mediante altas presiones hidrostáticas los quesos a diferentes estadios de maduración con distintas combinaciones de presión (400 ó 600 MPa, 7 min). El efecto del tratamiento se evaluó tras la aplicación del procesado y al final de la maduración. Se realizaron dos tipos de ensayos para la consecución de este objetivo, utilizando quesos con las presentaciones comerciales más frecuentes en este tipo de quesos (sin recubrimiento y con recubrimiento de pimentón):

Ensayo II.- Tratamiento de APH en quesos sin recubrimiento en corteza a diferentes momentos de maduración (día 1, 30 y 50) y evaluación del efecto a día 2, 31 y 60, con el fin de determinar el efecto de APH en quesos con distinto estado de maduración. Las analíticas llevadas a cabo aparecen en el siguiente cuadro derecho.

52 quesos (=lote)
(1 quesería; 2 intensidades de tratamiento en 3 estados de maduración)

- Análisis microbiológico
- Composición fisicoquímica
- Color instrumental
- Análisis del perfil de textura (APT)
- Índices de proteolisis secundaria
- Cuantificación de ácidos grasos libres
- Oxidación de lípidos y proteínas
- Identificación de compuestos volátiles

Ensayo III.- Tratamiento de APH en quesos con recubrimiento en corteza (pimentón) al final de la maduración (día 60) y evaluación del efecto a día 60 y 90, para conocer el efecto del APH en quesos maduros pimentonados durante el almacenamiento. El cuadro de abajo a la derecha refleja las analíticas realizadas a estos quesos.

24 quesos (=lote)
(1 quesería; 2 intensidades de tratamiento al final de la maduración)

- Análisis microbiológico
- Composición fisicoquímica
- Color instrumental
- Análisis del perfil de textura (APT)
- Índices de proteolisis secundaria
- Identificación de compuestos volátiles
- Evaluación sensorial

Para facilitar la presentación y discusión de los resultados, la tesis ha sido dividida en 8 capítulos, agrupados de acuerdo a los objetivos a alcanzar con el diseño experimental propuesto.

Objetivo 1.- Estudios a lo largo de la maduración el Queso Ibores:

Capítulo I. "Proteolysis, colour and texture of a raw goat milk cheese throughout the maturation"

Capítulo II. "Free fatty acids and oxidative changes of a raw goat milk cheese through maturation"

Capítulo III. "Formation of the aroma of a raw goat milk cheese along maturation analysed by SPME–GC–MS"

Objetivo 2.- Efectos del tratamiento de APH sobre el Queso Ibores

2a.- Efecto de la aplicación de APH durante la maduración en quesos sin recubrimiento en corteza:

Capítulo IV. "Changes in microbiology, proteolysis, texture and sensory characteristics of raw goat milk cheeses treated by high-pressure at different stages of maturation"

Capítulo V. "Influence of high pressure treatment on free fatty acids and oxidation processes throughout maturation of a raw goat milk cheese"

Capítulo VI. "Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation"

2b.- Efecto de la aplicación de APH en quesos maduros con recubrimiento en corteza (pimentón). Cambios durante el almacenamiento:

Capítulo VII. "Changes during storage on microbiology, proteolysis, texture and sensory evaluation of a mature raw goat milk high-pressure treated cheese with paprika on rind"

Capítulo VIII. “Effect of high-pressure treatment on the volatile profile of a mature raw goat milk cheese with paprika on rind”

4. MATERIALES Y MÉTODOS

4.1. MATERIALES

4.1.1. Ensayo I: quesos empleados para el estudio a lo largo de la maduración

Los quesos se elaboraron a partir de leche de cruda de cabra en 3 queserías de la Denominación de Origen Protegida (DOP) Queso Ibores: "Berrocales Trujillanos", "S.A.T. Quesería de las Villuercas" y "Quesería Almonte". Para facilitar el proceso de acidificación, se empleó una mezcla de cultivos mesófilos liofilizados (R-704, 50 units; Chr. Hansen, Hørsholm, Denmark) que contenía *Lactococcus lactis subsp. cremoris* y *L. lactis subsp. lactis*. La leche se coaguló a una temperatura de 28–32 ºC en 60–90 minutos con la adición de cuajo animal (Naturen Plus 175, 20-25 mL per 100 L; Chr Hansen, Hørsholm, Denmark). La cuajada se cortó en granos de tamaño medio (1–2 cm). Los quesos de forma cilíndrica, con un peso de 0.7–1.2 kilogramos, se prensaron durante 3–8 h, se salaron en salmuera y pasaron a las cámaras de maduración (8–12 ºC y humedad relativa del 80%). La figura 1 muestra el diseño experimental del ensayo con indicación de tiempos de muestreo y número de muestras analizadas a cada tiempo de análisis.

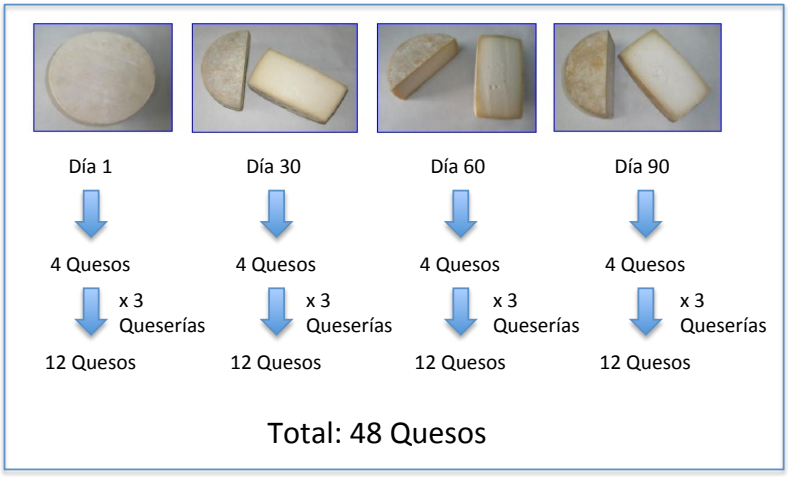

Figura 1. Muestreo de los quesos utilizados para el estudio a lo largo de la maduración.

Para la realización del estudio, se tomaron 4 quesos procedentes de cada una de las 3 queserías mencionadas de la DOP en cuatro estados de maduración diferentes (1, 30, 60 y 90 días). Por tanto, un total de 48 quesos fueron analizados, 12 de cada momento de la maduración (Figura 1). Durante los primeros 60 días de maduración los quesos permanecieron en cada quesería. El último mes los quesos se mantuvieron en refrigeración a 5 ºC. Los quesos se dividieron en cuñas que fueron almacenadas a -20 ºC hasta su análisis.

4.1.2. Ensayo II: quesos sin recubrimiento de pimentón en corteza tratados por APH a lo largo de la maduración

Para el estudio del efecto de la APH sobre quesos sin recubrimiento de pimentón en corteza se adquirieron un total de 52 quesos de una misma quesería, "Berrocales Trujillanos" (Tabla 1). El proceso de elaboración fue idéntico al descrito en el apartado anterior. Los quesos (4 quesos por lote) se trataron mediante APH a 400 ó 600 MPa durante 7 minutos a tres tiempos diferentes de maduración (día 1, 30 y 50). Los quesos se analizaron un día después del tratamiento de APH y al final de la maduración (día 60), excepto los quesos tratados con 50 días de maduración, que se mantuvieron envasados al vacío hasta su análisis a día 60 para simular las verdaderas condiciones de maduración si el tratamiento de APH fuese considerado interesante para los productores. Los quesos no tratados se usaron como control (n=4) en cada momento de análisis. Por lo tanto, a día 1, se analizaron 12 quesos: control (n=4), 400 MPa (n=4), 600 MPa (n=4); a día 30, otros 12 quesos fueron analizaron: control (n=4), 400 MPa (n=4), 600 MPa (n=4); y a día 60, se analizaron 28 quesos: control (n=4) y tratados a día 1, 30 y 50 a 400 MPa y 600 MPa (n=24; 3 días x 2 tratamientos x 4 quesos por lote).

4.1.3. Ensayo III: quesos recubiertos con pimentón en corteza tratados con APH al final de la maduración

Para el estudio del efecto del tratamiento de APH sobre quesos recubiertos con pimentón, se adquirieron 24 quesos de la quesería "Berrocales Trujillanos" al final de la

maduración (60 días). El proceso de elaboración fue similar al descrito en el apartado 3.1.1., a excepción de la aplicación manual de pimentón sobre su corteza al final de la maduración. Al final de la maduración, día 60, los quesos se dividieron en dos lotes de 8 quesos cada uno y se trataron por alta presión a dos condiciones de trabajo 400MPa 7 min (n=8) y 600 MPa 7min (n=8). Una vez tratados por alta presión, los quesos fueron divididos en dos lotes de 4 quesos cada uno y analizados a dos tiempos diferentes, 1. tras el tratamiento de APH y 2. a día 90, tras 30 días de almacenamiento refrigerado (6 ± 1 ºC), simulando las condiciones habituales de conservación en la industria. Se utilizó un grupo de quesos sin tratar (n=4) como control que fue analizado en cada uno de los tiempos de ensayo fijados en el diseño experimental.

Tabla 1. Resumen de los quesos tratados por APH a lo largo de la maduración.

Lote	Condiciones P-t*	Quesos/lote	Aplicación APH	Muestreo	Total muestras
Quesos tratados por APH	400 MPa 7min 600 MPa 7min	4	Día 1	Día 2, día 60 (n=16)	n= 40
			Día 30	Día 31, día 60 (n=16)	
			Día 50	Día 60 (n=8)	
Quesos control (no tratados)	----	4	-----	día 2, 31 y 60	n= 12
				TOTAL QUESOS	n= 52

* P: Presión, t: tiempo de presurización

4.1.4. Tratamiento de los quesos por Alta Presión Hidrostática

Para desarrollar este trabajo se utilizó un equipo comercial de altas presiones hidrostáticas de HYPERBARIC Wave 6000/55, fabricada en Burgos (España) (Figura 2). El equipo tiene un diseño y fiabilidad industrial, con una capacidad de 55L, que aplica un tratamiento máximo de 600 MPa. Dispone de una línea de carga y descarga

automatizada (Tabla 2). Es un equipo ideal para producciones pequeñas y para el desarrollo de nuevos productos.

Figura 2. Equipo de APH modelo Wave 6000/55 de NC Hyperbaric.

Tabla 2. Características técnicas del equipo industrial de altas presiones hidrostáticas, Hyperbaric Wave 6000/55.

Características	Especificaciones
Diámetro de la cámara	200 mm
Longitud de la cámara	2000 mm
Volumen útil de la cámara	55 L
Presión máxima de trabajo	6000 bar
Fluido transmisor de presión	Agua *SIN* aditivos
Temperatura del agua de proceso	5-30ºC
Tiempo de presurización	Inferior a 5min
Sistema carga / descarga contenedores	Automático

Para el tratamiento de APH los quesos fueron envasados al vacío en bolsas de poliamida/polietileno 20/70 con un tamaño de 30x40 cm y 90 µ de espesor, con una permeabilidad al oxígeno de 50 cm^3/m^2 en 24 h a 1 atm, 23 ºC y 75% de humedad

relativa (Eurobag & film S.L., Málaga, España) y tratados en el equipo de APH. La temperatura inicial del agua fue de 10 °C. Las intensidades de tratamiento aplicadas fueron de 400 MPa ó 600 MPa, durante 7 min. Los tiempos para alcanzar 600 MPa y 400 MPa fueron 3 min 50 segundos y 2 min 54 segundos, respectivamente.

4.2. MÉTODOS

4.2.1. Análisis microbiológico

Se realizó un análisis microbiológico de los quesos (en fresco) tratados por APH con y sin recubrimiento de pimentón, y en los pertinentes controles de los ensayos II y III para comprobar la efectividad del tratamiento de APH. Se determinaron los recuentos de microorganismos mesófilos totales, psicrotrofos, enterobacterias, bacterias del ácido láctico (BAL) y micrococcaceas. Se tomaron 10 g de queso que se depositaron en una bolsa para homogenizador a la que se añadieron 90 mL de citrato sódico (2%) y se homogeneizó en un Stomacher (IUL Instruments, Königswinter, Deutschland) durante 5 minutos. A partir de esta solución se hicieron diluciones decimales seriadas utilizando medio Ringer, de las cuales se sembró 1mL en placa por inmersión, a excepción para la determinación de enterobacterias que se sembró en doble capa. Se determinaron los siguientes microorganismos:

i. Mesófilos totales. Como medio de cultivo se empleó Plate Count Agar (PCA), medio de cultivo exento de sustancias inhibidoras y de indicadores, concebido esencialmente para la determinación del número total microorganismos aerobios mesófilos. Para la determinación de mesófilos totales las placas se incubaron durante 72±3 h a 30±1 ºC en aerobiosis.

ii. Psicrótrofos. Se utilizó el mismo medio que para la determinación de mesófilos, PCA, pero con unas condiciones de incubación de 4 ó 5 días a 16 ºC.

iii. Enterobacterias. Para el crecimiento de Enterobacterias se empleó el medio Violet-red Bile Dextrose Agar to Mossel (VRBD Agar), que es un agar selectivo para el

aislamiento y quantificación de microorganismos totales del género *Enterobacteriaceae* en alimentos, según Mossel y cols. (1962, 1963). Las placas se incubaron durante 24 horas a 37 ºC en aerobiosis. Las colonias de color rojo, con halo de precipitación rojizo correspondían a enterobacterias.

iv. Bacterias del ácido láctico (BAL). El medio empleado fue el Man, Rogosa and Sharpe (MRS Broth), un medio para el enriquecimiento, cultivo y aislamiento de todas las especies de *Lactobacillus*, a partir de todo tipo de materiales de investigación, según de Man y cols. (1960). Las placas deben incubarse durante 3 días a 35 ºC en una atmósfera con 5% de CO_2 en campana de anaerobiosis (Merck Anaerocult® C).

v. Micrococaceas. Para su determinación se utilizó el medio Manitol Salt Phenol-red Agar (MSA), un agar selectivo para micrococaceas en alimentos y otros materiales objetos de investigación, según Chapman (1945), modificado. Las placas se incubaron 3 días a 35 ºC en aerobiosis.

vi. *Listeria* spp. A diferencia de las determinaciones anteriores, se añadieron 25 g de queso a 225 ml de caldo Fraser semi (Fraser selective Medium ½) y se homogeneizaron en un Stomacher (IUL Instruments, Königswinter, Deutschland) durante 5 min. El homogeneizado resultante se preincubó durante 20 min a temperatura ambiente, realizando posteriormente diluciones decimales seriadas en caldo Fraser, de las cuales se tomó 1ml para su siembra en placa con el medio Chromogenic Listeria Agar (ISO) Base (Oxoid). Una vez solidificadas se incubaron a 37 ºC durante 24-48h.

En este medio, las colonias de *L. monocytogenes* aparecen rodeadas por un halo opaco. Aunque no se detectaron colonias con dicho fenotipo, alguna de ellas fueron analizadas mediante la determinación de su "huella metabólica" con el empleo de un test discreto usando un equipo BIOLOG (Biolog Inc., California, USA). Después de la inoculación e incubación, los 96 pocillos de las microplacas, que contienen distintas fuentes de carbono, se leyeron mediante la unidad Micro-Station Reader. El patrón metabólico generado por el microorganismo y obtenido a partir de la cuantificación de

las reacciones de color producidas en los pocillos, fue comparado con el de cientos de perfiles de identificación en la base de datos del BIOLOG.

4.2.2. Composición fisico-química

a. pH. El pH se midió directamente en el centro del queso (en fresco) utilizando un pH-metro perforador Crison 20 mod. 507 (Crison Instruments, Barcelona, España).

b. Extracto seco. La materia seca se determinó mediante un método gravimétrico secando las muestras de queso a 102 ºC (IDF 4A/1982).

c. Grasa. El contenido en grasa se analizó por el método acidobutirométrico según Van Gulik empleando un butirómetro con escala calibrada desde 0 a 40% de grasa (IDF 5B/1986).

d. Proteína bruta. El nitrógeno se determinó mediante el método Kjeldahl con la conversión a contenido en proteína bruta utilizando un factor de conversión de 6,38 (IDF 20B/1993).

4.2.3. Color instrumental

Los parámetros de color se determinaron en muestras en fresco con un colorímetro Minolta CR–200 (Minolta Camera Co., Osaka, Japan), con un diámetro de área de exposición de 8 mm y con el empleo de una fuente de iluminación D65, realizando las medidas con un ángulo de 0º. Antes de utilizarse el colorímetro fue calibrado con una placa blanca (Y= 93,2; x= 0,3159; y= 0,3324 para D65). Los valores CIE L*, a* y b* se emplearon para definir las coordenadas de color en el espacio. CIE L* indica luminosidad, con un rango de valor de 0 (completamente opaco o “negro”) a 100 (completamente luminoso o “blanco”); un valor positivo de CIE a* indica color verde y el negativo color verde; por su parte, un valor positivo de CIE b* nos revela color amarillo y el negativo tonos azules. Los quesos se dividieron en mitades de igual tamaño y se realizaron 5 medidas en cada muestra y en distintas localizaciones

aleatorias de las mismas, tomando la media de los valores como dato para el análisis estadístico.

4.2.4. Análisis del Perfil de Textura (APT)

El análisis de la textura se realizó en muestras en fresco con un texturómetro TA-XT2i (Stable Micro Systems S.L., Aname, España). Las muestras se cortaron en cubos de 1 cm de lado. La temperatura de ensayo fue de 15±2 ºC. Se aplicaron dos ciclos de compresión con un 25% de deformación. La velocidad pre-ensayo fue de 3 mm/seg y la de ensayo 1 mm/seg. Se realizaron 8 réplicas de cada muestra, descartando los valores máximos y mínimos. En la Figura 3 se representa una curva de APT y se identifican los parámetros reológicos obtenidos: la dureza (N) definida por el pico máximo de fuerza del primer ciclo de compresión (F1) y que en ocasiones también se denomina firmeza; la adhesividad (N seg) que es el área negativa situada entre ambos ciclos de compresión (A2); la cohesividad, calculada como el cociente del área del segundo ciclo de compresión (A3) entre el área del primer ciclo (A1); la elasticidad (cm), que resulta de la división del tiempo que transcurre desde el inicio al final del ciclo 2 (t2) entre el tiempo transcurrido del inicio al final de ciclo 1 (t1); la gomosidad (N), obtenida multiplicando dureza y cohesividad; y la masticabilidad (N cm), resultado de la multiplicación de la gomosidad y la elasticidad.

4.2.5. Índices de proteolisis secundaria: fracciones de Nitrógeno y aminoácidos libres

Para el análisis de fracciones de Nitrógeno se realizó en primer lugar una dispersión de queso en citrato tri-sódico 0,5 M de la cual se tomaron alícuotas para analizar el nitrógeno total (NT) y el nitrógeno soluble (NS) a pH 4,4. A partir de una muestra del NS se calcula el nitrógeno soluble no proteico (NNP) en ácido tricloroacético al 12%. Todas las fracciones anteriores se determinaron por valoración mediante el método Kjeldahl (Ardö, 1999). Los resultados se expresaron como porcentaje de NS/NT y NNP/NT. Otras fracciones calculadas en porcentaje fueron el nitrógeno caseínico (NC= NT-NS) y el nitrógeno polipeptídico (NP= NS-NNP) (Tejada y cols., 2008).

Para el análisis de aminoácidos libres se preparó una fracción de nitrógeno soluble en agua según Folkertsma y Fox (1992) de la forma siguiente: a 2 g de queso picado se le añadieron 10 mL de agua destilada en una bolsa de Stomacher. La mezcla se homogenizó en un Stomacher durante 5 min. Después, la bolsa con el contenido se mantuvo a 40 ºC durante 1 hora y el homogenizado se centrifugó a 3000 *g* durante 30 min a 4 ºC. Posteriormente el sobrenadante se filtró a través de papel de filtro Whatman (nº 41; 150 mm Ø).

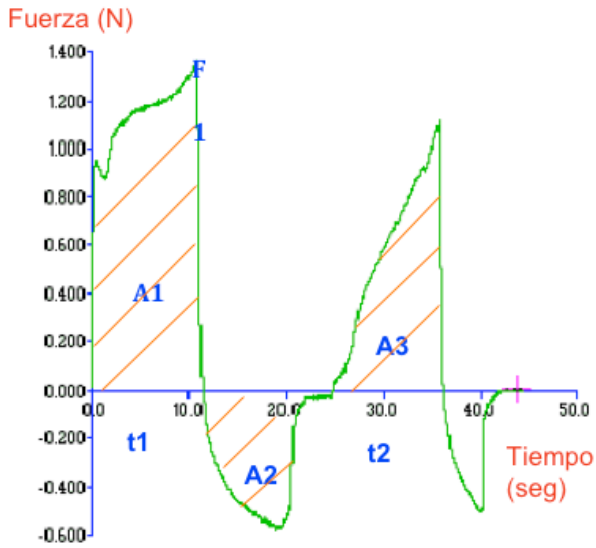

Figura 3. Curva del Análisis de Perfil de Textura.

A continuación se empleó el método de Cd-ninhidrina descrito también por Folkertsma y Fox (1992). Una muestra de nitrógeno soluble (20–100 µL, dependiendo de la concentración de aminoácidos esperada) se llevó a 1 mL con agua destilada. A este volumen se le añadieron 2 mL del reactivo Cd-ninhidrina (se disolvieron 0,8 g de ninhidrina en una mezcla de 80 mL de etanol al 99,5% y 10 mL de ácido acético, seguido de la adición de 1 g de $CdCl_2$ disuelto en 1 mL de agua destilada). La mezcla se calentó a 84 ºC durante 5 min y se enfrió rápidamente con hielo, midiendo en último

término la absorbancia a 507 nm en un espectrofotómetro UV-VIS Shimadzu mod. UV-2450 (Kyoto, Japan). También se preparó un blanco (reactivo sin nitrógeno soluble en agua). Se elaboró una curva con L-Leucina para las concentraciones de 15 a 60 mg L^{-1}.

4.2.6. Cuantificación de ácidos grasos libres (AGL) mediante cromatografía de gases-detector de ionización de llama

Los AGL se extrajeron siguiendo el método de de Jong y Badings (1990) modificado. El queso (1 g), molido con 3 g de Na_2SO_4 anhidro en una batidora analítica, se colocó en un tubo falcon y se añadieron 0,3 mL de H_2SO_4 (2,5 M) y 50 µL de una solución de estándar interno (ácido nonanoico a una concentración de 8 mg/mL). A continuación se añadieron 3 mL de dietil éter/heptano (1:1 v/v) y se agitó durante 3 min empleando un agitatubos. El sobrenadante fue transferido a un tubo de tapón de rosca que contenía 1 g de Na_2SO_4 anhidro. Esta operación fue repetida 3 veces. El extracto lipídico fue fraccionado utilizando una columna de aminopropilo (AccuBondIISPE, Agilent Technologies, United Kingdom) que fue acondicionada con 10 mL de heptano. Para eluir los triglicéridos se añadieron 10 mL de cloroformo/2-propanol (2:1 v/v), mientras que los AGL se eluyeron con 5 mL de dietil éter conteniendo el 2% de ácido fórmico. Se realizó una inyección directa de esta disolución (1 µL) para los análisis de cromatografía de gases. Para cada muestra se realizaron dos extracciones. Los AGL extraídos se analizaron en un cromatógrafo de gases HP 6890 equipado con un inyector HP 7683 (Agilent Technologies, Palo Alto, United States), una columna FFAP (Agilent Technologies, 30 m x 0,25 mm ID x 0,25 µm) y un detector de ionización de llama. Las muestras fueron analizadas antes de las 24 horas posteriores a la extracción debido a la volatilidad del solvente empleado. Se empleó un muestreador automático HP 7683 (Agilent Technologies, Palo Alto, United States) para la inyección de los AGL. Las condiciones cromatográficas fueron las siguientes: temperatura inicial de 50 ºC mantenida 1 min, hasta una temperatura final de 240 ºC a un rango de 5 ºC/min mantenida 20 min. El flujo de He fue de 2 mL/min y la relación de split 1:10. Para la cuantificación de cada ácido graso se realizó una curva de regresión mediante el

análisis del incremento de las cantidades de los ácidos grasos individuales (Sigma-Aldrich, St. Louis, MO) con cantidades fijas de estándar interno. Se calcularon ratios entre las diferentes fracciones que fueron empleados como variables para el análisis: ácidos grasos de cadena corta (AGCC, C4:0–C8:0), ácidos grasos de cadena media (AGCM, C10:0–C14:0), ácidos grasos de cadena larga (AGCL, C15:0–C18:2 n-6) y ácidos grasos insaturados (AGI, C16:1 n-9, C18:1 n-9 and C18:2 n-6). Los resultados se expresaron como mg AGL/Kg muestra.

4.2.7. Determinación de la oxidación de lípidos y proteínas

La oxidación de lípidos se realizó por duplicado mediante el método del ácido 2-tiobarbitúrico de Salih y cols. (1987). 2 g de muestra fueron homogeneizados con 7,5 mL de ácido perclórico (3,86%) y 0,25 mL de butilhidroxitolueno (BHT; 4,2 % en etanol) para minimizar el desarrollo de reacciones oxidativas durante la determinación analítica. El homogeneizado se filtró y centrifugó a 3000 rpm durante 2 min. Después de esto, se tomaron alícuotas (en duplicado) y se mezclaron con ácido tiobarbitúrico (0,02 M) y calentaron a 90 ºC durante 30 min en un baño de agua caliente. Después de enfriarlos, los tubos se centrifugaron a 3000 rpm durante 2 min y la absorbancia se midió a 508 nm, 532 nm y 600 nm mediante espectrofotometría. Se realizó una curva estándar utilizando diferentes diluciones de una solución de 1,1,3,3-tetraetoxipropano (TEP). Los valores de TBA-RS (en castellano, especies reactivas al ácido tiobarbitúrico) se calcularon a partir de una curva estándar y se expresaron como mg de malondialdehido Kg^{-1} de queso.

La oxidación de proteínas se midió mediante la estimación de grupos carbonilo formados durante la incubación con 2,4-dinitrofenilhidrazina (DNPH) en 2N HCl siguiendo el método descrito por Oliver y cols. (1987). Los homogeneizados (1 g de muestra en 10 mL de tampón KCL 0,15 M) se dividieron en dos alícuotas de 0,1 mL, y se colocaron en eppendorfs. Se precipitaron las proteínas en ambas alícuotas con 1 mL de ácido tricloroacético al 10% y centrifugado a 2240 g durante 5min. Se añadió 1 mL de

HCL 2N a uno de los eppendorf para medir la concentración de proteínas y 1 mL de 2,4-dinitrofenilhidrazina (DNPH) al 0,2% en HCL 2N al otro para medir la concentración de carbonilos. Ambas muestras fueron incubadas a temperatura ambiente durante 1h. Después, las muestras fueron otra vez precipitadas con 1 mL de ácido tricloroacético al 10% y lavadas con 1 mL de etanol:etil acetato (1:1). Finalmente, se añadieron 1,5 mL de guanidina HCl 6 M con tampón fosfato sódico 20 mM. La concentración de carbonilos se determinó en la muestra tratada mediante la medida de DNHP incorporados en la muestra tratada en base a la absorción de 21 mM^{-1} cm^{-1} a 370 nm para hidrazonas proteicas. La oxidación de proteínas se expresó como nmoles de carbonilos/mg de proteína. La concentración de proteínas se calculó mediante espectrofotometría a 280 nm utilizando albúmina de suero bovino como estándar.

Los resultados se expresaron como mg de Leucina liberados por g de queso.

4.2.8. Identificación de compuestos volátiles mediante microextracción en fase sólida y cromatografía de gases acoplada a espectrometría de masas

Para la extracción de compuestos volátiles se empleó el método de Lee y cols. (2003). Se utilizaron 10 g de queso picado que se añadieron a un vial de 50 mL junto con 10 mL de NaH_2PO_4. La mezcla se agitó durante 30 min a 50 ºC para acelerar el equilibrio de los compuestos volátiles entre el espacio de cabeza y la matriz del queso. Entonces, se llevó a cabo la extracción de los volátiles por inyección de una fibra de Divinilbenceno/Carboxen/Polidimetilsiloxano (Supelco, Bellefonte, PA) en el vial que se mantuvo en el espacio de cabeza durante otros 30 min a 50 ºC. Las muestras fueron directamente desorbidas en el puerto de inyección del cromatógrafo de gases a 250 ºC. Los análisis de los compuestos volátiles fueron realizados en un cromatógrafo de gases VARIAN CP-3800 acoplado a un detector selectivo de masas VARIAN modelo Saturn 2200 (Varian Inc., Palo Alto, CA). Los compuestos volátiles se separaron utilizando una columna capilar HP Innowax (Agilent, Santa Clara, CA) de 60 metros de longitud, 0,25 mm de diámetro interno y 0,50 µm de grosor. El gas transportador fue He con un flujo

de 1 mL/min. La temperatura del programa fue isoterma a 40 ºC durante 10 min, entonces fue aumentada a 240 ºC al rango de 5 ºC min^{-1} y mantenida durante 11 min. La temperatura de la línea de transferencia del GC/MS fue de 280 ºC. El espectrómetro de masas operó en modo de impacto electrónico con una energía de impacto electrónico de 70 eV y un rango de recolección de datos fue de 0,7 scan seg^{-1} sobre un rango de masas de 40-650 m/z. Los compuestos fueron identificados por comparación de sus tiempos de retención con compuestos comerciales de referencia (Sigma-Aldrich, St. Louis, MO) y por comparación de sus espectros de masa con los contenidos en las librerías NIST y Varian.

4.2.9. Evaluación sensorial

i. Test cuantitativo descriptivo con panel de cata entrenado (ensayo II y III)

En el caso de los quesos sin recubrimiento en la corteza tratados por APH, un panel entrenado constituido por 10 catadores del INTAEX (Instituto Tecnológico Agroalimentario de Extremadura) realizaron diferentes sesiones. Los quesos se presentaron al azar en cada grupo. En cada sesión se presentaron 2 cuñas triangulares de unos 0,2 cm de grosor de cada muestra (6-7 muestras por sesión y día). A cada catador se le presentaron las muestras individualmente, codificadas con códigos de una letra y dos cifras elegidos al azar para identificar cada una de las muestras que se presentaron en un plato de plástico. Además se les proporcionó agua mineral (~200 mL) y un poco de pan. Se aplicó un test cuantitativo descriptivo en una escala lineal no estructurada con valores de 0-10. Los análisis se realizaron en cabinas de cata perfectamente equipadas para la realización de evaluaciones sensoriales (Figura 4). Los parámetros que se evaluaron se muestran en la tabla 3.

ii. Test de consumidores (ensayo III).

Los quesos recubiertos con pimentón tratados por APH a día 60 se realizaron 2 catas a un total de 39 consumidores, una a día 60 y otra a día 90. En las dos catas los quesos se

eligieron al azar dentro de cada grupo y se dividieron en 6 cuñas triangulares, eliminando posteriormente la corteza y cortando muestras de unos 0,2 cm de grosor. A cada catador se les presentaron 3 muestras, una de cada grupo, en un plato de plástico y se les proporcionó agua mineral y pan. Los atributos se valoraron en una escala lineal de 10 cm, pudiéndose obtener puntuaciones mínimas de 0 y máximas de 10. Los atributos evaluados fueron aspecto, aroma, textura y sabor. Por último, los consumidores eligieron aquellas muestras que mejor cualidades sensoriales presentaban.

Tabla 3. Parámetros sensoriales y descripción de los mismos evaluados en la cata de quesos Ibores.

Aspecto	
Color	Intensidad que presenta el queso (blanco claro → amarillo oscuro)
Presencia de ojos	Intensidad de la presencia de ojos en el queso (pocos-muchos)
Aroma	
Olor	Intensidad del olor del queso antes de degustarlo (poco-mucho)
Textura	
Dureza	Firmeza percibida durante la masticación (poca-mucha)
Elasticidad	Elasticidad del queso durante la masticación (poca-mucha)
Friabilidad	Capacidad para mantener su estructura durante la masticación (poca-mucha)
Flavor	
Intensidad flavor	Intensidad del aroma del queso percibido durante la masticación (poco-mucho)
Gusto	
Sabor salado	Intensidad sabor salado (poco-mucho)
Sabor ácido	Intensidad sabor ácido (poco-mucho)
Sabores extraños	Intensidad sabores extraños (poco-mucho)

4.2.10. Análisis estadístico

Las diferencias entre los parámetros fueron analizadas mediante un análisis de la varianza (ANOVA) utilizando el programa estadístico SPSS, versión 14.0. Cuando el

ANOVA resultó estadísticamente significativo, las muestras fueron comparadas mediante el Test de Tukey (P<0,05). Se han calculado los valores medios y las desviaciones estándar o errores estándar de la media, según el caso. Las relaciones entre parámetros se calcularon mediante correlaciones de Pearson y análisis de componentes principales.

Figura 4. Catador realizando la evaluación sensorial en la cabina de cata.

Referencias

Ardö, Y. (1999). Evaluating proteolysis by analysing the N content of cheese fractions. Bulletin of International Dairy Federation Nº 337, pp. 4-9.

Chapman, G.H. (1945). The significance of sodium chloride in studies of Staphylococci. Journal of Bacteriology, 50, 201–203.

Coda, R., Brechany, E., De Angelis, M., De Candia, S., Di Cagno, R., & Gobbetti, M. (2006). Comparison of the Compositional, Microbiological, Biochemical, and Volatile Profile Characteristics of Nine Italian Ewes' Milk Cheeses. Journal of Dairy Science, 89, 4126–4143.

de Jong, C., & Badings, H. T. (1990). Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up, and capillary gas chromatographic analysis. Journal of High Resolution Chromatography, 13, 94–98.

de Man, J.D., Rogosa, M., & Sharpe, M.E. (1960). A Medium for the Cultivation of Lactobacilli. Journal of Applied Bacteriology, 23, 130–135.

Folkertsma, B. & Fox, P.F. (1992). Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. Journal of Dairy Research, 59, 217 224.

Guillén, M.D., Ibargoitia, M.L., Sopelana, P., Palencia, G., & Fresno, N. (2004). Components Detected by Means of Solid-Phase Microextraction and Gas Chromatography/Mass Spectrometry in the Headspace of Artisan Fresh Goat Cheese Smoked by Traditional Methods. Journal of Dairy Science, 87, 284–299.

Hayaloglu, A.A., & Brechany, E. Y. (2007). Influence of milk pasteurization and scalding temperature on the volatile compounds of Malatya, a farmhouse Halloumi-type cheese. Le Lait, 87, 39–57.

IDF. 1982. Cheese and processed cheese: moisture determination, determination of the total solids content (Standard 4A) International Dairy Federation: Brussels.

IDF. 1986. Fat, Cheese and processed cheese: determination of fat content, Schmid-Bondzynskiratzlaff method (Standard 5B) International Dairy Federation: Brussels.

IDF. 1988. Cheese and processed cheese: salt determination, determination of chloride content, potentiometric titration method (Standard 88A) International Dairy Federation: Brussels.

IDF. 1993. Protein determination, milk: determination of N content, Kjeldahl method, and calculation of crude protein content (Standard 20 B) International Dairy Federation: Brussels.

Kataoka, H., Lord, H. L., & Pawliszyn, J. (2000). Applications of solidphase microextraction on food analysis. Journal of Chromatography A, 880, 35–62.

Lecanu, L., Ducruet, V., Jouquand, C., Gratadoux, J.J., & Feigenbaum, A. (2002). Optimization of headspace solid-phase microextraction (SPME) for the odor

analysis of surface-ripened cheese. Journal of Agricultural and Food Chemistry, 50, 3810–3817.

Lee, J.H., Diono, R., Kim, G.Y., & Min, D. (2003). Optimization of solid phase microextraction analysis for the headspace volatile compounds of Parmesan cheese. Journal of Agricultural and Food Chemistry, 51, 1136–1140.

Mossel, D.A.A., Mengerink, W.H.J., & Scholts, H.H.A. (1962). Use of a modified MacConkey agar medium for the selective growth and enumeration of all Enterobacteriaceae. Journal of Bacteriology, 84; 381.

Mossel, D.A.A., & Cornelissen, A.M.R. (1963). The examination of foods for Enterobacteriaceae using a test of the type generally adopted for the detection of Salmonellae. Journal of Application Bacteriologic, 26, 444–452.

Oliver, C.N., Ahn, B.W., Moerman, E.J., Goldstein, S., & Satadtman, E.R. (1987). Aged-related changes in oxidized proteins. Journal of Biological Chemistry, 262, 5488–5491.

Salih, A.M., Smith, D.M., Price, J.F., & Dawson, L.E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poultry Science, 66, 1483–1489.

Tejada, L., Abellán, A., Cayuela, J.M., Martínez-Cachá, A., & Fernández-Salguero, J. (2008). Proteolysis in goats' milk cheese made with calf rennet and plant coagulant. International Dairy Journal, 18, 139–146.

Wilkes, J.G., Conte, E.D., Kim Y., Holcomb, M., Sutherland, J.B., & Miller, D.W. (2000). Sample preparation for the analysis of flavours and off-flavours in foods. Journal of Chromatography A, 880, 3–33.

5. RESULTADOS

Capítulo 1

Proteolysis, texture and colour of a raw goat milk cheese throughout the maturation

Enviado para su publicación en[1]: *European Food Research and Technology*

ABSTRACT

Changes on chemical and textural parameters were studied throughout maturation of PDO Ibores cheese. NCN/TN (non casein nitrogen/total nitrogen) values were significantly modified ($P<0.001$) throughout maturation and significantly lower values were observed at day 1 than at day 30, 60 and 90. NPN/TN (non-protein nitrogen/total nitrogen) was not significantly changed during the ripening process ($P>0.05$). Therefore, proteolysis extent (casein degradation and "ripening depth") took place at initial stage and was limited throughout Ibores cheese maturation. In addition, since Casein N decreased during the first month of ripening without a simultaneous increase of NPN/TN, this likely indicates that the large fragments of caseins liberated during this period are not further degraded into smaller products. Primary proteolysis (resulting from the action of endoproteases on caseins) is thus far more important than secondary proteolysis during this period. Moreover secondary proteolysis remains limited along the 90 days. Polypeptide nitrogen significantly increased at day 30 from casein degradation compared with day 1 while Free Amino Acids (FAA) content significantly increased during maturation process. In addition, hardness and adhesiveness values significantly increased, but cohesiveness and springiness significantly decreased up to day 60. Variables such as dry matter, NCN/TN, Polypeptide nitrogen and FAA showed high correlations with textural parameters. Principal component analysis of variables divided the Ibores cheeses according to their ripening: early, middle or late ripening.

[1] Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (Submitted). Proteolysis, texture and colour of a raw goat milk cheese throughout the maturation. *European Food Research and Technology. EFRT-11-0221*

1. INTRODUCTION

It is well known that proteolysis is the most complex and main primary event that takes place in cheese. The caseins are initially hydrolysed by residual coagulant activity retained in the curd and by plasmin and perhaps by other indigenous proteolytic enzymes to a range of intermediate-sized peptides, which are hydrolyzed by proteinases and peptidases from the starter lactic acid bacteria, non-starter lactic acid bacteria and secondary microflora to shorter peptides and amino acids (Upadhyay et al., 2004). Proteolysis during cheese maturation plays an important role on the flavour and texture of cheese (Fox & McSweeney, 1996; McSweeney & Sousa, 2000). Proteolysis affects the formation of flavour and off-flavour compounds in cheese by releasing peptides and free amino acids that could undergo secondary reactions. Moreover, breakage of protein network causes structural alterations on cheese matrix, which could also affect textural characteristics.

The Texture Profile Analysis (TPA) method is an instrumental texture measurement using double bite compression and was developed to imitate the compressing action of molar teeth during food masticating (Bourne, 1978). Despite using texture analysers is not considered a complete substitute for sensory evaluation, significant correlations between sensory textural parameters and TPA parameters (e.g., hardness, cohesiveness and adhesiveness) have been reported in cheese (Antoniou et al., 2000; Halmos, 2000; Pinho et al., 2004). Although instrumental methods alone cannot be used to determine consumer acceptance, their value resides in their ability to detect small changes in physical characteristics and their objectivity.

Spain was the first goat milk European producer in 2008 (592.800 metric tons), followed by France and Greece (FAOSTAT, 2008). In Spain, consumption of goat milk is reduced and an important part of milk is used for cheese making. Goat cheeses (fresh, lactic, semi-hard or hard) are valued products for consumers owing to their sensorial quality, slight creaminess and medium-price (in comparison to cow and ewe cheeses).

Among Spanish goat cheeses, PDO Ibores cheese is an artisanal product produced from raw goat milk (from Serrana, Verata and Retinta goat breeds) and animal rennet. Ibores cheese is a fatty and semi-hard cheese, with sweet aroma but a strong taste, slightly tangy and salty. The use of raw milk for cheese making is unusual but milk pasteurization can give rise to "low-flavour" cheeses because of the enzyme inactivation and loss of native flora induced by pasteurization (Grappin & Beuvier 1997; Atasoy & Türkoglu, 2009). For food safety, Ibores cheese is consumed after 60 days of ripening.

Few current data are available on the proteolytic changes throughout maturation of goat cheeses despite its influence on textural parameters and, therefore, on consumer acceptance of the product. Franco et al. (2003) studied the physico-chemical characteristics and proteolysis throughout the ripening of a traditional Spanish goat lactic acid curd cheese variety (Babia-Laciana). Tejada et al. (2008) evaluated the proteolytic changes during maturation of a goat milk cheese (PDO Murcia al Vino) made with either plant coagulant or calf rennet. In this cheese, Ferrandini et al. (2011) determined the influence of an artisan lamb rennet paste on proteolysis and textural properties. Previous studies have also analysed the biochemical changes during ripening of goat cheeses (Martín-Hernández et al., 1992; Medina et al., 1992; Carballo et al., 1994; Fresno et al., 1996; Freitas et al., 1997).

The aim of the present study is to determine the changes on chemical and textural properties during maturation process of Ibores raw goat milk cheese and their relationships. Data reported could be relevant to understand which proteolytic or physical-chemical indexes are related to textural variations during raw goat cheese ripening.

2. MATERIALS AND METHODS

2.1. Manufacture and sampling

Cheeses were made from raw goat milk in dairies of Ibores PDO. One lyophilized direct-to-vat mesophilic mixed culture (R-704, 50 units; Chr. Hansen, Hørsholm, Denmark), containing *Lactococcus lactis subsp. cremoris* and *L. lactis subsp. lactis* were used to facilitate the acidification process. Milk was coagulated at 28–32ºC in 60–90 min with commercial animal rennet (Naturen Plus 175; activity ~175 IMCU mL^{-1}; 22–25 mL per 100 L; Chr. Hansen, Hørsholm, Denmark). The coagulum was cut to medium-size (1–2 cm) grains. Cheeses of flat cylindrical shape, weighing 0.7–1.2 kg, were pressed for 3–8 h, brine-salted for a maximum of 24 h (18–20 ºBaume) and ripened at 8–12 ºC and 80% relative humidity.

Four cheeses from 3 different producers from the PDO were analysed at four different stages of ripening (1, 30, 60 and 90 days): a total of 48 cheeses were thus analysed, 12 in each stage of maturation. For each producer, 4 cheeses of the same batch were collected in each time of ripening. During the first 60 days of ripening, cheeses were ripened in each dairy. The last month, cheeses were stored under refrigeration at 5 ºC.

2.2. Physico-chemical analysis

pH was measured directly in the middle of cheeses using a portable puncture pH-meter Crison mod. 507 (Crison Instruments, Barcelona, Spain). Dry-matter was assessed by a gravimetric method drying cheese samples at 102 ºC (FIL-IDF 4A/1982). Fat content was analysed by acidbutyrometric method according to Van Gulik using a butyrometer (FIL-IDF 5B/1986); and nitrogen by Kjeldahl method with conversion to protein content using a factor of 6.38 (FIL-IDF 20B/1993).

Colour parameters were determined with a Minolta CR–200 colorimeter (Minolta Camera Co., Osaka, Japan) with illuminant D65, an 8 mm port/viewing area and a 0º viewing angle. Before use, the colorimeter was standardized with a calibration plate

(Y= 93.2; x= 0.3159; y= 0.3324 to D65). The following colour parameters were determined: lightness (CIE L*), redness (CIE a*: + red, - green) and yellowness (CIE b*: + yellow, - blue). The measurements were repeated at five randomly selected locations on each sample and average data were reported.

2.3. Nitrogen fractions and free aminoacids (FAA) measurements

A 0.5 M tri-sodium citrate dispersion with cheese was used for Kjeldahl analysis of total nitrogen (TN) and soluble nitrogen compounds at pH 4.4 (non casein nitrogen (NCN). Soluble non-protein nitrogen (NPN) in 12% trichloroacetic acid (TCA) was analyzed from the NCN fraction (Ardö, 1999). Nitrogen fractions were calculated by difference of the experimental data obtained in the previously described fractions as follows: Casein nitrogen= TN-NCN; Polypeptide nitrogen= (NCN-NPN)*100.

For FAA determination, water-soluble extract was prepared according to Cd-ninhydrin method described by Folkertsma and Fox (1992) as follows: 2 g grated cheese were added to 10 mL distilled water in a Stomacher bag and homogenized for 3-4 min. After 1 h at 40ºC, the homogenate was centrifuged at 3000 *g* for 30 min at 4ºC. The centrifugal supernatant was filtered through Whatman filter (number 41; 150 mm diameter). A sample (20-100 µL, depending on the concentration of amino acids expected) of water-soluble extract was diluted to 1 mL with distilled water was taken and 2 mL Cd-ninhydrin reagent were added (0.8 g ninhydrin dissolved in a mixture of 80 mL of 99.5% ethanol and 10 mL acetic acid, followed by the addition of 1 g $CdCl_2$ dissolved in 1 mL of distilled water). The mixture was heated at 84 ºC for 5 min, cooled and the absorbance at 507 nm was determined in a spectrophotometer (Shimadzu UV-2450, Japan). Analysis of 0.015 to 0.06 mg mL^{-1} L-leucine solutions allowed constructing the standard curve. The results were expressed as mg leucine released per g cheese.

2.4. Texture profile analysis (TPA)

Texture analysis was performed in a texturometer TA-XT2i (Stable Micro Systems LTD, Godalming, UK). For the development of the texture profile analyses (TPA) samples

were diced (1cm x 1cm x 1cm). Texture was measured at constant temperature (15±2ºC). A cylinder probe was used (Aluminium cylinder probe P/25, 25 mm diameter; Stable Micro Systems LTD, Godalming, UK). The test consisted of two successive axial compression ramps to a value of 25% of the unloaded specimen height. The following texture parameters were measured from force–deformation curve: hardness, adhesiveness, cohesiveness, gumminess, springiness and chewiness (Bourne, 1978). Determinations were repeated 8 times per sample.

2.5. Statistical analysis

Analysis of Variance (ANOVA) was performed to establish the effect of ripening. Highest significant difference (HSD) Tukey's test was applied to compare the mean values of parameters. Mean values and standard error of mean are shown. The relationship between parameters was studied by the calculation Pearson's correlation analysis. A multivariate analysis was also performed (PCA, Principal Components Analysis). SPSS software version 14.0 was used to statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Changes on physico-chemical composition

Changes in the physico-chemical composition of Ibores cheese during ripening are presented in Table 1. pH significantly decreased during the first 30 days of ripening, probably owing to the metabolism of lactic acid bacteria (LAB) which metabolizes lactose releasing lactic acid. Lower values of pH were reported than in other similar goat cheeses (Martín-Hernández et al., 1992; Ferrandini et al., 2011) probably due to a soft pressing which facilitates the whey retention in curd and its subsequent acidification. However, pH values at the end of ripening were similar to those reported by Fresno et al. (1996), Psoni et al. (2006) and Serhana et al. (2010) in Armada, Batzos and Darfiyeh goat cheeses, respectively. Dry matter significantly (P<0.05) increased at the end of ripening (day 60–90). The increase of dry-matter was caused by the natural

and progressive loss of moisture throughout maturation. The highest fat and protein contents (in dry matter) were found at the end of ripening (day 60–90). At day 60, levels of dry matter, fat and protein were in the range demanded by PDO (DOE, 2003) and comparable to those found in the literature for goat milk cheese (Martín-Hernández et al., 1992; Franco et al., 2003; Ferrandini et al., 2011).

Table 1. Physico-chemical composition (pH, dry matter, fat and protein content) and instrumental colour measurements of Ibores cheese during ripening.

	Day 1	Day 30	Day 60	Day 90	SEM	P-value
pH	5.18a	4.95b	4.88b	4.98b	0.03	0.001
Dry matter (%)	55.82b	56.70b	63.28a	62.70a	0.57	0.001
Fat (%) in DM	55.55b	56.65ab	56.53ab	57.58a	0.26	0.048
Protein (%) in DM	32.74ab	28.41b	34.65ab	36.28a	1.03	0.036
CIE L*	93.43b	95.63ab	95.98a	97.02a	0.37	0.003
CIE a*	-1.53c	0.10b	1.09a	0.76ab	0.18	0.001
CIE b*	8.17a	2.32b	1.32b	0.81b	0.57	0.001

a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean. DM= Dry Matter.

Regarding instrumental colour parameters, lightness (CIE L*) significantly increased throughout ripening process; a similar trend was found for redness (CIE a*), but up to day 60; and yellowness (CIE b*) showed an important decrease during cheese ripening. Therefore, results indicate that Ibores cheese gained lightness and lost yellowness throughout maturation, getting its typical whitish colour at the end of ripening. The opposite tendency was reported by Juan et al. (2008) in control ewes' milk cheese throughout the ripening (day 1, 15, 30, 60 and 90).

3.2. Changes on Nitrogen fractions and Free Amino Acids (FAA)

Table 2 shows nitrogen fractions and free amino acids (FAA) values throughout cheese maturation. The soluble nitrogen fraction (NCN) includes proteins (excluding all caseins), peptides, amino acids and smaller N compounds, such as amines, urea and ammonium. The ratio of soluble nitrogen and total nitrogen (NCN/TN) has frequently been used as a "ripening index" for cheese as it reflects the proteolysis extent. SN/TN values were significantly modified (P<0.001) throughout maturation; however, statistically significant lower levels were reported at day 1 while values were similar at 30, 60 and 90 days. Non-protein nitrogen (NPN) or soluble nitrogen in 12% TCA fraction (NPN/TN), which contains medium-sized to small peptides, amino acids and smaller N compounds, was not significantly changed during the ripening process (P>0.05). Results showed that high-molecular weight peptides principally released during the first 30 days by proteolysis were not gradually broken down to lower molecular weight peptides which are soluble in TCA. This proteolytic pattern has not been reported in other studies on goat cheeses in which NPN/TN increased throughout cheese ripening (Martín-Hernández et al., 1992; Tejada et al., 2008; Ferrandini et al., 2011). Differences might be due to i. type of animal rennet (artisanal or commercial) and/or ii. proteolytic microbial enzymes present in each type of cheese and maturation conditions. Other important factors are the low pH values and moisture levels (less than 45% after 1 day and 37% from day 60 to 90) reported during Ibores cheese ripening which may be limiting factors to the enzymatic activity (Sousa et al., 2001).

On the other hand, casein nitrogen showed a significant decreased during the first 30 days of ripening (due to casein proteolysis) and constant values were reported later (Table 2). Polypeptide nitrogen values significantly increased at day 30 from casein degradation but similar values were found at 60 and 90 days. FAA content significantly rose during maturation process (P<0.001), which shows the few small peptides present are degraded to FAA. Overall, these results indicate that proteolysis extent (casein degradation and "ripening depth") was limited throughout Ibores cheese maturation

compared with data reported in other goat milk cheeses (Martín-Hernández et al., 1992; Tejada et al., 2008; Ferrandini et al., 2011).

Table 2. Evolution in the nitrogen (N) fractions (expressed as grams per 100g of total nitrogen) and free amino acids (FAA, expressed as mg Leu g^{-1} cheese) during Ibores cheese ripening.

	Day 1	Day 30	Day 60	Day 90	SEM	P-value
SN/TN	12.14b	20.94a	20.65a	21.50a	0.87	0.001
NPN/TN	6.88	6.76	7.28	8.61	0.30	0.108
Casein N	3.28b	8.88a	11.32a	11.05a	0.57	0.001
Polypeptide N	0.37c	0.97bc	1.74ab	2.42a	0.15	0.001
FAA	12.14b	20.94a	20.65a	21.50a	0.87	0.001

a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test P<0.05). SEM: Standard Error of Mean. SN: soluble nitrogen. TN: total nitrogen. NPN: non protein nitrogen. FAA: free amino acids.

3.3. Changes on the Texture Profile Analysis (TPA)

Textural parameters changes are shown in Table 3. Hardness and adhesiveness values significantly increased (P<0.05) during ripening, probably due to the progressive loss of moisture throughout cheese maturation (Table 1). Hardness decreased during maturation of other cheeses such as Monterey Jack goat milk cheese (Van Hekken et al., 2004) or Torta del Casar raw ewe milk cheese (Delgado et al., 2010) but increased during ripening of Terrincho raw ewe milk cheese (Pinho et al., 2004). Differences could be attributed to different final textures in each type of cheese (hard or soft paste). Cohesiveness and springiness significantly decreased up to day 60 in Ibores cheese. Cohesiveness probably decreased via casein network breakage and springiness by the loss of moisture and the increase of cheese fat. Gumminess significantly increased throughout maturation while chewiness values were remained constant.

Table 3. Textural parameters calculated from Texture Profile Analysis (TPA) during Ibores cheese ripening.

	Day 1	Day 30	Day 60	Day 90	SEM	P-value
Hardness (N)	4.22c	7.60b	9.46b	12.40a	0.57	0.001
Adhesiveness (N sec)	1.91b	2.33ab	2.60ab	3.37a	0.16	0.010
Cohesiveness	1.02a	0.75b	0.65c	0.59c	0.02	0.001
Springiness (cm)	0.90a	0.79b	0.67c	0.70c	0.01	0.001
Gumminess (N)	4.35b	5.79ab	6.36ab	7.41a	0.35	0.017
Chewiness (N cm)	3.96	4.56	4.41	5.28	0.27	0.372

a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test P<0.05). SEM: Standard Error of Mean.

The relationships between chemical and textural parameters were established by Pearson's correlation coefficients including results of parameters of cheese during maturation. Hardness and adhesiveness had significant positive correlation with dry matter (r= +0.697 and r= +0.482, respectively; P<0.001). In contrast, cohesiveness and springiness showed a significant negative correlation with Polypeptide nitrogen (r= -0.836 and r= -0.820, respectively; P<0.001), FAA (r= -0.716 and r= -0.687, respectively; P<0.001), dry matter (r= -0.658 and r= -0.769, respectively; P<0.001) and NCN/TN (r= -0.614 and r= -0.567, respectively; P<0.001). NPN/TN was not significantly correlated (P>0.05) with texture parameters. In contrast with these results, Van Hekken et al. (2004) reported a positive correlation between cohesiveness and springiness with peptides and negative with caseins in Monterey Jack pasteurised goat milk cheese and, therefore, correlations may be cautiously carried out for each type of cheese. Gumminess and chewiness did not show significant correlations with other parameters evaluated in Ibores cheese. Therefore, the increases in hardness and adhesiveness found throughout Ibores cheese maturation were exclusively related to the increase of dry matter (or the decrease of moisture). The decreases in cohesiveness and

springiness were related to the increases of Polypeptide nitrogen and FAA, and the decrease of Casein nitrogen which happened during ripening process. Creamer and Olson (1982) indicated that high acidity in cheese and high protein and total solids contents generally make the cheese harder and with less capacity of deformation, which is in line with our results. However, proteolysis reactions did not affect so much texture formation in this cheese.

3.4. Multivariate analysis

A principal component analysis (PCA) was applied to explain the variance of data. 39.7% and 25.5% of the variability were explained by principal components (PC) 1 and 2, respectively. Figure 1 shows the loadings of the different variables for the two first principal components (PC1 and PC2). Redness (CIE a*), dry matter, Protein nitrogen and NCN/TN are placed in the positive axis on the PC1 whereas cohesiveness, springiness and yellowness (CIE b*) are in the negative axis of PC1. Regarding PC2, chewiness, adhesiveness and gumminess are found in the positive axis. Therefore, most of the variation of data can be explained by the changes of TPA parameters throughout cheese maturation, and to a lesser degree by chemical parameters.

The scores of data showed 3 separated groups of points by the PC1 (Figure 2) related to the days of maturation. The 1 day-old cheeses are located in the negative area of PC1 associated with cohesiveness and springiness values. The 30 days-old cheeses are closely placed in the central area of plane. Cheeses with 60 and 90 days of ripening are found in the positive area of PC1 but their distinction is difficult. This result is not unexpected since cheeses were stored at 5 ºC during the last 30 days and proteolysis extent is limited under refrigeration conditions. Therefore, the combination between chemical and textural parameters is a useful tool to determinate the ripening time of Ibores cheese (early, middle or late).

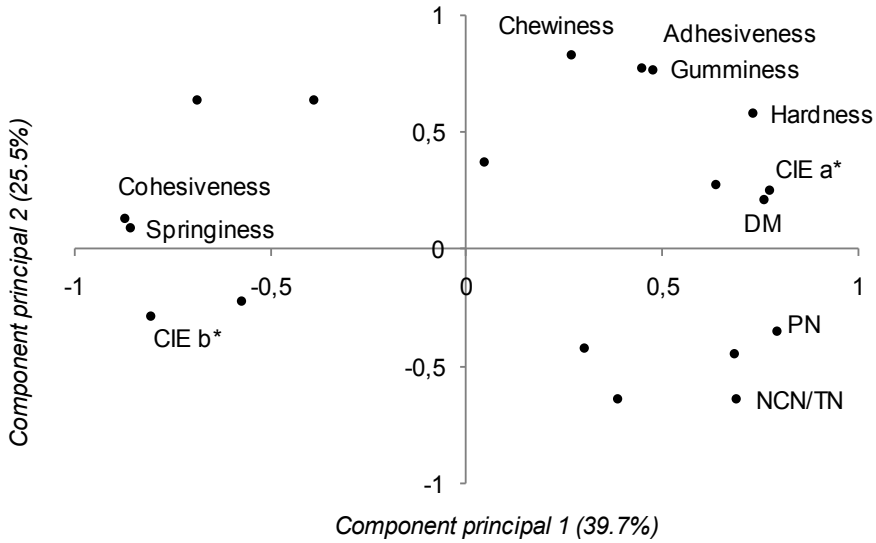

Figure 1. Loadings plots after principal components analysis of the variables in the plane defined by the two first principal components (PC1 and PC2). (CIE a*; CIE b*; DM: dry matter; PN: Polypeptide N).

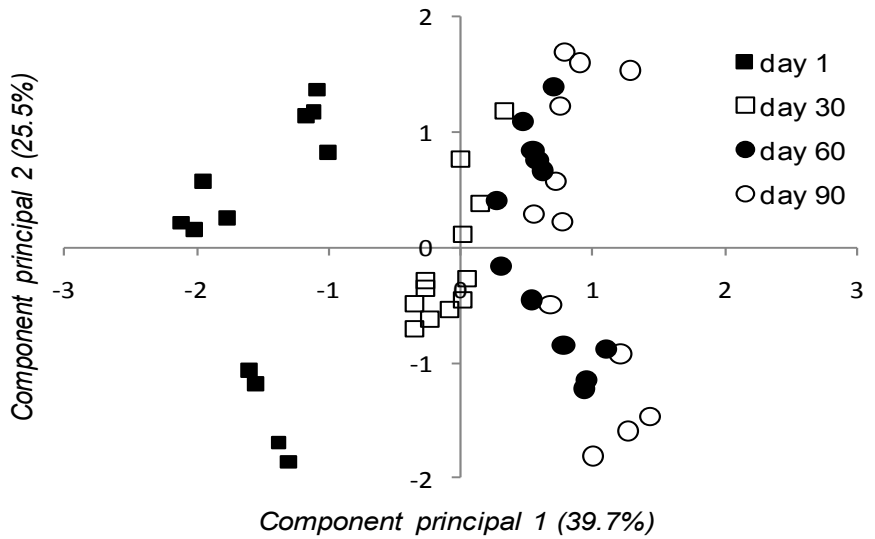

Figure 2. Scores plot after principal component analysis of the variables according to days of ripening defined by the two first principal components (PC1 and PC2).

4. CONCLUSIONS

During the first 30 days of maturation, Ibores cheese showed a more intense initial proteolytic event than at the end of maturation principally by realising of high-molecular weight peptides, but they underwent a weak subsequent degradation. The increase of free amino acids denoted a gradual degradation of small peptides present which could be relevant for cheese flavour. Therefore, proteolysis was mainly early and limited and its relationship with cheese texture formation was less intense than in other cheeses. However, the chemical composition of cheese such as dry matter content showed high correlations with some instrumental texture parameters.

5. Acknowledgements

This work has been financed thanks to European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). We thank the PDO dairies for providing the cheeses.

6. References

Antoniou, K. D., Petridis, D., Raphaelides, S., Ben Omar, Z., & Kesteloot, R. (2000). Texture assessment of French cheeses. Journal of Food Science, 65, 168–172.

Ardö, Y. (1999). Evaluating proteolysis by analysing the N content of cheese fractions. Bulletin of International Dairy Federation Nº 337, 4–9.

Atasoy, A. F., & Türkoglu, H. (2009). Lipolysis in Urfa cheese produced from raw and pasteurized goats' and cows'milk with mesophilic or thermophilic cultures during ripening. Food Chemistry, 115, 71–78.

Bourne, M. C. (1978). Texture profile analysis. Food Technology, 32, 62–66.

Carballo, J., Fresno, J. M., Tuero, J. R., Prieto, J. G., Bernardo, A., & Martín-Sarmiento, R. (1994). Characterization and biochemical changes during the ripening of a Spanish hard goat cheese. Food Chemistry, 49, 77–82.

Creamer, L. K., & Olson, N. F. (1982). Rheological evaluation of maturing Cheddar cheese. Journal of Food Science, 47, 631–647.

Delgado, F. J., Rodríguez-Pinilla, J., González-Crespo, J., Ramírez, R., & Roa, I. (2010). Proteolysis and texture changes of a Spanish soft cheese ("Torta del Casar") manufactured with raw ewe milk and vegetable rennet during ripening. International Journal of Food Science & Technology, 45, 512–519.

DOE (2003). Diario Oficial de Extremadura (Regional Legislation of Extremadura), nº 61, 17th May 2003. Decreto 69/2003, de 20 de mayo, por el que se aprueba el Reglamento de la Denominación de Origen Protegida "Queso de Ibores" y su Consejo Regulador.

FAOSTAT (2008). Food and Agricultural commodities production: goat milk, whole, fresh. Food and Agriculture Organization of the United Nations Statistical Databases.

Ferrandini, E., López, M.P., Castillo, M., & Laencina, J. (2011). Influence of an artisanal rennet paste on proteolysis and textural properties of Murcia al Vino cheese. Food Chemistry, 124, 583–588.

Folkertsma, B. & Fox, P.F. (1992). Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. Journal of Dairy Research, 59, 217–224.

Fox, P. F., & McSweeney, P. L. H. (1996). Proteolysis in cheese during ripening. Food Reviews International, 12, 457–509.

Franco, I., Prieto, B., Bernardo, A., González, J., & Carballo, J. (2003). Biochemical changes throughout the ripening of a tradicional Spanish goat cheese variety (Babia-Laciana). International Dairy Journal, 13, 221–230.

Freitas, A. C., Fresno, J. M., Prieto, B., Malcata, F. X., & Carballo, J. (1997). Effects of ripening time and combination of ovine and caprine milks on proteolysis of Picante cheese. Food Chemistry, 60, 219–229.

Fresno, J. M., Tornadijo, M. E., Carballo, J., González-Prieto, J., & Bernardo, A. (1996). Characterization and biochemical changes during the ripening of a Spanish craft goat's milk cheese (Armada variety). Food Chemistry, 55, 225–230.

Grappin, R., & Beuvier, E. (1997). Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese. International of Dairy Journal, 7, 751–61.

Halmos, A.L. (2000). Relationships between instrumental texture measurements and sensory attributes. In Nishinari K. (Ed.), Hydrocolloids - Part 2 (pp. 431-444). Elsevier, Amsterdam.

IDF. (1982). Cheese and processed cheese: moisture determination, determination of the total solids content (Standard 4A) International Dairy Federation: Brussels.

IDF. (1986). Fat, Cheese and processed cheese: determination of fat content, Schmid-Bondzynskiratzlaff method (Standard 5B) International Dairy Federation: Brussels.

IDF. (1993). Protein determination, milk: determination of N content, Kjeldahl method, and calculation of crude protein content (Standard 20 B) International Dairy Federation: Brussels.

Juan, B., Ferragut, V., Guamis, B., & Trujillo, A.J. (2008). The effect of high-pressure treatment at 300 MPa on ripening of ewes' milk cheese. International Dairy Journal, 18, 129−138.

Martín-Hernández, M. C., & Juárez, M. (1992). Biochemical characteristics of three types of goat cheese. Journal of Dairy Science, 75, 1747–1752.

Medina, M., Gaya, P., & Nuñez, M. (1992). Gredos goat's milk cheese: Microbiological and chemical changes throughout ripening. Journal of Dairy Research, 59, 563–566.

McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening. A review. Lait, 80, 293–324.

Pinho, E. M., Alves, M. M., & Ferreira, I. M. (2004). Chemical, physical, and sensorial characteristics of "Terrincho" ewe cheese: changes during ripening and intravarietal comparison. Journal of Dairy Science, 87, 249–257.

Psoni, N. T., & Litopoulou-Tzanetaki, E. (2003). Microbiological characteristics of Batzos, a traditional Greek cheese from raw goat's milk. Food Microbiology, 20, 575–582.

Serhana, M., Lindera, M., Hosrib, C., & Fannia, J. (2010). Changes in proteolysis and volatile fraction during ripening of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. Small Ruminant Research, 90, 75–82.

Sousa, M. J., Ardö, Y., & McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. International Dairy Journal, 11, 327–345.

Tejada, L., Abellán, A., Cayuela, J. M., Martínez-Cacha, A., & Fernández-Salguero, J. (2008). Proteolysis in goats' milk cheese made with calf rennet and plant coagulant. International Dairy Journal, 18, 139–146.

Upadhyay, V. K., McSweeney, P. L. H., Magboul, A. A. A., & Fox, P. F. (2004). Proteolysis in cheese during ripening. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.), Cheese: chemistry, physics and microbiology (3rd ed., pp. 391–433). Amsterdam: Elsevier Academic Press.

Van Hekken, D. L., Tunick, M.H., & Park, Y. W. (2004). Rheological and proteolytic properties of Monterey Jack goat milk cheese during 6 months aging. Journal of Agriculture and Food Chemistry, 52, 5372–5377.

Capítulo 2

Free fatty acids and oxidative changes of a raw goat milk cheese through maturation

Publicado en[1]: *Journal of Food Science, 76, pp. 669-673 (2011)*

ABSTRACT

Free fatty acids (FFA) and lipid and protein oxidation changes were studied throughout maturation process of a raw goat milk cheese with protected designation of origin (PDO). Cheeses were analysed at four different times of maturation, at 1, 30, 60 and 90 days. All free fatty acids significantly increased during maturation and the relative increase was higher for long-chain than medium or short-chain FFA. At the end of maturation, oleic (C18:1 n9), butyric (C4:0) and palmitic (C16:0) acids were the most abundant. The higher levels of short-chain fatty acids (SCFA) regarding total FFA obtained at the end of Ibores cheese ripening compared with other raw goat milk cheeses, highlights the notable role of SCFA on the flavour of this cheese owing to their low odour thresholds. Lipid oxidation values significantly increased during maturation process but low levels of MDA were reported; however, protein oxidation did not significantly change during ripening.

[1] Francisco J. Delgado, José González-Crespo, Ramón Cava, and Rosario Ramírez (2011). Free Fatty Acids and Oxidative Changes of a Raw Goat Milk Cheese through Maturation. *Journal of Food Science*, **76**, pp. 669-673.

1. INTRODUCTION

The cheese flavour is the result of biochemical reactions that take place during maturation process, caused by the interaction of starter bacteria, enzymes from the milk and rennet, lipases and secondary flora (Urbach, 1997). The compounds implicated in cheese aroma are formed by three main catabolic pathways namely i) lactose and lactate, ii) proteins and iii) lipids (Molimard & Spinnler, 1996). Free fatty acids (FFA) released by lipolysis, principally short and medium chain FFA (C4:0–C8:0 and C10:0–C14:0, respectively), have a great influence on cheese flavour (Urbach, 1993; McSweeney & Sousa, 2000). In contrast, long-chain FFA (>14 carbon atoms) have a minor role on cheese flavour owing to the fact that they have high perception thresholds (Molimard & Spinnler, 1996). Additionally, FFA also act as substrate for several catabolic pathways, producing flavour and aroma compounds, such as methyl ketones, lactones, esters, alkanes and secondary alcohols (McSweeney & Sousa, 2000).

Milk pasteurization normally lead to cheeses with less flavour development compared with those manufacture from raw milk, because of the enzyme inactivation as well as the loss of native microbial flora present in raw milk (Grappin & Beuvier 1997). Atasoy and Türkoglu (2009) reported that pasteurization of milk reduced the level of lipolysis throughout ripening cheese and also the relative amounts of short chain FFA. Therefore, cheeses made from raw milk have unique flavours and are popularly sold in many parts of the world. The agents which cause the lipolysis in raw milk cheeses, like Ibores cheese, are i) indigenous milk lipase, ii) raw milk microflora and iii) non-starter lactic acid bacteria. Commercial animal rennet, used for milk enzymatic coagulation of this goat cheese, is normally free of lipolytic activity (Collins et al., 2003).

The cheese with the protected designation of origin (PDO) “Queso Ibores” is manufactured in the SW of Spain. Ibores cheese is a fatty cheese, produced from raw milk from goats of the breeds Serrana, Verata, Retinta and crosses thereof. Very few goat cheeses use raw milk for their manufacture. Its aroma varies between sweet and

mild, typical of goat's cheese made from raw milk. It has a strong taste: slightly tart, tangy and salty, with a slight aftertaste of goat's milk. Few references are available about FFA profile of goat milk cheeses. Buffa et al. (2001) studied the FFA profiles of cheeses made from raw, pasteurized and HP-treated goat's milk. Franco et al. (2003) analysed lipolysis pattern of Babia-Laciana cheese throughout ripening. This cheese is a traditional Spanish goat cheese made from raw milk, but with milk lactic coagulation. Lipolysis during ripening of Urfa cheese produced in Turkey from raw and pasteurized goats' and cows' milk has also been described (Atasoy & Türkoglu, 2009). Lipolysis of some Spanish pasteurized goat milk cheeses have been studied (Fontecha et al., 2006; Poveda & Cabezas, 2006). However, no previous studies have addressed lipolysis in this raw goat milk cheese during maturation.

In many processed products, lipid oxidation causes changes on sensorial attributes such as texture and aroma. However, lipid oxidation is not a significant process in some cheese (Delgado et al., 2009), probably due to its low redox potential and the presence of natural antioxidants (Erickson et al., 1964; McSweeney & Sousa, 2000); so lipid oxidation could have a slight influence on cheese flavour. However, a parallel study about the volatile profile of Ibores cheese (Delgado et al., unpublished results) showed a notable increase of FFA during cheese maturation which could undergo oxidation processes.

Protein oxidation could modify the cheese quality due to it has been related to the reduction of protein digestibility and the production of toxic substances (Moreaux & Birlouez-Aragon, 1997; Naranjo et al., 1998). The oxidation reactions can be transferred from lipids to proteins due to interactions between them. However and despite the importance that these reactions could have in some cheeses, according to our knowledge, only two study has reported protein and lipid oxidative during cheese ripening (Fedele & Bergamo, 2001; Delgado et al., 2009).

Due to the lack of studies about the lypolitic and the oxidative processes during cheese maturation, and especially in raw goat milk cheeses, the main objectives of this paper

are the characterization of the lipolytic pattern and lipid and protein oxidation changes in the raw goat milk cheese Ibores throughout maturation.

2. MATERIALS AND METHODS

2.1. Cheesemaking

Cheeses were made from raw goat milk in dairies of Ibores PDO. Milk was coagulated at 28–32 ºC for 60–90 min with animal rennet. The curd was cut to medium-size (1–2 cm) grains. Cheeses weight between 0.7–1.2 kg and were pressed for 3–8 h, brine-salted and ripened (at 8–12 ºC and 80% relative humidity). According to the regulation of the PDO, the physicochemical properties must be a minimum of 50% of fat content in dry matter, a minimum of 50% of dry matter, a pH of 5.0–5.5 and a maximum of 4% NaCl (DOE, 2003).

Four cheeses from 3 different PDO dairies were studied in four different time of ripening (1, 30, 60 and 90 days). A total of 48 cheese were analysed, 12 in each stage of maturation. For each producer, four cheeses of the same batch were taken in each time of ripening. Cheeses were matured in each dairy for 60 days. The last month, cheeses were stored under refrigeration conditions at 5 ºC. Cheeses were sampled and stored at -20 ºC.

2.2. Free fatty acids analysis

FFA were extracted according to the modified method of De Jong and Badings (1990) described by Delgado et al. (2009). FFA analyses were performed by Gas Chromatography and Flame Ion Detector (GC–FID) according to Delgado et al. (2009). Ratios between the different fractions, namely short chain fatty acids (SCFA, C4:0–C8:0), medium chain fatty acids (MCFA, C10:0–C14:0), long chain fatty acids (LCFA, C15:0–C18:2 n-6), unsaturated fatty acids (UFA, C16:1 n-9, C18:1 n-9 and C18:2 n-6) were calculated and used as variables for the analyses.

2.3. Lipid oxidation

Lipid oxidation analysis was performed by the 2-thiobarbituric acid (TBA) method of Salih et al. (1987). TBA–RS values were calculated from the standard curve and expressed as mg malondialdehyde Kg^{-1} cheese.

2.4. Protein oxidation

Protein oxidation was measured by quantification of carbonyl groups produced during incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl following the method described by Oliver et al. (1987). Protein oxidation was expressed as nmol carbonyls mg^{-1} protein.

2.5. Statistical analysis

The effect of ripening on the studied variables was analysed by the Analysis of Variance (ANOVA) using the SPSS statistic software, version 14.0. HSD Tukey's test was performed to compare the mean values of FFA and oxidative markers throughout maturation. Mean values and standard error of mean are reported.

3. RESULTS AND DISCUSSION

3.1. Free fatty acids analysis

Figure 1 shows the evolution of short chain fatty acids (SCFA, C4:0–C8:0), medium chain fatty acids (MCFA, C10:0–C14:0) and long chain fatty acids (LCFA, C15:0–C18:2 n-6) throughout maturation process of Ibores cheese. SCFA, MCFA and LCFA amounts increased throughout maturation, with the highest levels at the end of ripening (day 60) and after 30 days of refrigerated storage (day 90). SCFA, with a significant role on flavour cheese, had the lowest increment (81.5%) compared to MCFA (250%) and LCFA (250%).

The highest amounts of SCFA were found after 90 days of maturation. MCFA amounts significantly (P<0.001) increased at the end of maturation. Similarly, LCFA significantly

increased (*P*<0.001) during whole maturation process. The highest contents of LCFA could be caused by the higher affinity of milk lipoprotein lipase for long chain triacylglycerols (Deckelbaum et al., 1990). Previous studies in raw goat milk cheeses have also found important increases of FFA during cheese maturation (Buffa et al., 2001; Franco et al., 2003; Atasoy & Türkoglu, 2009). Similarly, although in a pasteurised goat milk cheese, Fontecha et al. (2006) reported FFA increases throughout maturation in Majorero cheese.

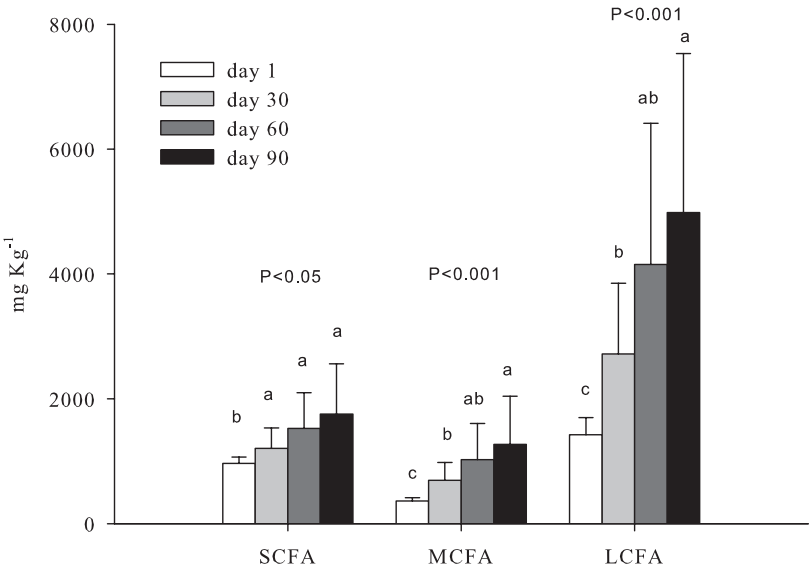

Figure 1. Evolution of free fatty acids contents (mg Kg^{-1}) during ripening of Ibores cheese. a, b, c: Different letters (a,b,c) in the same group of fatty acids indicate significant statistical differences (Tukey's Test, *P*<0.05). SCFA (Short Chain Fatty Acids), MCFA (Medium Chain Fatty Acids), LCFA (Long Chain Fatty Acids).

Table 1 shows the concentrations (mg Kg^{-1}) of individual FFA throughout maturation of Ibores cheese. The amounts of total FFA were similar to that reported by Buffa et al. (2001) in goat cheese made from raw milk, but higher than values found in other regionally-produced Spanish goat cheeses by Poveda and Cabezas (2006), probably due to the pasteurized milk used to make these cheeses, which can inactive milk lipase reducing cheese lipolysis. The content of most FFA increased during ripening (*P*<0.05),

and this increase was particularly noticeable (~5–fold times higher) for caprilic (C8:0), capric (C10:0) and oleic (C18:1 n-9) acids. Franco et al. (2003) reported a higher increase of oleic acid (~10–fold times) in Babia-Laciana cheese, a raw goat milk cheese with lactic coagulation, but amounts of caprilic and capric acids did not significantly increase. The differences detected between both could be associated to the different coagulation process (enzymatic vs. lactic) and also to the goat milk type (breed, feeding and others).

At day 1 of maturation, cheese FFA profile showed high levels of butyric (C4:0), palmitic (C16:0) and oleic (C18:1 n-9) acids (Table 1). The LCFA levels found in Ibores cheese at day 1 could be due to the fact that palmitic and oleic acids are the predominant FFA in goat milk fat (Park et al., 2007). The LCFA amounts are consistent with the results reported for other raw goat milk cheeses, even though the levels of butyric acid were higher in Ibores cheese at the beginning of maturation (Buffa et al., 2001; Franco et al., 2003; Atasoy & Türkoglu, 2009).

Cheeses showed a lower rate of lipolysis the last 30 days of ripening (see Figure 1), which may be caused by a diminished enzymatic activity due to microenvironmental changes in cheese (e.g., moisture or pH). In matured cheeses (60-90 days), LCFA were the main FFA such as oleic (C18:1 n-9) and palmitic (C16:0) acids which showed high amounts, followed by stearic (C18:0) and linoleic (C18:2 n-6) acids. Similar results have been reported in Babia-Laciana cheese (raw goat milk) (Franco et al., 2003) and other pasteurised goat milk cheeses (Poveda & Cabezas, 2006) at the end of ripening. In spite of the quantitative importance of LCFA, they are not the main contributors to cheese flavour (Freitas & Malcata, 1998).

Calculated lipolysis index (FFA released per day) showed that LCFA had the highest degree of lipolysis (39.56 mg Kg^{-1} day^{-1}) compared to MCFA (10.06 mg Kg^{-1} day^{-1}) and SCFA (8.75 mg Kg^{-1} day^{-1}). These results disagrees with those previously reported by the authors in Torta del Casar, a raw ewe milk cheese, where SCFA showed the highest value for FFA released per day and SCFA were the most abundant FFA at the end of

ripening (Delgado et al., 2009). Differences in hydrolysis rate can be attributed to the variations in i. specific lipases found in each milk type (ewe vs goat) and/or ii. endogenous microflora of each cheese and/or iii. the different fatty acids and triacylglicerols composition of the milk fat.

Table 1. Free fatty acids contents (mg Kg^{-1}) isolated in Ibores cheese during ripening.

	Time of ripening (day)					
Free fatty acids	1	30	60	90	SEM	*P–value*
Butyric (C4:0)	787.3c	911.2bc	1109.1ab	1251.6a	48.4	0.001
Caproic (C6:0)	128.3b	166.1ab	211.4ab	242.2a	12.7	0.005
Caprilic (C8:0)	50.5b	127.2ab	205.5ab	260.0a	24.6	0.011
Capric (C10:0)	127.2b	300.6ab	472.1ab	594.5a	50.8	0.004
Lauric (C12:0)	90.0c	145.9bc	201.8ab	242.3a	13.1	0.001
Myristic (C14:0)	144.8c	248.7bc	352.7ab	430.5a	22.9	0.001
Pentadecanoic (C15:0)	85.0b	87.9ab	94.8a	96.8a	1.4	0.004
Palmitic (C16:0)	454.0c	711.3bc	1031.7ab	1241.3a	62.9	0.001
Palmitoleic (C16:1)	0.0b	7.8ab	13.9a	0.0b	1.5	0.001
Margaric (C17:0)	0.0b	142.7a	148.4a	150.1a	9.4	0.001
Stearic (C18:0)	279.1c	433.5bc	621.9ab	767.8a	42.9	0.001
Oleic (C18:1)	479.4c	1079.4bc	1818.8ab	2500.0a	196.2	0.001
Linoleic (C18:2)	125.3b	257.2ab	421.2a	227.3b	27.8	0.001
Total FFA	2751.0c	4619.6bc	6703.2ab	8004.4a	489.3	0.001

a, b, c: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard Error of the Mean.

Throughout maturation process, most FFA are synthesized from the breakdown of amino acids or from lipolysis predominantly by the action of milk native lipases which remains active in raw milk cheeses (Urbach, 1993) like in Ibores cheese. In addition, SCFA can also be synthesised from ketones, esters and aldehydes by oxidation (Molimard & Spinnler, 1996). Due to the low perception thresholds of SCFA and MCFA they are considered important for the cheese flavour providing characteristic aromatic notes to cheese (Collins et al., 2003). For example, butyric acid has a rancid odour and is a key flavour compound of several international cheeses (reviewed by Curioni & Bosset, 2002). SCFA level could be important for Ibores cheese flavour since their concentrations at the end of maturation (1526–1754 mg Kg^{-1}) were higher than those reported in other types of cheese elaborated from raw goat milk (Franco et al., 2003; Atasoy & Türkoglu, 2009). Poveda and Cabezas (2006) reported that butyric acid correlated with bitterness, brine odour and goat milk odour in regionally-produced Spanish goat cheeses and it was the main short-chain FFA detected. Other SCFA detected in this cheese, such as caproic (C6:0) and caprilic (C8:0) acids, may also have an important influence on the final aroma of Ibores cheese since their amounts were higher than those obtained in Babia-Laciana cheese (Franco et al., 2003) and other pasteurised goat milk cheeses (Poveda & Cabezas, 2006). Regarding MCFA, capric acid was the most abundant FFA in each time of sampling which is in line with results reported by Nájera et al. (1993) for goat milk cheeses. Capric (C10:0), lauric (C12:0) and myristic (C14:0) acids are responsible of the milky, rind and sweat odours in Roncal raw ewe milk cheese (Curioni & Bosset, 2002).

Table 2 shows the mean values for the ratios of SCFA, MCFA and LCFA to total FFA and the rates SCFA/LCFA, MCFA/LCFA, SCFA/MCFA and UFA/LCFA observed throughout maturation of Ibores cheese. Significant differences ($P<0.05$) were found for all indexes, except for MCFA/LCFA throughout maturation. The rate SCFA/total FFA decreased throughout maturation and reached the lowest value at 90 days (SCFA/total FFA= 0.22). However, LCFA/total FFA ratio increased throughout maturation and the

highest values were found at the end of ripening. MCFA/total FFA ratio increased during the first 30 days of maturation and remained constant until the end of ripening. The significant decrease of SCFA was reflected by the decreases in SCFA/LCFA and SCFA/MCFA ratios as well, while MCFA/LCFA maintained constant levels throughout cheese maturation.

Table 2. Free fatty acid (FFA) indexes calculated through ripening of Ibores cheese.

	Time of ripening (days)					
FFA index	1	30	60	90	SEM	*P–value*
SCFA/total FFA	0.35a	0.27b	0.24bc	0.22c	0.01	0.001
MCFA/total FFA	0.13b	0.15a	0.15a	0.15a	0.01	0.001
LCFA/total FFA	0.51c	0.58b	0.61ab	0.62a	0.01	0.001
SCFA/LCFA	0.70a	0.47b	0.40bc	0.36c	0.02	0.001
MCFA/LCFA	0.26	0.26	0.25	0.25	0.01	0.388
SCFA/MCFA	2.70a	1.82b	1.62bc	1.50c	0.08	0.001
UFA/LCFA	0.42b	0.47ab	0.50a	0.52a	0.01	0.005

a, b, c: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard Error of the Mean. SCFA (Short Chain Fatty Acids, C4:0–C8:0), MCFA (Medium Chain Fatty Acids, C10:0–C14:0), LCFA (Long Chain Fatty Acids, C15:0–C18:2 n-6), UFA (Unsaturated Fatty Acids, C16:1 n-9, C18:1 n-9 and C18:2 n-6).

At the end of ripening, the most FFA were LCFA (61–62%), followed by SCFA (22–24%) and MCFA (15%). Among LCFA, half of them were UFA. According to Nájera et al. (1993), goat cheeses presented the 59.5% of LCFA, 28.6% of MCFA and 10.5% of SCFA, regarding total FFA. Atasoy & Türkoglu (2009) reported in Urfa cheese, a raw goat milk, that LCFA comprised the 61–62% of FFA, while MCFA were the 33% and SCFA the 5–7% at the end of maturation (day 60–90). Therefore, the higher percentage of SCFA found in Ibores cheese at the end of ripening compared with other raw goat cheeses could highlight the notable role of SCFA on the flavour of this cheese owing to their low

odour thresholds. Differences could be attributed to several factors related to i. the goat breeds and ii. the specific diet from each geographical localisation, which could give rise to a milk fat with unique composition and/or iii. enzymes and the native microbiota.

3.2. Lipid and protein oxidation analysis

Table 3 shows protein and lipid oxidation changes throughout Ibores cheese maturation. Lipid oxidation values significantly increased ($P<0.05$) during the first and the last 30 days of maturation. The low concentrations of MDA reported are lower than those reported by Fedele and Bergamo (2001) in Grana Padano cheese, probably because this cheese is made with pasteurized milk. However, in Torta del Casar cheese (raw ewe milk soft cheese) higher values of TBA-RS were reported than in Ibores cheese. In this case, the lipid oxidation processes could be associated to the highest levels of linolenic acid (C18:3) in that cheese than in Ibores cheese, in which this easily-oxidized polyunsaturated fatty acid was not detected. Even though lipid oxidation is an key quality problem of processed dairy products, mainly during storage (Kristensen & Skibsted, 1999), our results prove that lipid oxidation does not happen to a notable extent during Ibores cheese ripening, which is in line with previous studies in raw ewe milk cheese (Fedele & Bergamo, 2001; Delgado et al., 2009).

Protein oxidation did not significantly ($P>0.05$) change throughout maturation but slightly increased. Concentrations of carbonyls from protein oxidation were lower than those reported by Fedele and Bergamo (2001) for Grana Padano cheese but similar than those obtained by Balestrieri et al. (2002) for mozzarella cheese made from buffalo milk and higher than in Torta del Casar cheese (Delgado et al., 2009), probably due to the different properties of each type of cheese and maturation conditions. The same as for lipid oxidation, the low protein oxidation degree can be due to the fact that milk was not pasteurised during cheese-making of Ibores cheese, since thermal treatment is a pro-oxidant process.

Fedele and Bergamo (2001) found a positive correlation between heat intensity and oxidative damage but they did not find correlation between ripening time and oxidation extent in Grana Padano cheese. Consequently, the use of raw milk to make Ibores cheese could be the cause of the low level of oxidative markers. Oxidative processes are relevant to aroma formation in ripened product like cheese, but in excess, it can be negative by means of the off-flavour formation (Serra et al., 2008). Further research is needed to determine the contribution of oxidation of lipids and proteins to the characteristics of Ibores cheese.

Table 3. Protein (nmol carbonyls mg^{-1} protein) and lipid oxidation (mg MDA Kg^{-1}) changes during cheese ripening.

	Time of ripening (days)				SEM	*P–value*
	1	30	60	90		
Protein oxidation	1.23	1.25	1.40	2.0	0.14	0.145
Lipid oxidation	0.04b	0.05ab	0.05ab	0.08a	0.01	0.012

a, b: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard Error of the Mean.

4. CONCLUSIONS

The free fatty acids relative increase was higher in long-chain than in medium or short-chain free fatty acids. The higher levels of short-chain free fatty acids regarding total free fatty acids found at the end of Ibores cheese maturation compared with other raw goat cheeses highlight the notable role of short-chain free fatty acids could play on the flavour of this cheese owing to their low odour thresholds. In comparison with lipolytic changes, oxidation of lipids and proteins are not important processes in the maturation of Ibores cheese. Carbonyls formation from oxidation of proteins is not of importance in Ibores cheese maturation. Instead, lipid oxidation could participate in the formation of volatile compounds that contribute to the flavor of Ibores cheese.

5. Acknowledgements

This work has been financed thanks to European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). We thank the PDO dairies for providing the cheeses.

6. References

Atasoy, A.F., & Türkoglu, H. (2009). Lipolysis in Urfa cheese produced from raw and pasteurized goats' and cows' milk with mesophilic or thermophilic cultures during ripening. Food Chemistry, 115, 71–78.

Balestrieri, M., Spagnuolo, M.S., Cigliano, L., Storti, G., Ferrara, L., Abrescia, P., & Fedele E. (2002). Evaluation of oxidative damage in mozzarella cheese produced from bovine or water buffalo milk. Food Chemistry, 77, 293–299.

Buffa, M.N., Guamis, B., Pavia, M., & Trujillo, A.J. (2001). Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 175–179.

Collins, Y.F., McSweeney, P.L.H., & Wilkinson, M.G. (2003). Lipolysis and free fatty acid catyabolism in cheese: A review of current knowledge. International Dairy Journal, 13, 841–866.

Curioni, P.M.G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

de Jong, C., & Badings, H.T. (1990). Determination of free fatty acids in milk and cheese. Procedures for extraction, clean up, and capillary gas chromatographic analysis. Journal of High Resolution Chromatography, 13, 94–98.

Deckelbaum, R.J., Hamilton, J.A., Moser, A., Bengtsson-Olivecrona, G., Butbul, E., Carpentier, Y.A., Gutman, A. and Olivecrona, T. (1990). Medium-chain versus longchain triacylglycerol emulsion hydrolysis by lipoprotein lipase and hepatic

lipase: implications for the mechanisms of lipase action. Biochemistry, 29, 1136–1142.

Delgado, F.J., González-Crespo, J., Ladero, L., Cava, R., & Ramírez, R. (2009). Free fatty acids and oxidative changes of a Spanish soft cheese (PDO "Torta del Casar") during ripening. International Journal of Food Science & Technology, 44, 1721–1728.

Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (unpublished results). Formation of the aroma volatile profile of a raw goat milk cheese analysed by SPME–GC–MS.

DOE (2003). Diario Oficial de Extremadura (Regional Legislation of Extremadura), nº 61, 17th May 2003. Decreto 69/2003, de 20 de mayo, por el que se aprueba el Reglamento de la Denominación de Origen Protegida "Queso de Ibores" y su Consejo Regulador.

Erickson, D.R., Dunkley, W.L., & Smith, L.M. (1964). Tocopherol Distribution in Milk Fractions and Its Relation to Antioxidant Activity. Journal of Food Science, 29, 269–275.

Fedele, E., & Bergamo, P. (2001). Protein and Lipid Oxidative Stresses During Cheese Manufacture. Journal of Food Science, 66, 932–935.

Fontecha, J., Castillo, I., Blasco, L., Alonso, L., & Juárez, M. (2006). Effect of artisanal kid rennet paste on lipolysis in semi-hard goat cheese. Food Chemistry, 98, 253–259.

Franco, I., Prieto, B., Bernardo, A., González-Prieto, J., & Carballo, J. (2003). Biochemical changes throughout the ripening of a traditional Spanish goat cheese variety (Babia-Laciana). International Dairy Journal, 13, 221–230.

Freitas, A.C., & Malcata, F.X. (1998). Lipolysis in Picante cheese: influence of milk type and ripening time on free fatty acid profile. Lait, 78, 251–258.

Grappin, R., & Beuvier, E. (1997). Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese. International of Dairy Journal, 7, 751–61.

Kristensen, D., & Skibsted, L.H. (1999). Comparison of three methods based on electron spin resonance spectrometry for evaluation of oxidative stability of processed cheese. Journal of Agricultural and Food Chemistry, 47, 3099–3104.

McSweeney, P.L.H., & Sousa, M.J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening. A review. Lait, 80, 293–324.

Molimard, P., & Spinnler, H.E. (1996). Review: Compounds involved in the flavour of surface mould-ripened cheeses: Origins and properties. Journal of Dairy Science, 79, 169–184.

Moreaux, V., & Birlouez-Aragon, I. (1997). Degradation of tryptophan in heated αlactoglobulin-lactose mixtures is associated with intense Maillard reaction. Journal of Agricultural and Food Chemistry, 45, 1905–1910.

Nájera, A.I., Barron, L.J.R., & Barcina, Y. (1993). Review: Lipid fraction composition of cow's, sheep's and goat's cheese, and the influence on its quality. Science and Food Technology Spanish Journal, 33, 345–363.

Naranjo, G.B., Malec, L.S., & Vigo, M.S. (1998). Reducing sugars effect on available lysine loss of casein by moderate heat treatment. Food Chemistry, 62, 309–313.

Oliver, C.N., Ahn, B.W., Moerman, E.J., Goldstein, S., & Satadtman, E.R. (1987). Aged-related changes in oxidized proteins. Journal of Biological Chemistry, 262, 5488–5491.

Park, Y.W., Juárez, M., Ramos, M., & Haenlein, G.F.W. (2007). Physico-chemical characteristics of goat and sheep milk. Small Ruminant Research, 68, 88–113.

Poveda, J.M., & Cabezas, L. (2006). Free fatty acid composition of regionally-produced Spanish goat cheese and relationship with sensory characteristics. Food Chemistry, 95, 307–311.

Salih, A.M., Smith, D.M., Price, J.F., & Dawson, L.E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poultry Science, 66, 1483–1489.

Serra, M., Trujillo, A.J., Pereda, J., Guamis, B., & Ferragut, V. (2008). Quantification of lipolysis and lipid oxidation during cold storage of yogurts produced from milk treated by ultra-high pressure homogenization. Journal of Food Engineering, 89, 99–104.

Urbach, G. (1993). Relations between cheese flavour and chemical composition. International Dairy Journal, 3, 389–422.

Urbach, G. (1997). The flavour of milk and dairy products: II. Cheese: contribution of volatile compounds. International Journal of Dairy Technology, 50, 79–89.

Capítulo 3

Formation of the aroma of a raw goat milk cheese along maturation analysed by SPME-GC–MS

Artículo en prensa [1]: *Food Chemistry*

ABSTRACT

The volatile profile of the Spanish goat raw milk cheese of the protected designation of origin (PDO) "Queso Ibores" was studied at four stages of maturation (day 1, 30, 60 and 90) by the method of SPME–GC–MS to determinate the characteristic volatile compounds of this cheese. Ibores cheese aroma varies between sweet and mild and it has a strong taste, slightly tart. The characteristic compounds of Ibores cheese aroma were butanoic, hexanoic and octanoic acids, some alcohols (2-butanol and 2-heptanol), ethyl esters of hexanoic and butanoic acids, numerous methyl ketones (2-butanone, 2-pentanone and 2-heptanone) and δ-decalactone. A total of 64 compounds were detected: 14 acids, 18 alcohols, 13 esters, 6 ketones and 13 compounds which could not be classified in these groups. Carboxylic acids were the most abundant volatile compounds in the headspace of Ibores cheese. Content of volatile compounds was significantly modified (P<0.05) during ripening. The relative total amounts of acids, esters and ketones increased during the first 60 days of maturation.

[1] Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (In Press). Formation of the aroma of a raw goat milk cheese along maturation analysed by SPME-GC–MS. *Food Chemistry*. FOODCHEM-D-10-03088R1

1. INTRODUCTION

The characteristic flavour of cheeses is one quality component of particular importance for cheese producers because of among the many organoleptic quality components such as colour, or rheologic properties, the flavour takes a particular place, i.e. the odour and taste sensations received when eating. Thus the presence, contents and composition of volatile compounds in food have a substantial influence on its quality. The unique flavour of a cheese variety is the result of a complex balance among volatile and non-volatile chemical compounds, originating during the ripening process from milk fat, protein and carbohydrates (Fox & Wallace, 1997). These changes are followed and/or overlapped by a serie of secondary catabolic reactions, which are responsible for the unique aroma profile of a particular variety of cheese (Marilley & Casey, 2004). The characteristic flavour of goats' milk cheeses is mostly due to volatile compounds (Medina & Nuñez, 2004). In addition, the use of raw milk in the manufacture process enhances the volatiles in cheese, especially acids, alcohols and esters (Hayaloglu & Brechany, 2007), probably due to heat treatment inactive enzymes and microorganisms present in milk which are related to formation of aroma compounds.

Volatile compounds are generally analysed by gas chromatography (GC) coupled to mass spectrometry (MS). GC–MS is a useful method for identifying flavour substances, but it is always necessary to include a prior step involving the extraction of the volatile fraction. The SPME (solid-phase micro-extraction) technique permits to isolate volatile analytes from solid and liquid matrices in short time and in a simple way. Nowadays SPME is commonly used for the extraction of flavour compounds in cheese (Lecanu et al., 2002; Lee et al., 2003; Guillén, et al., 2004; Coda et al., 2006; Hayaloglu & Brechany, 2007; Delgado et al., 2010).

Due to the growing interest in the characterization of traditional products protected by a designation of origin (PDO) the volatile fractions of some Spanish cheeses have been studied in the last year: ewes milk cheese such as Manchego (Martínez-Castro et al.,

1991; Villaseñor et al., 2000), Roncal (Izco & Torre, 2000), Idiazabal (Larráyoz et al., 2001), La Serena (Carbonell et al., 2002), Zamorano (Fernández-García et al., 2004) and Torta del Casar (Delgado et al., 2010); goat milk cheeses such as Palmero (Guillén et al., 2004) and Majorero (Castillo et al., 2007); and cow milk cheese such as Mahón (Mulet et al., 1999).

Ibores cheese with Protected Designation of Origin (PDO) is produced in Spain from raw goat milk from the breeds Serrana, Verata, Retinta and crosses thereof. Aroma varies between sweet and mild, typical of goat's cheese made from raw milk. Typically, it has a strong taste: slightly tart, tangy and salty, with a slight aftertaste of goat's milk. Legislation does not allow the consumption of these cheeses before 60 days of ripening since they are made with raw milk (DOE, 2003).

Three other Spanish cheeses with PDO, Majorero, Murcia and Palmero cheeses, made from pasteurised goats' milk have been extensively studied (Guillén et al., 2004; Castillo et al., 2007). However, there are few studies about the biochemical changes of raw goat milk cheeses throughout ripening (i.e. Le Quéré et al., 1998). In addition, Sabio and Vidal-Aragon (1996) only studied the volatile compounds of 60 days Ibores cheese by Purge and Cold Trap Injection (PCTI) procedure, before Ibores cheese was PDO designation. The main advantages of SPME upon PCTI are principally the higher sensitive and rapidity, smaller sample amount and direct injection in GC. At present, producers and consumers are interesting in volatile compounds of PDO products because they are linked to food quality.

The main objective of this paper is to understand mechanism underlying of aroma formation from volatile profile evolution of Ibores cheese. There is a physico-chemical and microbial control of Queso Ibores PDO to guarantee the quality and safety food of this cheese. In this sense, changes on volatile compounds could be an additional tool for quality-control of Ibores cheese. In addition, incorporation of cheese volatile profile to PDO regulation could give a high added value product.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Milk was coagulated at 28-32 ºC for 60-90 min with animal rennet (Naturen Plus 175, 22-25 mL per 100 L; Chr Hansen, Hørsholm, Denmark). The coagulum was cut to medium-size (1-2 cm) grains. Cheeses (fiat cylindrical shape), weighting 0.7-1.2 kg, were pressed for 3-8 h, brine-salted and ripened (at 8–12 ºC and 80% relative humidity) for at least 2 months. According to the regulation of the PDO, the physicochemical properties must be a minimum of 50% of dry matter containing 50% of fat, a pH of 5.0–5.5 and a maximum of 4% NaCl (DOE, 2003).

Four cheeses from 3 different producers PDO were analysed in four different stages of ripening (1, 30, 60 and 90 days). A total of 48 cheeses were analysed, 12 cheeses in each stage of maturation. For each producer, four cheeses of the same batch were sampled in each time of ripening. During the first 60 days of ripening, cheeses were matured in each dairy in which were produced. The last month, cheeses were stored under refrigeration at 5 ºC. From each cheese, samples were taken from the inner part of the cheese and were stored at -20 ºC until they were analysed (within 2-3 weeks).

2.2. SPME-GC-MS analysis

Volatile compounds were extracted according to the method of Lee et al. (2003). The external part of the cheese was removed (1 cm). Ten grams of cheese were placed in a 50-mL vial and then 10 mL NaH_2PO_4 (25%, w/v) were added. The sample was stirred for 30 min at 50 ºC to accelerate equilibrium of headspace volatile compounds between the cheese matrix and the headspace. Then, volatile compounds were extracted by placing a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane SPME fibre (Supelco, Bellefonte, PA) into the vial and exposing it to the headspace for 30 min at 50 ºC. Chromatographic conditions are described in Delgado et al. (2010). The volatile compounds were identified by comparison with commercial reference compounds provided by Sigma–Aldrich (St. Louis, MO) by comparison of retention index with those

described in the literature and by comparison of their mass spectra with those contained in the NIST and Varian libraries.

2.3. Statistical analysis

The differences in the volatile profiles during ripening were analysed using the one-way analysis of variance (ANOVA). HSD Tukey's test was applied to compare the mean values of the volatile compounds at the different days of ripening. Mean values and standard error of the mean (SEM) are reported. The relationship between variables was assessed by the principal component analysis (PCA). All these statistical treatments were performed using the SPSS program for Windows, version 14.0 (SPSS Inc., Chicago, IL).

3. RESULTS AND DISCUSSION

3.1. General

Volatiles compounds (area units, AU, $x10^4$) isolated from the Spanish raw goat cheese Ibores by SPME–GC–MS along maturation are shown in Table 1. A total of 64 compounds were detected: 14 acids, 18 alcohols, 13 esters, 6 ketones and 13 compounds which could not be classified in these groups. Sabio and Vidal-Aragon (1996) only detected 29 volatile compounds in Ibores cheese but the volatile isolation technique was different (SPME vs. PCTI). These volatile compounds were esters, metylketones and alcohols; terpenes, an aldehyde and two aromatic hydrocarbons were also identified.

Figure 1 shows the changes of the main chemical groups of volatile compounds during ripening. Compared to day 1, an important increase of total area units was found in the first 60 days of ripening, while in the last 30 days (days 60-90) the amount of acids, alcohols and, above all, ketones tended to decrease. Therefore, when cheese is commercialized (day 60), most volatile compounds grouped in chemical families (except esters) have reached their highest level. Total carboxylic acid content tended

increased during the first 60 days of maturation, and the last 30 days they slightly decreased. Amounts of alcohols decreased the first 30 days, probably due to alcoholysis or esterification reactions, and increased at the end of ripening. Area units of esters increased during the four stages of ripening. Volatile ketones followed a similar trend as described for acids, but its reduction was higher at day 90. Finally, the levels of other compounds were relatively constant during ripening.

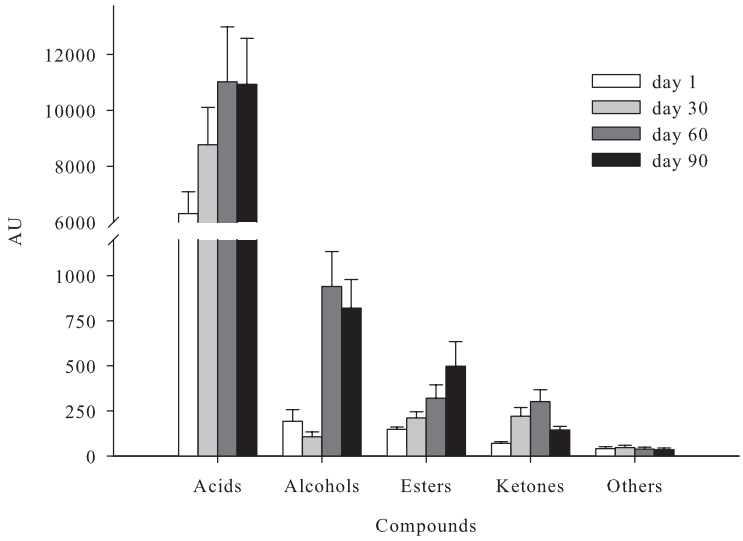

Figure 1. Evolution of volatile compounds (Area Units x 10^4) grouped in the main chemical families isolated in Ibores cheese through of maturation.

3.2. Carboxylic acids

The evolution of carboxylic acids according to their most probable origin is showed in Figure 2. During the ripening of cheeses, carboxylic acids can be originated from 3 main biochemical pathways: i. lipolysis (hydrolysis of triglycerides into free fatty acids), ii. proteolysis (breakage of caseins into peptides and amino acids) and iii. lactose fermentation (reviewed by Curioni & Bosset, 2002). Lactose is metabolized to lactate in all cheese varieties and lactate may also be further metabolized to cheese flavour compounds such as acetic and propanoic acids by microbial metabolism (McSweeney

& Sousa, 2000). Previously, we argued about the most probable origin of different carboxylic acids according to the literature (Delgado et al., 2010). Amounts of carboxylic acids with origin in lipolysis (Σ Area Units of butanoic, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic and undecanoic acids), which were considerably the most abundant volatile acids, tended to increase along ripening especially in the first two month of maturation. Carboxylic acids with origin in lactate metabolism (microbial origin–principally lactic acid bacteria-; Σ Area Units of acetic and propanoic acids) and from free amino acids (Σ Area Units of 2-methylpropanoic and 3-methylbutanoic acids) were less abundant compared to those from lipolysis. The level of carboxylic acids from amino acids increased along maturation, but the last 30 days slightly decreased. However, carboxylic acids from lactate metabolism importantly increased in the first 30 days of ripening, and then, the content tended to decrease. This indicates that microbial activity responsible of lactate metabolism could be more intense at the beginning of ripening of this cheese. Throughout the entire maturation process, most of carboxylic acids detected probably came from lipolysis of triglycerides followed by those produced from lactate metabolism while carboxylic acids from free amino acids were the less abundant. Therefore, lipolysis could be the main pathway responsible for the release of carboxylic acids in Ibores cheese. As this cheese is made from raw milk, lipolysis principally occurs by the action of indigenous lipases from the milk. In a previous study developed in the raw ewe milk cheese Torta del Casar (Delgado et al., 2010), a different pattern of formation of acid volatiles was reported according to their origin. The differences detected between both types of cheeses in the acid volatile formation which could be associated to i. different coagulation pattern (use of vegetable coagulant increased the volatile acid from amino acids in Torta del Casar because cardosin has a higher proteolytic activity than chymosin), ii. type of milk (goat vs. ewe), iii. maturation conditions (a higher temperature and lower humidity in Ibores cheese) and iv. natural microflora associated to the milk.

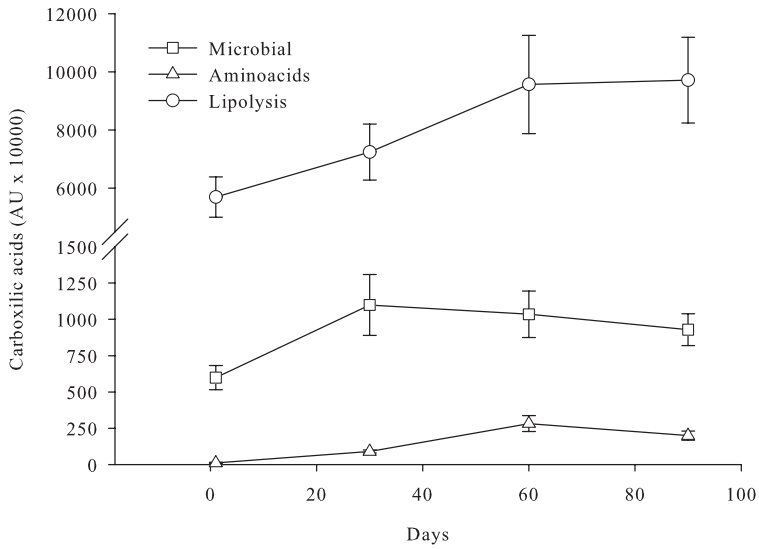

Figure 2. Evolution of carboxylic acids along ripening acording to their most probable origin. Carboxylic acids from lactate metabolism (microbial origin; Σ Area Units of acetic and propanoic acids), lipolysis (Σ Area Units of butanoic, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, decanoic and undecanoic acids) and amino acids (Σ Area Units of 2-methylpropanoic and 3-methylbutanoic acids).

Carboxylic acids were the most abundant volatile compounds in the headspace of Ibores cheese (Figure 1). Acid compounds at day 60 constituted the 41% of the total area units of volatile compounds isolated from the Ibores cheese. Statistically significant differences (P<0.05) for all acids were found during maturation, except in acetic and octanoic acids. Hexanoic acid was the most abundant acid isolated. Butanoic, octanoic and decanoic acids, in decreasing order, were also very abundant. Acetic, propanoic and 3-methylbutanoic acids were also present; they are very volatile compounds owing to their smaller molecular size. Other carboxylic acids detected in Ibores cheese are listed in Table 1.

Carboxylic acids are not only aroma compounds by themselves but also they are precursors of other compounds, such as methyl ketones, alcohols, lactones, aldehydes and esters (Collins et al., 2003). Due to their low aroma thresholds, short and medium-chain carboxylic acids (C4:0–C8:0 and C10:0–C14:0, respectively) are considered to be

important contributors to the flavour profile in a wide variety of cheeses (Woo & Lindsay, 1982; Zerfiridis et al., 1984; Moio & Addeo, 1998; Pinho et al., 2003; Tavaria et al., 2004; Kraggerud et al., 2008; Delgado et al., 2010). Acids are important, or even predominant, components of the aroma of many goat cheeses (Le Quéré et al., 1998; Castillo et al., 2007; Poveda et al., 2008), but Sabio and Vidal-Aragon (1996) did not identify any acid compounds in Ibores cheese. This difference could be explained by the different headspace extraction method used (purge and trap vs. direct adsorption on SPME fibre). We also reported that acids are the most important aroma compounds in Torta del Casar ewe raw milk cheese (Delgado et al., 2010).

Hexanoic acid was the most abundant acid found in headspace of Ibores cheese (Table 1). This acid was the second major acid isolated in the volatile fraction of a traditional Sainte-Maure type soft goat cheese made from raw milk by lactic coagulation and with one month of ripening (Le Quéré et al., 1998) and in Majorero goat cheese artisanal produced with 60 days (Castillo et al., 2007). This compound originated by lipolysis, contributes significantly to goat cheese odour and has been identified as the main odorant in different cheese types such as aged Cheddar (Christensen & Reineccius, 1995) and Grana Padano (Moio & Addeo, 1998). For this reason, hexanoic acid, a short-chain carboxylic acid, could importantly contribute to the typical aroma of Ibores cheese. The other major acid detected, butanoic acid, has a rancid cheese-like odour and plays an important role in the flavour of many cheese types such as Camembert, Cheddar (aged, regular and low fat), Grana Padano, Gruyère, Pecorino, Ragusano, Roncal (reviewed by Curioni & Bosset, 2002) and Majorero cheese (Castillo et al., 2007). Octanoic and decanoic acid are also listed among the major odorants of the latter type of cheese (reviewed by Curioni & Bosset, 2002). The fatty acids hexanoic, octanoic and decanoic acids have been widely recognized as the responsible for the characteristic aroma of goat cheeses, giving rise to the popular terms caproic, caprilic and capric acids, respectively (Poveda & Cabezas, 2006), and its contribution to aroma of Ibores cheese has been showed in the present study as well.

Table 1. Mean and standard error media of volatile compounds (Area Units x 10^4) isolated in Ibores cheese at different stages of ripening. (See footnote Table 1 cont)

Compounds	Id. Method [z]	Day 1 (n=12)	Day 30 (n=12)	Day 60 (n=12)	Day 90 (n=12)	SEM	Sign.
Acids							
Acetic acid	RF,TI	594.9	977.1	898.1	800.8	61.6	0.144
Propanoic acid	RF,TI	3.9b	121.8a	137.2a	128.0a	16.8	0.010
Propanoic acid, 2-methyl	RF,TI	2.2b	17.1b	44.9a	36.9a	3.1	0.001
Butanoic acid	RF,TI	1437.9ab	1116.4b	2088.4a	2172.9a	118.4	0.001
Butanoic acid, 3-methyl	RF,TI	8.9c	73.0bc	237.5a	162.3ab	18.5	0.001
Pentanoic acid	RF,TI	10.5b	12.8b	22.4a	22.2a	1.1	0.001
Hexanoic acid	RF,TI	2665.8b	2684.2b	4489.2a	4595.4a	304.6	0.019
3-Hexenoic acid	TI	0.0	0.0	2.0	2.2	0.3	0.024
Heptanoic acid	TI	22.6	33.8	47.2	46.0	3.5	0.034
Octanoic acid	RF,TI	919.3	1641.7	1768.8	1769.1	165.1	0.207
2,4-Hexadienoic acid	TI	0.0b	341.3a	137.5ab	83.4ab	44.9	0.043
Nonanoic acid	TI	9.8b	14.1ab	23.6a	21.1ab	1.9	0.031
Decanoic acid	RF,TI	614.8b	1599.4a	1064.2ab	1045.3ab	102.1	0.005
Undecanoic acid	TI	12.7b	139.8a	63.9ab	44.4b	12.6	0.001
Alcohols							
2-Propanol	TI	0.6bc	0.0c	1.5ab	1.6a	0.2	0.001
Ethanol, 2-methoxy	TI	12.9b	11.0b	33.1a	40.8a	3.0	0.001
2-Butanol	RF,TI	109.7b	73.8b	727.3a	602.4a	60.6	0.001
1-Propanol	RF,TI	0.0b	0.0b	5.8a	5.2a	0.6	0.001
2-Pentanol	RF,TI	0.0b	1.1b	39.0a	27.2ab	4.7	0.002
1-Butanol	RF,TI	0.0b	0.3b	4.8a	7.7a	0.6	0.001
1-Butanol, 3-methyl	TI	37.5a	4.5b	30.2a	22.5ab	3.2	0.001
2-Hexanol	TI	0.0b	0.0b	1.5a	0.0b	0.2	0.003
1-Pentanol	RF,TI	1.6a	0.0b	0.7b	0.4b	0.1	0.001
3-Buten-1-ol, 3-methyl	TI	2.7a	0.0b	2.1a	1.3ab	0.3	0.005
2-Heptanol	TI	2.4b	6.6b	71.5a	84.3a	9.4	0.001
3-Penten-2-ol	TI	5.7a	0.9b	2.8b	2.0b	0.4	0.001
2-Nonen-1-ol	TI	8.4a	2.6b	3.0b	1.8b	0.5	0.001
Cyclohexanol, 2-methyl	TI	7.5	1.3	4.2	4.7	1.1	0.258
2-Nonanol	TI	0.0c	3.8bc	8.0ab	13.1a	1.1	0.001
2,4-Hexadien-1-ol	TI	0.0b	0.0b	3.1ab	4.6a	0.6	0.017
2-Furanmethanol	RF,TI	0.0c	0.0c	0.7a	0.4b	0.1	0.001
1-Butanol, 4-butoxy	TI	3.2a	1.4b	1.2b	0.0c	0.2	0.001

Table 1 (continuation). Mean and standard error media of volatile compounds (Area Units x 10^4) isolated in Ibores cheese at different stages of ripening.

Compounds	Id. Method [z]	Day 1 (n=12)	Day 30 (n=12)	Day 60 (n=12)	Day 90 (n=12)	SEM	Sign.
Esters							
Ethyl acetate	RF,TI	5.1ab	0.7b	2.8ab	6.6a	0.7	0.008
Propyl acetate	TI	0.0b	0.0b	0.3ab	1.0a	0.1	0.001
Butanoic acid, ethyl ester	RF,TI	62.6ab	19.2b	64.6ab	76.9a	7.2	0.022
Butanoic acid, propyl ester	TI	0.0b	0.8b	8.6ab	13.5a	1.6	0.004
Butanoic acid, butyl ester	TI	0.0b	0.9b	30.3ab	41.0a	4.9	0.001
Hexanoic acid, ethyl ester	RF,TI	42.0b	43.3b	110.4ab	173.7a	16.5	0.008
Propanoic acid, 2-methyl-3-methylbutyl ester	TI	0.2b	0.4b	2.1ab	2.8a	0.3	0.006
4-Hexenoic acid, ethyl ester	TI	0.0	1.1	4.4	3.5	0.8	0.221
Hexanoic acid, butyl ester	TI	0.0b	6.0b	36.1ab	77.2a	7.1	0.001
Octanoic acid, methyl ester	TI	4.8ab	7.8a	0.4b	0.7b	0.8	0.001
Octanoic acid, ethyl ester	RF,TI	24.1c	58.1a	34.5bc	54.2ab	3.4	0.001
Decanoic acid, ethyl ester	RF,TI	8.9c	73.7a	25.5c	47.2b	4.5	0.001
Propanoic acid, 2-methyl, ethyl ester	TI	0.0	0.5	1.1	0.8	0.2	0.101
Ketones							
2-Butanone	TI	0.3b	111.9a	160.1a	74.5ab	14.1	0.001
2-Pentanone	RF,TI	13.0b	9.6b	30.8a	6.3b	2.6	0.002
2-Heptanone	TI	32.8ab	36.5ab	73.1a	30.9b	5.8	0.026
2-Octanone	RF,TI	0.0b	0.8a	0.6ab	0.4ab	0.1	0.022
2-Nonanone	RF,TI	24.4b	58.2a	35.2ab	29.1b	3.7	0.004
2-Undecanone	TI	0.0c	3.4a	2.6b	2.9ab	0.2	0.001
Others							
Butanal, 3-methyl	TI	0.4	0.0	0.4	0.2	0.1	0.106
2,4-Hexadienal	TI	0.0	0.6	1.7	1.7	0.3	0.068
Methanethiol	TI	0.0b	0.0b	0.3a	0.2a	0.03	0.001
Toluene	TI	3.4a	1.2b	4.1a	3.0a	0.2	0.001
Camphene	TI	8.6	0.6	7.5	4.5	1.4	0.158
p-Xylene	TI	13.5a	4.3b	0.7c	0.3c	0.8	0.001
Styrene	TI	1.0b	3.0a	0.6b	0.8b	0.2	0.001
Limonene	RF,TI	6.7a	1.8b	2.3b	0.8b	0.6	0.001
Cymene	TI	1.3a	0.3b	0.5ab	0.3b	0.1	0.022

Compounds	Id. Method [z]	Day 1 (n=12)	Day 30 (n=12)	Day 60 (n=12)	Day 90 (n=12)	SEM	Sign.
Copaene	TI	1.3	1.3	1.3	1.2	0.3	0.998
Cadinene	TI	1.4	5.0	2.2	2.1	0.7	0.223
δ-Decalactone	TI	3.5c	8.5a	6.5b	6.1b	0.4	0.001
Dodecalactone	TI	0.0b	20.9a	10.5ab	13.3ab	2.4	0.014

z Method of identification: RF, mass spectrum and retention time identical with a reference compound; TI, tentative identification by mass spectrum. SEM: Standard Error Media. a,b,c: different letters in the same row indicate significant statistical differences (Tukey's Test, P<0.05).

Acetic acid originates from different reactions by the action of lactic acid bacteria – e.g., *Lactococcus and Streptococcus*– (Ur-Rehman et al., 2000) and propanoic acid has its origin in the metabolism of lactate by *Propionibacterium spp* (Steffen et al., 1987). Acetic and propanoic acids could be associated with the slight tart taste of Ibores cheese.

The branched-chain fatty acids (BCFA) are characteristic impact odour active compounds of goat and sheep cheeses. 2-methylpropanoic (isobutyric) and 3-methylbutanoic (isovaleric) acids are derived from the metabolism of Valine and Leucine, respectively (Molimard & Spinnler, 1996). 3-methylbutanoic acid was the most abundant BCFA found in Ibores cheese and it provides a rancid cheese and sweaty odours (Yvon & Rijnen, 2001). Poveda et al. (2008) reported that 3-methylbutanoic acid was the fatty acid perceived with the highest intensity by the sniffers in a study about semi-hard Spanish goat cheeses. Although the presence of 4-methyl and 4-ethyl octanoic acids has been considered to be a distinctive characteristic of goat cheese (reviewed by Curioni & Bosset, 2002) these compounds were not isolated in the samples of Ibores cheese. Le Quéré et al. (1998) found these BFCA in Sainte-Maure type soft goat cheese, but yet in other Spanish goat cheeses they either were not detected (Castillo et al., 2007) or appeared in traces (Guillén et al., 2004; Poveda et al., 2008). This fact could be attributable to the composition of the milk depending on the goat breed and its feed (Guillén et al., 2004). Likewise, the presence of microorganisms (*Lactococcus* and non starter lactic acid bacteria) with different amino acid catabolic

activities might also play a key role in these differences (Castillo et al., 2007). In this case, as raw goat milk is used for cheese manufacture the native microorganisms of non-pasteurized milk may have a significant contribution on its final volatile profile.

3.3. Alcohols

Alcohols were the second most abundant chemical family (35% of the total relative area units at day 60) isolated from the headspace of the Ibores cheese at the end of ripening. In general, the strong reducing conditions in cheese favour the formation of alcohols from aldehydes and ketones, following reaction pathways which involve alcohol dehydrogenases (Molimard & Spinnler, 1996). Levels of alcohols were significantly affected ($P<0.05$) by the maturation process. The decrease of alcohols the first and last month of ripening is probably due to the reaction of alcohols with acids to form esters. 2-butanol, 2-heptanol and 2-pentanol were the most abundant alcohols at the end of ripening (Table 1). 3-methyl-1-butanol was also detected in the headspace of this cheese.

Secondary alcohols are formed by enzymatic reduction (alcohol dehydrogenase) of the corresponding methyl ketones (Molimard & Spinnler, 1996). 2-butanol was the highest secondary alcohol isolated in the artisanal Manchego cheese (Gómez-Ruiz et al., 2002). Other secondary alcohol such as 2-heptanol has been identified as a key odorant of Gorgonzola and Grana Padano cheeses (Curioni & Bosset, 2002), and it was the alcohol detected in the highest concentrations in semi-hard Spanish goat cheeses (Póveda et al., 2008). On the other hand, 2-pentanol and 3-methyl-1-butanol have lower detection thresholds (Carbonell et al., 2002) and thus they are probably playing an important role in the aroma of this cheese variety. The presence of branched-chain primary alcohols, such as 3-methyl-1-butanol, indicates the reduction of the aldehyde produced from Leucine. It was identified in other goat cheeses (Le Quéré et al., 1998; Castillo et al., 2007) and confers a pleasant aroma of fresh cheese (Moio et al., 1993).

3.4. Esters

Amounts of esters were significantly modified (P<0.05) after 90 days of ripening, except 4-hexenoic acid ethyl ester and propanoic acid, 2-methyl, ethyl ester. Esters were the unique chemical family which increased in every stage of ripening (Figure 1). This increase could be due to the esterification of acids and alcohols.

Hexanoic acid and butanoic acid ethyl ester were the most abundant esters isolated in the Ibores cheese. These esters are obtained from esterification reactions occur between hexanoic and butanoic acids, respectively, and primary and secondary alcohols derived from lactose fermentation or from amino acid catabolism. Butanoic acid ethyl ester has been identified as one of the most potent odorants of Cheddar, Emmental, creamy Gorgonzola, Grana Padano and Pecorino cheeses, whereas hexanoic acid ethyl ester plays an important role in the aroma profiles of aged Cheddar, natural Gorgonzola, Grana Padano, Pecorino and Ragusano cheeses (reviewed by Curioni & Bosset, 2002). These two esters, in addition to ethyl acetate, were the majority esters identified by Castillo et al. (2007) in Majorero goat cheese and were considered as key constituents of the aroma of this cheese variety.

Esters are common cheese volatiles and most esters encountered are described as having sweet, fruity and floral notes, and especially ethyl esters are known for their important role in the formation of a fruity character in cheese (Curioni & Bosset, 2002). Some of these esters have a very low perception threshold: e.g., butanoic and hexanoic acid ethyl ester have a nasal threshold of 28 and 40 μg Kg^{-1}, and retro nasal of 3.5 and 20 μg Kg^{-1}, respectively. Therefore, esters could provide fruity notes to Ibores cheese that minimise the strong aroma produced by carboxylic acids.

3.5. Ketones

Most of the ketones in Ibores cheese were methylketones (Table 1), as occurred in other goat cheese varieties (Le Quéré et al., 1998; Castillo et al., 2007; Poveda et al., 2008). Amounts of methylketones were significantly reduced (P<0.05) after 1 month at

refrigeration conditions (day 60-90). This reduction was probably due to the decrease of the microbial activity during this conservation time because free fatty acids arising from the lipolysis are generally catabolised to methylketones by the microorganisms. 2-Butanone and 2-heptanone were present in larger amounts than any other ketone at day 60 of ripening. Similar results have been found in other regional raw milk cheeses with PDO (Carbonell et al., 2002; Delgado et al., 2010), so they could play an important role in the final aroma of these cheeses made from raw milk.

Ketones are abundant constituents of most dairy products and they have typical odors and low perception thresholds (nasal of 1.5–3.4 mg Kg^{-1}, and retro nasal of 1.5–2.4 mg Kg^{-1}; reviewed by Curioni & Bosset, 2002). Methylketones are produced from free fatty acids by an alternative pathway to the β-oxidation (McSweeney & Sousa, 2000). 2-butanone, with a butterscotch odor, was identified as main odorant in Cheddar cheese (Arora et al., 1995) and 2-heptanone, with a herbaceous odor, is an important flavour compound of Emmental and natural and creamy Gorgozola cheeses (reviewed by Curioni & Bosset, 2002). Fruity, floral and musty notes are associated with various methylketone such as 2-octanone, 2-nonanone and 2-undecanone, so the presence of these volatile compounds can be considered positive to flavour cheese.

3.6. Others

Two aldehyde (3-methyl butanal and 2,4-hexadienal), one sulfur compound (methanethiol), three hydrocarbons (toluene, p-xylene and styrene), five terpenes (camphene, limonene, cymene, copaene and cadiene) and two lactone (δ-decalactone and dodecalactone) were also detected in Ibores cheese. The relative abundance of five of them was not significantly modified (P>0.05) after 90 days of cheese maturation (Table 1). Aldehydes are transitory compounds in cheese because they are rapidly reduced to primary alcohols or even oxidized to the corresponding acids. 3-methyl butanal was identified as a potent odorant in Cheddar, Emmental and Gruyère cheese varieties (reviewed by Curioni & Bosset, 2002). Volatile sulfur compounds greatly

contribute to the flavor of many cheeses (Molimard & Spinnler, 1996; McSweeney & Sousa, 2000) and they can interact with each other and with other compounds in cheese, generating a diversity of volatile flavor compounds (Juan et al., 2007). Moreover, their perception thresholds are very low. Methanethiol is an important sulphur-containing compound. It derives from Metinone and may be a precursor for further sulphur compounds such as dimethyldisulphide (DMDS) and dimethyltrisulphide (DMTS) via oxidative reactions. Methanethiol is present in Camembert cheese as well and, together with other sulphur compounds, is responsible for the garlic note which can be found in well-ripened Camembert cheese (Adda et al., 1998) and from our results it could be an important volatile compound of Ibores cheese. Concerning aromatic hydrocarbons, toluene, which provides nutty odor, was the most abundant and was already identified at high levels in Feta-type (Bintis & Robinson, 2004) and Spanish ewe's milk semihard cheeses (Mariaca et al., 2001). In traditional cheeses manufactured in alpine regions terpenes are important volatile compounds with origin in plants that constitute the forage mixture of the pastures (Mariaca et al., 1997). Although, the majority of terpenes identified in Ibores cheese have been isolated in other cheese types, the importance of terpenes in the formation of flavour of cheese remains controversial (Curioni & Bosset, 2002). Finally, one of the most common and important lactones identified in Ibores cheese is the δ-decalactone. This compound is a key odorant of Camembert and Emmental cheese varieties (reviewed by Curioni & Bosset, 2002). Both δ-decalactone and dodecalactone could provide coconut notes to the background aroma of Ibores cheese.

3.7. Principal Component Analysis (PCA)

PCA is a statistical analysis for resolving sets of data into orthogonal components, whose linear combinations (principal components) approximate the original data to any desired degree of accuracy. In most cases, two principal components are sufficient to explain a great proportion of the variation in the original parameters. Table 2 shows

the most important loadings and the percentage accounted by the two first principal components (PC#1 and PC#2) after PCA.

Table 2. Results of the principal component analysis on the volatile composition through maturation of Ibores cheese showing the most important loadings and the percentage variance accounted for by the first two principal components (PC#1 and PC#2).

Compounds	Principal Component	
Acids	PC1	PC2
Acetic acid	0.815	0.007
Propanoic acid	0.850	0.243
Hexanoic acid	0.902	0.082
3-Hexenoic acid	0.830	-0.020
Heptanoic acid	0.953	-0.083
Octanoic acid	0.959	-0.173
Nonanoic acid	0.911	-0.098
Decanoic acid	0.863	-0.250
Alcohols		
2-Butanol	0.308	0.868
1-Propanol	0.119	0.914
2-Pentanol	-0.191	0.830
2-Heptanol	-0.107	0.953
2,4-Hexadien-1-ol	0.813	-0.008
Esters		
Propyl acetate	0.001	0.872
Butanoic acid, ethyl ester	-0.422	0.808
Butanoic acid, propyl ester	0.021	0.923
Butanoic acid, butyl ester	0.082	0.927
Hexanoic acid, ethyl ester	-0.097	0.918
Propanoic acid, 2-methyl-3-methylbutyl ester	0.125	0.860
Hexanoic acid, butyl ester	0.235	0.865
Propanoic acid, 2-methyl, ethyl ester	0.921	-0.095
Percentage of variance explained	28	25

The 28% and 25% of the variability was explained by PC#1 and PC#2, respectively. With positive loadings on PC#1 are located most acids, one alcohol (2,4-hexadien-1-ol) and one ester (propanoic acid, 2-methyl, ethyl ester). Regarding PC#2, most alcohols and esters had positive loadings and also explained an important part of the variation detected.

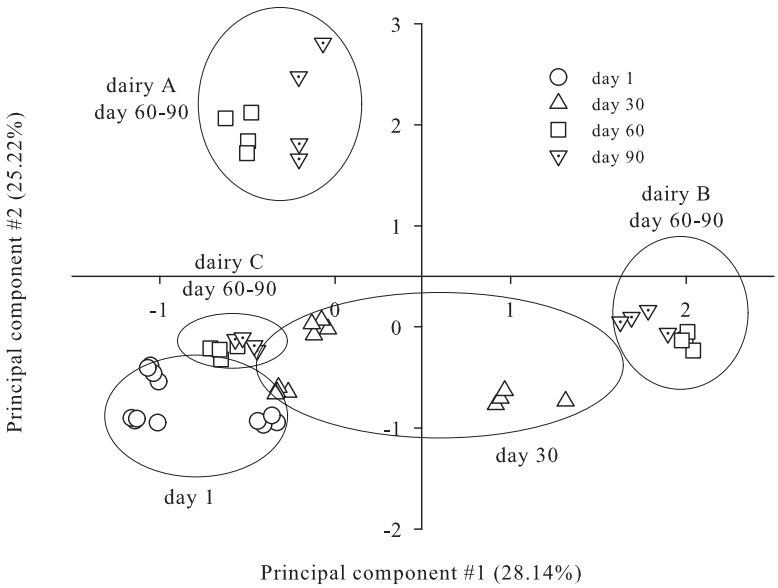

Figure 3. Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC#1: principal component 1; PC#2: principal component 2).

The distribution of the scores on the first two PCs is showed in Figure 3. Cheeses at day 1 are located in the negative area of both PC#1 and PC#2, and cheeses at day 30 are near of the axis origin. Cheeses with 60 and 90 days were located in three different sectors defined by the two PC which correspond to the three dairies which manufactured the cheeses. Thus, dairy A cheeses are located at the positive end of PC#2 (related to a higher content in alcohols and esters), dairy B cheeses are at the positive end of PC#1 (related to a higher content in acids) and dairy C cheeses are at

the negative area of PC#1 (lower content in acids). PC#1 explained part of the differences between cheeses belonging to dairy B and the other dairies at the end of ripening (day 60 and 90) and PC#2 separated cheeses of dairy A from the dairies B and C. These results indicate that initial reactions involved in the release of volatile compounds of Ibores cheese principally were similar at the beginning of ripening but different aromatic profiles were formed at the end of maturation in each dairy. Inside the typical and characteristic flavour of Ibores cheese, the differences for volatile compounds showed between dairies can be considered positive because it offers to consumer products with a particular background aroma.

4. CONCLUSIONS

The characteristic compounds of Ibores cheese aroma were butanoic, hexanoic and octanoic acids, some alcohols (2-butanol and 2-heptanol), ethyl esters of hexanoic and butanoic acids, numerous methyl ketones (2-butanone, 2-pentanone and 2-heptanone) and δ-decalactone. Volatile acids were the most abundant compounds isolated from the headspace of Ibores cheese. Those acids with origin in lipolysis had the highest importance in the aromatic profile of this cheese. The pattern of volatile acids formation according to their most probably origin could be associated to the differential and typical characteristics in each type of cheese and could be an interesting pathway to go further in the characterization of the aromatic volatile profile of cheeses. In addition, volatile profile could be applied to characterization of Ibores cheese PDO and to guarantee the quality-control of this cheese. Complementary sensory analyses should be made in a future.

5. Acknowledgements

This work has been financially supported thanks to European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). We thank the PDO dairies for providing the cheeses.

6. References

Adda, J., Czulak, J., Mocquot, G., & Vassal, L. (1998). In Cross H.R., Overby A.J. (Eds.), Cheese, Meat Science, Milk Science and Technology (pp. 373–392). The Netherlands: Elsevier Science Publications.

Arora, G., Cormier, F., & Lee, B. (1995). Analysis of odor-active volatiles in Cheddar cheese headspace by multidimensional GC/MS sniffing. Journal of Agricultural and Food Chemistry, 43, 748–752.

Bintis, T., & Robinson, R. K. (2004). A study of the effects of adjunct cultures on the aroma compounds of Feta-type cheese. Food Chemistry, 88, 435–441.

Carbonell, M., Núñez, M., & Fernández-García, E. (2002). Evolution of the volatile components of ewes raw milk La Serena cheese during ripening. Correlation with flavour characteristics. Lait, 82, 683–698.

Castillo, I., Calvo, M. V., Alonso, L., Juárez, M., & Fontecha, J. (2007). Changes in lipolysis and volatile fraction of a goat cheese manufactured employing a hygienized rennet paste and a defined strain starter. Food Chemistry, 100, 590–598.

Coda, R., Brechany, E., De Angelis, M., De Candia, S., Di Cagno, R., & Gobbetti, M. (2006). Comparison of the compositional, microbiological, biochemical, and volatile profile characteristics of nine Italian ewes' milk cheeses. Journal of Dairy Science, 89, 4126–4143.

Collins, Y. F., McSweeney, P. L. H., & Wilkinson, M. G. (2003). Review: Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. International Dairy Journal, 13, 841–866.

Christensen, K. R., & Reineccius, G. A. (1995). Aroma extract dilution analysis of aged Cheddar cheese. Journal of Food Science, 60, 218–220.

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

Delgado, F.J., González-Crespo, J., Cava, R., García-Parra, J. & Ramírez, R. (2010). Characterization of the volatile profile of a Spanish ewe raw milk soft cheese P.D.O. Torta del Casar during ripening by SPME-GC-MS. Food Chemistry, 118, 182–189.

DOE (2003). Diario Oficial de Extremadura (Regional Legislation of Extremadura), nº 61, 17th May 2003. Decreto 69/2003, de 20 de mayo, por el que se aprueba el Reglamento de la Denominación de Origen Protegida "Queso de Ibores" y su Consejo Regulador.

Fernández-García, E., Carbonell, M., Gaya, P., & Nuñez, M. (2004). Evolution of the volatile components of ewes raw milk Zamorano cheese. Seasonal variation. International Dairy Journal, 14, 701–711.

Fox, P. F., & Wallace, J. M. (1997). Formation of flavour compounds in cheese. Advances in Applied Microbiology, 45, 17–85.

Gómez-Ruiz, J. A., Ballesteros, C., González-Viñas, M. A., Cabezas, L., & Martínez-Castro, I. (2002). Relationships between volatile compounds and odour in Manchego cheese: comparison between artisanal and industrial cheeses at different ripening times. Lait, 82, 613–628.

Guillén, M. D., Ibargoitia, M. L., Sopelana, P., Palencia, G., & Fresno, N. (2004). Components detected by means of solid-phase microextraction and gas chromatography/mass spectrometry in the headspace of artisan fresh goat cheese smoked by traditional methods. Journal of Dairy Science, 87, 284–299.

Hayaloglu, A. A., & Brechany, E. Y. (2007). Influence of milk pasteurization and scalding temperature on the volatile compounds of Malatya, a farmhouse Halloumi-type cheese. Le Lait, 87, 39–57.

Izco, J. M., & Torre, P. (2000). Characterisation of volatile flavour compounds in Roncal cheese extracted by the ''purge and trap'' method and analysed by GC–MS. Food Chemistry, 70, 409–417.

Juan, B., Barron, L. J. R., Ferragut, V., & Trujillo, A. J. (2007). Effects of High Pressure Treatment on Volatile Profile During Ripening of Ewe Milk Cheese. Journal of Dairy Science, 90, 124–135.

Kraggerud, H., Skeie, S., Høy, M., Røkke, L., & Abrahamsen, R. K. (2008). Season and ripening temperature influence fatty acid composition and sensory properties of semi-hard cheese during maturation. International Dairy Journal, 18, 801–810.

Larráyoz, P., Addis, M., Gauch, R., & Bosset, J. O. (2001). Comparison of dynamic headspace and simultaneous distillation extraction techniques used for the analysis of the volatile components in the three European PDO ewe's milk cheeses. International Dairy Journal, 11, 911–926.

Le Querré, J.L., Pierre, A., Riaublanc, A., & Demaizières, D. (1998). Characterization of aroma compounds in the volatile fraction of soft goat cheese during ripening. Lait, 78, 279–290.

Lecanu, L., Ducruet, V., Jouquand, C., Gratadoux, J. J., & Feigenbaum, A. (2002). Optimization of headspace solid-phase microextraction (SPME) for the odor analysis of surface-ripened cheese. Journal of Agricultural and Food Chemistry, 50, 3810–3817.

Lee, J. H., Diono, R., Kim, G. Y., & Min, D. (2003). Optimization of solid phase microextraction analysis for the headspace volatile compounds of Parmesan cheese. Journal of Agricultural and Food Chemistry, 51, 1136–1140.

Mariaca, R. G., Berger, T. F. H., Gauch, R., Imhof, M. I., Jeangros, B., & Bosset, J. O. (1997). Occurrence of volatile mono- and sesquiterpenoids in highland and lowland plant species as possible precursors for flavor compounds in milk and dairy products. Journal of Agricultural and Food Chemistry, 45, 4423–4434.

Mariaca, R. G., Fernández-García, E., Mohedano, A. F., & Núñez, M. (2001). Volatile fraction of ewe's milk semi-hard cheese manufactured with and without the addition of a cysteine proteinase. Food Science and Technology, 212, 239–246.

Marilley, L., & Casey, M. G. (2004). Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. International Journal of Food Microbiology, 90, 139–159.

Martínez-Castro, I., Sanz, J., Amigo, L., Ramos, M., & Martín-Alvarez, P. (1991). Volatile components of Manchego cheese. Journal of Dairy Research, 58, 236–239.

McSweeney, P.L.H., & Sousa, M.J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Le Lait, 80, 293–324.

Medina, M., & Nuñez, M. (2004). Cheeses made from ewes' and goats' milk. In P.F. Fox, P.L.H. McSweeney, T.M. Cogan, & T.P. Guinee (Eds.), Cheese: Chemistry, Physics and Microbiology, Vol. 2: Major Cheese Groups (3rd ed.) (pp. 279–299). London, UK: Elsevier Academic Press.

Moio, L., Langlois, D., Etievant, P. X., & Addeo, F. (1993). Powerful odorants in water buffalo and bovine Mozzarella cheese by use of extract dilution sniffing analysis. Italian Journal of Food Science, 3, 227–237.

Moio, L., & Addeo, F. (1998). Grana Padano cheese aroma. Journal of Dairy Research, 65, 317–333.

Molimard, P., & Spinnler, H. (1996). Review: Compounds involved in the flavour of surface mold-ripened cheeses: origins and properties. Journal of Dairy Science, 79, 169–184.

Mulet, A., Escriche, I., Rossello, C., & Tarrazó, J. (1999). Changes in the volatile fraction during ripening of Mahón cheese. Food Chemistry, 65, 219–225.

Pinho, O., Ferreira, I. M. P. L. V. O., & Ferreira, M. A. (2003). Quantification of shortchain free fatty acids in ''Terrincho" ewe cheese: intravarietal comparison. Journal of Dairy Science, 86, 3102–3109.

Poveda, J.M., & Cabezas, L. (2006). Free fatty acid composition of regionally-produced Spanish goat cheese and relationship with sensory characteristics. Food Chemistry, 95, 307–311.

Poveda, J.M., Sánchez-Palomo, E., Pérez-Coello, M.S., & Cabezas, L. (2008). Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat cheeses. Dairy Science & Technology, 88, 355–367.

Sabio, E., & Vidal-Aragon, M. C. (1996). Analysis of the volatile fraction of Ibores cheese. Alimentaria, 278, 101–103.

Steffen, C., Flueckiger, E., Bosset, J. O., & Ruegg, M. (1987). Swisstype varieties. In P. F. Fox (Ed.), Cheese: Chemistry, physics and microbiology (pp. 93–120). London: Elsevier Applied Science.

Tavaria, F. K., Silva Ferreira, A. C., & Malcata, F. X. (2004). Volatile free fatty acids as ripening indicators for Serra da Estrela cheese. Journal of Dairy Science, 87, 4064–4072.

Ur-Rehman, S., Banks, J. M., Bechany, E. Y., Muir, D. D., McSweeney, P. L. H., & Fox, P. F. (2000). Influence of ripening temperature on the volatiles profile and flavour of Cheddar cheese made from raw or pasteurised milk. International Dairy Journal, 10, 55–65.

Villaseñor, M. J., Valero, E., Sanz, J., & Martínez-Castro, I. (2000). Analysis of volatile components of Manchego cheese by dynamic headspace followed by atomic thermal desorption-GC–MS. Milkchwissenschaft, 55, 378–382.

Woo, A. H., & Lindsay, R. C. (1982). Rapidmethod for quantitative analysis of individual free fatty acids in Cheddar cheese. Journal of Dairy Science, 65, 1102–1109.

Yvon, M., & Rijnen, L. (2001). Cheese flavor formation by amino acids catabolism. International Dairy Journal, 11, 185–201.

Zerfiridis, G. K., Vafopoulou-Mastrogiannaki, A., & Lipoulou-Tzanetaki, E. (1984). Changes during ripening of commercial Gruyère cheese. Journal of Dairy Science, 67, 1397–1405.

Capítulo 4

Changes in microbiology, proteolysis, texture and sensory characteristics of raw goat milk cheeses treated by high-pressure at different stages of maturation

Enviado para su publicación en[1]: *Food Research International*

ABSTRACT

The influence of high-pressure (HP) treatment (400 MPa or 600 MPa for 7 min) on microbiology, proteolysis, instrumental texture and sensory parameters was investigated in the Ibores raw goat milk cheese. Treatments were applied at three different stages of ripening (1, 30 or 50 days) and analyses were carried out after the treatment or at the end of maturation. Treatments at 600 MPa at the three stages of cheese maturation could be interesting to decrease the counts of undesirable microorganisms in mature Ibores cheese (day 60), such as some psychrotrophics bacteria, *Enterobacteriaceae* and *Listeria spp*. SN/TN (soluble nitrogen/total nitrogen) was increased after HP treatment at day 1 (accelerating effect on cheese maturation), in contrast to the decrease of SN/TN level found after pressurisation at day 30 (arresting effect on cheese maturation). However this effect was diluted throughout cheese maturation. On the other hand, mature cheeses (day 60) pressurized at the beginning of ripening showed a higher variation of texture profile analysis. In the sensory analysis, cheeses treated at day 1 showed a significant change of appearance, odour and texture. They had less "eyes", odour intensity and hardness and more springiness than the other ones while the treatments applied at the end of maturation did not affect sensory characteristics. In general HP treatments at 600 MPa at middle (day 30) or at the end of ripening (day 50) would be suitable for both reduction of undesirable microorganisms and a minimum modification of typical characteristics of Ibores cheese.

[1] Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (Submitted). Changes in microbiology, proteolysis, texture and sensory characteristics of raw goat milk cheeses treated by high-pressure at different stages of maturation. *Food Research International. FOODRES-D-11-00975*

1. INTRODUCTION

High pressure (HP) treatment is an innovative technique for food preservation that inactivates pathogens and, spoilage microorganisms by means of pressure. At industrial scale, HP processing is in the range of 400–600 MPa and temperatures lower than 45 ºC. For most commercial applications, products are pressurized for less than 10 min. In principle, HP allows the preservation of most foods with minimal effects on taste, texture, appearance or nutritional value (Balasubramaniam et al., 2008; Ramírez et al., 2009). In addition, HP processing should be applied on dairy foods where heat treatment would negatively affect product quality; i.e., in raw milk cheese, like Ibores cheese, and milk pasteurization reduces key microorganisms involved in the suitable cheese ripening.

HP is a useful tool to inactivate microorganisms, as it induces changes in the morphology, biochemical reactions, genetic mechanisms and cell membrane and wall of microorganisms (Hoover et al., 1989). This fact would be significant for cheeses manufactured from raw milk where HP could reduce contaminant microorganisms increasing the product stability and food safety. The effect of HP on cheese microorganisms, principally pathogenic, has been extensively studied (Capellas et al., 1996; O'Reilly et al., 2000; Saldo et al., 2000a; Carminati et al., 2004; Arqués et al., 2005; López-Pedemonte et al., 2007; Voigt et al., 2010). Nevertheless, as far as we know there are no studies on the effect of HP on microorganisms present in raw goat milk cheeses.

Proteolysis is an important event which occurs during cheese ripening and it has influence on the textural changes of the cheese matrix (Mcsweeney & Sousa, 2000; Sousa et al., 2001). The influence of HP treatment on the proteolytic indexes (pH 4.4 soluble nitrogen –SN–, non protein nitrogen –NPN– and free amino acids –FAA–) is controversial in cheese. Results depend on several factors such as the cheese type, the stage of cheese ripening, pressure intensity, treatment temperature and/or treatment

holding time; e.g., in Cheddar cheese, O'Reilly et al. (2000) reported that there was an instantaneous increase in SN, expressed as % total nitrogen (TN), and FAA after HP treatment at 50 MPa for 3 days at 25ºC at day 2 of maturation. However, this effect decreased with cheese age. These results suggested that the ripening acceleration of HP-treated Cheddar cheese postulated by Yokoyama et al. (1992) was a more complex process non-applicable in all cheeses that is dependent of several factors such as cheese type, treatments, etc. Other researchers found that HP treatment at pressures ≥ 400 MPa could arrest the cheese ripening by decreasing FAA levels in ewe's milk cheese treated at 12 ºC for 10 min (Juan et al., 2004) and Cheddar cheese treated at 25 ºC for 5 min (Wick et al., 2004). In addition, in a recent study, Voigt et al. (2010) reported that the levels of SN and NPN increased immediately after HP treatment at 400 and 600 MPa on a mature 42-day-old Irish blue-veined cheese; however, after 28 days of storage, values were lower in HP-treated cheeses than control ones. Therefore, it is necessary to evaluate the changes on proteolytic indexes ("ripening indexes") induced by HP treatment of different cheese types.

The influence of HP on texture and other sensory properties of cheese has also been studied in the scientific literature. Messens et al. (2000) reported a significant effect on viscoelastic properties of HP-treated (225 and 400 MPa) Gouda cheese after pressurisation. However, these differences disappeared after 42 days of ripening. Similarly, Saldo et al. (2000b) found that differences between pressurized and control cheese were reduced throughout maturation time in HP-treated goat's milk cheeses. Pressurized cheeses were less crumbly and more elastic than control ones. Juan et al. (2007a) and Juan et al. (2008) evaluated the influence of HP treatment (from 200 to 500 MPa for 10 min at 12 ºC) on texture and sensory characteristics of ewe's milk cheeses treated at day 1 and 15 of maturation. They concluded that moderate pressures (200 and 300 MPa) increased the firmness of cheese; however, higher pressures increased the deformability and decreased the fracturability and rigidity. Regarding to sensory analysis, HP treatment at 500 MPa for 10 min produced the

softer, more elastic and less crumbly cheeses. However, as far as we know a Texture Profile Analysis (TPA) has not previously been carried out to evaluate the HP effect on cheese texture. TPA method is an instrumental texture measurement using double bite compression that was developed to imitate the compressing action of molar teeth during food masticated. Although instrumental methods cannot be used as a substitute of sensory evaluation, their value resides in their ability to detect small changes in physical characteristics and their objectivity.

The aim of this work is to know the viability of using HP treatment on raw goat milk cheeses at different stages of ripening by evaluation of the HP effect on microbiology, proteolysis, texture and sensory characteristics of Ibores cheese. Ibores cheese is a regionally produced cheese with protected denomination of origin (PDO) in the south west of Spain, manufactured from raw goat milk. HP treatment could inactivate pathogens initially present in milk, and also microorganisms which appear throughout cheese-making and cheese maturation. However, HP may also affect the characteristics of Ibores cheese, reducing its acceptability by consumers; this effect could depend on the pressure intensity and the moment of maturation in which HP is applied.

2. MATERIAL AND METHODS

2.1. Cheese samples and HP treatment

Raw goat milk cheeses were manufactured in a dairy plant of Spanish dairy of “Ibores” Protected Designation of Origen (PDO). One lyophilized direct-to-vat mesophilic mixed culture (R-704, 50 units; Chr. Hansen, Hørsholm, Denmark), containing *Lactococcus lactis subsp. cremoris* and *L. lactis subsp. lactis* were used to facilitate the acidification process. Milk was heated at 28-32 ºC and animal rennet was added (Naturen Plus 175, 22–25mL per 100L, CHR Hansen, Hørsholm, Denmark). After ~90 min, the curd was cut into grains (1–2 cm). Cheeses (0.7-1.2 kg) were pressed for 3-8 h, brine-salted and ripened (at 8–12 ºC and 80% relative humidity).

A total of 52 cheeses were taken at three different stages of maturation (day 1, 30 and50) and HP-treated at 400 or 600 MPa for 7 min at 10 ºC. Cheeses were vacuum-packed in plastic nylon/polyethylene bags (50 mL O_2 per m^2 per 24 h at 0 °C) and processed by HP in a semi-industrial hydrostatic high pressure unit NC Hyperbaric Wave 6000/55 (55–L, Burgos, Spain). Times to reach 400 MPa and 600 MPa were 2 min 54 sec and 3 min 50 sec, respectively. Cheeses were analysed after HP-treatment and at the end of maturation, except cheeses treated with 50 days of maturation, which were ripened vacuum-packed until day 60, when they were analysed. After HP treatment, half of cheeses treated (n=4) were unpacked and followed the normal ripening in the dairy while the other half were analysed (n=4). Therefore, at day 1, 12 cheeses were analysed: control (n=4), 400 MPa (n=4), 600 MPa (n=4); at day 30, 12 cheeses were also analysed: control (n=4), 400 MPa (n=4), 600 MPa (n=4) and, at day 60, 28 cheeses were analysed: control (n=4) and cheeses treated at days 1, 30 and 50 at 400 MPa or 600 MPa (n=24; 3 days x 2 treatments x 4 cheeses per batch).

2.2. Microbiological analysis

10 g of cheese were placed into a sterile Stomacher bag with 90 mL of sterile 2% (w/v) tri-sodium citrate solution and blended for 5 min in a Stomacher (IUL instruments, Germany) at room temperature. Serial dilutions were made using Ringers solution and microorganisms were grown in different media (Merck, Germany) and incubation conditions: Plate Count Agar (PCA) for mesophilic aerobic and psychrotrophic, incubated at 30 and 16 ºC for 72 and 96–120 h, respectively; Violet-red Bile Dextrose (VRBD) for *Enterobacteriaceae*, incubated at 37 ºC for 24 h; Man, Rogosa and Sharpe (MRS) for Lactic Acid Bacteria (LAB) incubated at 35 ºC for 72 h in an atmosphere with 5% CO_2 (Merck Anaerocult® C); Manitol Salt Phenol-red Agar (MSA) for *Micrococcaceae*, incubated at 35 ºC for 72 h.

In order to evaluate *Listeria spp.* levels, 25 g of cheese were homogenised in a Stomacher with 225 mL of Half-Fraser Broth (Fraser Selective Medium ½). After 20 min

of pre-incubation at room temperature, serial dilutions were made using Fraser medium and microorganisms were grown in Chromogenic Listeria Agar (ISO) Base (Oxoid Ltd, UK) at 37 ºC for 24–48 h. In this medium, the chromogenic compound X-glucoside is added as substrate for the detection of β-glucosidase, resulting in blue-coloured listeria colonies. Through the production of a phosphatidylinositol-specific phospholipase C in *L. monocytogenes* strains and the addition of a specific purified substrate for the enzyme to the medium, an opaque halo surrounds the colonies. This could allow a first identification of *L. monocytogenes* which in case of presumptive detection was tried to be confirmed by means of the "metabolic fingerprint" from discrete test reactions performed within 96 well Micro-plate, using Biolog equipment (Biolog Inc., California, USA). After inoculation and incubation, the Micro-plate is placed into the Micro-Station Reader for analysis. 95 different carbon compounds including sugars, carboxylic acids, amino acids and peptides for the microorganism identification were used to provide discriminating biochemical characterizations. The unique metabolic pattern generated by the organism is recorded and compared to hundreds of identification profiles in a corresponding Biolog Database.

2.3. Physico-chemical analysis

pH was measured directly in the middle of cheeses using a portable puncture pH-meter Crison mod. 507 (Crison Instruments, Barcelona, Spain). Dry-matter was assessed by a gravimetric method drying cheese samples at 102ºC (FIL-IDF 4A/1982). Fat content was analysed by acidbutyrometric method according to Van Gulik using a butyrometer with a calibrated range from 0 to 40% fat (FIL-IDF 5B/1986); and protein by Kjeldahl method with conversion to protein content using a factor of 6.38 (FIL-IDF 20B/1993).

Colour parameters were determined with a Minolta CR–200 colorimeter (Minolta Camera Co., Osaka, Japan) with illuminant D65, an 8 mm port/viewing area and a 0º viewing angle. Before use, the colorimeter was standardized with a calibration plate (Y= 93.2; x= 0.3159; y= 0.3324 to D65). The following colour parameters were

determined: lightness (CIE L*), redness (CIE a*: + red, - green) and yellowness (CIE b*: + yellow, - blue). The measurements were repeated at five randomly selected locations on each sample and average data were reported.

2.4. Nitrogen fractions and Free Amino Acids (FAA) measurements

5 g of cheese were homogenised in a Stomacher with 25 mL of a tri-sodium citrate solution (0.5 M; 40 ºC). From this solution, total nitrogen (TN) and soluble nitrogen (SN) compounds at pH 4.4 were measured by Kjeldahl analysis. Soluble non-protein nitrogen (NPN) in 12% trichloroacetic acid (TCA) was analysed from the pH 4.4-SN fraction (Ardö, 1999). Other parameters were calculated: Casein nitrogen= TN-SN; Polypeptide nitrogen= SN-NPN.

For FAA determination, water-soluble nitrogen (WSN) was made according to Cd-ninhydrin method described by Folkertsma and Fox (1992) modified by Delgado et al. (2010).

2.5. Texture Profile Analysis (TPA)

Texture analysis was performed in a texturometer TA-XT2i (Stable Micro Systems LTD, Godalming, UK). For the development of the texture profile analyses (TPA) samples were diced (1cm x 1cm x 1cm). Texture was measured at constant temperature (15±2 ºC). A cylinder probe was used (Aluminium cylinder probe P/25, 25 mm diameter; Stable Micro Systems LTD, Godalming, UK). In each analysis the cube was axially compressed twice at a 25% of deformation. The following texture parameters were measured from force–deformation curve: hardness, adhesiveness, cohesiveness, gumminess, springiness and chewiness (Bourne, 1978). Determinations were repeated 8 times per sample.

2.6. Sensory analysis

Sensory evaluation of control and HP-treated cheeses was carried out by 10 trained panellists from the Technological Agri-food Institute. A profile of 10 sensory attributes

of cheese grouped in appearance, odour, texture, flavour and taste were analysed. Analyses were developed in tasting rooms with the conditions specified in UNE regulation. All sessions were conducted at ambient temperature in a sensory panel room equipped with white fluorescent lighting. The software used for record scores in sensory sessions was FIZZ Network (version 1.01: Biosystemes, France). The panel sessions were held mid-morning, about 3 h after breakfast. In each group, cheeses were presented individuality and at random. Cheeses were cut in triangular shape and with 0.2 cm of thickness. Mineral water and bread were supplied in each session. A quantitative descriptive test was applied by a 10 cm long non-structured scale 0-10. The attributes evaluated by panellists were: appearance (colour –from whitish to yellow– and presence of "eyes"), odour (odour intensity), texture (hardness, springiness and friability), flavor (flavor intensity) and taste (salty, sharp and strange tastes) (Table 1).

2.7. Statistical analysis

Differences between control and HP-treated cheeses were evaluated by means of the one-way analysis of variance (ANOVA). Tukey's test was applied to compare the mean values of variables at the different days of maturation. The relationship between TPA parameters and sensory analysis was studied by the calculation Pearson's correlation analysis. Mean values and standard error of mean (SEM) are reported. SPSS software version 14.0 (SPSS Inc., Chicago, IL) was used in the statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Changes on microbial composition of Ibores raw goat milk cheese induced by HP treatment

Microbial counts of cheeses pressurized at day 1, 30 and 50 are presented in Table 2. In cheese treated at day 1 and 30 and analysed after pressurisation (day 2 and 31, respectively), HP significantly reduced the counts of psychrotrophics, mesophilic

aerobics, *Enterobacteriaceae*, LAB and *Listeria spp.*, especially at 600 MPa of pressure. *Micrococcaceae* counts (e.g., *Micrococcus spp.*, *Staphylococcus spp.*) were only significantly reduced in cheeses treated at 600 MPa at day 30. At day 60 of analysis, psychrotrophics were significantly reduced by HP treatment, except in the treatments carried out at 400 MPa at day 1 and 50. Mesophilic aerobics only showed a significant decrease when treatments were applied at 600 MPa on 30 and 50 day-old cheeses. *Enterobacteriaceae* counts were significantly lower in all HP-treated cheeses. LAB population was significantly reduced at 600 MPa of pressure, especially when HP treatment was applied at the middle and the end of cheese maturation.

Table 1. Sensory attributes evaluated by the tasting panel in the sensory analyses of HP- treated and control raw goat milk cheeses.

Appearance	
colour	Cheese colour (light white-dark yellow)
presence of "eyes"	Level of "eyes" in the cheese (low number-high number)
Odour	
odour intensity	Intensity of odour before eating (odourless - very intense odour)
Texture	
hardness	Firmness perception during chewing (very tender- very firm)
springiness	Perception of elasticity during chewing (not elastic-very elastic)
friability	Impression of crumbliness during chewing (not crumbly - very crumbly)
Flavour	
flavour intensity	Intensity of overall flavour (flavourless - very intense flavour)
Taste	
salty taste	Intensity of salt taste (not salty - very salty)
sharp taste	Intensity of sharp taste (not sharp- very sharp)
strange taste	Intensity of strange taste (not strange- very strange)

The reduction of LAB population in HP-treated cheeses could negatively affect to cheese maturation as they are responsible for the suitable fermentation of cheese and also influences microbial control by pH reduction (acidification process by the conversion of lactose into lactic acid). LAB are also related to some metabolic pathways

responsible for cheese flavor (Molimard & Spinnler, 1996; Mcsweeney & Sousa, 2000). Nevertheless, Saldo et al. (2000a) found that starter populations in HP-treated goat cheeses were recovered after 3 weeks of ripening. In Ibores cheese, LAB populations in cheeses pressurized at 400 MPa at day 1 were recovered at day 60 and in cheeses treated at day 50 at 400MPa the original level of LAB was maintained. *Micrococcaceae* were only affected by treatments applied on 30 day-old cheeses, which is positive as they participate in the maturation of cheese and in the formation of its final aroma (Mas et al., 1991). Finally, *Listeria spp.* showed a significant reduction in all HP-treated cheeses, except cheeses treated at day 30 at 400 MPa.

Treatments at 600 MPa at the three stages of maturation could be interesting to decrease the population of undesirable microorganisms in mature Ibores cheese (day 60), such as some psychrotrophics, *Enterobacteriaceae* and *Listeria spp*. However, LAB reduction induced by HP treatment at 600 MPa could produce a negative effect on sensory properties of Ibores cheese, although, in our sensory study on cheeses with 60 days, no relationship was found between LAB levels and sensory characteristics of cheese. Reduction of psychrotrophics (e.g., *Pseudomonas spp.*) and *Enterobacteriaceae* (e.g., *Enterobacter spp.*, *Escherichia coli*) could be interesting in Ibores cheese because they can cause defects on cheese, such as rancidity and gas production, respectively. Psychrotrophic bacteria may also contribute to lipolysis in cheese when they are present in high numbers (Mcsweeney & Sousa, 2000). *Enterobacter aerogenes* and *E. coli* are responsible for the appearance of "early blowing" and defects in texture and flavor (Lück & Dunkeld, 1981). Inactivation of theses bacteria could avoid the appearance of defects in cheese and the formation of unpleasant flavors. In addition, presence of *Enterobacteriaceae* is connected to hygienic quality of the product; high counts in milk and cheese indicate poor hygienic practices during the manufacture of cheese (Tornadijo et al., 2001). Moreover, *Enterobacteriaceae* family includes many familiar pathogens, such as *Salmonella spp.* and *E. coli* and they could be present in Ibores cheese. On the other hand, *Listeria monocytogenes* could be present in a raw

milk cheeses and this is a pathogen that causes listeriosis, a serious infection usually caused by eating food with *L. monocytogenes* at infectious levels in risk population (Lundén et al., 2004). Therefore, a decrease of theses microorganism groups induced by HP treatment could be important to guarantee the food safety in a raw milk cheese, like Ibores cheese. However, among the *Listeria spp.* isolated from in this study, we could not confirm any presumptive colony as *L. moncytogenes* by the production of their typical opaque halo of surrounding the green-blue colonies and also by biochemical methods ("metabolic fingerprint" by using the Biolog identification).

3.2. Changes on the physico-chemical composition of Ibores raw goat milk cheese induced by HP treatment

Table 3 shows the effect of HP treatment on the physico-chemical composition and colour parameters of Ibores cheese. At day 2 of analysis, the pH values between control and HP-treated cheeses were significantly different ($P<0.05$). pH value was higher in cheese treated at 400 MPa than control cheeses while cheeses treated at 600 MPa showed an intermediate value. Differences between control and 400 and 600MPa treated cheeses were 0.07 units and 0.03 respectively, therefore slight differences were found. The effect of HP treatment on the pH value of Ibores cheese was less intense than in other pressurized cheeses. Saldo et al. (2000a) reported that pasteurized goat's milk cheese pressurized at 400 MPa for 5 min had a pH value of 0.5 units higher than control cheese. Additionally, Juan et al. (2007a) found a higher pH level (0.36 units) in ewes' milk cheeses treated at 400 MPa for 10 min than in control cheeses with day 1 of maturation and analysed after pressurisation. According to these authors, this effect was probably due to the inactivation of LAB at the beginning of maturation. No effect on the pH was observed when HP treatment was applied at day 30 of maturation. However, at day 60 of analysis, cheeses HP-treated after 50 days of maturation and cheeses pressurized at 400 MPa at day 1 had similar pH values as control cheeses. In contrast, pressures at 600 MPa at day 1 and HP-treatments at the middle of maturation had a higher influence on pH level than the other treatments

applied. In Ibores cheese, HP treatment at 600 MPa caused a more important reduction of starter bacteria (Table 2), however, we have not found a clear relationship between LAB counts and pH changes at day 60, so other factors may be also involved (e.g. basification of cheese due to proteolysis).

No effects on the dry matter (DM), fat and protein contents were found after the application of HP treatment on 1-old-day chesses. In HP-treated cheeses at day 30 and analysed the next day, fat and protein in dry matter (DM) were significantly increased (P<0.05) by HP treatment at 400 MPa, while at 600 MPa only protein in DM was higher than in control cheeses. No explanation has been found to this fact. At day 60 of maturation, DM content and protein content in DM were significantly affected (P<0.05) by HP treatment. In general, DM content was higher in control cheeses than the HP-treated cheeses (except in cheeses treated at 400 MPa at day 30), probably due to a better water retention in HP-treated cheese. According to Messens et al. (2000) and Juan et al. (2008), HP-treated cheeses have better moisture retention compared with control cheeses, showing a lower DM content than control ones. Water retention was the highest in cheeses HP-treated at beginning of maturation (day 1) probably because they had higher moisture content at this maturation stage.

Regarding the instrumental colour of cheeses, at day 2 of analysis, there were significant differences (P<0.05) in CIE a* and b* values between control and HP-treated cheeses. CIE a*-value was significantly lower in cheese treated at 400 MPa than in the other ones. In addition, CIE b*-value was lower in cheese treated at 600 MPa. In cheese HP-treated at day 30 and analysed the next day, CIE L* and a* values were significantly modified (P<0.05) by HP treatment. CIE L*-value was significantly higher in cheeses treated at 400 MPa than in the other groups of cheeses, but CIE a* was similar between control and HP-treated cheeses although the effect on CIE a* was different depending on the pressure intensity (400 vs. 600 MPa). At day 60, CIE L*-value was similar in control and HP-treated cheeses; differences between treatments were found by Tukey's test. Therefore, at the end of maturation, the lightness of cheese was not

affected by HP treatment applied at different stages of cheese maturation. In addition, CIE a* was significantly higher in control than in the HP-treated cheeses and CIE b* was lower in control cheeses. Capellas et al. (2001) found a decrease in the lightness and an increase in the yellowness in goat milk fresh cheeses treated at 500 MPa for 5, 15 and 30 min. Juan et al. (2008) also found a higher yellowness in ewe's cheeses HP-treated at 300 MPa at day 1 and 15 and analysed at day 60 of ripening than untreated cheese; however, they reported lower significantly levels of lightness in HP-treated cheeses than in control ones. According to the literature, changes on the protein network induced by HP treatment could explain the colour differences between control and HP-treated cheeses, but the exact relationship between them is not known and it should be dependent on i. the type of cheese coagulation, ii. milk type and/or iii. other factors.

In this cheese, differences in instrumental colour could be detected by sensory analysis and, as a result, this could affect the consumer acceptance. The increase of the yellowness in HP-treated Ibores cheese is especially important since, in general, goat cheese has a typical whitish colour.

3.3. Changes on proteolysis of Ibores raw goat milk cheese induced by HP treatment

Table 4 shows the nitrogen fractions and FAA measured in control and HP-treated cheeses. Changes in proteolysis due to HP treatment could affect the normal velocity of maturation of the cheese, the final texture of cheese or could potentiate the appearance of certain undesirable tastes and/or aromas.

At day 2 of analysis, SN/TN and Polypeptide N significantly increased ($P<0.05$) after HP treatment. In contrast, Casein N and FAA levels were reduced in HP-treated cheeses. The relation SN/TN has traditionally been evaluated as a "ripening index" for cheese. The effect of HP treatment on SN/TN fraction could be caused by an increase of the residual coagulant action and/or milk proteinases activity (e.g., plasmin) by a destabilization of casein micelles induced by HP (Huppertz et al., 2004; Regnault et al., 2006), which could enhance their sensibility to proteolytic enzymes. This fact could

have caused a decline of Casein N and an increase in Polypeptide N, and, therefore, SN/TN was also enhanced in HP-treated cheeses. The increases in the levels of SN/TN after pressurisation have also been reported in other cheeses such as Cheddar and Irish blue-veined cheese (Rynne et al., 2008; Voigt et al., 2010). The reduction of FAA levels reported in Ibores cheese could be due to the inactivation of LAB and non-starter LAB peptidases induced by HP treatment. LAB and non-starter LAB peptidases are responsible for the degradation of short peptides and the production of FAA (Sousa et al., 2001). In this sense, Juan et al. (2007b) found that cheeses HP treated at 400 and 500 MPa showed lower levels of FAA than control cheeses immediately after treatment at day 1, and these results were correlated with the lower aminopeptidase activity observed.

In cheeses HP treated at day 30 and analysed after 1 day of storage (day 31), in general a significant decrease of SN/TN and FAA levels were found in HP-treated Ibores cheeses. On the other hand, Casein N value was higher in HP-treated cheeses than in control ones, owing to the fact that these caseins were not degraded to polypeptides. Therefore, SN/TN was reduced after HP treatment at day 30 (arresting of cheese maturation), in contrast to the increase of SN/TN level found after pressurisation at day 1 (acceleration of cheese maturation).

It is interesting to know the level of nitrogen fractions and FAA found in Ibores cheese analysed at the end of ripening (day 60) to know if changes in the proteolytic parameters due to HP treatment are maintained at the end of maturation. At day 60 of analysis, SN/TN value was only significantly higher in cheeses HP treated at 600 MPa at day 50 than in the other ones. These results indicate that the HP effect on SN/TN reported after Ibores cheese pressurisation at the beginning and the middle of maturation, was mitigated throughout cheese maturation process. Therefore, according to this ripening index (SN/TN), at day 60 of analysis we can assert that HP treatment did not cause a significant increase of Ibores cheese ripening; except in cheese HP treated at 600 MPa at day 50, although maybe the analysis of longer

maturation times than 60 days would show a similar effect. In agreement with our results, O'Reilly et al. (2000) found that there was an immediate increase after pressurisation of SN/TN in 2 day-old cheese, although this effect decreased during ripening time. In contrast, other researchers have postulated HP treatment could accelerate (Yokoyama et al., 1992; Saldo et al., 2000b) or arrest (Juan et al., 2004; Wick et al., 2004; Voigt et al., 2010) the cheese maturation. In addition, NPN/TN significantly increased (P<0.05) in HP-treated cheeses compared to control cheeses. This fraction had probably been increased by the release of medium-sized and/or small peptides, because HP could potentiate the release of intracellular proteinases/peptidases to the medium. The decrease of Casein N and polypeptide N in cheeses treated at 600MPa at day 50 would show the same trend as that reported for SN/TN increase; therefore, in order to obtain clear conclusions longer times of maturation (>60 days) should be analysed to know if this trend is maintained during cheese storage. Treatments at 600 MPa caused a lower level of FAA than at 400 MPa, probably due to a higher enzymatic inactivation. Reps et al. (2003) reported that aminopeptidases activity was entirely inactivated under pressure of 600 MPa in different cheese varieties HP treated in 3 cycles of 5 min.

3.4. Changes on textural parameters of Ibores raw goat milk cheese induced by HP treatment

Table 5 shows the results of the texture profile analysis (TPA) of control and HP-treated cheeses. At day 2 of analysis, hardness, gumminess and chewiness were significantly reduced in HP-treated cheeses. There were no differences between pressure intensities (400 vs 600 MPa). The reduction of theses TPA parameters may be owing to a weakening of casein network induced by HP treatment.

In contrast, treatments carried out on 30 day-old cheeses did not cause a significant effect on TPA parameters after pressurisation. Differences between both cheeses treated at day 1 and 30, could be related to different moisture contents between 1

day-old and 30 day-old cheeses (47.3% and 39.35%, respectively) and the own stability of the cheese curdle. In this sense, cheeses with higher moisture content (e.g., 1 day-old cheeses) could undergo significant modifications on textural properties when they are pressurized. In line with these results, Juan et al. (2008) reported a higher modification on some textural parameters (fracture stress, fracture strain and modulus) in ewe's cheeses treated at day 1 at 300 MPa than cheeses treated at day 15 at 300 MPa, in comparison with control cheeses.

Regarding to cheeses analysed at day 60, all TPA parameters were significantly modified (P<0.001) by HP treatment. Cheeses treated at the beginning of ripening (day 1) were responsible for the observed variation of hardness and springiness. Hardness was higher in samples treated at day 30 at 400 MPa than in the other groups of cheeses, which also showed the highest dry-matter content (Table 2), while cheeses treated at day 1 showed the lowest hardness. In fact, the hardness of cheeses showed a positive correlation with dry-matter (r= +0.609; P<0.001). In addition, all HP-treated cheeses showed a reduction of hardness proportional to pressure intensity. Springiness was significantly higher in cheeses treated at day 1, which had the highest value, than in HP-treated cheeses at day 50 and control cheeses. Pressurisation can induce modifications in the protein network of cheeses (Okpala et al., 2009), which, in Ibores cheeses, could have caused a fall of hardness (except in cheeses pressurized at day 30 at 400 MPa) and a rise of springiness in HP-treated cheeses. On the other hand, differences in the adhesiveness were reported between pressurized cheeses. Cohesiveness values were significantly higher in cheeses treated at day 1 at 600 MPa than in the other batches of cheeses; in addition, HP-treated at day 50 and control cheeses presented the lowest values. Finally, the highest values of gumminess and chewiness were found in 30 day-old cheeses pressurized at 400 MPa, and they showed significant differences compared with control cheeses. In conclusion, cheeses pressurized at the beginning (at 400 or 600 MPa) and at the middle (at 400 MPa) of ripening showed higher variations in certain TPA parameters than the other ones.

There are no previous studies about TPA values in pressurized cheeses; therefore we cannot compare our results with those reported by other researchers.

3.5. Changes on sensory attributes of Ibores raw goat milk cheese induced by HP treatment

Sensory analysis of 60 day-old cheeses are presented in Table 6. We found significant differences (P<0.05) in the appearance, odour and texture between control and HP-treated cheeses while flavour and taste parameters remained unchanged. An increase of yellowness in HP-treated could be negative for consumer acceptance; however, the sensory analysis did not show a clear effect of the HP on the colour of cheeses. The presence of "eyes" is another important attribute in Ibores cheese because it has irregularly distributed eyes. Cheeses pressurized at day 1 showed the lowest presence of "eyes", followed by cheeses pressurized at day 30 and 50, respectively. In general, we reported a higher number of eyes reduction when cheeses were treated by pressure intensity at 600 MPa than at 400 MPa (except for cheeses treated at day 30). The disappearance of eyes in HP-treated cheese is normal since air inside the cheese is compressed under HP.

Regarding the odour trait, odour intensity was higher in control cheese than in HP-treated cheeses, but only cheeses pressurized at day 1 showed significant differences compared with control cheeses. All textural parameters analysed by panellists (hardness, springiness and friability) in cheese were significantly modified by HP treatment at day 1. Hardness and friability were significantly lower in cheeses pressurized at day 1 than in the rest. In contrast, springiness was higher in cheeses treated at day 1. In line with our results, Saldo et al. (2000b) found that pasteurized goat milk cheeses pressurized (50, 400 and 400+50 MPa) at the beginning of ripening were less crumbly (friability) and more elastic (springiness) than control ones. In this sense, Reps et al. (1998) also reported that pressurized Gouda cheese was more elastic than control cheese. Unlike our results, Juan et al. (2007a) reported that cheeses

treated at day 1 at 400 MPa had a higher hardness than control cheeses at day 60 of ripening. In addition, these authors did not find significant differences for the elasticity between control and HP-treated (400 MPa) cheeses. Differences could be due to the different types of cheeses subjected to HP treatment (ewe vs goat cheese).

Finally, panellists did not find significant differences for the flavor and taste (salty, sharp and strange tastes) attributes between control and HP-treated cheeses which could be positive for a possible application of this treatment at commercial level. In general, as we reported for instrumental TPA, cheeses pressurized at day 1 showed more modifications on sensory parameters (appearance, odour and texture) than the others.

No differences among cheese flavour were perceived by the panelists, although they detected a reduction in the intensity of the odour perceived in HP-treated cheeses, especially when the treatment was applied at the beginning of maturation. This result is in line with a parallel study about the volatile profile of these cheeses (Delgado et al., 2011). We reported that HP-treatment reduced the total volatile content of the headspace of these cheeses, especially in those treated at day 1. In fact, odour intensity positively correlated with total volatile content (r= +0.558; $P<0.05$).

3.6. Correlation between TPA parameters and sensory attributes at day 60

TPA hardness showed positive correlations with sensory hardness and sensory friability (r= +0.70 and +0.62, respectively; $P<0.001$), and a negative correlation with sensory springiness (r= -0.63; $P<0.001$). Cohesiveness showed negative correlations with presence of “eyes”, sensory hardness and sensory friability (r= -0.79, -0.75 and -0.82, respectively; $P<0.001$), and a positive correlation with sensory springiness (r= +0.76; $P<0.001$). The same as cohesiveness, TPA springiness had negative correlations with presence of “eyes”, sensory hardness and sensory friability (r= -0.81, -0.66 and -0.74, respectively; $P<0.001$), and positive correlation with sensory springiness (r= +0.64; $P<0.001$).

Therefore, TPA parameters could be a useful and rapid tool to know sensory characteristics of control and HP-treated Ibores cheese. Despite using texture analysers is not considered a complete substitute for sensory evaluation, significant correlations between sensory textural parameters and TPA parameters (e.g., hardness, cohesiveness and adhesiveness) have been reported in cheese (Antoniou et al., 2000; Halmos, 2000). In this sense, Pinho et al. (2004) in a study on "Terrincho" ewe cheese, found that hardness on the mouth, friability when cutting with a knife and elasticity when pressing with the fingers, were positively correlated with TPA hardness, TPA friability and TPA springiness.

4. CONCLUSIONS

HP treatment at the three stages of ripening, especially at 600 MPa, decreased the microorganism counts in Ibores cheese. Reduction of psychrotrophics and *Enterobacteriaceae* could be interesting because they can cause defects on cheese. Moreover, *Enterobacteriaceae* family and *Listeria spp.* can include important pathogens, and thus HP treatment would enhance the food safety of this raw goat milk cheese. In general, at day 60 of analysis, HP treatment did not cause a significant increase of the ripening index (SN/TN); therefore, pressurisation could be used to reduce undesirable or pathogenic microorganisms in Ibores cheese, without an alteration of its stage of ripening. In addition, HP treatments carried out at day 1 showed the highest effect on instrumental texture and sensory attributes (related to appearance, odour and texture) analysed at the end of cheese ripening (day 60). According to the results, pressurisations at 600 MPa at middle (day 30) or at the end of ripening (day 50) could be suitable for both reduction of undesirable microorganisms and a minimum modification of the typical characteristics of Ibores raw goat milk cheese.

5. Acknowledgements

This work has been financed thanks to European Regional Development Fund (ERDF).

F. J. Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). R. Ramírez thanks INIA and ERDF for the contract conceded (BOE, 10.01.07). We thank the PDO dairy "Berrocales Trujillanos" for providing the cheeses. We also thank to Dolores Valcárcel and Jonathan Delgado for the microbial analyses.

6. References

Antoniou, K.D., Petridis, D., Raphaelides, S., Ben Omar, Z., & Kesteloot, R. (2000). Texture assessment of French cheeses. Journal of Food Science, 65, 168–172.

Ardö, Y. (1999). Evaluating proteolysis by analysing the N content of cheese fractions. Bulletin of International Dairy Federation Nº 337, 4–9.

Arqués, J.L., Rodríguez, E., Gaya, P., Medina, M., & Nuñez, M. (2005). Effect of combinations of high-pressure treatment and bacteriocin-producing lactic acid bacteria on the survival of Listeria monocytogenes in raw milk cheese. International Dairy Journal, 15, 893–900.

Balasubramaniam, V.M., Farkas, D., & Turek, E.J. (2008). By destroying pathogenic and spoilage organisms while keeping food chemistry basically intact, high-pressure technology enables pasteurization of foods with minimal effects on taste, texture, appearance, or nutritional value. Food Technology, 11, 32–38.

Bourne, M.C. (1978). Texture profile analysis. Food Technology, 32, 62–66.

Capellas, M., Mor-Mur, M., Sendra, E., Pla, R., & Guamis, B. (1996). Populations of Aerobic Mesophiles and Inoculated E. coli During Storage of Fresh Goat's Milk' Cheese Treated with High Pressure. Journal of Food Protection, 59, 582–587.

Capellas, M., Mor-Mur, M., Sendra, E., & Guamis, B. (2001). Effect of high-pressure processing on physico-chemical characteristics of fresh goats' milk cheese (Mató). International Dairy Journal, 11, 165–173.

Carminati, D., Gatti, M., Bonvini, B., Neviani, E., & Mucchetti, G. (2004). High pressure processing of Gorgonzola cheese: influence on Listeria monocytogenes

inactivation and on sensory characteristics. Journal of Food Protection, 67, 1671–1675.

Delgado, F.J., Rodríguez-Pinilla, J., González-Crespo, J., Ramírez, R., & Roa, I. (2010). Proteolysis and texture changes of a Spanish soft cheese ("Torta del Casar") manufactured with raw ewe milk and vegetable rennet during ripening. International Journal of Food Science & Technology, 45, 512–519.

Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic pressure at different stages of ripening. International Dairy Journal, 21, 135–141.

Folkertsma, B., & Fox, P.F. (1992). Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. Journal of Dairy Research, 59, 217–224.

Halmos, A.L. (2000). Relationships between instrumental texture measurements and sensory attributes. In: Nishinari K. (Ed.), Hydrocolloids, Part 2, Elsevier, Amsterdam.

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.F., & Knorr, D. (1989). Biological Effects of High Hydrostatic Pressure on Food Microorganisms. Food Technology, 43, 99–107.

Huppertz, T., Fox, P.F., & Kelly, A.L. (2004). Dissociation of caseins in high pressure-treated bovine milk. International Dairy Journal, 14, 675–680.

IDF (1982). Cheese and processed cheese: moisture determination, determination of the total solids content (Standard 4A) International Dairy Federation, Brussels

IDF (1986). Fat, Cheese and processed cheese: determination of fat content, Schmid-Bondzynskiratzlaff method (Standard 5B) International Dairy Federation, Brussels

IDF (1993). Protein determination, milk: determination of N content, Kjeldahl method, and calculation of crude protein content (Standard 20 B) International Dairy Federation, Brussels

Juan, B., Ferragut, V., Guamis, B., Buffa, M., & Trujillo, A.J. (2004). Proteolysis of a high pressure-treated ewe's milk cheese. Milchwissenschaft, 59, 616–619.

Juan, B., Trujillo, A.J., Guamis, V., Buffa, M., & Ferragut, V. (2007a). Rheological, textural and sensory characteristic of high-pressure treated semi-hard ewes' milk cheese. International Dairy Journal, 17, 248–254.

Juan, B., Ferragut, V., Buffa, M., Guamis, B., & Trujillo, A. J. (2007b). Effects of high pressure on proteolytic enzymes in cheese: Relationship with the proteolysis of ewe milk cheese. Journal of Dairy Science, 90, 2113−2125.

Juan, B., Ferragut, V., Guamis, B., & Trujillo, A.J. (2008). The effect of high-pressure treatment at 300 MPa on ripening of ewes' milk cheese. International Dairy Journal, 18, 129–138.

López-Pedemonte, T., Roig-Sagués, A., De Lamo, S., Hernández-Herrero, M., & Guamis, B. (2007). Reduction of counts of Listeria monocytogenes in cheese by means of high hydrostatic pressure. Food Microbiology, 24, 59–66.

Lück, H., & Dunkeld, M. (1981). Enterobacteriaceae in cheese. South African Journal of Dariy Technology, 13, 9–14.

Lundén, J., Tolvanen, R., & Korkeala, H. (2004). Human listeriosis outbreaks linked to dairy products in Europe. Journal of Dairy Science, 87, 6–11.

Mas, M., Timón, J., & González-Crespo, J. (1991). Queso de los Ibores: caracterización productiva, fisicoquímica y microbiológica. Archivos de Zootecnia, 40, 103–113.

McSweeney, P.L.H., & Sousa, M.J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Le Lait, 80, 293-324.

Messens, W., Van de Walle, D., Arevalo, J., Dewettinck, K., & Huyghebaert, A. (2000). Rheological Properties of High-pressure-treated Gouda Cheese. International Dairy Jorunal, 10, 359–367.

Molimard, P., & Spinnler, H.E. (1996). Compounds involved in the flavor of surface mold-ripened cheeses: origins and properties. Journal of Dairy Science, 79, 169–184.

O'Reilly, C.E., O'Connor, P.M., Kelly, A.L., Beresford, T.P., & Murphy, P.M. (2000). Use of Hydrostatic Pressure for Inactivation of Microbial Contaminants in Cheese. Applied and Environmental Microbiology, 66, 4890–4896.

Okpala, C.O.R., Piggott, J.R., & Schaschke, C.J. (2009). Effects of high-pressure processing (HPP) on the microbiological, physico-chemical and sensory properties of fresh cheeses: A review. African Journal of Biotechnology, 8, 7391–7398.

Pinho, E.M., Alves, M.M., & Ferreira, I.M. (2004). Chemical, physical, and sensorial characteristics of "Terrincho" ewe cheese: changes during ripening and intravarietal comparison. Journal of Dairy Science, 87, 249–257

Ramírez, R., Saraiva, J., Pérez Lamela, C., & Torres, J.A. (2009). Reaction Kinetics Analysis of Chemical Changes in pressure assisted treatment processing. International Critical Reviews, 1, 16-30

Regnault, S., Dumay, E., & Cheftel, J.C. (2006). Pressurization of raw skim milk and of a dispersion of phosphocaseinate at 9 or 2 ºC: Effects on the distribution of minerals and proteins between colloidal and soluble phases. Journal of Dairy Research, 73, 91–100.

Reps, A., Kolakowski, P., & Dajnowiec, F. (1998). The effect of high pressure on microorganism and enzymes of ripening cheeses. In N. S. Isaacs (Ed.), High pressure food science, bioscience and chemistry (pp. 265–270). Cambridge, UK: Royal Society of Chemistry.

Reps, A., Kolakowski, P., Wisniewska, K., & Krasowska, M. (2003). The effect of high pressures on proteolytic enzymes in ripened cheeses. Milchwissenschaft, 58, 138–140.

Rynne, N.M., Beresford, T.P., Guinee, T.P., Sheehan, E., Delahunty, C.M., & Kelly, A.L. (2008). Effect of high-pressure treatment of 1 day-old full-fat Cheddar cheese on subsequent quality and ripening. Innovative Food Science & Emerging Technologies, 9, 429−440.

Saldo, J., McSweeney, P.L.H., Sendra, E., Kelly, A.L., & Guamis, B. (2000a). Changes in Curd Acidification Caused by High Pressure Treatment. Irish Journal of Agriculture Food Research, 39, 169.

Saldo, J., Sendra, E., & Guamis, B. (2000b). High hydrostatic pressure for accelerating ripening of goat's milk cheese: Proteolysis and texture. Journal of Food Science, 65, 636–640.

Sousa, M.J., Ardö, Y., & McSweeney, P.L.H. (2001). Advances in the study of proteolysis during cheese ripening. International Dairy Journal, 11, 327–345.

Tornadijo, M.E., García, M.C., Fresno, J.M., & Carballo, J. (2001). Study of Enterobacteriaceae during the manufacture and ripening of San Simón cheese. Food Microbiology, 18, 499–509.

Voigt, D.D., Chevalier, F., Qian, M.C., & Kelly, A.L. (2010). Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innovative Food Science and Emerging Technologies, 11, 68–77.

Wick, C., Nienabert, U., Anggraeni, O., Shellhammer, T.H., & Courtney, P.D. (2004). Texture, proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure. Journal of Dairy Research, 71, 107–115.

Yokohama, H., Swamura, N., & Motobyashi, N. (1992). Method for accelerating cheese ripening. European Patent Application EP 0 469 857 A1.

Table 2. Microbial counts (log cfu g^{-1}) of control and HP-treated cheeses at day 1, 30 and 50 of maturation, and analysed at day 2, 31 and 60.

			HP-treated day 1		HP-treated day 30		HP-treated day 50			
	Day of analysis	Control	400MPa	600MPa	400MPa	600MPa	400MPa	600MPa	SEM	P-value
Psychrotrophic	2	8.0a	6.9b	3.2c	---	---	---	---	0.62	0.001
	31	7.7a	---	---	6.5b	3.5c	---	---	0.54	0.001
	60	5.8b	6.5a	4.3c	4.3c	4.1c	5.6b	4.7c	0.18	0.001
Mesophilic aerobic	2	8.0a	7.2b	4.6c	---	---	---	---	0.43	0.001
	31	6.7a	---	---	5.0b	2.6c	---	---	0.54	0.001
	60	5.9a	6.1a	5.5ab	5.7ab	4.1c	5.9a	5.1b	0.13	0.001
Enterobacteriaceae	2	4.4a	3.0b	1.8c	---	---	---	---	0.34	0.001
	31	nd	---	---	nd	nd	---	---	-	-
	60	1.7	nd	nd	nd	nd	nd	nd	0.06	0.001
Lactic Acid Bacteria	2	6.8a	5.7b	1.0c	---	---	---	---	0.77	0.001
	31	7.2a	---	---	3.5b	1.0c	---	---	0.8	0.001
	60	6.7a	6.3ab	4.7c	5.5bc	2.0e	6.7a	3.7d	0.32	0.001
Micrococcaceae	2	4.8a	5.1a	2.4b	---	---	---	---	0.37	0.001
	31	4.0	---	---	4.0	3.5	---	---	0.16	0.444
	60	5.2a	4.7abc	4.7abc	3.9d	4.2bc	5.2a	4.9ab	0.11	0.001
Listeria spp.	2	3.9a	2.7b	1.3c	---	---	---	---	0.32	0.001
	31	3.2a	---	---	3.0a	2.0b	---	---	0.16	0.001
	60	2.66a	1.50bc	nd	2.25ab	nd	1.33bc	0.65cd	0.20	0.001

a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean. nd: non-detected (nd: non-detected, below detection limit <10 cfu g^{-1}).

Table 3. Physic-chemical composition (pH, dry matter, fat and protein content) and instrumental colour measurements of control and HP-treated cheeses at day 1, 30 and 50 of maturation, and analysed at day 2, 31 and 60.

			HP-treated day 1		HP-treated day 30		HP-treated day 50			
	Day of analysis	Control	400MPa	600MPa	400MPa	600MPa	400MPa	600MPa	SEM	P-value
pH	2	5.14b	5.21a	5.18ab	---	---	---	---	0.01	0.008
	31	4.93	---	---	4.96	4.97	---	---	0.01	0.268
	60	4.71cd	4.68d	5.12a	4.80b	4.77bc	4.69d	4.70d	0.03	0.001
Dry matter	2	56.30	56.30	57.13	---	---	---	---	0.37	0.609
	31	60.65	--	---	59.17	61.04	---	---	0.41	0.142
	60	64.43ab	60.84bc	59.03c	66.35a	61.67abc	62.50abc	60.83bc	0.56	0.002
Fat in DM	2	54.19	56.19	55.64	---	---	---	---	0.54	0.318
	31	55.25b	---	---	58.12a	56.94ab	---	---	0.49	0.037
	60	55.51	56.51	57.61	54.98	54.75	55.22	56.12	0.34	0.272
Protein in DM	2	35.28	36.28	36.63	---	---	---	---	1.13	0.900
	31	22.41b	---	---	35.97a	33.42a	---	---	2.08	0.003
	60	22.41bc	29.58a	29.44a	19.49cd	23.18bc	25.57ab	16.12d	0.94	0.001
CIE L*	2	97.55	96.80	98.05	---	---	---	---	0.24	0.085
	31	95.16b	---	---	96.99a	95.62b	---	---	0.28	0.006
	60	97.26abc	96.39bc	96.21c	96.48abc	96.30c	97.52ab	97.60a	0.14	0.002
CIE a*	2	0.03a	-1.76b	0.43a	---	---	---	---	0.29	0.001
	31	-0.50ab	---	---	-0.27a	-0.78b	---	---	0.08	0.022
	60	0.18a	-0.34b	-0.26ab	-0.40b	-0.44b	-1.06c	-1.14c	0.09	0.001
CIE b*	2	1.80a	2.05a	0.33b	---	---	---	---	0.25	0.001
	31	3.22	---	---	2.71	3.69	---	---	0.17	0.056
	60	2.10c	2.69bc	3.84a	3.27ab	2.95abc	2.31bc	2.51bc	0.13	0.001

a, b, c, d: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean. DM: dry matter

Table 4. Nitrogen fractions and free amino acids (FAA, expressed as mg Leu g cheese^{-1}) of control and HP-treated cheeses at day 1, 30 and 50 of maturation, and analysed at day 2, 31 and 60.

			HP-treated day 1		HP-treated day 30		HP-treated day 50			
	Day of analysis	Control	400MPa	600MPa	400MPa	600MPa	400MPa	600MPa	SEM	P-value
SN/TN	2	9.35c	12.87a	11.46b	---	---	---	---	0.46	0.001
	31	21.25a	---	---	17.15b	18.61ab	---	---	0.65	0.014
	60	18.35b	21.98ab	22.51ab	21.97ab	22.26ab	18.51ab	23.27a	0.50	0.013
NPN/TN	2	5.75	5.54	4.59	---	---	---	---	0.29	0.240
	31	7.42	--	---	5.17	7.35	---	---	0.46	0.056
	60	6.53b	9.57a	10.40a	10.46a	9.52a	8.72ab	10.70a	0.33	0.002
Casein N	2	90.65a	87.13c	88.54b	---	---	---	---	0.08	0.001
	31	78.75b	---	---	82.85a	81.39ab	---	---	0.65	0.014
	60	81.65a	78.02ab	77.49ab	78.03ab	77.74ab	81.49ab	76.73b	0.50	0.013
Polypeptide N	2	2.80b	5.95a	5.64a	---	---	---	---	0.53	0.009
	31	7.39	---	---	9.98	9.14	---	---	0.59	0.196
	60	9.77a	8.89ab	8.26abc	5.78bc	7.14abc	6.13abc	4.83c	0.43	0.004
FAA	2	0.37a	0.26b	0.22b	---	---	---	---	0.02	0.001
	31	0.53a	---	---	0.44ab	0.40b	---	---	0.02	0.022
	60	0.70ab	0.78ab	0.58b	0.89a	0.71ab	0.85a	0.70ab	0.02	0.003

a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean. SN: Soluble Nitrogen. TN: Total Nitrogen. NPN: Non Protein Nitrogen. N: Nitrogen. FAA: Free Amino Acids.

Table 5. Textural parameters of control and HP-treated cheeses at day 1, 30 and 50 of maturation, and analysed at day 2, 31 and 60.

			HP-treated day 1		HP-treated day 30		HP-treated day 50			
	Day of analysis	Control	400MPa	600MPa	400MPa	600MPa	400MPa	600MPa	SEM	P-value
Hardness (N)	2	5.15a	2.86b	2.81b	---	---	---	---	0.36	0.001
	31	41.94	---	---	40.49	42.13	---	---	1.19	0.855
	60	54.16ab	31.33c	26.07c	59.35a	42.97abc	44.50abc	37.28bc	2.56	0.001
Adhesiveness (N sec)	2	2.04	1.45	1.05	---	---	---	---	0.20	0.106
	31	12.70	---	---	11.77	11.45	---	---	0.58	0.702
	60	14.05ab	11.10b	11.57b	19.76a	12.24b	11.28b	9.22b	0.80	0.003
Cohesiveness	2	1.14	1.15	1.15	---	---	---	---	0.01	0.752
	31	0.37	---	---	0.40	0.37	---	---	0.01	0.479
	60	0.33c	0.69b	0.94a	0.64b	0.52bc	0.35c	0.36c	0.04	0.001
Springiness (cm)	2	0.94	0.94	0.92	---	---	---	---	0.01	0.115
	31	0.67	---	---	0.63	0.63	---	---	0.01	0.052
	60	0.63d	0.78ab	0.85a	0.76abc	0.74abcd	0.65cd	0.70bcd	0.02	0.001
Gumminess (N)	2	5.89a	3.30b	3.25b	---	---	---	---	0.41	0.001
	31	15.72	---	---	16.13	15.84	---	---	0.70	0.976
	60	17.88b	21.73b	24.35ab	38.18a	22.43b	15.70b	13.56b	1.79	0.001
Chewiness (N cm)	2	5.56a	3.09b	3b	---	---	---	---	0.39	0.001
	31	10.73	---	---	10.20	10.12	---	---	0.53	0.899
	60	11.33b	17.04ab	20.75ab	29.30a	17.38ab	10.34b	9.51b	1.55	0.001

a, b, c, d: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean

Table 6. Values of sensory attributes (scale from 0 to 10 points) of control and HP-treated cheeses at day 1, 30 and 50 of maturation, and analysed at day 60.

		HP-treated day 1		HP-treated day 30		HP-treated day 50			
	Control	400MPa	600MPa	400MPa	600MPa	400MPa	600MPa	SEM	P-value
APPEARANCE									
Colour	3.32	4.1	4.36	3.57	3.62	3.40	3.46	0.10	0.034
Presence of "eyes"	4.05a	0.83cd	0.56d	1.83bc	1.76bc	2.47b	2.03b	0.22	0.001
ODOUR									
Odour intensity	5.83a	4.80bc	4.40c	5.50ab	5.78a	5.21abc	5.70ab	0.12	0.001
TEXTURE									
Hardness	7.21a	4.24b	3.98b	6.41a	6.35a	6.25a	6.54a	0.23	0.001
Springiness	0.96b	4.28a	4.67a	1.10b	1.04b	1.07b	0.96b	0.31	0.001
Friability	7.33	3.70b	3.13b	6.46a	6.62a	7.17a	6.65a	0.31	0.001
FLAVOR									
Flavor intensity	5.78	6.07	5.85	5.74	5.81	6.22	5.91	0.08	0.725
TASTE									
Salty	5.48	5.48	5.68	5.70	5.76	5.54	5.48	0.06	0.739
Sharp	4.99	5.85	4.56	4.78	5.15	5.15	5.22	0.12	0.100
Strange tastes	0.81	0.52	0.53	0.55	0.93	0.70	0.88	0.05	0.070

a, b, c, d: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean

Capítulo 5

Influence of high-pressure treatment on free fatty acids and oxidation processes throughout maturation of a raw goat milk cheese

Enviado para su publicación en[1]: *Journal of Dairy Research*

ABSTRACT

The effect of high-pressure (HP) treatment on free fatty acids (FFA) profile and lipid and protein oxidation of a raw milk goat cheese with "Queso Ibores" protected designation of origin (PDO) was evaluated. HP treatment (400 or 600 MPa for 7 min) was applied at three different times of maturation (1, 30 or 50 days). FFA profile was studied on HP-treated and control samples after treatment and at the end of maturation by GC–FID. HP treatment applied at the beginning of maturation (day 1) significantly decreased the concentrations of most FFA in cheeses analysed after HP processing. However, in cheeses treated at 400 MPa and analysed at intermedious times of ripening (day 30), FFA content was increased and significant differences between pressure intensities effects (400 and 600 MPa) were found. Nevertheless, neither the time of HP treatment (day 1, 30 or 50) nor pressure intensity (400 or 600 MPa) had influence on FFA profile at the end of the ripening (day 60). Finally, lipid oxidation showed a significant increase in HP-treated cheeses while no differences were found in protein oxidation development.

[1] Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (Submitted). Influence of high-pressure treatment on free fatty acids and oxidation processes throughout maturation of a raw goat milk cheese. *Journal of Dairy Research*. JDR 3297.

1. INTRODUCTION

Current progress in High Pressure (HP) technology, combined with a growth in the consumers' demand for high quality and minimally processed foods have increased the interest of HP in food processing (Rastogi et al., 2007). HP processing has a lower impact on food properties such as nutritional value or flavour than thermal treatment since small molecules such as amino acids or vitamins are slightly affected by HP (Balci & Wilbey, 1999).

The application of HP could be interesting to increase the safety of raw milk cheeses which could present some microbial risks (Trujillo et al., 2002). Another application is the possible use of HP to accelerate the cheese maturation process, which was first described in a patent by Yokoyama et al. (1992) where Cheddar and Parmesan cheeses were HP treated from 5 to 300 MPa for 3 days at 25 ºC and the effect of HP on proteolysis and flavour development were studied; they reported that HP treatment at 50 MPa for 3 days at 25 ºC gave rise to a HP-treated cheese similar to a 6 month-old control cheese. Consequently, an important reduction in maturation times of these cheeses could be obtained through HP treatment. However, in this patent ten times more starter bacteria were used and these results could not be replicated by others. On the other hand, ripening processes of other types of cheese may be unaffected by HP. For example, lipolysis in Camembert cheese was only slightly affected by HP treatment of up to 500 MPa for 4 h, while in Gouda cheese maturation processes were not modified (Kolakowski et al., 1998). Most recently, some authors have reported that HP-treatment of cheese resulted in decelerated FFA production (Saldo et al., 2003) or arrested the cheese riepening (Wick et al., 2004; Voigt et al., 2010). Therefore, the effect of HP treatment on ripening is controversial and there are different factors involved in the evolution and intensity of the maturation changes, e.g., holding time, pressure intensity and the moment of treatment and/or cheese type.

Recent studies (unpublished data) indicated an increase in TBA-RS formation over the course of ripening of Ibores cheeses, so it could influence the final quality of cheeses at the end of ripening. Changes in this reaction could modify the original aroma and even could be associated with the appearance of off-flavours. Protein oxidation has also been related with the reduction of protein bio-availability and the formation of toxic compounds (Moreaux & Birlouez-Aragon, 1997; Naranjo et al., 1998). Nevertheless, very few studies have evaluated the changes in oxidative markers throughout cheese maturation (Fedele & Bergamo, 2001; Delgado et al., 2009) and no studies have evaluated oxidative changes after HP treatment. In addition, there are no studies regarding the effect of HP on free fatty acids (FFA) and on the lipolysis pathways during ripening of raw milk cheeses. Buffa et al. (2001) studied the FFA profiles of cheeses made from raw, pasteurized or pressure-treated (500 MPa, 15 min, 20ºC) goats' milk to assess the effect of milk treatment on cheese lipolysis. Juan et al. (2007) analysed the effect of HP treatment (200, 300, 400 or 500 MPa for 10 min) at two stages of ripening (after 1 and 15 days of maturation) of cheese made from pasteurised ewes milk. For this reason, the aims of the present study were to determine the effect of HP treatments at 400 or 600 MPa for 7 min and the influence of the maturation stage at which the treatment is applied (1, 30 and 50 days) on free fatty acids profile and lipid and protein oxidation changes of a raw milk goat cheese.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Raw goat milk cheeses were manufactured in one Spanish dairy of "Ibores" PDO: "Berrocales Trujillanos" (Trujillo, Cáceres, Spain). One lyophilized direct-to-vat mesophilic mixed culture (R-704, 50 units; Chr. Hansen, Hørsholm, Denmark), containing *Lactococcus lactis subsp. cremoris* and *L. lactis subsp. lactis* were used to facilitate the acidification process. Milk was heated at 28-32 ºC and animal rennet was added (Naturen Plus 175, 22-25 mL per 100L, CHR Hansen, Hørsholm, Denmark). After

~90 min, the curd was cut into grains (1–2 cm). Cheeses (0.7-1.2 kg) were pressed for 3-8 h, brine-salted and ripened (at 8–12 ºC and 80% relative humidity).

2.2. Sampling and High-Pressure Treatment

A total of 52 commercial PDO cheeses were selected at three different stages of ripening (day 1, 30 and 50) and HP-treated at 400 or 600 MPa for 7 min at 10°C. Cheeses were vacuum-packed in plastic bags and processed by HP in a semi-continuous high pressure unit NC Hyperbaric Wave 6000/55 (55–L, Burgos, Spain). The pressure increase to 400 MPa or 600 MPa at 2 min 54 sec and 3 min 50 sec, respectively; the pressure decrease was instantaneous (1 sec). Pressure transmitting media was running water. Cheeses were analysed after HP-treatment and at the end of maturation, except cheeses treated with 50 days of maturation, which were ripened vacuum-packed until day 60, when they were analysed. These cheeses were maintained vacuum-packed in order to simulate real maturation conditions that would be applied in case HP treatment was considered interesting for dairies. Following HP-treatment at each ripening point one part of the selected PDO cheese were analysed, while the other half was ripened up to 60 days and then analysed. Therefore, at day 1, 12 cheeses were analysed: control (n=4), 400 MPa (n=4), 600 MPa (n=4); at day 30, 12 cheeses were also analysed: control (n=4), 400 MPa (n=4), 600 MPa (n=4) and, at day 60, 28 cheeses were analysed: control (n=4) and cheeses treated at days 1, 30 and 50 at 400 MPa or 600 MPa (n=24; 3 days x 2 treatments x 4 cheeses per batch). Cheeses were stored at -80ºC until analysis.

2.3. Moisture content

Moisture content was measured by a gravimetric method drying cheese samples at 102ºC (FIL-IDF 4A/1982).

2.4. Free fatty analysis

FFA were extracted and analysed according to Delgado et al. (2009). Ratios between the different fractions were obtained: short chain fatty acids (SCFA, C4:0–C8:0), medium chain fatty acids (MCFA, C10:0–C14:0), long chain fatty acids (LCFA, C15:0–C18:2 n-6) and unsaturated fatty acids (UFA, C18:1 n-9 and C18:2 n-6).

2.5. Lipid oxidation

Lipid oxidation analysis was measured by the 2-thiobarbituric acid (TBA) method of Salih et al. (1987). TBA–RS levels were expressed as mg malondialdehyde Kg^{-1} cheese.

2.6. Protein oxidation

Protein oxidation was performed by quantification of carbonyl groups formed during incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl according to the method described by Oliver et al. (1987). Protein oxidation was expressed as nmol carbonyls mg^{-1} protein.

2.7. Statistical analysis

A one-way analysis of variance (ANOVA) was performed to evaluate the differences between control and HP-treated cheeses. When differences were significant, a post hoc Tukey's test was applied to compare mean values. A principal component analysis (PCA) was used for a better understanding of results. Statistical analysis was performed by SPSS 14.0 software (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of high-pressure processing on moisture content.

Moisture content (Table 1) was not significantly affected by HP- treatment on unripened cheeses at day 1 and 30. However, at day 60 moisture content of cheeses was significantly modified by HP- treatment. Cheeses treated at day 1 at 600 MPa had

the highest moisture content when the lowest was found in those cheeses treated at day 30 at 400 MPa. The rest of batches showed intermediate values. Other authors (Saldo et al. 2000) have found a similar behavior in cheeses after HP application.

Table 1. Moisture content (%) found in control and HP-treated raw goat milk cheeses at day 1, 30 and 50.

		HP-treated day 1		HP-treated day 30		HP-treated day 50			
Day of analysis	Control	400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa	SEM	*P-value*
2	43.7	43.7	42.87					0.37	0.609
31	39.35			40.83	38.96			0.41	0.142
60	35.57bc	39.16ab	40.97a	33.65c	38.33abc	37.5abc	39.17ab	0.56	0.002

a, b, c: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard Error of the Mean.

3.2. Effect of high-pressure processing at day 1 on free fatty acids profile and oxidative changes

The level of FFA in control and HP-treated cheeses at day 1, are shown in Table 2. Contents of FFA such us butyric (C4:0), caproic (C6:0), caprilic (C8:0), capric (C10:0), lauric (C12:0), pentadecanoic (C15:0) palmitic (C16:0) and linoleic (C18:2 n-6) acids significantly ($P<0.05$) decreased after HP treatment. These results disagree with those reported by Juan et al. (2007) in pasteurized ewes' milk cheese, where capric, lauric and linoleic acids significantly increased after HP treatment at 400 MPa for 10 min at day 1. Moreover, these authors reported an increase in FFA content immediately after HP treatment.

Overall, the pressure intensity did not significantly change the FFA profile of Ibores cheese. According to Juan et al. (2007) pressure intensities (200, 400 or 500 MPa for 10 min) did not have a significant effect on FFA content in 1 day-old ewes' cheeses after

their pressurization. However, they found a significant increase in cheeses HP-treated at 300 MPa.

The significant reduction of butyric (C4:0), caproic (C6:0) and caprilic (C8:0) acids (–short chain fatty acids–SCFA) content after HP treatment could be negative for cheese flavour because SCFA are compounds with low odor thresholds and characteristic tastes, e.g., butyric acid, the most abundant FFA in this cheese, has a rancid odour and an important influence on the flavour of many cheeses (Curioni & Bosset, 2002). In our case, no differences of moisture content were found at day 1, so differences on FFA could be linked with other factors such as the inactivation of certain enzymes and/or microorganisms. The FFA reduction could favor a less development of flavour in HP-treated cheeses at the end of ripening due to the role of FFA as precursors of volatile compounds.

Table 2. Contents of free fatty acids (mg Kg^{-1}) found in control and HP-treated raw goat milk cheeses at day 1.

Free fatty acids	Control	400 MPa	600 MPa	SEM	*P–value*
Butyric (C4:0)	744.1a	677.9b	669.4b	11.6	0.002
Caproic (C6:0)	120.7a	107.9b	106.3b	2.1	0.001
Caprilic (C8:0)	43.8a	35.5b	35.2b	1.3	0.001
Capric (C10:0)	122.1a	104.0b	102.6b	3.2	0.005
Lauric (C12:0)	97.0a	86.8b	86.2b	1.8	0.003
Myristic (C14:0)	156.9	145.8	143.3	2.6	0.054
Pentadecanoic (C15:0)	85.5a	79.3b	78.3b	1.1	0.002
Palmitic (C16:0)	524.5a	440.1b	439.4b	15.4	0.014
Stearic (C18:0)	292.3	257.8	253.9	8.7	0.140
Oleic (C18:1 n-9)	465.0	498.2	463.2	14.8	0.598
Linoleic (C18:2 n-6)	134.2a	121.4ab	119.8b	2.6	0.026
Total FFA	2786.1	2554.7	2497.6		

a, b: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard Error of the Mean.

On the other hand, oxidative markers were not significantly (P>0.05) modified after HP processing. Protein oxidation values were 0.042, 0.035 and 0.034 nmol carbonyls mg^{-1} protein and lipid oxidation levels were 1.01, 1.42 and 0.92 mg MDA Kg^{-1} (data not tabulated), in control, HP-treated at 400 MPa and 600 MPa cheeses, respectively. Therefore, presurisation neither 400 MPa nor 600 MPa applied at day 1 did not cause changes in cheese oxidation immediately after treatment.

3.3. Effect of high-pressure processing at day 30 on free fatty acids profile and oxidative changes

FFA concentrations (mg Kg^{-1}) found in HP-treated at day 30 and control cheeses are shown in Table 3. HP treatment at 400 MPa significantly (P<0.05) increased levels of FFA in Ibores cheeses in contrast to day 1 (except for caproic acid which showed a higher amount in control ones). In cheeses treated at 600 MPa, lauric (C12:0), myristic (C14:0), palmitic (C16:0), and oleic (C18:1 n-9) acids contents increased with respect to control ones but the increases were not statistically significant. In the same way contents of FFA in treated cheeses at 600 MPa were significantly lower than those found in cheeses treated at 400 MPa indicating a significant effect of pressure on FFA release. The highest FFA content in HP-treated cheeses could be attributed to the faster and better interaction of microbial lipases with the substrate due to the lysis produced by HP treatment as reported Juan et al. (2008). The differences on the effect of the intensity of pressures applied (400 and 600MPa) could be caused by the higher enzymatic inactivation at 600 MPa than at 400 MPa. In this sense, Seyderhelm et al. (1996) reported that lipase was inactivated above 700 MPa at 45 ºC for 10 min in tris buffer, although after a treatment at 600 MPa and room temperature it retained the 60% of its activity. Therefore, HP processing could increase the levels of lipolysis due to physical changes in cheese matrix during compression process; however, treatments at 600 MPa could partially inactive lipolytic enzymes, and both effects will be counteracted. For these reasons, the levels of FFA were higher after treatments at 400

MPa than at 600 MPa. Differences in the effect between pressures intensities on FFA profile were also reported by Voigt et al. (2010) in a mature blue-veined cheese HP-treated (400 or 600 MPa) and analysed after 28 days of storage. As we reported to 1 day-old cheeses, lipid and protein oxidation levels were not changed by HP treatment at day 30. Protein oxidation values were 0.027, 0.029 and 0.030 nmol carbonyls mg^{-1} protein and lipid oxidation levels were 1.25, 1.09 and 1.04 mg MDA Kg^{-1} (data not tabulated), in control, HP-treated at 400 MPa and 600 MPa cheeses, respectively. Thus, HP-treated cheeses maintained its "oxidative status" after HP processing at day 30.

Table 3. Contents of free fatty acids (mg Kg^{-1}) found in control and HP-treated raw goat milk cheeses at day 30.

Free fatty acids	Control	400 MPa	600 MPa	SEM	*P–value*
Butyric (C4:0)	746.3	734.5	723.4	4.8	0.154
Caproic (C6:0)	124.6a	122.5ab	118.7b	0.9	0.008
Caprilic (C8:0)	65.4	67.7	62.0	1.4	0.259
Capric (C10:0)	179.1	201.5	182.5	4.9	0.134
Lauric (C12:0)	126.4b	142.8a	131.0ab	2.9	0.043
Myristic (C14:0)	209.1b	247.1a	222.3ab	6.0	0.011
Pentadecanoic (C15:0)	84.6b	87.1a	83.9b	0.5	0.014
Palmitic (C16:0)	625.8b	795.0a	691.9b	24.5	0.003
Stearic (C18:0)	298.9c	410.7a	344.8b	14.9	0.001
Oleic (C18:1 n-9)	619.9b	875.5a	731.6b	36	0.001
Linoleic (C18:2 n-6)	198.7	231.0	204.5	6.5	0.083
Total FFA	3278.8	3915.4	3496.6		

a, b, c: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard Error of the Mean.

3.4. Effect of high-pressure processing on free fatty acids profile and oxidative changes in raw goat milk cheeses at the end of ripening (day 60)

Table 4 shows the FFA concentrations (mg Kg^{-1}) and FFA indexes calculated in control

and HP-treated cheeses at 1, 30 and 50 days and analysed at the end of ripening (day 60). At day 60, only the content of three FFA was significantly affected (P<0.05) by HP treatment. Butyric (C4:0) and stearic (C18:0) acids were highest in control cheeses while linoleic (C18:2 n-6) acid was highest in HP-treated cheeses. Therefore, the HP effect showed at day 1 and 30 after treatment could be reduced during the maturation process. Butyric and stearic acids concentrations were higher in control cheese than in treated ones, unlike linoleic acid which level was reduced. A similar effect was found on these fatty acids in ewe' milk cheeses treated at 400 MPa at day 1 and analysed at day 60 (Juan et al., 2007), but a higher number of FFA were affected by HP treatment.

In general, there were no significant differences for FFA content between pressure intensities (400 and 600 MPa) and the maturation stage when the treatment was applied. This fact could be linked with the internal biochemical or enzymatic regulation processes which tend to maintain a similar FFA content at the end of ripening. These results could be very interesting for cheese-makers because they could choose the ripening moment and pressure intensities for HP treatment without a relevant modification of cheese FFA profile which is important to maintain its sensory characteristics.

SCFA content had a significant reduction in treated cheese owing to the fact that HP treatment reduced the butyric acid concentration. Despite everything, SCFA reduction was not very large in quantitative terms (see Table 4) and, although high levels of butyric acid are undesirable because of its strong flavour, a significant reduction of this extremely volatile acid could affect to the original flavour of Ibores cheese. MCFA, LCFA, UFA and total FFA were not significantly affected by HP treatment. Juan et al. (2007) reported a more intense effect of HP treatment on ewe' milk cheeses treated at 400 MPa at day 1 and 15 of ripening and analysed at day 60 since the levels of SCFA, LCFA and total FFA were significantly modified (P<0.05) at the end of ripening. In contrast to our results, these authors concluded that pressures ≥400 MPa for 10 min

decelerated the lipolysis in treated ewe' cheeses. Differences could be explained by a higher holding time of treatment (7min compared with 10 min) and the different cheese type studied (goat compared with ewe).

Regarding FFA indexes, no significant differences (P>0.05) were observed in HP-treated and control cheeses (Table 3), except for UFA/LCFA. The maintenance of these indexes could be positive due to ratios between FFA were constant despite HP processing and treated cheeses could keep its characteristic flavour.

Protein and lipid oxidation markers in HP-treated cheeses at 1, 30 and 50 days and analysed at day 60 are presented in Table 5. Protein oxidation was not significantly affected by HP treatment, but significant differences were obtained for lipid oxidation. The higher TBA-RS values were found in treated cheeses at day 1 and 50. This fact could be connected with the higher content of linoleic acid in treated cheeses compared with control cheeses (see Table 3) because lipid oxidation usually involves the reaction of unsaturated fatty acids with molecular oxygen. This process gives rise to the formation of off-flavours products known as fatty acid hydroperoxides; however, these compounds are unstable and can yield volatile flavour products such as aldehydes (Serra et al., 2008). Other compounds originated by lipids oxidation are some ketones, alcohols and hydrocarbons. In a parallel study we reported that HP treatment increased the relative abundance of ketones when the treatment was applied at the beginning and middle of maturation (Delgado et al., 2011) and, therefore, these results could be related with lipid oxidation development of Ibores cheese. Despite all, lipid oxidation levels detected were lower than in other cheeses (Balestrieri et al., 2002; Delgado et al., 2009), and, in a parallel study in three dairies of PDO cheese Ibores, similar levels of TBA-RS (~0.05 mg MDA Kg^{-1}) were found than those reported for HP-treated cheeses. However, lipid oxidation development should be taken into account for HP processing of cheeses with high initial oxidation levels such as pasteurised milk cheeses.

Table 4. Contents of free fatty acids –FFA– (mg Kg^{-1}) and FFA indexes calculated in control and HP-treated raw goat milk cheeses at 1, 30 and 50 days and analysed at day 60

		Day 1		Day 30		Day 50			
Free fatty acids	Control	400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa	SEM	*P*-value
Butyric (C4:0)	834.5a	781.2ab	729.4b	802.7ab	793.4ab	785.3ab	761.2ab	8.1	0.009
Caproic (C6:0)	138.4	131.5	122.2	130.6	130.2	130.4	127.8	1.3	0.057
Caprilic (C8:0)	80.0	79.1	69.5	77.8	78.4	79.6	78.3	1.4	0.492
Capric (C10:0)	226.6	231.6	203.9	228.9	229.0	234.1	226.8	3.9	0.511
Lauric (C12:0)	154.5	160.0	145.2	157.2	158.5	159.6	154.8	2.1	0.589
Myristic (C14:0)	269.4	282.8	256.7	274.0	276.4	282.9	275.0	3.6	0.523
Pentadecanoic (C15:0)	92.2	89.6	85.8	88.3	89.7	88.7	86.3	0.6	0.102
Palmitic (C16:0)	944.9	883.5	811.1	849.2	858.8	897.0	866.7	12.4	0.114
Stearic (C18:0)	501.4a	435.7ab	412.0b	412.5b	416.6b	447.2ab	419.5b	7.9	0.010
Oleic (C18:1 n-9)	906.2	995.4	998.4	958.2	947.6	1028.1	991.6	14.5	0.374
Linoleic (C18:2 n-6)	217.7b	279.6a	260.1ab	271.5ab	276.7a	278.9a	269.7ab	5.6	0.017
SCFA	1052.9a	991.9ab	921.1b	1011.0ab	1002.0ab	995.3ab	967.3ab	10.4	0.021
MCFA	650.4	674.4	605.8	660.1	663.9	676.6	656.6	9.6	0.545
LCFA	2662.4	2683.9	2567.4	2579.8	2589.4	2739.9	2633.8	32.4	0.816
UFA	1123.9	1275.0	1258.5	1229.7	1224.3	1307.0	1261.3	19.0	0.232
Total FFA	4365.7	4350.2	4094.4	4251.0	4255.3	4411.8	4257.6	45.8	0.653

		Day 1		Day 30		Day 50			
Free fatty acids	Control	400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa	SEM	*P*-value
SCFA/total FFA	0.24	0.23	0.23	0.24	0.24	0.23	0.23	0.00	0.203
MCFA/total FFA	0.15	0.15	0.15	0.16	0.16	0.15	0.15	0.00	0.230
LCFA/total FFA	0.61	0.62	0.63	0.61	0.61	0.62	0.62	0.00	0.255
SCFA/LCFA	0.40	0.37	0.36	0.39	0.39	0.36	0.37	0.00	0.228
MCFA/LCFA	0.24	0.25	0.24	0.26	0.26	0.25	0.25	0.00	0.288
SCFA/MCFA	1.63	1.48	1.52	1.53	1.52	1.47	1.47	0.02	0.139
UFA/LCFA	0.42c	0.47b	0.49a	0.48ab	0.47b	0.48ab	0.48ab	0.00	0.001

a, b, c: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard Error of the Mean. SCFA (Short Chain Fatty Acids), MCFA (Medium Chain Fatty Acids), LCFA (Long Chain Fatty Acids), UFA (Unsaturated Fatty Acids).

Table 5. Protein (nmol carbonyls mg^{-1} protein) and lipid oxidation (mg MDA Kg^{-1}) markers in HP-treated raw goat milk cheeses at 1, 30 and 50 days and analysed at day 60.

		Day 1		Day 30		Day 50			
	Control	400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa	SEM	*P*-value
Protein	1.26	1.04	1.33	1.49	1.46	1.89	1.10	0.08	0.081
Lipid	0.03b	0.05a	0.04a	0.04ab	0.04ab	0.05a	0.04a	0.00	0.001

a, b: Different letters in the same row indicate statistically significant differences (Tukey's Test, $P<0.05$). SEM: Standard error of the mean.

3.5. Principal Component Analysis of data at the end of ripening (day 60)

Table 6 shows the most important loadings and the percentage accounted by the first two principal components (PC1 and PC2) after PCA. The 46.8% and 29.5% of the variability was explained by PC1 and PC2, respectively. With positive loadings on PC1 are caprilic, capric, lauric and myristic acids and MCFA, followed by palmitic acid and LCFA. Regarding PC2, SCFA/LCFA and SCFA/total FFA and butyric acid had positive loadings, and LCFA/total FFA, UFA and oleic acid had negative loadings.

The distribution of the scores on the first two PCs is presented in Figure 1. Results could not be grouped according to the HP processing time and/or the pressure intensity. HP processing did not affect FFA content when Ibores cheeses were treated at day 1, 30 and 50 and analysed at day 60, and as a result, cheeses treated at different stages of ripening showed a similar FFA profile than untreated cheeses at the end of maturation. These results disagree with those reported by us in a study about the effect of HP on profile volatile of Ibores cheese, where the volatile profile was significantly influenced by the time of application of HP processing during maturation and by the pressure intensity (Delgado et al., 2011). Probably, the subsequent metabolism of FFA released by lipolysis could be modified by HP treatment and, for

this reason, the volatile profile was different in HP-treated cheeses. Maybe the study of the enzymes involved in these reactions could clarify these results.

Table 6. Loading plots after principal components analysis of the variables defined by the two first principal components (PC1 and PC2).

Compounds	Principal Component	
	PC1	PC2
Butyric (C4:0)	0.609	0.729
Caproic (C6:0)	0.736	0.615
Caprilic (C8:0)	0.944	0.227
Capric (C10:0)	0.978	0.116
Lauric (C12:0)	0.979	0.089
Myristic (C14:0)	0.986	-0.045
Pentadecanoic (C15:0)	0.732	0.539
Palmitic (C16:0)	0.857	0.027
Stearic (C18:0)	0.420	-0.086
Oleic (C18:1)	0.617	-0.756
Linoleic (C18:2)	0.677	-0.454
SCFA	0.694	0.674
MCFA	0.987	0.051
LCFA	0.838	-0.418
UFA	0.669	-0.711
FFA	0.957	-0.132
SCFA/total FFA	-0.322	0.926
MCFA/total FFA	0.612	0.315
LCFA/total FFA	0.015	-0.918
SCFA/LCFA	-0.225	0.941
MCFA/LCFA	0.421	0.610
SCFA/MCFA	-0.672	0.593
UFA/LCFA	0.008	-0.671
Lipid OXID	-0.063	-0.545
Protein OXID	0.196	0.013
Percentage of variance	46.8%	29.5%

FFA (total Free Fatty Acids), SCFA (Short Chain Fatty Acids), MCFA (Medium Chain Fatty Acids), LCFA (Long Chain Fatty Acids). UFA (Unsaturated Fatty Acids).

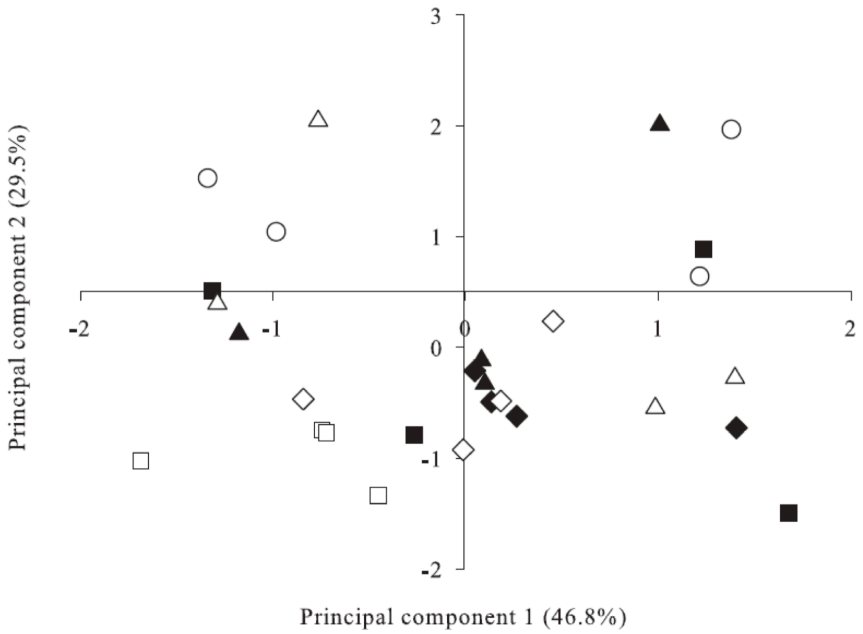

Figure 1. Scores plot after principal component analysis of the variables in the plane defined by the two first principal components (PC1 and PC2) in HP-treated raw goat milk cheeses at 1, 30 and 50 days and analysed at day 60: ○, control; ■, 400 MPa (day 1); □, 600 MPa (day 1); ▲, 400 MPa (day 30); △, 600 MPa (day 30); ◆, 400 MPa (day 50); ◇, 600 MPa (day 50).

4. CONCLUSIONS

HP treatment did not altered FFA profiles at day 60 in treated cheeses. Therefore, FFA concentration at the end of maturation could be independent of the time of application (day 1, 30 or 50) and HP treatment conditions –pressure intensity–. However, changes were more intense when cheeses were analysed after processing at day 1 and 30. HP treatment applied at day 1 caused a decrease of FFA in cheeses analysed the same day, but, in contrast, HP treatment at day 30 had a differential effect dependent on the pressure intensities (400 and 600 MPa) applied. These differences in FFA concentration showed after processing were reduced throughout of ripening process. Regarding oxidative markers, protein oxidation was not significantly affected by HP treatment at the end of ripening, but lipid oxidation showed a

significant increase in HP-treated cheeses. Further studies about the effect of high pressure treatment on lipid oxidation of cheese, and the relationship with sensory properties are necessary to evaluate the impact of these changes.

5. Acknowledgements

This work has been financed thanks to European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). We thank the PDO dairy "Berrocales Trujillanos" for providing the cheeses.

6. References

Balci, A.T., & Wilbey, R.A. (1999). High Pressure Processing of Milk: the First 100 Years in the Development of a New Technology. International Journal of Dairy Technology, 52, 149–155.

Balestrieri, M., Spagnuolo, M.S., Cigliano, L., Storti, G., Ferrara, L., Abrescia, P., & Fedele E. (2002). Evaluation of oxidative damage in mozzarella cheese produced from bovine or water buffalo milk. Food Chemistry, 77, 293–299.

Buffa, M.N., Guamis, B., Pavia, M., & Trujillo, A.J. (2001). Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 175–179.

Curioni, P.M.G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

Delgado, F.J., González-Crespo, J., Ladero, L., Cava, R., & Ramírez, R. (2009). Free fatty acids and oxidative changes of a Spanish soft cheese (PDO "Torta del Casar") during ripening. International Journal of Food Science & Technology, 44, 1721–1728.

Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation. International Dairy Journal, 21, 135–141.

Fedele, E., & Bergamo, P. (2001). Protein and Lipid Oxidative Stresses During Cheese Manufacture. Journal of Food Science, 66, 932–935.

IDF (1982). Cheese and processed cheese: moisture determination, determination of the total solids content (Standard 4A) International Dairy Federation: Brussels.

Juan, B., Ferragut, V., Buffa, M., Guamis, B., & Trujillo, A. J. (2007). Effects of high-pressure treatment on free fatty acids release during ripening of ewes' milk cheese. Journal of Dairy Research, 74, 438–445.

Juan, B., Ferragut, V., Guamis, B., & Trujillo, A.J. (2008). The effect of high-pressure treatment at 300 MPa on ripening of ewes' milk cheese. International Dairy Journal, 18, 129−138.

Kolakowski, P., Reps, A., & Babuchowski, A. (1998). Characteristics of Pressure Ripened Cheeses. Polish Journal of Food Nutritional Science, 48, 473–483.

Moreaux, V., & Birlouez-Aragon, I. (1997). Degradation of tryptophan in heated αlactoglobulin-lactose mixtures is associated with intense Maillard reaction. Journal of Agricultural and Food Chemistry, 45, 1905–1910.

Naranjo, G.B., Malec, L.S., & Vigo, M.S. (1998). Reducing sugars effect on available lysine loss of casein by moderate heat treatment. Food Chemistry, 62, 309–313.

Oliver, C.N., Ahn, B.W., Moerman, E.J., Goldstein, S., & Satadtman, E.R. (1987). Aged-related changes in oxidized proteins. Journal of Biological Chemistry, 262, 5488–5491.

Rastogi, N.K., Raghavarao, K.S.M.S., Balasubramaniam, V.M., Niranjan, K., & Knorr, D. (2007). Opportunities and challenges in high pressure processing of foods. Critical Reviews in Food Science and Nutrition, 47, 69−112.

Saldo, J., Sendra, E., & Guamis, B. (2000). High hydrostatic pressure for accelerating ripening of goat's milk cheese: proteolysis and texture. Journal of Food Science, 65, 636–640.

Saldo, J., Fernández, A., Sendra, E., Butz, P., Tauscher, B., & Guamis, B. (2003). High pressure treatment decelerates the lipolysis in a caprine cheese. Food Research International, 36, 1061–1068.

Salih, A.M., Smith, D.M., Price, J.F., & Dawson, L.E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. Poultry Science, 66, 1483–1489.

Serra, M., Trujillo, A.J., Pereda, J., Guamis, B., & Ferragut, V. (2008). Quantification of lipolysis and lipid oxidation during cold storage of yogurts produced from milk treated by ultra-high pressure homogenization. Journal of Food Engineering, 89, 99–104.

Seyderhelm, I., Boguslawski, S., Michaelis, G., & Knorr, D. (1996). Pressure induced inactivation of selected food enzymes. Journal of Food Science, 61, 308–310.

Trujillo, A.J., Capellas, M., Saldo, J., Gervilla, R. & Guamis, G. (2002). Applications of high-hydrostatic pressure on milk and dairy products: a review. Innovative Food Science and Emerging Technologies, 3, 295–307.

Voigt, D.D., Chevalier, F., Qian, M.C., & Kelly, A.L. (2010). Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innovative Food Science and Emerging Technologies, 11, 68–77.

Wick, C., Nienaber, U., Anggraeni, O., Shellhammer, T.H., & Courtney, P.D. (2004). Texture proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure. Journal of Dairy Research, 71, 107−115.

Yokoyama, H., Sawamura, N., & Motobayashi, N. (1992). Method for accelerating cheese ripening. European patent application EP 0 469857 A1.

Capítulo 6

Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation

Publicado en[1]: *International Dairy Journal, 21, pp. 135–141 (2011)*

ABSTRACT

The effect of high-pressure (HP) treatment (400 or 600 MPa for 7 min) applied at three different stages of ripening (1, 30 or 50 days) on the volatile profile of a raw milk goat cheese was investigated. Volatile compounds were studied on treated and untreated samples after the treatment and at the end of maturation by SPME–GC–MS. In general, fifty compounds were identified in the cheeses: 12 acids, 16 alcohols, 5 esters, 8 ketones and 9 other compounds. HP treatment applied at the beginning of maturation decreased the relative abundance of most volatile compounds, but enhanced the formation of ketones and other compounds. Changes were less intense when treatment was applied at the end of maturation. Therefore, HP treatment does not maintain the original flavour of cheese, but further changes can be minimized when treatments are applied when the maturation process is concluded. Changes in volatile profile are not necessarily negative, and cheeses with an acceptable modified aroma can be obtained.

[1] Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation. *International Dairy Journal*, **21**, pp. 135–141.

1. INTRODUCTION

The volatile profile of dairy products is an important characteristic for their organoleptic quality which determines consumer's acceptability. For this reason, dairies try to maintain the typical aroma of their cheeses. Three pathways are responsible for release of volatile compounds in cheese during maturation, i) metabolism of lactose and lactate, ii) lipolysis and iii) proteolysis (McSweeney & Sousa, 2000). Ibores cheese with Protected Designation of Origin (PDO) is produced in Spain from raw goat milk from the breeds Serrana, Verata, Retinta and crosses thereof. Its aroma varies between sweet and mild, and has a strong taste, with a slight aftertaste of goat milk.

High pressure (HP) is a novel technology for preserving food in which a product is processed under high isostatic pressure (400–900MPa), leading to the inactivation of certain enzymes and microorganisms. It is an alternative method to the thermal treatment since avoids the undesirable changes produced in food after heating. HP processing can be an interesting method for raw milk cheeses to increase food safety; however, HP could modify the biochemical routes involved in the development of the quality characteristics typical of each cheese.

Few studies have investigated the effect of HP treatment on the volatile profile of cheeses at different stages of maturation. Some researchers have studied the changes in the volatile composition of HP-treated raw ewe milk cheese; e.g., Arqués et al. (2007) reported the variation of volatile compounds of La Serena cheese HP-treated at two different stages of ripening (day 2 and 50), while Juan et al. (2007) investigated the effects of HP (treatment at 200, 300, 400 or 500 MPa) on volatile profile of ewe pasteurized milk cheeses applied at two stages of ripening (after 1 and 15 days of manufacturing). As far as we know, only one paper has studied the fraction volatile of a goat milk cheese treated by HP at 400 MPa for 5 min at two stages of ripening, i.e. 21 and 60 days (Saldo et al., 2003). However, this study was carried out on cheeses made

from pasteurized milk and lower pressures were applied. For this reason, the aim of the present work was to establish the effect of HP treatments at 400 and 600 MPa for 7 min and the possible influence of the ripening stage at which the treatment is applied (1, 30 and 50 days) on the headspace volatile profile of a raw milk goat cheese produced in an industrial dairy immediately after the treatment and at the end of maturation.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Cheeses were made from raw goat milk in one Spanish dairy of "Ibores" PDO. Milk was coagulated at 28-32 ºC in 60-90 min with animal rennet (Naturen Plus 175, 22-25 mL per 100 L, CHR Hansen, Hoersholm, Denmark). The coagulum was cut to medium-size (1-2 cm) grains. Cheeses of fiat cylindrical shape, weighing 0.7-1.2 kg, were pressed for 3-8 h, brine-salted and ripened (at 8–12 ºC and 80% relative humidity) for at least 60 days. According to legislation, raw milk cheeses can be consumed after two months of maturation. PDO regulator council establishes physicochemical criterions to certify the cheeses, a minimum of 50% of fat content in dry matter, a minimum of 50% of dry matter and a maximum of 4% NaCl (DOE, 2003). At the end of maturation, dry matter was 64%, fat 55% and pH 4.7.

2.2. Sampling and high pressure treatment

A total of 52 cheeses were taken from the dairy at different days of maturation to apply HP treatment. Cheeses (4 cheeses per batch) were HP-treated at 400 or 600 MPa for 7 min at three different stages of ripening (day 1, 30 or 50). Cheeses were vacuum-packed in plastic bags and HP-treated in a 55-L capacity discontinuous hydrostatic press at NC Hyperbaric Wave 6000/55 (Burgos, Spain), water temperature was 10°C. Times to reach 600 MPa and 400 MPa were 3min 50sec, and 2 min 54 sec. respectively. Cheeses were stored at -80ºC until analysis.

Cheeses were analysed after HP-treatment and at the end of maturation, except cheeses treated with 50 days of maturation, which were ripened vacuum-packed until day 60, when they were analyzed. These cheeses were maintained vacuum-packed in order to simulate real maturation conditions that would be applied in case HP treatment was considered interesting for dairies. Untreated cheeses were used as control (n=4) in each stage of ripening. After HP treatment, half of cheeses treated (n=4) were unpacked and followed the normal ripening in the dairy while the other half were analyzed (n=4).Therefore, at day 1, 12 cheeses were analysed: control (n=4), 400 MPa (n=4), 600 MPa (n=4); at day 30, 12 cheeses were also analysed: control (n=4), 400 MPa (n=4), 600 MPa (n=4) and, at day 60, 28 cheeses were analysed: control (n=4) and cheeses treated at days 1, 30 and 50 at 400 MPa or 600 MPa (n=24; 3 days x 2 treatments x 4 cheeses per batch).

2.3. Solid phase microextraction and gas chromatography-mass spectrometry

For extraction of volatile compounds, the solid phase microextraction method of Lee et al. (2003) was followed. Volatile compounds analysis by Gas Chromatography–Mass Spectrometry was performed according to Delgado et al. (2010).

2.4. Statistical analysis

A one-way analysis of variance was carried out to establish the differences between control and HP-treated cheeses throughout maturation. A post host test (Tukey's test) was applied when means differences were significant (P<0.05). Principal component analysis (PCA) was performed to reduce the data in two dimensions and identify patterns of variation in the results. SPSS 14.0 software was used for statistical analysis (SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

3.1. Effect of high pressure treatment on cheeses with 1 day of maturation

Table 1 shows volatile compounds (Area Units x 104) measured in the headspace of HP-treated cheeses at day 1. Eight compounds were isolated: 6 acids and 2 alcohols. The levels of most volatile compounds, except decanoic acid and 1-butanol, 4-butoxy were modified after HP treatment. The intensity of the HP treatment (600 or 400 MPa) did not modify cheese volatile profiles as similar intensity of changes were found. Levels of most volatile compounds were reduced after HP treatment (reduction of 26% compared with control). The exception was that a small amount of 1-butanol, 4-butoxy was formed after HP treatment.

Table 1. Levels of volatile compounds (area units x 10^4) isolated from HP-treated raw goat milk cheeses at day 1.

		Control		400 MPa		600 MPa		
Compounds	Id. Method[a]	Mean	SD	Mean	SD	Mean	SD	*P*-value
Ethanol, 2-methoxy	TI	2.8a	1.1	1.2b	0.0	1.3b	0.2	0.010
Lactic acid	TI	6.1a	1.3	0.6b	0.2	0.6b	0.2	0.001
Acetic acid	RF,TI	74.1a	11.7	40.1b	6.3	40.7b	10.9	0.001
Butanoic acid	RF,TI	29.5a	5.5	18.9b	1.5	16.2b	2.8	0.002
1-Butanol, 4-butoxy	TI	0.0b	0.0	0.6a	0.1	0.7a	0.2	0.001
Hexanoic acid	RF,TI	20.4	7.8	14.5	2.4	15.4	3.6	0.260
Octanoic acid	RF,TI	44.3a	9.6	36.8ab	1.4	32.2b	1.8	0.044
Decanoic acid	RF,TI	93.6	9.5	88.5	6.4	90.4	8.1	0.681
Total		270.8		201.2		197.5		

a Method of identification: RF, mass spectrum and retention time identical with a reference compound; TI, tentative identification by mass spectrum. a, b: Different letters in the same row indicate statistically significant differences (Tukey's Test, P<0.05).

Lactic acid and acetic acid were significantly reduced after HP treatment. Lactic acid is not usually considered a volatile compound, but it is usually present in the cheeses at

day 1. Mesophilic starter bacteria produce lactic acid in the curd, which is quickly metabolized by secondary microorganisms (Mcsweeney & Sousa, 2000). Acetic acid is not produced by lipolysis, it is considered that is originated from the fermentation of lactate by microorganisms but also from reactions amino acids (Fox et al., 1993). In a parallel study, we found that HP caused a reduction of lactic acid bacteria (LAB) at the beginning of cheese maturation and, for this reason, lactic and acetic acids could have decreased after HP treatment. In addition, four other volatile free fatty acids (FFA) were detected: butanoic, hexanoic, octanic and decanoic acids. Levels of hexanoic and decanoic acids did not significantly differ ($P>0.05$) between control and treated cheeses. At day 1, cheeses had high moisture content and a soft structure and HP treatment induced notable physical modification (mainly compacting and lost of whey) and this probably may reduce levels of volatile compounds due to losses in fluid removed.

It is likely that the lower levels of acids isolated from HP-treated cheeses could contribute to a lower development of aroma compounds at the end of ripening, due to the role of these compounds as volatile precursors. Fatty acids are important aroma compounds in cheese (Christensen & Reineccius, 1995; Rychlik & Bosset, 2001; Delgado et al., 2011) and they also serve as precursors of other volatiles of cheese (Curioni & Bosset, 2002).

3.2. Effect of high-pressure treatment on cheeses with 30 days of maturation

Volatile compounds (Area Units x 10^4) isolated in HP-treated raw goat milk cheeses at day 30 of maturation are shown in Table 2; eight acids and one alcohol were identified. Three new acids were detected at day 30 compared to 1 day-old treated cheeses: propanoic acid, propanoic acid 2-methyl (isobutyric) and butanoic acid 3-methyl (isovaleric). The last compounds are originated from amino acids metabolism, concretely from valine and leucine. (Molimard & Spinnler, 1996), and also, they are frequent volatile compounds of goat and sheep cheeses (reviewed by Curioni & Bosset,

2002). Levels of these acids were not affected by HP treatment as; in general, HP treatment at day 30 of maturation did not modify cheese volatile profile as much as when the treatment was applied at the beginning of maturation.

Table 2. Levels of volatile compounds (area units x 10^4) isolated from HP-treated raw goat milk cheeses at day 30.

Compounds	Id. Method[a]	Control		400 MPa		600 MPa		*P*-value
		Mean	SD	Mean	SD	Mean	SD	
Ethanol, 2-methoxy	TI	2.6	0.7	2.9	0.6	2.6	0.5	0.756
Acetic acid	RF,TI	88.7	37.1	109.4	30.4	86.1	23.1	0.527
Propanoic acid	RF,TI	1.0a	0.1	0.8a	0.1	0.6b	0.2	0.002
Propanoic acid, 2-methyl	RF,TI	1.0	0.4	1.3	0.2	0.9	0.3	0.231
Butanoic acid	RF,TI	43.1	8.0	47.6	12.1	31.5	12.8	0.165
Butanoic acid, 3-methyl	RF,TI	2.5	0.9	3.2	0.6	2.1	0.6	0.185
Hexanoic acid	RF,TI	33.3	6.7	30.1	5.3	31.0	7.3	0.779
Octanoic acid	RF,TI	55.8a	3.5	51.2ab	4.6	47.9b	2.6	0.041
Decanoic acid	RF,TI	130.5	22.4	122.2	21.1	107.7	12.6	0.285
Total		385.5		368.7		310.4		

a Method of identification: RF, mass spectrum and retention time identical with a reference compound; TI, tentative identification by mass spectrum. a, b: Different letters in the same row indicate statistically significant differences (Tukey's Test, P<0.05).

The volatile profile of cheese did not significantly differ between control and treated cheeses at 30 days of ripening, except for propanoic and octanoic acids (P<0.05). These results are in agreement with those reported by Saldo et al. (2003) in 21-day-old Garrotxa cheese treated at 400 MPa for 5 min as they did not find differences in most volatile fatty acids between control and treated cheese. In the present study, when cheeses were treated at 600 MPa the levels of propanoic and octanoic acid were significantly reduced. Both have a different origin, propanoic acid could be formed from the fermentation of lactose and lactic acid whereas the origin of octanoic acid is the lipolysis (Molimard & Spinnler, 1996). The reduction in the level of these acids

could modify the final cheese volatile profile at the end of maturation, as these acids are precursors of other volatiles formed after maturation.

3.3. Effect of high pressure treatment on cheeses at the end of maturation (60 days)

Table 3, 4 and 5 shows the levels of volatile compounds found in HP-treated raw goat milk cheeses at 1, 30 and 50 days and analysed at day 60 of maturation. Fifty compounds were identified in cheeses at day 60: 12 acids, 16 alcohols, 5 esters, 8 ketones and 9 other compounds. Four compounds were only isolated in HP-treated cheeses: 4-heptanone, cyclohexanone 2-methyl, dimethyl trisulfide and ethylbenzene. Dimethyl trisulfide (DMTS) provides odour notes of cooked cabbage, broccoli or cauliflower, and it is an important odorant in Gruyére cheese (Rychlik & Bosset, 2001). Amounts of DMTS increased in cheese treated at higher pressure (600 MPa) after 1 and 30 days of maturation. The increase of this sulfur compound could affect Ibores cheese aroma because it has very low perception thresholds (Curioni & Bosset, 2002).

In general, HP treatment decreased the relative abundance (AU) of most volatile compounds, except ketones, when the treatment was applied at the beginning and the middle of maturation (day 1 and 30). Treatments at 1 day of ripening caused important changes in the volatile profile of cheeses, while the effect was less intense when cheeses were treated after 30 days of maturation. In contrast, when cheeses were treated after 50 days of ripening, the volatile profile was slightly modified. Treatments applied at days 1 and 30, at higher pressures (600 compared to 400 MPa) had more effect on cheese volatile profile, while a different trend was found when treatment was applied at day 50.

Acids were the main volatile compounds of this cheese and were significantly modified by HP, except for nonanoic and undecanoic acids (Table 3). Volatile contents were lower in HP-treated cheeses than in control cheese when cheeses were treated at the beginning (day 1) of maturation. However, when cheeses were treated after 50 days of maturation at the highest pressure (600 MPa) no significant changes were found while,

at the lowest pressure (400 MPa), acid content was increased. Thus, the level of propanic, butanoic, pentanoic, hexanoic, heptanoic, octanoic and decanoic acids was significantly higher in cheeses HP-treated at 400 MPa at day 50 than in the other batches.

The relative abundance of branched fatty acids detected at day 60, such as propanoic acid 2-methyl and butanoic acid 3-methyl was higher in cheeses treated at day 30 at 600 MPa, so treatment at 600 MPa could facilitate the release of branched fatty acids, which play a important role in the flavour goat cheese due to they are potent odorants.

HP treatment of cheeses at the beginning of maturation could influence overall cheese aroma due to a reduction in FFA contents with goaty flavor, e.g., hexanoic, octanoic and nonanoic acids (Le Quéré et al., 1998). In contrast, the increase in acids in cheeses treated at 400 MPa at day 50 could enhance the aromatic notes provided by these aroma compounds. No explanation has been found for the more intense increase in FFA at 400 MPa than at 600 MPa. It is possible that the application of lower pressures might enhance certain biochemical lipolytic pathways, and the effect could be dependent on the pressure applied.

Alcohols were the second most abundant compounds identified in these cheeses (Table 4). Most volatile alcohols were significantly modified (P<0.05) by HP treatment. The decline in alcohol levels was more intense when treatment was applied at the beginning of maturation than at the middle (day 30). In addition, modifications were more intense at higher pressures applied. As for acid compounds, levels of alcohols decreased in HP-treated cheese, probably due to the enzymatic inactivation following HP treatment. Pressure can affect most biochemical reactions, since they often involve changes in volume and control certain enzymatic reactions (Rastogy et al., 2007). In contrast with changes when HP was applied at the beginning of maturation, HP treatment at the end of maturation did not modify the volatile alcohol profile, probably because these compounds were completely formed after 50 days of maturation.

Table 3. Levels of volatile acids in cheese made from raw goat milk, HP- treated after 1, 30 and 50 days of ripening, and analysed at day 60[a].

Acids	Id. Method	Control	Day 1		Day 30		Day 50	
			400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa
Acetic acid	RF,TI	494±81a	227±16d	119±20e	383±23ab	318±17c	406±46b	365±38bc
Propanoic acid	RF,TI	7±0a	4±1c	3±0d	5±0bc	4±0c	7±0a	6±0b
Propanoic acid, 2-methyl	RF,TI	31±9bcd	17±10d	20±10cd	39±8ab	53±5a	31±2bcd	35±6bc
Butanoic acid	RF,TI	1418±85ab	1032±31d	722±26e	1294±112bc	1159±46cd	1500±62a	1266±67c
Butanoic acid, 3-methyl	RF,TI	139±53bc	70±54c	72±51c	211±55ab	281±27a	148±15bc	158±29bc
Pentanoic acid	RF,TI	14±1abc	13±0c	10±1d	15±1ab	13±0c	16±1a	14±0bc
Hexanoic acid	RF,TI	2175±89b	2009±21bc	1603±79d	2198±169b	1899±94c	2440±230a	2134±72bc
Heptanoic acid	TI	28±2ab	26±1b	24±1b	27±3ab	24±1b	31±4a	26±2ab
Octanoic acid	RF,TI	802±38ab	753±25b	722±23b	810±67ab	729±15b	906±121a	788±33ab
Nonanoic acid	TI	13±4	12±1	10±2	13±1	10±1	14±2.1	11±1
Decanoic acid	RF,TI	631±36ab	599±14b	594±36b	664±62ab	582±18b	724 ±84a	626±31ab
Undecanoic acid	TI	36±5	34.7±2.4	42±2	36±5	34±2	36±7	35±3
Total		5788	4798	3939	5695	5106	6258	5464

[a] Values (Area Units x 10^4) are given as means ± standard deviation; different letters in the same row indicate statistically significant differences (Tukey's Test, $P \leq 0.05$). The method of identification is denoted as: RF, mass spectrum and retention time identical with a reference compound; TI, tentative identification by mass spectrum.

Table 4. Levels of volatile alcohols in cheese made from raw goat milk, HP- treated after 1, 30 and 50 days of ripening, and analysed at day 60a. (See Table 3 footnote)

	Id.		Day 1		Day 30		Day 50	
Alcohols	Method	Control	400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa
2-Propanol	TI	2.4±0.3a	0.4±0.1c	0.2±0.0c	2.0±0.2ab	1.8±0.1b	2.4±0.2a	2.0±0.2ab
Ethanol, 2-methoxy	TI	53.4±11.3a	19.7±2.6c	13.0±0.7c	24.6±2.9bc	33.4±7.4b	49.4±7.2a	47.4±3.8a
2-Butanol	RF,TI	145.3±13.8a	0.0±0.0c	0.0±0.0c	0.0±0.0c	0.0±0.0c	50.7±14.3b	39.1±5.6b
1-Propanol	RF,TI	2.6±0.4a	0.0±0.0c	0.0±0.0c	0.0±0.0±	0.0±0.0c	1.4±0.1b	1.2±0.1b
2-Pentanol	RF,TI	12.2±7.8a	12.7±4.2a	3.9±2.1b	10.5±0.4ab	8.7±1.1ab	14.0±3.3a	13.9±2.4a
1-Butanol	RF,TI	1.0±0.7a	0.8±0.1a	0.0±0.0b	0.0±0.0b	0.0±0.0b	1.1±0.3a	0.9±0.1a
1-Butanol, 3-methyl	TI	46.9±8.9a	36.5±3.2bd	30.4±6.2c	42.5±2.9ab	46.3±1.6a	47.4±3.3a	47.2±3.3a
1-Pentanol	RF,TI	1.2±0.1	1.3±0.1	1.5±0.4	1.4±0.2	1.5±0.1	1.4±0.3	1.1±0.0
3-Buten-1-ol, 3-methyl	TI	3.7±2.5	4.7±1.1	3.8±0.7	4.7±0.7	4.8±0.2	5.0±0.5	5.2±1.7
2-Heptanol	TI	23.4±13.7abc	12.3±9.0bc	7.9±3.8c	30.4±7.5a	25.6±2.0a	36.1±7.5a	32.0±4.3a
3-Penten-2-ol	TI	7.8±0.6a	7.8±0.2a	4.2±0.3c	6.8±0.7ab	6.6±0.7b	7.6±0.4ab	6.7±0.3b
2-Nonen-1-ol	TI	3.2±1.0	3.8±0.8	4.7±1.3	3.5±2.0	3.1±1.3	3.4±0.7	3.0±0.6
Cyclohexanol, 2-methyl	TI	14.2±6.2	15.6±0.6	17.4±1.0	16.8±1.3	16.4±0.5	18.1±1.8	17.2±0.5
2-Nonanol	TI	5.9±0.3ab	1.4±0.6c	1.2±0.6c	6.7±3.5ab	4.0±0.6bc	7.9±1.4a	5.3±0.5ab
2-Furanmethanol	RF,TI	0.9±0.3	0.8±0.1	0.5±0.1	0.6±0.2	0.8±0.4	1.9±2.0	1.1±0.3
1-Butanol, 4-butoxy	TI	1.5±0.6a	0.6±0.1b	0.6±0.2b	0.4±0.0b	0.4±0.0b	0.8±0.1b	0.7±0.1b
Total		325.6	118.4	89.3	150.9	153.4	248.6	224

Table 5. Levels of volatile esters, ketones and some other compounds in cheese made from raw goat milk, HP- treated after 1, 30 and 50 days of ripening, and analysed at day 60[a].

Compounds	Id. Method	Control	Day 1		Day 30		Day 50	
			400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa
Esters								
Acetic acid, et.es	RF,TI	3.3±0.2a	2.1±0.4c	1.5±0.2d	2.8±0.3b	2.7±0.2b	3.4±0.1a	2.6±0.3b
Butanoic acid, et.es	RF,TI	38.1±2.8a	16.7±3.0d	5.8±0.6e	26.5±3.7b	21.7±1.5c	36.4±2.6a	30.3±1.0b
Hexanoic acid, et.es	RF,TI	43.1±7.2a	17.8±3.7c	8.2±1.1d	30.6±4.0b	23.0±1.1c	43.4±4.4a	34.8±0.8b
Octanoic acid, et.es	RF,TI	28.4±11.3a	12.3±1.6cd	7.8±0.6d	20.9±3.3abc	17.5±1.4bcd	28.2±3.9a	24.7±1.9ab
Decanoic acid, et.es	RF,TI	18.7±1.5bc	10.7±1.4d	6.9±0.7d	18.9±2.8bc	15.2±1.6c	23.8±3.4a	20.8±3.0ab
Total		131.6	59.6	30.2	99.7	80.1	135.2	113.2
Ketones								
2-Butanone	TI	78.2±7.9a	2.2±0.6c	2.0±0.2c	2.1±0.8c	2.2±0.3c	17.5±5.0b	17.9±7.9b
2-Pentanone	RF,TI	27.4±9.8cd	204.3±47.4a	145.5±50.6ab	81.1±46.6bcd	105.7±24.1bc	9.6±3.2d	30.9±44.0bc
4-Heptanone	RF,TI	0.0±0.0b	0.9±0.7ab	3.2±1.3a	1.4±0.5ab	2.9±0.6a	3.6±3.0a	0.0±0.0b
2-Heptanone	TI	30.7±5.7d	165.8±24.8a	162.3±41.9a	94.6±36.2bc	108.2±24.6ab	22.9±7.1d	44.1±39.0cd
2-Octanone	RF,TI	0.4±0.2c	1.7±0.4a	2.0±0.6a	1.4±0.6a	1.2±0.2ab	0.5±0.1bc	0.3±0.4c
Cyclohexanone, 2-	TI	0.0±0.0c	4.1±0.6a	4.5±0.3a	1.6±0.2b	1.4±0.3b	0.0±0.0c	0.0±0.0c
2-Nonanone	RF,TI	30.6±3.1b	64.2±10.3a	58.2±12.9ab	56.6±27.5ab	52.6±7.2ab	33.4±5.9b	30.2±4.1b
2-Undecanone	TI	2.7±0.5abc	2.4±0.4bc	1.7±0.3c	3.5±0.7ab	2.9±0.2ab	3.7±0.7a	3.0±0.6ab
Total		170	445.6	379.4	242.3	277.1	91.2	126.4

Compounds	Id. Method	Control	Day 1		Day 30		Day 50	
			400 MPa	600 MPa	400 MPa	600 MPa	400 MPa	600 MPa
Others								
Butanal, 3-	TI	1.2±0.2c	1.8±0.3bc	3.1±0.5a	1.6±0.2bc	2.6±0.2ab	1.9±1.2abc	1.5±0.6bc
Methanethiol	TI	0.5±0.1	0.6±0.0	0.4±0.2	0.6±0.2	0.3±0.2	0.6±0.2	0.5±0.3
Dimethyl	TI	0.0±0.0b	0.0±0.0b	3.3±2.1a	0.0±0.0b	1.7±0.2b	0.0±0.0b	0.0±0.0b
Toluene	TI	4.6±0.8c	5.4±0.9c	6.4±0.9bc	10.7±4.1a	10.0±1.8ab	7.9±1.6abc	6.4±0.9bc
Ethylbenzene	TI	0.0±0.0c	1.0±0.5ab	0.6±0.3bc	1.3±0.7a	1.1±0.2ab	0.0±0.0c	0.0±0.0c
p-Xylene	TI	0.4±0.3c	4.3±2.1abc	3.7±1.1abc	7.8±4.7a	5.3±1.7ab	1.3±0.6bc	0.6±0.1c
Styrene	TI	0.4±0.3b	0.7±0.2ab	0.6±0.2ab	1.1±0.5a	0.8±0.1ab	1.0±0.2a	0.7±0.1ab
Limonene	RF,TI	0.5±0.6b	2.2±0.3ab	3.9±1.6ab	4.2±3.8a	1.5±0.3ab	1.1±0.9ab	1.3±0.3ab
δ-Decalactone	TI	5.5±0.5c	6.3±0.6bc	7.1±0.7abc	8.5±0.9ab	6.9±1.0abc	8.9±1.8a	7.8±1.3ab
Total		13.1	22.3	29.1	35.8	30.2	22.7	18.8

[a] Values (Area Units x 10^4) are given as means±standard deviation; different letters in the same row indicate statistically significant differences (Tukey's Test, $P \leq 0.05$). The method of identification is denoted as: RF, mass spectrum and retention time identical with a reference compound; TI, tentative identification by mass spectrum.

The most abundant alcohol detected in control cheeses, 2-butanol, was not isolated in cheeses treated at day 1 and 30. Alcohols can contribute to cheese flavor because of their ability to form esters with acids (Gripon et al., 1991).

The lower level of branched-chain primary alcohols such as 3-methyl 1-butanol in HP-treated cheeses at the beginning of maturation could reduce the pleasant aroma of fresh cheese (Moio et al., 1993). Reduction of the aldehyde produced from Leu is considered as the origin of 3-methyl 1-butanol, so the enzymatic or microbiological mechanism involved in this chemical reaction could be inactivated by HP.

Ester contents were modified by HP in a similar way as previously described for some acids and alcohols (Table 5). HP applied at the end of maturation (day 50) at the lower pressure (400 MPa) had the least effect on the cheese volatile profile. In contrast, HP treatment at the beginning of maturation (day 1) at the highest pressure (600 MPa) had the greatest effect on the cheese volatile profile. These results contrast with those of Saldo et al. (2003), who did not find changes in these compounds after HP treatment of 21-days Garrotxa cheese. Modifications in esters could have a great influence in the total aroma of this raw goat milk cheese since they add fruity notes and have low perception threshold (Curioni & Bosset, 2002).

The effect of HP on all ketones was statistically significant (P<0.05). In general, HP treatment applied at the beginning of maturation increased the levels of ketones, so the highest levels of 2-pentanone, 4-heptanone, 2-heptanone, 2-octanone and 2-nonanone were found in cheeses treated at day 1. Changes in ketones due to HP were not as intense when treatment was applied at the end of ripening. It is probable that the increase in methyl ketones in cheese treated at day 1 could be caused because some ketones did not undergo reduction of alcohols, since lower levels of alcohols were found in HP-treated cheeses than control ones. Consistent with these findings, Juan et al. (2007) reported that HP treatment at ≥400 MPa applied on the first day of ripening of pasteurized ewe milk cheese could affect the enzyme activity responsible for degrading methyl ketones to alcohols, thus increasing levels of methyl ketones and

decreasing alcohols. On the other hand, HP could also facilitate the production of methyl ketones from free fatty acids, although additional studies are needed for clarifying this hypothesis. The ketone 2-butanone derives from 2,3-butanedione, which is produced of fermentation lactose and metabolism of citrate. HP treatments reduced the amounts of 2-butanone in a similar way as described by Butz et al. (2000) in Gouda cheese treated by HP as a result of variations in the enzyme activity due to treatment. According to the review of Curioni and Bosset (2002), methyl ketones such as 2-heptanone impairs Blue cheese notes, other methyl ketones like 2-octanone, 2-nonanone, 2-decanone and 2-undecanone provide fruity, floral and musty odors, so an increase in these compounds can be considered positive.

One aldehyde (3-methyl butanal), two sulfur compounds (methanethiol and dimethyl trisulfide), four hydrocarbons (toluene, ethylbenzene, p-xylene and styrene), one terpene (limonene) and one lactone (δ-decalactone) were also detected in this cheese. The abundance of 3-methyl butanal was significantly increased by HP treatment. This compound has been isolated in several cheese varieties and it could be an intense odorant (reviewed by Curioni & Bosset, 2002). Aromatic hydrocarbons such as toluene, ethylbenzene, xylene and styrene were increased when HP was applied at 30 days. Toluene has also been isolated from the headspace of other cheeses at high levels (Mariaca et al., 2001; Bintis & Robinson, 2004). One of the most common and important lactones identified in cheese is δ-decalactone, which is is a key odorant of different varieties of cheese (reviewed by Curioni & Bosset, 2002). HP treatment increased δ-decalactone when treatment was applied at the end of maturation, and could provide a more intense coconut odor to the background aroma of these cheeses.

3.4. Principal Component Analysis of data at the end of maturation (60 days)

Principal component analysis showed that 2 first principal components (PC1 and PC2) accounted the 58.9% of the total variability, being explained the 44% and 14.9% of the variability by the PC1 and PC2, respectively. The allocation of the variables in the space

defined by the two first components showed that some representative acids such as butanoic, propanoid and acetic acids and esters such as butanoic acid, ethyl ester and decanoic acid, ethyl ester were distributed along the positive axis of the PC1. In contrast, ketones (cyclohexanone 2-methyl, 2-heptanone, 2-octanone and 2-pentanone) did on the negative axis of PC1. Concerning distribution of volatile compounds in the PC2, hydrocarbons, δ-decalactone and 2-undecanone were situated on the positive axis of the PC2 in opposition to 1-butanol 4-butoxy and 2-butanone that were allocated in the negative portion of the PC2. Therefore, the distribution and contrast of the different volatiles can be explained the differences found among cheese treated by hydrostatic high pressure at different stages of maturation.

The distribution of the scores on the first two principal components (Figure 2) shows 7 separate groups of points, corresponding to the different treatments and mostly separated by PC1. Control and day-50-treated cheeses were located in the positive area of PC1 while in the negative area were those which were treated at day 1. In the middle of the plane are located cheeses HP-treated at day 30. PC1 cannot explain differences between control and day 50/400 MPa treated cheeses; however, PC2 could explain it. Cheeses HP-treated at 400 MPa at day 50 are in the upper part of the plane and control cheeses are in the low part of the plane. Treatment at the beginning of cheese ripening and at the higher pressure produced the greatest modification of volatile compounds in the headspace of this raw goat milk cheese, compared with untreated cheeses. However, both treatments (400 and 600 MPa) applied at the end of maturation (day 50) resulted in similar modifications in volatile profile, so both treatments could be suitable for the increase of microbial safety in this raw goat milk cheeses with minimum changes in cheese volatile profiles. In line with this, Arqués et al. (2007) concluded that HP treatment applied at the end of maturation could be a valuable tool for controlling undesirable microorganisms without altering the sensory characteristics of cheese.

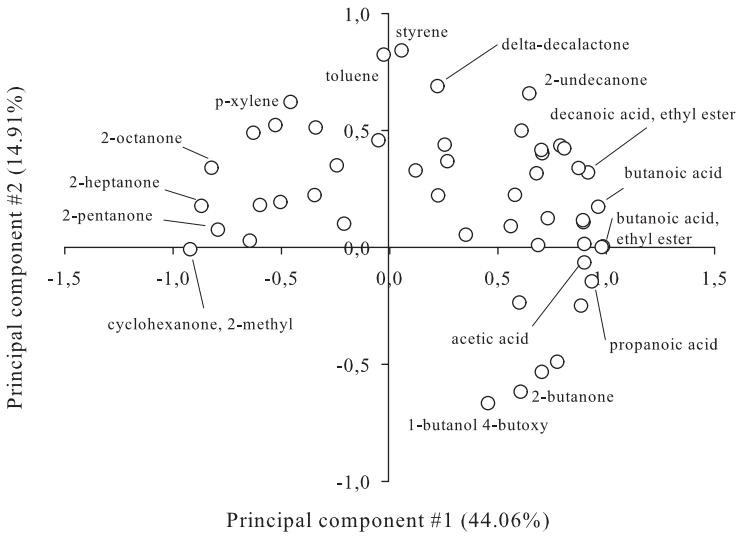

Figure 1. Loading plots after principal component analysis of the variables in the plane defined by the two first principal components (PC1 and PC2) in raw goat milk cheeses HP-treated at 1, 30 and 50 days and analysed at day 60.

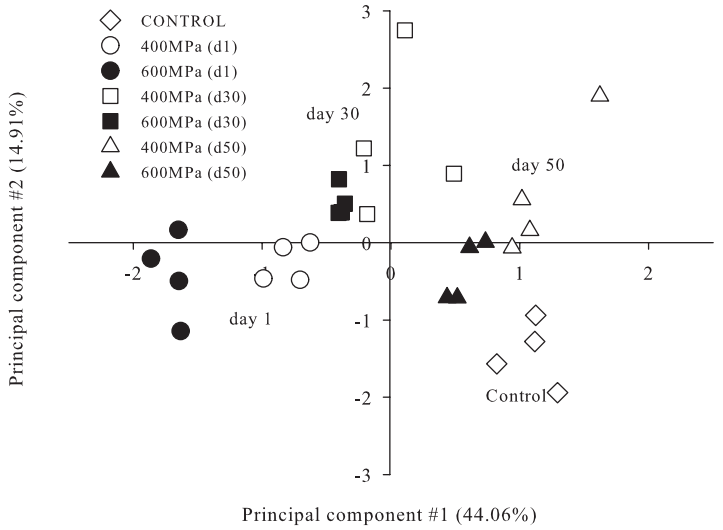

Figure 2. Scores plot after principal component analysis of the variables in the plane defined by the two first principal components (PC1 and PC2) in raw goat milk cheeses HP-treated at 1, 30 and 50 days and analysed at day 60. d is day.

4. CONCLUSIONS

High-pressure treatment induced important changes in the volatile profile of treated cheeses, especially when applied at the beginning of maturation; changes were less intense when HP treatment was applied at the end of maturation. HP treatment applied early decreased the relative abundance of the most volatile compounds, but enhanced the formation of ketones, hydrocarbons and δ-decalactone. Nevertheless, it is possible to reduce the changes if treatments are applied at the end of the ripening, but the pressure applied must be taken into account. It is important to note that changes in volatile profiles are not necessarily negative for consumer perception; however, the characteristic aroma of cheese will not be completely maintained after HP treatment.

5. Acknowledgements

This work has been financed thanks to European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). We thank the PDO dairy "Berrocales Trujillanos" for providing the cheeses.

6. References

Arqués, J. L., Garde, S., Fernández-García, E., Gaya, P., & Núñez, M. (2007). Volatile Compounds, Odor, and Aroma of La Serena Cheese High-Pressure Treated at Two Different Stages of Ripening. Journal of Dairy Science, 90, 3627–3639

Bintis, T., & Robinson, R. K. (2004). A study of the effects of adjunct cultures on the aroma compounds of Feta-type cheese. Food Chemistry, 88, 435–441.

Butz, P., Fernández, A., Koller, W. D., Messens, W., & Tauscher, B. (2000). Effects of high pressure treatment on fermentation process during ripening of Gouda cheese. High Pressure Research, 19, 37–41.

Christensen, K. R., & Reineccius, G. A. (1995). Aroma extract dilution analysis of aged Cheddar cheese. Journal of Food Science, 60, 218–220.

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

Delgado, F.J., González-Crespo, J., Cava, R., García-Parra, J., & Ramírez, R. (2010). Characterization of the volatile profile of a Spanish ewe raw milk soft cheese P.D.O. Torta del Casar during ripening by SPME-GC-MS. Food Chemistry, 118, 182–189.

DOE (2003). Diario Oficial de Extremadura (Regional Legislation of Extremadura), nº 61, 17th May 2003. Decreto 69/2003, de 20 de mayo, por el que se aprueba el Reglamento de la Denominación de Origen Protegida "Queso de Ibores" y su Consejo Regulador.

Gripon, J. C., Monnet, V., Lamberet, G., & Desmazeaud, M. J. (1991). Microbial enzymes in cheese ripening. In P. F. Fox (Ed.). Food Enzymology (Vol. 1, pp. 131–168). London: Elsevier Applied Science.

Juan, B., Barron, L. J. R., Ferragut, V., & Trujillo, A. J. (2007). Effects of High Pressure Treatment on Volatile Profile During Ripening of Ewe Milk Cheese. Journal of Dairy Science, 90, 124–135.

Lee, J. H., Diono, R., Kim, G. Y., & Min, D. (2003). Optimization of solid phase microextraction analysis for the headspace volatile compounds of Parmesan cheese. Journal of Agricultural and Food Chemistry, 51, 1136–1140.

Le Querré, J.L., Pierre, A., Riaublanc, A., & Demaizières, D. (1998). Characterization of aroma compounds in the volatile fraction of soft goat cheese during ripening. Lait, 78, 279–290.

Mariaca, R. G., Fernández-García, E., Mohedano, A. F., & Nuñez, M. (2001). Volatile fraction of ewe's milk semi-hard cheese manufactured with and without the addition of cysteine proteinase. Food Science & Technology International, 7, 131–139.

McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening: A review. Le Lait, 80, 293–324.

Moio, L., Langlois, D., Etiévant, P. X., & Addeo, F. (1993). Powerful odorants in water buffalo and bovine Mozzarella cheese by use of extract dilution sniffing analysis. Italian Journal of Food Science, 3, 227–237.

Molimard, P., & Spinnler, H. E. (1996). Compounds involved in the flavor of surface mold-ripened cheeses: origins and properties. Journal of Dairy Science, 79, 169–184.

Rastogy, N. K., Raghavarao, K. S. M. S., Balasubramaniam, V. M., Niranjan, K., & Knorr, D. (2007). Opportunities and Challenges in High Pressure Processing of Foods. Food Science and Nutrition, 47, 69–112.

Rychlik, M., & Bosset, J.O. (2001). Flavour and off-flavour compounds of Swiss Gruyère cheese. Evaluation of potent odorants. International Dairy Journal, 11, 895–901.

Saldo, J., Fernández, A., Sendra, E., Butz, P., Tauscher, B., & Guamis, B. (2003). High pressure treatment decelerates the lipolysis in a caprine cheese. Food Research International, 36, 1061–1068.

Capítulo 7

Changes during storage on microbiology, proteolysis, texture and sensory evaluation of a mature raw goat milk high-pressure treated cheese with paprika on rind

Enviado para su publicación en[1]: *Journal of Food Science*

ABSTRACT

The effect of high-pressure (HP) treatment (400 or 600 MPa for 7 min) on microbiology, proteolysis, texture and sensory parameters was investigated in a mature raw goat milk cheese after HP-treatment and during storage. Cheeses were treated at the end of ripening (day 60), and analysed after pressurisation and after 30 days of refrigerated storage (day 90). All microorganism groups were significantly decreased by HP treatment at day 60. A higher decrease was found in cheeses pressurized at 600 MPa than in those treated at 400 MPa. At day 90, there were still significant differences for all microbial counts between control and HP-treated cheeses, except for *Listeria spp* and *Enterobacteriaceae*, which were at very low levels. pH value was not affected by HP treatment, independently of the pressure intensity applied and the day of analysis. Regarding to instrumental colour parameters, CIE L* and CIE a* values were significantly modified (P<0.05) by HP treatment at the two moments of analysis. In general, nitrogen fractions were significantly modified (P<0.05) after HP treatment at day 60 at 600 MPa compared with control cheeses, but after 30 days of storage this effect disappeared. On the other hand, HP treatment caused a significant increase of some TPA parameters such as adhesiveness, gumminess and chewiness. However, neither trained panellists not consumers found significant differences between control and HP-treated cheeses after the treatment or after the storage.

[1] Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (Submitted). Changes during storage on microbiology, proteolysis, texture and sensory evaluation of a mature raw goat milk high-pressure treated cheese with paprika on rind. *Journal of Food Science JFS-2011-0669.*

1. INTRODUCTION

Consumers now demand for fresh products without the negative effect often associated with other methods such as irradiation, heat treatment or additives. High Pressure (HP) processing is an emerging food treatment that increases food safety, while allow food to retain many of its original qualities and healthy attributes. HP is a non-thermal preservation technique that causes slight or no change in the organoleptic and nutritional quality of the HP-treated product, unlike most conventional heat treatments. Generally, HP–treated food shows better retention of nutrients, flavor, and colour than thermally treated products (San Martín-González et al., 2006).

HP involves exposing a product to high pressures (from 100 to 1000 MPa) for a given time, although pressures used in commercial systems commonly range between 400 and 600 MPa. Hydrostatic pressure is applied to food products through a liquid medium, generally water, which surrounds the product. The hydrostatic pressure is transmitted to food products in the same way from all sides (isostatically). This equal distribution of pressure is the reason because, generally, solid foods retain their original shape. Hydrostatic pressure also has little effect on covalent bonds (O'Reilly et al., 2001), and as a result, the HP-treated foods do not undergo significant chemical transformations.

Researches about HP-treated cheeses have been mainly focused on the acceleration or deceleration of cheese maturation (Saldo et al., 2003; Arqués et al., 2007; Juan et al., 2007) as well as to increase the cheese microbial safety (Buffa et al., 2001; Kheadr et al., 2002; López-Pedemonte et al., 2007). Besides acceleration or deceleration of cheese ripening and microbial safety of HP-treated cheeses, the use of HP treatment to stop the ripening when cheese is matured to prolong their shelf-life has also been described (Wick et al., 2004; Voigt et al., 2010).

Pressurisation of mature cheeses is a good commercial option because cheeses are vacuum-packed, pressurized and directly sold in their plastic bags. The effect of HP

processing on cheese has been mostly evaluated after the treatment applied, however, only a few studies (eg. Voigt et al., 2010) have evaluated if the possible produced changes are maintained during the storage period.

Ibores cheese is a raw goat milk cheese regionally produced in the south-west of Spain under Designation of Origen (PDO). In the market, two types of cheese can be purchased by consumers: with or without red paprika on rind. The most typical presentation of this cheese is with paprika on rind since consumers easily identify the red colour with this characteristic type of cheese.

HP treatment at the end of raw goat cheese ripening (day 60) could inactivate or reduce pathogens (e.g., *Enterobacteriaceae, Listeria spp.*) and/or undesirable microorganisms present in raw milk cheese, increasing the food safety and shelf-life. However, it is important to know the effect of HP treatment on chemical, textural and, principally, sensory properties of HP-treated cheeses. For this reason, the aim of the present study was to establish the effect of the HP treatment at 400 or 600 MPa for 7 min on microbiology, proteolysis, texture and sensory evaluation (trained panel and consumers) of a mature raw goat milk cheese (60 day-old) after HP treatment and after 30 days of refrigerated storage (day 90).

2. MATERIALS AND METHODS

2.1. Cheese samples and HP treatment

Raw goat milk cheeses from a dairy of "Ibores" Protected Designation of Origen (PDO) were studied. One lyophilized direct-to-vat mesophilic mixed culture (R-704, 50 units; Chr. Hansen, Hørsholm, Denmark), containing *Lactococcus lactis subsp. cremoris* and *L. lactis subsp. lactis* were used to facilitate the acidification process. Milk was coagulated at 28-32 ºC for 60–90 min with animal rennet (Naturen Plus 175, 22–25 mL per 100 L, CHR Hansen, Hørsholm, Denmark). The curd was cut into medium-size (1–2 cm) grains. Cheeses of fiat cylindrical shape, weighing 1–1.2 kg, were pressed for 3-8 h (1–2 Kg

cm^2), brine-salted and ripened (at 8–12ºC and 80% relative humidity) for 2 months. The cheese surface was rubbed with red-dried paprika and olive oil at the end of ripening as this is a typical presentation of this type of cheese. At the end of ripening, the physicochemical properties were 56% of fat content in dry matter, 63% of dry matter and 2.5% NaCl.

At the end of the ripening (60 days), 24 cheeses were pressurized. 8 cheeses were HP treated at day 60 at 2 intensities of treatment, 400 or 600 MPa, for 7 min (n= 8x2= 16). Cheeses were analysed after HP-treatment (day 60) and after 30 days of refrigerated storage (day 90). 4 untreated cheeses were used as control in each stage of ripening (n= 4x2= 8). Refrigerated cheeses were not unpacked to follow a real process in the case of the HP treatment was applied for an industrial commercialization. Cheeses were vacuum-packed in plastic nylon/polyethylene bags (50 ml O_2 per m^2 per 24 h at 0 °C) and HP treated in a 55-L capacity discontinuous hydrostatic press at NC Hyperbaric Wave 6000/55 (NC Hyperbaric, Burgos, Spain). Initial water temperature was 10°C. After HP treatments, the half of cheeses treated was kept at refrigeration conditions (n= 12; 4 control cheeses, 4 cheeses treated at 400 MPa and 4 cheeses treated at 600 MPa; stored at 6±1 ºC) and the other half were analysed (n= 12).

2.2. Microbiological analysis

10 g of cheese were placed into a sterile Stomacher bag with 90 mL of sterile 2% (w/v) trisodium citrate solution and blended for 5 min in a Stomacher (IUL instruments, Germany) at room temperature. Serial dilutions were made using Ringers solution and microorganisms were grown in different media (Merck, Germany) and incubation conditions: Plate Count Agar (PCA) for mesophilic aerobic and psychrotrophic, incubated at 30 and 16 ºC for 72 and 96–120 h, respectively; Violet-red Bile Dextrose (VRBD) for *Enterobacteriaceae*, incubated at 37 ºC for 24 h; Man, Rogosa and Sharpe (MRS) for Lactic Acid Bacteria (LAB) incubated at 35 ºC for 72 h in an atmosphere with

5% CO_2 (Merck Anaerocult® C); Manitol Salt Phenol-red Agar (MSA) for *Micrococcaceae*, incubated at 35 ºC for 72 h.

In order to evaluate *Listeria spp.* levels, 25 g of cheese were homogenised in a Stomacher with 225 mL of Half-Fraser Broth (Fraser Selective Medium ½). After 20 min of pre-incubation at room temperature, serial dilutions were made using Fraser medium and microorganisms were grown in Chromogenic Listeria Agar (ISO) Base (Oxoid Ltd, UK) at 37 ºC for 24–48 h. In this medium, the chromogenic compound X-glucoside is added as substrate for the detection of β-glucosidase, resulting in blue-coloured listeria colonies. Through the production of a phosphatidylinositol-specific phospholipase C in *L. monocytogenes* strains and the addition of a specific purified substrate for the enzyme to the medium, an opaque halo surrounds the colonies. This could allow a first identification of *L. monocytogenes* which in case of presumptive detection was tried to be confirmed by means of the "metabolic fingerprint" from discrete test reactions performed within 96 well Micro-plate, using Biolog equipment (Biolog Inc., California, USA). After inoculation and incubation, the Micro-plate is placed into the Micro-Station Reader for analysis. 95 different carbon compounds including sugars, carboxylic acids, amino acids and peptides for the microorganism identification were used to provide discriminating biochemical characterizations. The unique metabolic pattern generated by the microorganism was recorded and compared to hundreds of identification profiles in a corresponding Biolog Database.

2.3. pH and colour measurements

pH was measured directly in the middle of cheeses using a portable puncture pH-meter Crison mod. 507 (Crison Instruments, Barcelona, Spain). Colour parameters were determined with a Minolta CR–200 colorimeter (Minolta Camera Co., Osaka, Japan) with illuminant D65, an 8 mm port/viewing area and a 0º viewing angle. Before using, the colorimeter was standardized with a calibration plate (Y= 93.2; x= 0.3159; y= 0.3324 to D65). The following colour parameters were determined: lightness (CIE L*),

redness (CIE a*: + red, - green) and yellowness (CIE b*: + yellow, - blue). The measurements were repeated at five randomly selected locations on each sample and average data were reported.

2.4. Nitrogen fractions and Free Amino Acids (FAA) measurements

5 g of cheese were homogenised in a Stomacher with 25 mL of a trisodium citrate solution (0.5 M; 40 ºC). From this solution, total nitrogen (TN) and soluble nitrogen (SN) compounds at pH 4.4 were measured by Kjeldahl analysis. Soluble non-protein nitrogen (NPN) in 12% trichloroacetic acid (TCA) was analysed from the pH 4.4-SN fraction (Ardö, 1999). Other parameters were calculated: Casein nitrogen= TN-SN; Polypeptide nitrogen= SN-NPN.

For FAA determination, water-soluble nitrogen (WSN) was determined according to Cd-ninhydrin method described by Folkertsma and Fox (1992) modified by Delgado et al. (2010).

2.5. Texture Profile Analysis (TPA)

Texture analysis was performed in a texturometer TA-XT2i (Stable Micro Systems LTD, Godalming, UK). For the development of the texture profile analyses (TPA) samples were diced (1cm x 1cm x 1cm). Texture was measured at constant temperature (15±2 ºC). A cylinder probe was used (Aluminium cylinder probe P/25, 25 mm diameter; Stable Micro Systems LTD, Godalming, UK). In each analysis the cube was axially compressed twice at a 25% of deformation. The following texture parameters were measured from force–deformation curve: hardness, adhesiveness, cohesiveness, gumminess, springiness and chewiness (Bourne, 1978). Determinations were repeated 8 times per sample.

2.6. Sensory analysis

Sensory evaluation of control and HP-treated cheeses was carried out by 10 trained panellists from the Technological Agri-Food Institute. A profile of 10 sensory attributes

of cheese grouped in appearance, odour, texture, flavour and taste were analysed. Analyses were developed in tasting rooms with the conditions specified in UNE regulation. All sessions were conducted at room temperature in a sensory panel room equipped with white fluorescent lighting. The software used for recording scores in sensory sessions was FIZZ Network (version 1.01: Biosystemes, France). The panel sessions were held mid-morning, about 3 h after breakfast. In each group, cheeses were presented individually and at random. Cheeses were cut in triangular shape and with 0.2 cm of thickness. Mineral water and bread were supplied in each session. A quantitative descriptive test was applied by a 10 cm long non-structured scale 0-10. The attributes evaluated by panellists are described in the Table 1.

Table 1. Sensory attributes evaluated by the tasting panel in the sensory analyses of HP- treated and control raw goat milk cheeses.

Appearance	
colour	Cheese colour (light white-dark yellow)
presence of "eyes"	Level of "eyes" in the cheese (low number-high number)
Odour	
odour intensity	Intensity of odour before eating (odourless - very intense odour)
Texture	
hardness	Firmness perception during chewing (very tender- very firm)
springiness	Perception of elasticity during chewing (not elastic-very elastic)
friability	Impression of crumbliness during chewing (not crumbly - very crumbly)
Flavour	
flavour intensity	Intensity of overall flavour (flavourless - very intense flavour)
Taste	
salty taste	Intensity of salt taste (not salty - very salty)
sharp taste	Intensity of sharp taste (not sharp- very sharp)
strange taste	Intensity of strange taste (not strange- very strange)

In addition, a consumer's sensory evaluation was also performed to evaluate the compliance of consumers with the cheese after processing and after the storage period. A total of 39 consumers took part in sensory analysis: 15 men and 24 women

participated at day 60; and 17 men and 22 women participated at day 90. A hedonic test was carried out by a 10 cm long non-structured linear scale (values from 0= dislike to 10= like very much). The attributes evaluated were the degree of conformity with the appearance, odour, texture and flavour of the HP treated and control cheeses. The tests were carried out in two different days (one for cheeses of day 60 and the other for cheeses of day 90). Three triangular pieces of cheese (control, 400 and 600 MPa cheeses) were provided to each person in each day of analysis. Finally consumers were also asked to choose their favourite cheese/s among the three types of cheese provided (Test of preference).

2.7. Statistical analysis

Differences between control and HP-treated cheeses were established by means the one-way analysis of variance (ANOVA). Tukey's test was applied to compare the mean values of variables. Mean values and standard error of mean (SEM) are reported. SPSS software version 14.0 (SPSS Inc., Chicago, IL) was used in the statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Effect of high-pressure treatment on the microbial counts of Ibores raw goat milk cheese

The effect of HP treatment on the microbiology of Ibores raw goat milk cheese is presented in Table 2. All groups of microorganisms were significantly decreased after pressurisation at 600 MPa at day 60. Mesophilic aerobic, *Enterobacteriaceae*, LAB and *Listeria spp.* showed a significant reduction ($P<0.05$) after pressurisation at 400 MPa at day 60 compared with control cheeses while only psychrotophic bacteria and *Micrococaceae* remained unchanged after the treatment. Therefore, both 400 and 600 MPa treatments would significantly reduce the groups of microorganisms (*Enterobacteriaceae* and *Listeria spp*) which could include pathogens (e.g., *Escherichia coli* or *Listeria monocytogenes*). In this sense, numerous researchers have

demonstrated that HP treatment at 400 or 600 MPa (1–15 min) could reduce the counts of the pathogenic microorganisms mentioned above, but in different types of inoculated cheeses (Capellas et al., 1996; O'Reilly et al., 2001; Carminati et al., 2004; Arqués et al., 2005; López-Pedemonte et al., 2007).

Microbial counts reduction was proportional to the pressure intensity applied, since higher decreases were found in cheeses treated at 600 MPa than in those treated at 400 MPa. Voigt et al. (2010) reported that total aerobic bacteria were not reduced by HP treatments at 400 MPa or 600 MPa for 10 min. in a mature blue-veined cheese (42 day-old) after pressurisation. In addition, they found that counts of *Lactococci* were similar in both cheeses treated at 400 and 600 MPa of pressure. Differences could be due to a higher pH value reported in control and HP-treated blue-veined cheeses (pH= 5.6–6) than those found in Ibores cheese (pH= 5.08–5.23; Table 2), because a reduced pH value generally has a synergic effect with pressure for the elimination of microorganisms (Balasubramaniam et al., 2008). Other important factors such as differences in cheese composition and the different microorganism baroresistance in each type of cheese could explain these differences.

After 30 days of refrigerated storage (day 90), there were still significant differences between control and HP-treated cheeses for microbial counts, except for *Enterobacteriaceae* and *Listeria spp.*, but their levels were very low in pressurized cheeses (<1 log cfu g^{-1}). In fact, listeriosis is unlikely in dairy products with counts of *L. monocytogenes* <2 log cfu g^{-1} (Lundén et al., 2004). However, neither in control not in HP-treated cheeses (at day 60 and 90 of analysis) we identified colonies of *L. monocytogenes* by using both cromogenic and "metabolic fingerprint" methods. In addition, *Enterobacteriacea* were below the limit of detection (<1 log cfu g^{-1}) at day 90 in control and in HP-treated cheeses. In general, the effect of HP treatment found at day 60 was maintained after 30 days of storage. However, psychrotrophic bacteria and *Micrococcaceae* were not significantly reduced at day 60 at 400 MPa of pressure compared with control cheeses, but after 30 days of refrigerated storage significant

differences were found between both cheeses. These results indicate that the damaged cells by pressurisation at 400 MPa at day 60, which grew in culture medium, were not viable at day 90. In line with our results, López-Pedemonte et al. (2007) demonstrated that the use of moderate HP treatments (400 MPa for 10 min) coupled at 30 days of storage at refrigerating temperatures, could improve the food safety of cheese.

Although after the refrigerated storage (day 90), low counts of microbial groups that include pathogenic microorganisms such as *Enterobacteriaceae* and *Listeria spp*. were detected in control cheese, the possibility of infection by consumption of this cheese is very low, but exists, especially in not very ripened cheeses. This possibility could be reduced with the application of HP-treatment. In addition, another option is pressurize the raw milk cheeses with 40-50 days of maturation, when cheese quality characteristics are almost formed, so producers could shorten the time to reach the market (at this moment by law in raw milk cheese is at least 60 days).

3.2. Effect of high-pressure treatment on the pH and colour values of Ibores raw goat milk cheese

Table 3 shows pH and instrumental colour parameters of Ibores cheese analysed after HP treatment at day 60 and after 30 days of storage. The pH was not affected by HP treatment, independently of both the pressure intensity and the day of analysis. In contrast with our results, Voigt et al. (2010) found that the pH of blue-veined cheese increased after pressurisation at 400 MPa at day 42 (mature cheese), but decreased after HP treatment at 600 MPa. After 28 days of storage, the blue-veined cheese showed a pH value significantly (P<0.001) higher in control cheeses than HP-treated cheeses. The different behaviour in pH changes could be associated to the intrinsic differences between both cheeses (raw milk, different milk type, different starters, maturation conditions, etc.). In a parallel study (Chapter 4) in raw goat milk cheeses

pressurized at different stages of maturation, the pH of 50 days-old cheeses remained unchanged after HP-treatment.

Regarding to instrumental colour parameters, CIE L* and CIE a* values were significantly modified by HP treatment at day 60 while CIE b* remained unchanged. Differences induced by HP treatment were maintained after 30 days of refrigerated storage. Changes on colour parameters in pressurized cheeses have been previously reported in the literature (Capellas et al., 2001; Juan et al., 2008; Voigt et al., 2010) without clear conclusions. In a parallel study about this raw goat milk cheese treated at different stages of maturation (Chapter 4), similar colour modifications as in this paper were observed in those cheeses treated at day 50 of ripening and analyzed at day 60. CIE L* was not statistically different but tended to be higher in HP-treated cheeses; while CIE a* was significantly lower in HP-treated cheeses and CIE b* was not modified. However these colour changes were not perceived by panellist at sensory level. The mechanisms responsible for these colour alterations are unknown. Researchers postulate that alterations within protein network caused by HP treatment could explain these differences (O'Reilly et al., 2001).

3.3. Effect of high-pressure treatment on the nitrogen fractions and free amino acids (FAA) of Ibores raw goat milk cheese

Nitrogen fractions and FAA levels found in control and HP-treated cheese are shown in Table 4. SN/TN and NPN/TN were significantly increased after HP treatment at day 60 at 600 MPa compared with control cheeses. Obviously, Casein N levels decreased. However, after 30 days of storage, the effect of HP at 600 MPa on these fractions disappeared. FAA contents were not influenced by HP treatment in both days of analysis. Therefore, HP treatment at 400 MPa did not affect to Ibores cheese proteolysis after pressurisation (day 60) or after 30 days of storage (except Polypeptide N). Moreover, pressurisation at 600 MPa only affected to cheeses analysed at day 60 and changes were diluted after 30 days of storage.

Table 2. Microbial counts (log cfu g^{-1}) of Ibores cheese treated at day 60 and analysed after treatment (day 60) and after 30 days of refrigerated storage (day 90).

	HP-Treatment applied at day 60					After 30 days of storage (day 90)				
	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
Psychrotrophic	7.5a	7.3a	4.7b	0.4	0.001	6.4a	3.7b	3.1b	0.5	0.001
Mesophilic aerobic	8.0a	7.7b	6.4c	0.2	0.001	7.7a	6.9a	4.8b	0.4	0.001
Enterobacteriaceae	4.3a	3.8b	3.2c	0.1	0.001	nd	nd	nd	–	–
Lactic Acid Bacteria	7.4a	6.7b	4.6c	0.4	0.001	6.8a	5.5b	3.2c	0.4	0.001
Micrococacceae	5.6a	5.2a	4.3b	0.2	0.002	3.7a	2.6b	2.6b	0.2	0.004
Listeria spp.	3.3a	2.7b	1.8c	0.2	0.001	1.4	0.8	0.4	0.2	0.245

a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test. P<0.05). SEM: Standard Error of Mean. nd: non-detected (nd: non-detected, below detection limit <10 cfu g^{-1}).

Table 3. Mean of pH and colour values of Ibores cheese treated at day 60 and analysed after treatment (day 60) and after 30 days of refrigerated storage (day 90).

	HP-Treatment applied at day 60					After 30 days of storage (day 90)				
	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
pH	5.23	5.08	5.22	0.04	0.233	5.17	5.10	5.04	0.04	0.552
CIE L*	95.02b	97.76a	97.57a	0.47	0.009	95.87b	98.06a	97.88a	0.33	0.001
CIE a*	0.43b	-0.51a	-1.19a	0.23	0.002	0.10a	-1.38b	-1.76b	0.25	0.001
CIE b*	1.44	0.45	1.89	0.39	0.369	1.75	2.25	3.03	0.26	0.114

a, b: Different letters in the same row indicate significant statistical differences (Tukey's Test. P<0.05). SEM: Standard Error of Mean.

Table 4. Nitrogen fractions and free amino acids (FAA, expressed as mg Leu g cheese^{-1}) found in Ibores cheese treated at day 60 and analysed after treatment (day 60) and after 30 days of refrigerated storage (day 90).

	HP-Treatment applied at day 60					After 30 days of storage (day 90)				
	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
SN/TN	14.26b	15.34ab	17.21a	0.48	0.017	19.54	20.30	20.25	0.75	0.918
NPN/TN	8.34b	9.34ab	10.10a	0.26	0.003	11.41	11.41	11.91	0.42	0.874
Casein N	85.74a	84.66ab	82.79b	0.48	0.017	80.45	79.70	79.75	0.75	0.918
Polypeptide N	3.78	3.40	3.99	0.13	0.159	4.79b	5.46a	4.72b	0.75	0.009
FAA	0.75	0.78	0.77	0.01	0.604	1.37	1.47	1.43	0.04	0.705

a, b: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean. SN: Soluble Nitrogen. TN: Total Nitrogen. NPN: Non Protein Nitrogen. N: Nitrogen. FAA: Free Amino Acids

In line with our results, Voigt et al. (2010) reported an increase of SN fraction after HP treatment at 600 MPa in mature blue-veined cheese but after 28 days of storage at 4 ºC they showed similar values compared to control cheese. In addition, NPN/TN increased in these cheeses after pressurisation at 400 or 600 MPa. In general, the scarce effect of HP on proteolysis of mature Ibores cheeses is positive, because proteolysis is a key process for the correct development of cheese flavor and texture (McSweeney and Sousa 2000; Sousa et al., 2001).

3.4. Effect of high-pressure treatment on the texture profile analysis (TPA) of Ibores raw goat milk cheese

Classification of the mechanical attributes of cheese texture was designed with the aim of integrating sensory data for foods evaluated by trained panels, with texture-profile data obtained on the same foods using compression testing, i.e., instrumental analysis. For this purpose, objective textural parameters were defined and are known as TPA parameters (O'Callaghan & Guinee, 2004).

Regarding TPA parameters (Table 5), HP treatment applied at day 60 caused a significant increase of adhesiveness, gumminess and chewiness. An increase of these parameters could result in a rise of the energy necessary to masticate and ingest the food. After 30 days of storage (day 90), this observed effect on textural properties of HP-treated Ibores cheese was only kept in cheeses pressurized at 600 MPa compared with control. Moreover, at day 90 a higher hardness was found in cheeses treated at 600 MPa than in the other ones. Therefore, modifications on cheese texture induced by HP (especially at 600 MPa) should be taken into account, because textural properties are important for consumer acceptance. The effect of HP treatment applied during maturation process has been assessed in cheese (Saldo et al., 2000; Juan et al., 2007); however, as far we know, there are no studies about the influence of HP treatment on texture of mature cheeses and their evolution during storage. However, when HP was applied on raw goat cheese with different stages of maturation, HP only

modified the texture of cheeses which were pressurized at the beginning of ripening (Chapter 4).

3.5. Effect of high-pressure treatment on the sensory analysis of Ibores raw goat milk cheese

Results of sensory analysis carried out by trained panellists are shown in Table 6. In general, there were no differences in the assessed attributes between control and HP-treated cheeses after pressurisation or after 30 days of refrigerated storage. Only friability was significantly higher in cheese treated at 600 MPa and analysed after pressurisation than in the other groups of cheeses, but these differences disappeared after 30 days of storage. Therefore, differences reported to TPA parameters (instrumental analysis) were not detected in this case in the sensory evaluation. These results are interesting because it is important to maintain the typical texture of Ibores cheese to preserve the original characteristics of cheese known by consumers. In contrast, in a recent study (Chapter 4) we have found that HP treatments at the beginning of ripening modified the texture of mature Ibores cheese (60 day-old). Those pressurized cheeses had less “eyes”, odour intensity, hardness and more springiness than control cheeses. In that study, a reduction in the level of eyes was perceived by panellist after the HP treatment. This trend was also observed in this study although differences were not significant, maybe the less number of “eyes” in these control cheese could explain the differences in the results.

On the other hand, neither odour not flavor intensity were affected by pressurisation, which is positive since cheese flavor is one of the most important criteria determining consumer choice and acceptance (Plutowska & Wardencki 2007). In line with results obtained for pressurized Ibores cheese, we reported that few volatile compounds were affected when HP treatment was applied at the end of Ibores cheese maturation (day 60) and differences found were lessen after 30 days of storage (Delgado et al., 2011). In that paper, we also postulated that maybe the effect of the addition of paprika on

rind would be enhanced after HP treatment as some volatile compounds with origin in paprika tended to be higher in HP-treated cheese. However, this effect has not also been perceived by trained panellists.

Consumers did not find significant differences between HP-treated and control cheeses in sensory evaluation (Table 7). They were asked not about the intensity of the parameters but about their hedonic perception of quality parameters (like- dislike) such as appearance, odour, texture and taste of the cheeses. In general, all cheeses were well valuated (around 7 points). Both trained panellists and consumers did not find sensory differences between analysed cheeses. In this sense, we can conclude that HP treatment at 400 or 600 MPa applied on mature Ibores cheese did not significantly modify the sensory characteristics of Ibores cheese after pressurisation or after 30 days of refrigerated storage.

In addition, consumers participated in a preference test in which they had to choose the best of three cheeses (control, 400 and 600 MPa) in each day of analysis (day 60 and 90). At day 60 of sensory evaluation, consumers preferred control cheeses (42 %), closely followed by cheeses pressurized at 600 MPa (34 %) and those pressurized at 400 MPa (24 %); however, after 30 days of storage, consumers chose cheeses treated at 600 MPa (45 %), followed by control (29 %) and treated at 400 MPa cheeses (26 %).

4. CONCLUSIONS

The counts of Enterobacteriacea and *Listeris spp.* showed a significant decrease after HP treatment (day 60), increasing the food safety of the Ibores raw goat milk cheese. In addition, HP treatment significantly affected instrumental colour and texture as well as affected proteolysis; however these differences were reduced after refrigerated storage and were not detectable at sensory level. In this sense, HP treatment of Ibores cheese could be applied to increase its food safety and shelf-life without compromising its sensory quality.

Table 5. Texture profile analysis (TPA) of Ibores cheese treated at day 60 and analysed after treatment (day 60) and after 30 days of refrigerated storage (day 90).

	HP-Treatment applied at day 60					After 30 days of storage (day 90)				
	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
Hardness (N)	26.92	33.08	33.11	1.21	0.065	30.12	30.84	36.80	1.27	0.042
Adhesiveness (N sec)	8.55b	10.89a	10.42ab	0.42	0.034	8.22b	8.61b	10.88a	0.46	0.019
Cohesiveness	0.44	0.50	0.48	0.01	0.062	0.41	0.41	0.43	0.01	0.636
Springiness (cm)	0.67	0.69	0.67	0.01	0.533	0.62	0.64	0.65	0.01	0.209
Gumminess (N)	11.83c	16.68a	15.26a	0.73	0.004	12.23b	12.76b	15.78a	0.59	0.010
Chewiness (N cm)	8.00c	11.54a	10.22a	0.53	0.005	7.65b	8.15b	10.26a	0.41	0.006

a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test. $P<0.05$). SEM: Standard Error of Mean

Table 6. Values of sensory attributes (scale from 0 to 10 points) of control and HP-treated at day 60 and after 90 days of storage.

	HP-Treatment applied at day 60					After 30 days of storage (day 90)				
	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
APPEARANCE										
Colour	3.62	3.16	3.33	0.14	0.459	4.30	4.42	4.81	0.17	0.345
Presence of “eyes”	1.56	1.17	1.35	0.09	0.203	2.2	1.52	1.62	0.18	0.256
ODOUR										
Odour intensity	6.75	6.65	6.29	0.09	0.051	5.82	5.64	5.29	0.10	0.099
TEXTURE										
Hardness	5.44	5.02	5.29	0.10	0.227	5.12	4.94	5.24	0.11	0.576
Springiness	1.86	1.93	2.32	0.11	0.172	2.58	2.99	2.66	0.10	0.258
Friability	5.25ab	4.72b	5.38a	0.11	0.023	4.86	4.63	4.95	0.10	0.487
FLAVOR										
Flavor intensity	6.59	6.54	6.53	0.08	0.962	6.33	6.34	6.14	0.08	0.607
TASTE										
Salty	5.48	5.57	5.82	0.07	0.078	5.63	5.38	5.64	0.07	0.199
Sharp	5.03	4.96	5.21	0.07	0.359	4.32	4.29	4.08	0.08	0.455

a, b: Different letters in the same row indicate significant statistical differences (Tukey’s Test. $P<0.05$). SEM: Standard Error of Mean

Table 7. Mean of attributes assessed by consumers (scale from 0 to 10 points) in a hedonic test of control and HP-treated at day 60 and after 90 days of storage.

	Treatment applied at day 60					After 30 days of storage (day 90)				
	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
Appearance	7.77	7.56	8.22	0.14	0.140	7.15	7.51	7.56	0.14	0.456
Odour	7.57	7.74	7.62	0.15	0.887	6.72	6.79	7.58	0.16	0.051
Texture	7.89	7.43	7.72	0.14	0.408	7.31	6.85	7.24	0.14	0.378
Taste	7.94	7.84	7.67	0.13	0.711	7.34	7.31	7.61	0.14	0.644
Total mean	7.79	7.64	7.81			7.13	7.11	7.50		

SEM: Standard Error of Mean.

5. Acknowledgements

This work has been financed thanks to European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). R. Ramírez thanks INIA and ERDF for the contract conceded (BOE, 10.01.07). We thank the PDO dairy "Berrocales Trujillanos" for providing the cheeses. We also thank to Dolores Valcárcel for microbial analyses.

6. References

Ardö, Y. (1999). Evaluating proteolysis by analysing the N content of cheese fractions. Bulletin of International Dairy Federation Nº 337, 4–9.

Arqués, J.L., Garde, S., Fernández-García, E., Gaya, P., & Nuñez, M. (2007). Volatile Compounds, Odor, and Aroma of La Serena Cheese High-Pressure Treated at Two Different Stages of Ripening. Journal of Dairy Science, 90, 3627–3639.

Balasubramaniam, V.M., Farkas, D. & Turek, E.J. (2008). By destroying pathogenic and spoilage organisms while keeping food chemistry basically intact, high-pressure technology enables pasteurization of foods with minimal effects on taste, texture, appearance, or nutritional value. Food Technology, 11, 32–38.

Bourne, M.C. (1978). Texture profile analysis. Food Technology, 32, 62–66.

Buffa, M., Guamis, B., Royo, C., & Trujillo A.J. (2001). Microbiological changes throughout ripening of goat cheese made from raw, pasteurized and high-pressure-treated milk. Food Microbiology, 18, 45–51.

Capellas, M., Mor-Mur, M., Sendra, E., & Guamis, B. (2001). Effect of high-pressure processing on physico-chemical characteristics of fresh goats' milk cheese (Mató). International Dairy Journal, 11, 165–173.

Carminati, D., Gatti, M., Bonvini, B., Neviani, E., & Mucchetti, G. (2004). High pressure processing of Gorgonzola cheese: influence on *Listeria monocytogenes*

inactivation and on sensory characteristics. Journal of Food Protection, 67, 1671–1675.

Delgado, F.J., Rodríguez-Pinilla, J., González-Crespo, J., Ramírez, R., & Roa, I. (2010). Proteolysis and texture changes of a Spanish soft cheese ("Torta del Casar") manufactured with raw ewe milk and vegetable rennet during ripening. International Journal of Food Science & Technology, 45, 512–519.

Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Effect of high-pressure treatment on the volatile profile of a mature raw goat milk cheese with paprika on rind. Innovative Food Science & Emerging Technology, 12, 98–103.

Folkertsma, B. & Fox, P.F. (1992). Use of the Cd-ninhydrin reagent to assess proteolysis in cheese during ripening. Journal of Dairy Research, 59, 217–224.

Juan, B., Ferragut, V., Buffa, M., Guamis, B., & Trujillo, A.J. (2007). Effects of high-pressure treatment on free fatty acids release during ripening of ewes' milk cheese. Journal of Dairy Research, 74, 438–445.

Juan, B., Ferragut, V., Guamis, B., & Trujillo, A.J. (2008). The effect of high-pressure treatment at 300 MPa on ripening of ewes' milk cheese. International Dairy Journal, 18, 129–138.

Kheadr, E.E., Vachon, J.F., Paquin, P., & Fliss, I. (2002). Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. International Dairy Journal, 12, 435–446.

López-Pedemonte, T.J., Roig-Sagués, A.X., De Lamo, S., Gervilla, R., & Guamis, B. (2007). High hydrostatic pressure treatment applied to model cheeses made from cow's milk inoculated with Staphylococcus aureus. Food Control, 18, 441–447.

Lundén, J., Tolvanen, R., & Korkeala, H. (2004). Human listeriosis outbreaks linked to dairy products in Europe. Journal of Dairy Science, 87, 6–11.

McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheeses during ripening. A review. Lait, 80, 293-324.

O'Callaghan, D.J. and Guinee, T.P. (2004). Rheology and texture of cheese. In: Cheese: Chemistry, Physics and Microbiology, 3rd Ed. Vol.1 (Eds. Fox, P.F., McSweeney, P.L.H., Cogan, T.M. and Guinee, T.P.). Elsevier, London, 511-540.

O'Reilly, C.E., Kelly, A.L., Murphy, P.M., & Beresford, T.P. (2001). High-pressure treatment: Applications in cheese manufacture and ripening. Trends in Food Science and Technology, 12, 51–59.

Plutowska, B., & Wardencki, W. (2007). Aromagrams – Aromatic profiles in the appreciation of food quality. Food Chemistry, 101, 845-872.

Saldo, J., Sendra, E., & Guamis, B. (2000). High hydrostatic pressure for accelerating ripening of goat's milk cheese: Proteolysis and texture. Journal of Food Science, 65, 636–640.

Saldo, J., Fernández, A., Sendra, E., Butz, P., Tauscher, B., & Guamis, B. (2003). High pressure treatment decelerates the lipolysis in a caprine cheese. Food Research International, 36, 1061–1068.

San Martín-González, M.F., Welti-Chanes, J., & Barbosa-Cánovas, G.V. (2006). Cheese Manufacture Assisted by High Pressure. Food Reviews International, 22, 275–289.

Sousa, M.J., Ardo, Y., & McSweeney, P.L.H. (2001). Advances in the study of proteolysis during cheese ripening. International Dairy Journal, 11, 327–345.

Voigt, D.D., Chevalier, F., Qian, M.C., & Kelly, A.L. (2010). Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innovative Food Science and Emerging Technologies, 11, 68–77.

Wick, C., Nienabert, U., Anggraeni, O., Shellhammer, T.H., & Courtney, P.D. (2004). Texture, proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure. Journal of Dairy Research, 71, 107–115.

Capítulo 8

Effect of high-pressure treatment on the volatile profile of a mature raw goat milk cheese with paprika on rind

Publicado en[1]: *Innovative Food Science and Emerging Technologies, 12, 98-103*

ABSTRACT

The effect of high pressure (HP) treatment at 400 MPa and 600 MPa on the volatile profile of mature raw goat milk cheeses with paprika on the rind was studied in cheese treated at day 60 and analysed after treatment (day 60) and after 30 days of refrigerated storage (day 90) by the method of SPME–GC–MS. A total of fifty-three volatile compounds were identified and no new volatile compounds could be identified after pressure treatment. Forty-one and forty-nine volatile compounds at day 60 and 90, respectively, were not significantly influenced by HP treatment. Among acids, the main chemical family of compounds isolated, only four acids were significantly modified by HP treatment at day 60 and those differences disappeared at day 90. Similarly, the differences found in some alcohols and esters after HP treatment decreased after one month of storage. Levels of ketones were not affected by HP treatment. In conclusion, few volatile compounds were affected when HP treatment was applied at the end of cheese maturation (day 60) and differences found were lessen after storage (day 90).

[1] Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Effect of high-pressure treatment on volatile profile of a mature raw goat milk cheese with paprika on rind. *Innovative Food Science and Emerging Technologies*, **12**, 98-103.

1. INTRODUCTION

High pressure (HP) processing is classified as a non-thermal technology because treatments do not rely on temperature to generate desirable changes, such as microbial inactivation or enzyme denaturation. Some potential advantages of high pressure treated food are that products demonstrate better retention of nutrients, flavor, and color than thermally treated products (San Martín-González et al., 2006). HP involves exposing a product to extremely high pressures in the range of 100 to 1000 MPa for a given time, although pressures used in commercial systems commonly range between 400 and 600 MPa. HP effect is independent on the size or shape of the product (Hoover et al., 1989; Knorr, 1993), since pressure is applied isostatically (equally in all directions), and instantaneously, so solid foods retain their original shape. HP studies on cheese have been mainly focused on the modifications of cheese maturation (deceleration or acceleration) (Saldo et al., 2003; Arqués et al., 2007; Juan et al., 2007b) as well as to increase the cheese microbial safety (Buffa et al., 2001; Kheadr et al., 2002; López-Pedemonte et al., 2007). But at this moment, there is no commercial cheeses treated by this technology in the market.

Among the many sensory quality components such as colour, rheological properties or packaging, the flavour takes a particular place, i.e. the odour and taste sensations received when eating. Thus the presence, contents and composition of volatile substances in food have a substantial influence on its quality. What is more, each product has a characteristic and unique composition of volatile components. The aroma of most food products consists of complicated mixtures. An analysis of odour (its identification and quantitative evaluation) can constitute a valuable source of information on the health quality of food, which includes both the organoleptic quality and the consumer's health safety (Plutowska & Wardencki, 2007). Aroma profiles found in cheese are the result of a huge number of reactions (principally metabolism of lactose and lactate, lipolysis and proteolysis) occurring between matrix/components of

food products. The character of the resulting aroma depends upon a number of factors: availability and structure of the reagents, participation of fat, aminoacids and saccharides, reaction conditions (temperature, duration, water activity, pH, oxygen level, etc.).

The cheese with the Protected Designation of Origin (PDO) Ibores is produced in the southwest of Spain. Ibores cheese is a full-fat cheese, produced exclusively with raw milk from goats of the breeds Serrana, Verata, Retinta and crosses thereof. The cheese surface is frequently rubbed with a mixture of oil and paprika at the end of ripening, which provides this cheese a differential and characteristic appearance. Aroma varies between sweet and mild, typical of goat's cheese made from raw milk. It has a strong taste: slightly tart, tangy and salty, with a slight aftertaste of goat's milk (DOE, 2003). Legislation does not allow the consumption of these cheeses before 60 days of ripening since they are made with raw milk.

Few studies have investigated the effect of the HP treatment on the volatile profile of cheese. Some researchers have studied the changes in the volatile composition of ewe milk cheese HP-treated: e.g., Arqués et al. (2007) reported the variation of volatile compounds of La Serena cheese HP treated at two different stages of ripening (day 2 and 50); and Juan et al. (2007a) investigated the effects of HP on volatile profile during ripening of ewe milk cheese: cheeses were submitted to 200, 300, 400 and 500 MPa at 2 stages of ripening (after 1 and 15 days of maturation) and volatile compounds were assayed at 15 and 60 days of ripening. As far as we know only one paper has studied the fraction volatile of a goat cheese treated by HP at 400 MPa for 5 min at two stages of ripening, 21 and 60 days (Saldo et al., 2003). However, this study was carried out on cheeses made from pasteurized milk and lower pressures intensities were applied. A parallel study (Delgado et al., 2011) studied the HP effect on the volatile profile formation during the maturation of Ibores cheese, and different HP treatments were applied at three ripening stages. However, that study did not evaluate the effect of the addition of paprika on the surface of the cheeses which adds differential aromatic

notes to this cheese. The presentation of Ibores cheeses with paprika in the surface is the most frequent commercial presentation of this cheese. The aromatic notes with origin in the paprika could be modified (increased/decreased) during HP as this aspect has never been evaluated on cheeses submitted to this treatment. For this reason, the aim of the present study was to establish the effect of the HP treatment at 400 and 600 MPa for 7 min at the end of cheese ripening (day 60) on the headspace volatile profile of the Ibores cheese after the treatment and after 30 days of refrigerated storage (day 90), in order to determine the effect the HP treatment on the volatile profile of this cheese.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Milk was coagulated at 28-32 ºC for 60-90 min with animal rennet. The curd was cut into medium-size (1-2 cm) grains. Cheeses of fiat cylindrical shape, weighing 0.7-1.2 kg, were pressed for 3-8 h (1–2 Kg cm^{-2}), brine-salted and ripened (at 8–12ºC and 80% relative humidity) for at least 2 months. The cheese surface was rubbed with paprika and olive oil at the end of ripening. According to the regulation of the PDO, the physicochemical properties must be a minimum of 50% of fat content in dry matter, a minimum of 50% of dry matter and a maximum of 4% NaCl (DOE, 2003).

2.2. High pressure treatment

At the end of ripening (60 days), 24 cheeses were taken from the dairy to HP treatment study. 8 cheeses (4 cheeses per HP treatment) were HP-treated at day 60 at 2 intensities of treatment (400 and 600 MPa for 7 min). Untreated cheeses were used as control (n=4) in each stage of ripening. Cheeses were analyzed after HP-treatment (day 60) and after 30 days of refrigerated storage (day 90). These cheeses were not unpacked to follow a real process in the case of the HP treatment was applied for an

industrial commercialization. Cheeses were vacuum-packed in plastic bags and HP-treated in a 55-L capacity discontinuous hydrostatic press at NC Hyperbaric Wave 6000/55, initial water temperature was 10°C. After HP treatments, the half of cheeses treated were kept at refrigeration conditions (n=4) and the other half were analyzed (n=4). Cheeses were stored at -80ºC until analysis.

2.3. SPME–GC-MS analysis

For the extraction of volatile compounds the method of Lee et al. (2003) was followed. The external part of the cheese was removed (1 cm) to avoid taking the surface with paprika of the cheese. Ten grams of cheese were placed in a 50-ml vial and then 10 ml NaH_2PO_4 (25%, w/v) were added. The sample was stirred for 30 min at 50 ºC to accelerate equilibrium of headspace volatile compounds between the cheese matrix and the headspace. Then, volatile compounds extraction was carried out by injecting a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane SPME fibre (Supelco, Bellefonte, PA) into the vial and exposing it to the headspace for 30 min at 50 ºC. Chromatographic conditions are described in Delgado et al. (2010). The volatile compounds were identified by comparison with commercial reference compounds provided by Sigma–Aldrich (St. Louis, MO) by comparison of RI with those described in the literature and by comparison of their mass spectra with those contained in the NIST and Varian libraries.

2.4. Statistical analysis

The differences in the volatile profiles were analyzed using the one-way analysis of variance (ANOVA) procedure of SPSS, Version 14.0 (SPSS Inc., Chicago, IL). HSD Tukey's test was applied to compare the mean values of the volatile compounds of different days. Mean values with standard error media (SEM) are reported. The relationships between parameters were assessed by principal component analysis (PCA).

3. RESULTS AND DISCUSSION

3.1. Volatile compounds

Fifty-three compounds were identified in the headspace of control and HP-treated raw goat milk cheeses with paprika rubbed surface. Table 1 shows the relative abundance (Area Units x 10^4) of volatile compounds in control and treated cheeses at day 60 and analyzed at day 60 and 90. A total of 13 acids, 11 alcohols, 8 esters, 7 ketones, 8 terpenes and 6 compounds which could not be classified in these groups, were detected. Forty-one and forty-nine volatile compounds at day 60 and 90, respectively, were not significantly influenced by HP treatment. Results are in line with a parallel study performed on this cheese (Delgado et al., 2011), that demonstrated that HP treatment modified less the cheese volatile profile when treatments are applied when maturation process was concluded. However, HP treatment carried out at day 60 decreased the levels of the volatile compounds after 30 days under refrigeration conditions (day 90) because treated cheese showed a lower relative abundance than control cheese at day 90.

In general, there were no significant differences in the volatile profile of cheese due to the pressure intensities applied (400 vs. 600 MPa). Therefore, when HP treatment is applied at the end of ripening (day 60) the effect of HP on profile volatile is independent of pressure level. This fact could be due to most volatile compounds have reached their higher amounts at the end of ripening and HP intensities did not affect the biochemical pathways for the production of volatile compounds.

Acids were the main chemical family of compounds isolated in this cheese. The contribution of acids to aroma development has been demonstrated in many matured cheese varieties (Saldo et al., 2003; Poveda et al., 2008; Delgado et al., 2010). Hexanoic, butanoic and octanoic acid were the most abundant acids in cheeses at day 60 and 90. Amounts of butanoic, hexanoic, heptanoic and decanoic acids were significantly modified (P<0.05) after HP treatment (day 60). At day 60, the abundance

of those acids was higher in HP-treated cheeses at 400 MPa than in control while those HP-treated at 600 MPa showed intermediate levels.

The level of some carboxylic acids which are important flavour compounds in goat cheese, such as butanoic acid (rancid cheese-like odour), hexanoic acids (goat odour), octanoic acid (rancid, pungent odour) and decanoic acid (sour, aged cheese odour) (Le Quéré et al., 1998; Poveda et al., 2008), tended (P>0.05) to be lower in HP-treated cheeses than in control cheeses at day 90. Voigt et al. (2010) reported that it was possible to reduce the further breakdown of free fatty acids (lineal carboxylic acids) by HP treatment at 400 or 600 MPa after 28 days of storage in a blue-veined cheese, leading to no further undesirable flavour development. Juan et al. (2007b) also reported that pressures ≥400 MPa produced a great decrease in free fatty acids levels, indicating that these HP conditions may be useful to arrest cheese ripening at the desired ripening stage. This fact could be positive to flavour cheese owing to the fact that large amounts of volatile acids are undesirable (Curioni & Bosset, 2002). In contrast, our results do not support that claim and showed that no significant differences were found in the levels of carboxylic acids in cheeses HP-treated at day 60 compared with control after 30 days of storage (day 90). In line with this, Seyderhelm et al. (1996) reported that lipase was inactivated above 700 MPa at 45 ºC for 10 min in tris buffer; although after a treatment at 600 MPa and room temperature it retained 60% of its activity.

Branched-chain fatty acids are characteristic impact compounds of goat and sheep cheeses (Curioni & Bosset, 2002). Three branched fatty acids have been detected, 2-methyl propanoic acid, 3-methyl butanoic acid and 2-ethyl hexanoic acid. Among them, 3-methyl butanoic acid (sweaty odour) was the most abundant and its content was not significantly modified by HP treatment (P>0.05). Saldo et al. (2003) also detected this acid in control and HP treated goat cheese, which plays an undeniable role on the flavour of goat cheese. HP neither affects the content of 2-methyl propanoic acid

(rancid, butter odour). For this reason we can conclude that HP does not affect to aromatic notes that these acids provide to cheese.

Regarding alcohols, HP treatment produced significant (P<0.05) changes in the abundance of 3-penten-2-ol and 4-butoxy 1-butanol at day 60, and 1-propanol, 2-pentanol and 3-penten-2-ol at day 90 (Table 1). The highest area unit of alcohols was found in control chilled cheeses (day 90). Alcohols such as 1-propanol and 2-pentanol decreased in HP-treated cheese after 30 days of storage (day 90), compared with control cheese at day 90.

2-butanol was the most abundant alcohol identified and was not significantly affected (P>0.05) by HP treatment. This result could be positive owing to the fact that alcohols are precursors of esters, volatile compounds relevant to cheese aroma (Molimard & Spinnler, 1996). Other secondary alcohol, 2-heptanol, was also abundant and its content was not significantly modified (P>0.05) by HP treatment. 2-heptanol was the only one alcohol detected in other HP-treated goat cheese (Saldo et al., 2003) and it has been identified as a key odorant in others cheese types (Moio & Addeo, 1998; Moio et al., 2000).

3 methyl 1-butanol was the second most abundant alcohol and HP did not change its relative amounts, so the pleasant aroma of fresh cheese that this compound provide to cheese could be maintained after the treatment (Moio et al., 1993). The presence of branched-chain primary alcohols indicates the reduction of the aldehydes produced from leucine (Curioni & Bosset, 2002).

HP did not significantly affect (P>0.05) ester content. At day 60, HP increased the total area of esters probably due to HP facilitated the esterification and/or alcoholysis, decreasing the alcohol content in treated and analyzed cheese at day 60. As we previously found to acids and alcohols, control cheese at day 90 showed the highest abundance of esters. Esters are important volatile compound isolated in cheese

because, in general, they have a low perception threshold (Molimard & Spinnler, 1996) and give fruity notes to different cheese types (reviewed by Curioni & Bosset, 2002).

Hexanoic acid ethyl ester was the most abundant ester isolated and plays an important role in the aroma profiles of many cheeses (Preininger & Grosch, 1994; Christensen & Reineccius, 1995; Moio & Addeo, 1998; Moio et al., 2000). Owing to the fact that ethyl esters can minimize the sharpness and the bitterness imparted by acids and amines, respectively (Gallois & Langlois, 1990), its reduction in HP-treated cheese after 30 days of storage of storage could be negative on flavour of this goat raw milk cheese. However, levels of acids were also reduced after one month of HP treatment.

Ketones were more abundant in treated cheese at 600 MPa at day 60 and analysed the same day than control and treated cheeses at 400 MPa. After 30 days of refrigerated storage, levels of ketones were very similar in control and HP-treated cheeses. In fact, only one ketone with low abundance (2-undecanone) was significantly affected ($P<0.05$) by HP treatment. Moreover, content of the most abundant ketones, 2-butanone, 2-heptanone and 2-nonanone, was not significantly modified by HP. These methyl ketones (except 2-butanone which derives from 2,3-butanedione) are produced from free fatty acids by an alternative pathway to the β-oxidation. They are common constituents of most dairy products and due to their typical odours and their low perception thresholds are important for cheese flavour. 2-butanone, with a butterscotch odor, was identified as main odorant in Cheddar cheese (Arora et al., 1995) and 2-heptanone, with a herbaceous odor, is an important flavour compound of Emmental and natural and creamy Gorgozola cheeses (Preininger & Grosch, 1994; Preininger et al., 1994 Moio et al., 2000). Therefore, a similar relative abundance of these compounds after HP treatment can be considered positive. In agreement whit our results, Saldo et al. (2003) reported that the effect of HP treatment was not noticeable on methyl ketones amount in a pasteurized goat milk cheese.

Table 1. Volatile compounds (Area Units x 10^4) isolated from the headspace of goat cheeses treated at day 60 and analysed after treatment (day 60) and after 30 days of refrigerated storage (day 90).

		Treatment applied at day 60					After 30 days of storage (day 90)				
Compounds	Id. Method[1]	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
Acids											
Acetic acid	RF,TI	521.1	554.9	505.4	16.1	0.485	596.3	610.6	701.5	22.6	0.112
Propanoic acid	RF,TI	135.9	80.3	128.5	12.8	0.154	157.1	98.0	100.2	19.5	0.414
Propanoic acid, 2-methyl	RF,TI	36.3	73.1	36.5	7.6	0.058	42.9	51.3	64.6	7.5	0.538
Butanoic acid	RF,TI	1373.8b	1780.0a	1432.5ab	73.1	0.028	2151.3	1961.3	1982.5	124.7	0.824
Butanoic acid, 3-methyl	RF,TI	241.1	431.5	252.5	40.4	0.084	356.5	402.6	475.9	54.4	0.707
Pentanoic acid	RF,TI	13.9	15.8	14.0	0.4	0.124	23.4	18.4	19.7	1.8	0.548
Hexanoic acid	RF,TI	1923.8b	2553.8a	2177.5ab	106.1	0.030	3728.8	3000.0	2918.8	289.7	0.498
Hexanoic acid, 2-ethyl	TI	2.9	1.1	1.4	0.3	0.064	1.1	1.1	1.3	0.1	0.690
Heptanoic acid	TI	15.1b	21.1a	16.4ab	1.1	0.047	39.1	20.9	21.6	6.3	0.447
Octanoic acid	RF,TI	1289.3	782.8	674.9	230.2	0.554	1109.8	837.4	830.6	105	0.509
Nonanoic acid	TI	7.8	11.0	9.7	0.9	0.395	14.8	9.7	9.4	1.9	0.471
Decanoic acid	RF,TI	424.3b	576.4a	476.4ab	25.2	0.022	731.1	629.1	628.0	48.6	0.654
Undecanoic acid	TI	28.0	27.4	17.8	2.4	0.142	43.2	28.9	31.4	4.3	0.389
Total		6013.1	6909.1	5743.4			8995.1	7669.4	7785.5		
Alcohols											
Ethanol, 2-methoxy	TI	14.9	17.9	20.7	1.1	0.073	25.6	24.0	20.6	1.5	0.407
2-Butanol	RF,TI	638.1	486.3	580.4	42.3	0.369	687.5	449.4	395.1	59.4	0.090
1-Propanol	RF,TI	1.9	1.7	1.7	0.1	0.757	1.7a	0.9b	0.8b	0.1	0.001
2-Pentanol	RF,TI	18.5	15.5	20.9	1.9	0.544	33.1a	17.8b	10.1b	3.3	0.002
1-Butanol, 3-methyl	TI	48.4	46.9	59.0	2.6	0.119	48.7	51.3	45.3	2.5	0.668
2-Heptanol	TI	46.9	34.2	63.8	8.4	0.390	141.0	69.2	76.1	15	0.083
3-Penten-2-ol	TI	1.7b	3.5a	1.8b	0.3	0.001	1.8ab	1.2b	3.1a	0.3	0.011

		Treatment applied at day 60					After 30 days of storage (day 90)				
Compounds	Id. Method[1]	Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
Cyclohexanol, 2-methyl	TI	14.7	11.2	14.7	1.3	0.480	12.5	11.6	11.4	1.8	0.969
2-Nonanol	TI	2.8	3.8	8.0	1.4	0.296	28.0	10.0	9.2	4.5	0.153
2-Furanmethanol	RF,TI	1.2	0.9	1.0	0.2	0.739	0.9	1.1	0.7	0.1	0.422
1-Butanol, 4-butoxy	TI	3.6a	1.8b	1.0b	0.4	0.006	–	–	–	–	–
Total		795.8	626.9	775.9			983.4	639.3	575.0		
Esters											
Acetic acid, ethyl ester	RF,TI	1.6	1.9	2.2	0.1	0.144	3.3	3.1	2.4	0.2	0.101
Butanoic acid, ethyl ester	RF,TI	33.5	39.1	39.3	1.6	0.257	44.4	46.9	37.7	2.3	0.258
Hexanoic acid, ethyl ester	RF,TI	51.3	50.0	57.4	2.8	0.546	93.2	78.2	61.7	6.7	0.155
Propanoic acid, 2-methyl, 3-methylbutyl ester	TI	2.7	1.9	2.9	0.3	0.405	6.9	3.6	3.0	1.0	0.250
Hexanoic acid, buthyl ester	TI	2.6	2.4	1.7	0.3	0.471	4.9	3.4	2.8	0.6	0.344
Octanoic acid, ethyl ester	RF,TI	27.2	26.3	30.3	1.5	0.559	45.5	39.5	35.8	2.5	0.297
Decanoic acid, ethyl ester	RF,TI	17.8	18.3	23.2	1.2	0.111	33.5	30.9	25.6	1.5	0.078
Propanoic acid, 2-methyl, ethyl ester	TI	1.0	1.3	1.6	0.2	0.396	0.8	1.7	1.6	0.3	0.364
Total		137.7	141.1	158.5			232.4	207.3	170.6		
Ketones											
2-Butanone	TI	1135.0	943.9	1751.3	164.2	0.100	898.9	1011.3	906.9	137.2	0.944
2-Pentanone	RF,TI	27.3	29.0	38.8	2.7	0.166	58.8	47.7	33.0	5.3	0.130
4-Heptanone	RF,TI	14.2	11.4	13.5	2.0	0.860	21.4	23.5	28.5	2.8	0.622
2-Heptanone	TI	106.1	71.7	146.4	15.9	0.160	209.9	181.8	172.2	21.2	0.788
2-Octanone	RF,TI	1.1	0.5	2.0	0.3	0.092	4.1	2.8	2.8	0.5	0.552
2-Nonanone	RF,TI	38.9	24.5	76.2	12.9	0.260	104.7	86.1	117.0	17.2	0.796
2-Undecanone	TI	2.0b	2.3ab	4.3a	0.4	0.023	4.9	4.3	4.4	0.4	0.799
Total		1324.5	1083.3	2032.4			1302.9	1357.3	1264.8		
Terpenes											
Camphene	TI	6.4	14.3	6.6	1.6	0.045	5.4	10.3	9.5	1.5	0.415
3-Carene	RF,TI	50.0	75.5	50.0	5.5	0.075	36.3	64.1	59.9	7.1	0.238
Limonene	RF,TI	3.1	6.9	3.0	0.8	0.044	3.7	5.7	6.1	0.7	0.362

Compounds	Id. Method[1]	Treatment applied at day 60					After 30 days of storage (day 90)				
		Control	400MPa	600MPa	SEM	*P*-value	Control	400MPa	600MPa	SEM	*P*-value
Cineole	TI	1.5	2.2	2.5	0.3	0.399	1.5	2.8	7.2	1.5	0.302
p-Cymene	RF,TI	1.4b	2.6a	2.4ab	0.2	0.045	2.2	2.6	1.9	0.3	0.478
o-Cymene	TI	1.0b	2.4a	1.5ab	0.2	0.019	1.0	1.9	2.0	0.3	0.362
Cadinene	TI	4.6	10.3	6.0	1.1	0.078	5.4	8.9	9.0	1.2	0.418
Fenchone	TI	–	–	–	–	–	0.3b	1.6ab	1.9a	0.5	0.040
Total		67.9	114.3	72.0			55.7	98.0	97.5		
Others											
Methanethiol	TI	0.7	0.6	0.6	0.1	0.696	0.3	0.3	0.4	0.04	0.276
Toluene	TI	2.5b	5.9a	4.3ab	0.6	0.044	1.6	9.3	5.2	1.4	0.057
Styrene	TI	0.9	0.8	1.1	0.1	0.322	2.2	2.8	2.4	0.3	0.713
Phenol	TI	3.2	3.1	2.9	0.1	0.656	2.6	2.6	2.7	0.1	0.919
p-Cresol	TI	3.4	2.4	3.9	0.4	0.258	5.7	4.3	5.1	0.5	0.482
δ-Decalactone	TI	5.0	6.1	5.4	0.2	0.194	6.0	6.0	6.3	0.4	0.927
Total		12.6	15.7	15.4			15.8	22.6	19.3		

[1] Method of identification: RF, mass spectrum and retention time identical with a reference compound; TI, tentative identification by mass spectrum. SEM: standard error media. a, b, c: Different letters in the same row indicate significant statistical differences (Tukey's Test, $P<0.05$).

Terpenes are important compounds due to their low odour thresholds and they have their origin in the plants that constitute the forage mixture of the animal pastures (Mariaca et al., 1997). However, in this cheese they have their origin from the paprika rubbed in the surface since in a parallel study which characterized the aromatic profile of Ibores cheese without paprika from several dairies they were not isolated from the cheese headspace. A wide variety of terpenes and their derivatives have been isolated in red paprika (Kocsis et al., 2002). HP treatment significantly affected (P<0.05) the content of camphene, limonene, p-cymene and o-cymene at day 60. Terpenes have well-defined odors in the literature, like limonene and carene which add lemon notes. These results are interesting since HP treatment at certain levels could increase the odour provided by the paprika on the surface increasing the migration of certain compounds from the surface to the inner part of the cheese. However, only the level of one terpene, fenchone, which add aromatic notes to mint, camphor and warm (Pourmortazavi & Hajimirsadeghi, 2007) was significantly modified in HP-treated cheeses after storage; although in general, the sum of the area units of terpenes was higher in HP-treated cheeses at day 90. In contrast, Arqués et al. (2007) did not find significant difference for terpenes between La Serena cheeses attributable to HP treatments, but those terpenes probably have a different origin.

One sulfur compound (methanethiol), four aromatic hydrocarbons (toluene, styrene, phenol and p-cresol) and one lactone (δ-decalactone) were also detected in this cheese. Methanethiol content was not significantly decreased by HP treatment and after 30 days of storage and this could be relevant for aroma cheese because this compound, derived from methionine and precursor for further sulphur compounds, appears to be one of the characteristic flavour compounds of international cheeses like Camembert or Cheddar (Christensen & Reineccius, 1995; Kubícková & Grosch, 1997). Levels of one hydrocarbon, toluene, was significantly modified by HP. This effect could be negative for background flavour of this cheese because aromatic hydrocarbons appear to make a positive contribution to cheese flavour at about threshold

concentration but tend towards an unpleasant note as their concentration increases (Curioni & Bosset, 2002). Other phenolic compound, p-cresol, originates from tyrosine was listed as the main odorant of British farmhouse Cheddar and thought to be responsible for it "cowy-barny" note (Suriyaphan et al., 2001). This compound has been also isolated from the Spanish paprika (Mateo et al., 1997) so, this could be the most probable origin in this cheese. HP did not affect its relative abundance and then its contribution to the typical aroma of this goat cheese. One of the most common an important lactones identified in cheese is δ-decalactone. This compound, for example, is a key odorant of Camembert and Emmental cheese varieties. HP treatment did not modified abundance of δ-decalactone, and this fact could be positive for cheese flavour.

3.2. Principal Component Analysis (PCA)

Table 2 shows the most important loadings and the percentage accounted by the first two first principal components (PC#1 and PC#2) after PCA. The 31% and 25% of the variability was explained by PC#1 and PC#2, respectively. With positive loadings on PC#1, explaining an important part of variation, are located some alcohols (2-heptanol), esters and ketones. However, some terpenes such as camphene and 3-carene had negative values for PC#1, which also explain part of variation. Regarding PC#2, some volatile compounds (mainly acids) had positive loadings and explained practically the rest of variation detected while others had negative loadings (2-methyl cyclohexanol and 2-butanone).

The distribution of the scores on the first two PCs (Figure 1) shows 2 separate groups of points, corresponding to the different days of analysis (day 60 and day 90). Most cheeses at day 60 are located in the negative area of PC#1 and PC#2, near of origin, while in the positive area of PC#1 are those cheeses stored under refrigeration (day 90). In general, cheeses analyzed at day 90 were characterized by higher content of acids, esters and ketones (except 2-butanone) than cheeses matured for 60 days. In

contrast, differences due to the HP treatment within the same day were not so evident, as the PCA only separated the cheeses according to the maturation extent (day 60 vs. day 90).

Table 2. Results of principal component analysis on the volatile composition showing the most important loadings and the percentage of variance accounted for by the first two principal components (PC#1 and PC#2).

Compounds	Principal Component	
Acids	PC#1	PC#2
Butanoic acid	0.05	0.97
Butanoic acid, 3-methyl-	-0.52	0.76
Pentanoic acid	0.26	0.85
Hexanoic acid	0.24	0.91
Heptanoic acid	0.22	0.74
Decanoic acid	0.15	0.94
Alcohols		
2-Heptanol	0.79	0.36
Cyclohexanol, 2-methyl-	0.49	-0.75
Esters		
Acetic acid, ethyl ester	0.79	0.25
Hexanoic acid, ethyl ester	0.89	0.29
Octanoic acid, ethyl ester	0.90	0.18
Decanoic acid, ethyl ester	0.87	0.25
Ketones		
2-Butanone	0.37	-0.75
2-Pentanone	0.80	0.09
2-Heptanone	0.90	0.00
2-Octanone	0.76	0.44
2-Undecanone	0.75	0.17
Terpenes		
Camphene	-0.78	0.44
3-Carene	-0.77	0.21
Others		
p-Cresol	0.82	-0.21
Percentage of variance explained	31%	25%

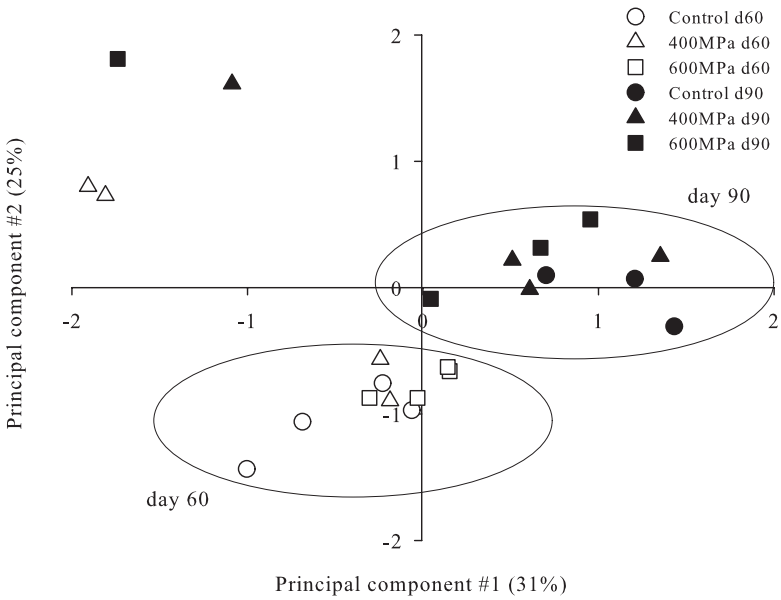

Figure 1. Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC#1 and PC#2).

4. CONCLUSIONS

Most volatile compounds isolated from the headspace of the raw goat milk cheese were not significantly affected by high pressure treatment when the treatment was applied at the end of the ripening (day 60) and the differences found were reduced after 30 days of refrigerated storage. In addition, high pressure treatment did not cause the apparition of new volatile compounds. On the other hand, HP treatment, especially at 400MPa, tended to increase the level of some terpenes from the paprika rubbed on the surface of the cheese. Nevertheless, further studies are necessary of evaluate the effect of high pressure processing on the sensory attributes of the cheese after the treatment.

5. Acknowledgements

This work has been financed thanks to European Regional Development Fund (ERDF). Francisco José Delgado thanks Junta de Extremadura for the grant received for the development of this work (DOE 10.10.07). We thank the PDO dairy "Berrocales Trujillanos" for providing the cheeses.

6. References

Arora, G., Cormier, F., & Lee, B. (1995). Analysis of odor-active volatiles in Cheddar cheese headspace by multidimensional GC/ MS sniffing. Journal of Agricultural and Food Chemistry, 43, 748–752.

Arqués, J.L., Garde, S., Fernández-García, E., Gaya, P., & Nuñez, M. (2007). Volatile Compounds, Odor, and Aroma of La Serena Cheese High-Pressure Treated at Two Different Stages of Ripening. Journal of Dairy Science, 90, 3627–3639.

Buffa, M., Guamis, B., Royo, C., & Trujillo A.J. (2001). Microbiological changes throughout ripening of goat cheese made from raw, pasteurized and high-pressure-treated milk. Food Microbiology, 18, 45–51.

Christensen, K.R., & Reineccius, G.A. (1995). Aroma extract dilution analysis of aged Cheddar cheese. Journal of Food Science, 60, 218–220.

Curioni, P.M.G., & Bosset, J.O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

Delgado, F.J., González-Crespo, J., Cava, R., García-Parra, J., & Ramírez, R. (2010). Characterization of the volatile profile of a Spanish ewe raw milk soft cheese P.D.O. Torta del Casar during ripening by SPME–GC–MS. Food Chemistry, 118, 182–189.

Delgado, F.J., González-Crespo, J., Cava, R., & Ramírez, R. (2011). Changes in the volatile profile of a raw goat milk cheese treated by hydrostatic high pressure at different stages of maturation. International Dairy Journal, 21, 135–141.

DOE (2003). Diario Oficial de Extremadura (Regional Legislation of Extremadura), nº 61, 17th May 2003. Decreto 69/2003, de 20 de mayo, por el que se aprueba el Reglamento de la Denominación de Origen Protegida "Queso de Ibores" y su Consejo Regulador.

Gallois, A., & Langlois, D. (1990). New results in the volatile odorous compounds of French cheeses. Le Lait, 70, 89–106.

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D.F., & Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. Food Technology, 43, 99–107.

Kheadr, E.E., Vachon, J.F., Paquin, P., & Fliss, I. (2002). Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. International Dairy Journal, 12, 435–446.

Knorr, D. (1993). Effect of high hydrostatic pressure processes on food safety and quality. Food Technology, 47, 156–161.

Kocsis, N., Amtmann, M., Mednyanszky, Z., & Korany, K. (2002). GC–MS Investigation of the Aroma Compounds of Hungarian Red Paprika (Capsicum annuum) Cultivars. Journal of Food Composition and Analysis, 15, 195–203.

Kubícková, J., & Grosch, W. (1997). Evaluation of potent odorants of Camembert cheese by dilution and concentration techniques. International Dairy Journal, 7, 65–70.

Juan, B., Barron, L.J.R., Ferragut, V., & Trujillo, A.J. (2007a). Effects of High Pressure Treatment on Volatile Profile During Ripening of Ewe Milk Cheese. Journal of Dairy Science, 90, 124–135.

Juan, B., Ferragut, V., Buffa, M., Guamis, B., & Trujillo, A.J. (2007b). Effects of high-pressure treatment on free fatty acids release during ripening of ewes' milk cheese. Journal of Dairy Research, 74, 438–445.

Lee, J.H., Diono, R., Kim, G. Y., & Min, D. (2003). Optimization of solid phase microextraction analysis for the headspace volatile compounds of Parmesan

cheese. Journal of Agricultural and Food Chemistry, 51, 1136–1140.

Le Quéré J.L., Pierre A., Riaublanc A., & Demaizières, D. (1998). Characterization of aroma compounds in the volatile fraction of soft goat cheese during ripening. Lait, 78, 279–290.

López-Pedemonte, T.J., Roig-Sagués, A.X., De Lamo, S., Gervilla, R., & Guamis, B. (2007). High hydrostatic pressure treatment applied to model cheeses made from cow's milk inoculated with Staphylococcus aureus. Food Control, 18, 441–447.

Mariaca, R.G., Berger, T.F.H., Gauch, R., Imhof, M.I., Jeangros, B., & Bosset, J.O. (1997). Occurrence of volatile mono- and sesquiterpenoids in highland and lowland plant species as possible precursors for flavor compounds in milk and dairy products. Journal of Agricultural and Food Chemistry, 45, 4423–4434.

Mateo, J., Aguirrezábal, M., Domínguez, C., & Zumalacárregui, J.M. (1997). Volatile Compounds in Spanish Paprika. Journal of Food Composition and Analysis, 10, 225–232.

Moio, L., Langlois, D., Etievant, P. X., & Addeo, F. (1993). Powerful odorants in water buffalo and bovine Mozzarella cheese by use of extract dilution sniffing analysis. Italian Journal of Food Science, 3, 227–237.

Moio, L., & Addeo, F. (1998). Grana Padano cheese aroma. Journal of Dairy Research, 65, 317–333.

Moio, L., Piombino, P., & Addeo, F. (2000). Odour-impact compounds in Gorgonzola cheese. Journal of Dairy Research, 67, 273–285.

Molimard, P., & Spinnler, H.E. (1996). Compounds involved in the flavor of surface mold-ripened cheeses: origins and properties. Journal of Dairy Science, 79, 169–184.

Plutowska, B., & Wardencki, W. (2007). Aromagrams – Aromatic profiles in the appreciation of food quality. Food Chemistry, 101, 845–872.

Pourmortazavi, S.M., & Hajimirsadeghi, S.S. (2007). Review: Supercritical fluid extraction in plant essential and volatile oil analysis. Journal of Chromatography A, 1163, 2–24.

Poveda, J.M., Sánchez-Palomo, E., Pérez-Coello, M.S., & Cabezas, L. (2008). Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat cheeses. Dairy Science & Technology, 88, 355–367.

Preininger, M., & Grosch, W. (1994). Evaluation of key odorants of the neutral volatiles of Emmentaler cheese by the calculation of odour activity values. Lebensmittel Wissenschaft und Technologie, 27, 237–244.

Preininger, M., Rychlik, M., & Grosch, W. (1994). Potent odorants of the neutral volatile fraction of Swiss cheese (Emmentaler). In H. Maarse, & D. G. van der Heij (Eds.), Trends in Flavour Research (pp. 267–270). Amsterdam: Elsevier.

Saldo, J., Fernández, A., Sendra, E., Butz, P., Tauscher, B., & Guamis, B. (2003). High pressure treatment decelerates the lipolysis in a caprine cheese. Food Research International, 36, 1061–1068.

San Martín-González, M.F., Welti-Chanes, J., & Barbosa-Cánovas, G.V. (2006). Cheese Manufacture Assisted by High Pressure. Food Reviews International, 22, 275–289.

Seyderhelm, I., Boguslawski, S., Michaelis, G., & Knorr, D. (1996). Pressure induced inactivation of selected food enzymes. Journal of Food Science, 61, 308–310.

Suriyaphan, O., Drake, M., Chen, X.Q., & Cadwallader, K.R. (2001). Characteristic aroma components of British farmhouse Cheddar cheese. Journal of Agricultural and Food Chemistry, 49, 1382–1387.

Voigt, D.D., Chevalier, F., Qian, M.C., & Kelly, A.L. (2010). Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innovative Food Science and Emerging Technologies, 11, 68–77.

6. DISCUSIÓN GENERAL

6. DISCUSIÓN GENERAL

6.1. Evaluación de los principales procesos de maduración relacionados con la formación de los parámetros de calidad del Queso Ibores

6.1.1. Parámetros físico-químicos y color y textura instrumental

Para caracterizar los principales cambios que se producen durante la maduración del queso de cabra Ibores se estudiaron quesos elaborados en tres queserías diferentes a distintos estadios madurativos (1, 30 y 60 días) y además se estudió su estabilidad tras 30 días de almacenamiento (día 90).

Durante los 30 primeros días de maduración del Queso Ibores, el pH disminuyó significativamente, debido a la formación de ácido láctico derivado del metabolismo de la lactosa por las bacterias ácido lácticas (BAL). En el Queso Ibores se detectaron niveles más bajos de pH que en otros quesos españoles de cabra (Martín-Hernández y cols., 1992; Ferrandini y cols., 2011), probablemente por el suave prensado del mismo que facilita la retención de suero con lactosa en la cuajada y su posterior acidificación. Sin embargo, al final de la maduración los valores de pH fueron muy parecidos a los obtenidos por Fresno y cols. (1996), Psoni y cols. (2006) y Serhana y cols. (2010) en el queso de cabra español Armada y en los quesos de leche cruda de cabra griego Batzos y libanés Darfiyeh, respectivamente.

Por otro lado, los valores de extracto seco se incrementaron significativamente al final de la maduración (día 60–90). A día 60, los niveles de extracto seco, y los niveles de grasa y proteína en extracto seco se encontraron dentro del rango fijado por la Denominación de Origen y fueron comparables a los valores reflejados en la literatura para otros quesos de cabra elaborados en España (Martín-Hernández y cols., 1992; Franco y cols., 2003; Ferrandini y cols., 2011).

Con respecto a los parámetros de color instrumental, la luminosidad (CIE L*) aumentó significativamente a lo largo del proceso de maduración. Por su parte, el parámetro CIE b*, que evalúa el color amarillo del queso, sufrió un importante descenso principalmente durante los primeros 30 días de maduración. Juan y cols. (2008) hallaron una tendencia opuesta en quesos de oveja a lo largo de 90 días de maduración. Estos cambios de color durante la maduración serían típicos de cada tipo de queso. Así, la formación del color del Queso Ibores, que es bastante blanquecino al final de la maduración, tendría lugar principalmente durante el primer mes de maduración. Por el contrario, en el queso de oveja se daría el proceso opuesto con la formación del color amarillento típico de este queso.

En cuanto a los parámetros obtenidos a partir del Análisis del Perfil de Textura (APT), los valores de dureza y adhesividad aumentaron significativamente a lo largo de la maduración. En otros tipos de quesos, como el queso de cabra Monterey Jack y la Torta del Casar, la dureza descendió durante la maduración (Van Hekken y cols., 2004; Delgado y cols., 2010a; respectivamente). Las diferencias entre estos quesos y el Queso Ibores son debidas al tipo de pasta en el Queso Ibores (semidura) en comparación con los anteriores (blanda), lo que explicaría las distintas tendencias. Por último, la cohesividad y la elasticidad descendieron significativamente hasta el día 60 de maduración, permaneciendo con un valor similar hasta el día 90, por lo que durante el almacenamiento no se produjeron modificaciones en estos parámetros.

6.1.2. Principales rutas metabólicas del proceso de maduración

i. Fenómenos proteolíticos

La evolución de la proteolisis durante la maduración del Queso Ibores se determinó mediante la medida de distintas fracciones de nitrógeno y el contenido en amino ácidos libres (AAL). El ratio nitrógeno soluble con respecto al nitrógeno total (NS/NT), considerado como un índice de maduración en queso, aumentó significativamente tras los 30 días iniciales de maduración en relación con el valor obtenido al comienzo de la

misma (día 1). Sin embargo, posteriormente su valor se mantuvo constante hasta el final del período de maduración (día 60–90). La relación de nitrógeno no proteico respecto al NT (NNP/NT) no varió significativamente a lo largo de la maduración. Este parámetro está con frecuencia relacionado con la "intensidad" del proceso proteolítico, es decir, en la proteolisis secundaria que sufren los productos liberados (polipéptidos) por la proteolisis primaria de las caseínas. Este patrón proteolítico no se ha encontrado en otros tipos de queso de cabra, en los que la relación NNP/NT se incrementó a lo largo de la maduración (Martín-Hernández y cols., 1992; Tejada y cols., 2008; Ferrandini y cols., 2011). Las diferencias encontradas con la bibliografía consultada pueden deberse i. al tipo de cuajo animal empleado (artesanal o comercial), ii. el tipo de enzimas microbianas presentes en cada tipo de queso y/o iii. las condiciones de maduración. Otro factor importante podría ser el bajo valor de pH en el Queso Ibores, que podría ser un factor limitante para la actividad enzimática (Sousa y cols., 2001). Por último, los valores de nitrógeno Polipeptídico se incrementaron fundamentalmente en el día 30 y los AAL aumentaron a lo largo de la maduración, lo que podría indicar una degradación de los péptidos de pequeño tamaño, presentes en bajas concentraciones, a AAL.

Para conocer las relaciones entre los parámetros fisicoquímicos, instrumentales y la proteolisis en el Queso Ibores, se calcularon las correlaciones entre ellos. La dureza y la adhesividad mostraron una correlación positiva con el extracto seco (r= +0,697, P<0,001; r= +0,482, P<0,05; respectivamente). Esto indicaría que los aumentos en la dureza y en la adhesividad del queso durante la maduración son debidos a las pérdidas de humedad del mismo, por un fenómeno de desecación. Además, la dureza correlacionó negativamente con el valor de pH (r= -0,452; P<0.05). En contraste, la cohesividad y la elasticidad mostraron correlaciones negativas con el nitrógeno Polipéptidico (r= -0,836 y r= -0,820, respectivamente; P<0,001), AAL (r= -0,716 y r= -0,687, respectivamente; P<0,001) y el extracto seco (r= -0,658 y r= -0,769, respectivamente; P<0,001). Creamer y Olson (1982) postularon que una elevada acidez

y un alto contenido en proteínas y sólidos totales dan lugar, generalmente, a un queso más duro y con una menor capacidad de deformación, lo que coincide con nuestros resultados. Estos resultados mostrarían la importancia de utilizar cultivos iniciadores adecuados en este tipo de queso para alcanzar las condiciones de pH suficientes que favorezcan todos los fenómenos madurativos del queso.

ii. Composición en ácidos grasos libres (AGL)

En el Queso Ibores, se determinaron los cambios lipolíticos que acontecen durante la maduración mediante la evolución de la composición de los AGL. Las concentraciones ($mg \cdot Kg^{-1}$ de queso) de ácidos grasos de cadena corta (AGCC: C4:0–C8:0), media (AGCM: C10:0–C14:0) y larga (AGCL: C15:0–C18:2 n-6) aumentaron a lo largo de la maduración (día 1, 30, 60) y durante el almacenamiento refrigerado (día 90). También se ha observado un importante aumento en los niveles de los ácidos grasos libres (AGL) en estudios previos realizados en quesos de leche cruda de cabra (Buffa y cols., 2001; Franco y cols., 2003; Atasoy y Türkoglu, 2009). Los ácidos caprílico (C8:0), cáprico (C10:0) y oleico (C18:1 n-9) fueron los que mostraron los incrementos más relevantes (x 5 veces) durante la maduración del Queso Ibores.

Los AGCL fueron los más abundantes y los que presentaron un incremento mayor durante la maduración. En el Queso Ibores maduro (día 60–90), los AGCL más abundantes fueron los ácidos oleico (C18:1 n-9) y palmítico (C16:0), seguidos por el esteárico (C18:0) y el linoleico (C18:2 n-6). Se han encontrado resultados similares al final de la maduración de otros tipos de queso de cabra (Franco y cols., 2003; Poveda y Cabezas, 2006). A pesar de la importancia cuantitativa de estos AGL, no se consideran compuestos muy relevantes para el aroma de los quesos debido a su elevado peso molecular y por ende su elevado umbral de percepción (Freitas y Malcata, 1998). En contraposición, los AGCC y AGCM contribuyen de manera importante al flavor del queso debido a sus bajos umbrales de percepción y a las notas aromáticas características que confieren al mismo (Collins y cols., 2003).

A pesar de no ser el grupo cuantitativamente mayor de AGL, los niveles de AGCC detectados al final de la maduración del Queso Ibores fueron mayores que los encontrados en otros tipos de quesos de cabra de leche cruda (Franco y cols., 2003; Atasoy y Türkoglu, 2009), por lo que serían compuestos relevantes para alcanzar el aroma y sabor típico de este queso. El más abundante de los AGCM fue el ácido cáprico (C10:0), lo que está en concordancia con los resultados obtenidos por Nájera y cols. (1993) en otros quesos de leche de cabra.

iii. Oxidación de lípidos y proteínas

En lo referente a los procesos oxidativos en el Queso Ibores, sólo la oxidación de lípidos mostró un incremento significativo durante la maduración; sin embargo, los valores de TBA-RS (en castellano, especies reactivas al ácido tiobarbitúrico) fueron inferiores a las descritos por Fedele y Bergamo (2001) en queso Grana Padano y por Delgado y cols. (2009) en la Torta del Casar. Por lo tanto, a pesar de que este proceso aumentó durante la maduración, su importancia en relación a otros cambios madurativos debe considerarse de escasa relevancia. En cuanto a la oxidación de proteínas, sus niveles también fueron bajos y no se modificaron significativamente durante el transcurso de la maduración. Los bajos niveles de oxidación encontrados en el Queso Ibores podrían estar relacionados, entre otros muchos factores, con la ausencia de tratamiento térmico (pasteurización) de la leche con la que se elabora este tipo de queso, debido al efecto prooxidante de la temperatura. De hecho, Fedele y Bergamo (2001) obtuvieron una correlación positiva entre la intensidad de calentamiento y el daño oxidativo en el queso Grana Padano. Los procesos oxidativos son positivos para la formación del aroma en productos madurados, no obstante, un exceso de oxidación puede dar lugar a la aparición de notas aromáticas negativas o desagradables (Serra y cols., 2008).

6.1.3. Perfil de compuestos volátiles en el espacio de cabeza del queso

El análisis del espacio de cabeza del Queso Ibores mostró la existencia de un total de 64 compuestos volátiles (14 ácidos, 18 alcoholes, 13 ésteres, 6 cetonas y otros 13

compuestos no clasificables dentro de los grupos químicos citados) que mostraron cambios en sus concentraciones y proporciones relativas durante la maduración. En este mismo tipo de queso, Sabio y Vidal-Aragón (1996) detectaron únicamente 29 compuestos volátiles, empleando como técnica de pre-concentración de compuestos volátiles el sistema de "purga y trampa", en lugar de la "micro-extracción de la fase sólida" (SPME, siglas en inglés), como en nuestro caso. Las diferencias pueden deberse a las ventajas de la técnica de SPME frente al sistema de purga. Las principales ventajas de la SPME son la mayor sensibilidad y rapidez, la menor cantidad de muestra necesaria y la inyección directa en el cromatógrafo de gases.

Durante los primeros 60 días de maduración del Queso Ibores se produjo un aumento importante de las cantidades (Unidades de Área –UA–) de la mayoría de los compuestos volátiles. De este modo, en los quesos con la maduración mínima exigida (60 días al tratarse de quesos elaborados con leche cruda) el perfil de compuestos volátiles estaba completamente formado.

Los compuestos volátiles más abundantes en el Queso Ibores fueron los ácidos carboxílicos. El más abundante fue el ácido hexanoico, seguido del butanoico, el octanoico y el decanoico. Otros ácidos aislados fueron los ácidos acético, propanoico y 3-metil butanoico, que son compuestos muy volátiles debido a su bajo peso molecular. Por sus bajos umbrales de percepción, los ácidos carboxílicos de cadena corta y media (C4:0–C8:0 y C10:0–C14:0, respectivamente) son compuestos relevantes en el perfil aromático de una gran variedad de quesos (Woo y Lindsay, 1982; Zerfiridis y cols., 1984; Moio y Addeo, 1998; Pinho y cols., 2003; Tavaria y cols., 2004; Kraggerud y cols., 2008; Delgado y cols., 2010b). Además, los ácidos son compuestos importantes o incluso predominantes en el aroma de muchos quesos de cabra (Le Quéré y cols., 1998; Castillo y cols., 2007; Poveda y cols., 2008). De hecho, los ácidos hexanoico, octanoico y decanoico, reciben la denominación de caproico, caprílico y cáprico (Poveda y Cabezas, 2006) por su abundancia en el queso de cabra.

Los bajos niveles de compuestos aromáticos relacionados con la proteolisis (los ácidos

carboxílicos de cadena sustituida) están en consonancia con los fenómenos proteolíticos descritos, donde parece que la proteolisis primaria de las caseínas tiene mayor importancia que la secundaria. En vista de los resultados obtenidos en el Queso Ibores se descarta la proteolisis como vía relevante en la formación de compuestos del aroma, mientras que la lipolisis y la fermentación llevada a cabo por los cultivos iniciadores desempeñan un papel fundamental en la formación de dichos compuestos.

La segunda familia química más relevante en el Queso Ibores fue la de los alcoholes. Destacaron por su abundancia los alcoholes secundarios, como el 2-butanol, 2-heptanol y 2-pentanol. Estos alcoholes han sido detectados previamente en otros tipos de quesos como los quesos de cabra semiduros elaborados a partir de leche pasteurizada en Castilla la Mancha, Extremadura, Andalucía y las Islas Canarias, donde el 2-heptanol fue el alcohol presente en mayores concentraciones (Póveda y cols., 2008).

Los ésteres fueron el único grupo químico de compuestos cuya cantidad relativa aumentó hasta el final del período de tiempo (día 90). Los ácidos hexanoico y butanoico etil éster fueron los más abundantes en el Queso Ibores. El ácido butanoico etil éster ha sido identificado como uno de los constituyentes más importantes del perfil aromático de los quesos Cheddar, Emmental, Gorgonzola cremoso, Grana Padano y Pecorino, mientras que el ácido hexanoico etil éster juega un papel importante en el perfil aromático de los quesos Cheddar añejo, Gorgonzola natural, Pecorino y Ragusano (revisado por Curioni y Bosset, 2002). Estos dos compuestos, junto con el ácido acético etil éster, fueron los ésteres mayoritarios aislados en el queso de cabra Majorero (Castillo y cols. 2007), y son considerados constituyentes clave del aroma de este tipo de queso. Además, el aroma afrutado asociado a los ésteres podría diluir el fuerte aroma que proporcionarían los ácidos carboxílicos al Queso Ibores.

La mayoría de las cetonas identificadas en el Queso Ibores fueron metil cetonas, al igual que en otros tipos de quesos de cabra (Le Quéré y cols., 1998; Castillo y cols.,

2007; Poveda y cols., 2008). A día 60, la 2-butanona y la 2-heptanona fueron las cetonas más abundantes. Estas cetonas también son compuestos relevantes del perfil aromático de otros quesos extremeños de leche cruda de oveja, como el queso de La Serena y la Torta del Casar (Carbonell y cols., 2002; Delgado y cols., 2010b). Las cetonas son constituyentes importantes de muchos productos lácteos y su importancia radica, en primer lugar, en sus bajos umbrales de percepción (nasal de 1,5–3,4 mg Kg^{-1}, and retro nasal de 1,5–2,4 mg Kg^{-1}; revisado por Curioni y Bosset, 2002) y, en segundo lugar, en las notas aromáticas florales y afrutadas que aportan al aroma y sabor de los quesos.

Por último, se identificaron 2 aldehídos, 1 compuesto azufrado, 3 hidrocarburos, 5 terpenos y 5 lactonas. De ellos, es destacable la presencia de metanotiol y δ-decalactona, que podrían participar en el aroma de fondo del Queso Ibores al igual que en otros tipos de queso (Adda y cols., 1998, Curioni y Bosset, 2002).

El análisis de la evolución del perfil de compuestos volátiles durante la maduración permitió asociar cada perfil aromático al estado de maduración del queso. Sin embargo, una vez superado el punto de madurez comercial (60 días) el perfil volátil de los quesos apenas se alteró. Esto indica que a día 60, el aroma de los quesos está completamente formado y que el periodo de almacenamiento no tiene un efecto significativo (al menos un almacenamiento corto, de 30 días, como el estudiado). Otros factores, como la quesería de partida podrían determinar las diferencias en el aroma de los quesos durante el periodo de comercialización.

El estudio de los procesos madurativos implicados en la formación de las características principales de calidad del Queso Ibores indica que las principales rutas involucradas en este proceso serían las implicadas en la liberación de ácidos grasos libres, que a su vez guardan una estrecha relación con los procesos de formación de compuestos aromáticos y por tanto tienen una influencia decisiva en el aroma del queso. Por el contrario, a la vista de los resultados obtenidos, la proteolisis parece no desempeñar un papel relevante en la formación de compuestos del aroma dado el

predominio de la proteolisis primaria respecto a la secundaria (que estaría más relacionada con la liberación de péptidos y aminoácidos implicados en la formación de ciertos compuestos aromáticos con gran influencia en el aroma). Por tanto, la proteolisis sería un proceso más ligado a la formación de la textura del Queso Ibores. En la formación de la textura característica del Queso Ibores también influirían los propios procesos de desecación del queso durante la maduración, dada la relevancia del nivel de extracto seco sobre los parámetros de textura.

Una característica muy importante que influye en este queso es el bajo pH del mismo, que parece alcanzarse por el propio sistema de elaboración de la cuajada del queso y por la adición de cultivos iniciadores que ayudan a mantener el pH al inicio de la maduración. El pH parece ser determinante en los fenómenos proteolíticos del queso y por tanto también en su textura final.

6.2. Efectos del tratamiento de alta presión hidrostática (APH) sobre el Queso Ibores

6.2.1. Quesos sin recubrimiento en corteza tratados con APH a diferentes estados de maduración

Con el fin de evaluar el efecto de la aplicación de tratamientos de APH sobre la maduración de quesos de leche cruda de cabra Ibores, se aplicaron tratamientos de APH (400 ó 600 MPa, durante 7 min) en quesos con diferente estado de maduración (día 1, 30 y 50 días). Se estudiaron los cambios tras el procesado y al final de la maduración de los quesos (60 días).

i. Microbiología

En primer término, se analizó el efecto inducido por el tratamiento de APH sobre los principales grupos microbianos presentes a lo largo de la maduración del Queso Ibores. En aquellos quesos tratados al inicio y mediados de la maduración (día 1 y 30) y analizados al día siguiente, se produjo una reducción significativa en los recuentos de

psicrótrofos, aerobios mesófilos, enterobacterias, BAL (bacterias del ácido láctico) y *Listeria spp*., especialmente en quesos procesados a presión más alta (600 MPa).

En general, en los análisis realizados al final de la maduración (día 60), los recuentos en quesos presurizados continuaron siendo inferiores a los de los quesos control. De forma global, los tratamientos realizados a 600 MPa de presión en los tres tiempos de maduración, se mostraron efectivos en la reducción de psicrótrofos, enterobacterias y *Listeria spp*., al final de la maduración del Queso Ibores (60 días). Los microorganismos psicrótrofos (ej., *Pseudomonas spp*.) y las enterobacterias (ej., *Enterobacter spp*., *Escherichia coli*) pueden causar defectos en los quesos, como son el enranciamiento y la producción de gas, respectivamente. Las especies *Enterobacter aerogenes* y *E. coli* pueden dar lugar al "hinchamiento temprano" de los quesos y a la aparición de defectos en la textura y el aroma (Lück y Dunkeld, 1981). Por ello, la reducción de los grupos microbianos a los cuales pertenecen dichas especies, disminuiría el riesgo de aparición de los citados defectos en el Queso Ibores.

En lo referente a la seguridad alimentaria del Queso Ibores, la familia *Enterobacteriaceae* incluye microorganismos patógenos como son *Salmonella spp*. y *E. coli*, que pueden estar presentes a lo largo de la maduración del queso. A este riesgo hay que añadir la posibilidad de encontrar *Listeria monocytogenes* en quesos de leche cruda, un patógeno causante de la listeriosis. La sensibilidad de *Listeria spp*. a los tratamientos por alta presión, reflejado en la reducción de sus recuentos, contribuye a incrementar la seguridad alimentaria del Queso Ibores. No obstante, ninguna de las colonias crecidas en medio cromogénico presentaron un fenotipo (color verde-azulado y halo de fosfolipolisis) atribuible a *L. monocytogenes*. De manera complementaria, algunas de las colonias aisladas fueron identificadas mediante métodos bioquímicos ("huella metabólica") con el empleo de un equipo Biolog, sin obtener en ningún caso coincidencias con la especie *L. monocytogenes*.

ii. Parámetros físico-químicos y color y textura instrumental

Otra serie de parámetros analizados fueron los relacionados con la composición físico-química de los quesos presurizados y control. El aumento del valor de pH inducido por la APH sobre los quesos de Ibores tratados y analizados al inicio de la maduración, fue mucho menor que el descrito en otros tipos de quesos presurizados (Saldo y cols., 2000a; Juan y cols., 2007a). En los quesos de Ibores analizados a día 60, los tratamientos a 600 MPa a día 1 provocaron un aumento significativo de 0,41 unidades de pH con respecto a quesos control. Este incremento en los valores de pH puede estar relacionado con la reducción de BAL, responsables del proceso de acidificación del queso que tiene lugar durante su maduración; sin embargo, no se encontró una correlación significativa entre la reducción de BAL y el aumento de pH, por lo que otros factores debían estar implicados en estos cambios (ej., neutralización del ácido láctico por los productos de la proteolisis).

Con respecto al resto de parámetros físico-químicos, merece ser destacado el contenido en extracto seco a los 60 días de maduración, que fue mayor en quesos control que en los tratados, a excepción de los quesos presurizados a 400 MPa a día 30 que presentaron un mayor contenido de extracto seco. Para dar una explicación a estos resultados, Messens y cols. (2000) y Juan y cols. (2008) postularon que en los quesos tratados mediante APH se incrementa la retención de humedad con respecto a los no tratados. En nuestro caso, la retención de agua fue mayor en los quesos presurizados al comienzo de la maduración por su mayor contenido en humedad.

El efecto del tratamiento de APH sobre el color instrumental del Queso Ibores maduro puede resumirse en el incremento del color amarillo del queso (CIE b*) inducido por la presurización. No existieron diferencias en la luminosidad (CIE L*) entre los quesos tratados y control. El efecto del incremento de color amarillo del queso inducido por la APH ha sido previamente descrito tanto en quesos de cabra presurizados a 500 MPa (Capellas y cols., 2001), como en quesos de oveja presurizados a 300 MPa (Juan y cols., 2008). Según la literatura, los cambios en la red proteica causados por el tratamiento

de APH explicarían las diferencias entre los quesos tratados y control; no obstante, la relación entre ellos es desconocida y dependería i. del tipo de queso, ii. tipo de leche y/o iii. otros factores.

También se determinó el efecto de la APH sobre la textura instrumental del Queso Ibores mediante un análisis del perfil de textura. La dureza, la gomosidad y la masticabilidad de los quesos presurizados al inicio de la maduración disminuyeron significativamente. Las modificaciones observadas fueron independientes de la intensidad de presión aplicada. En contraposición, los tratamientos realizados a la mitad del período de maduración no produjeron un efecto significativo sobre los parámetros de textura analizados tras la presurización. Las diferencias entre quesos con 1 y 30 días de maduración, pueden deberse a las diferencias entre el contenido de humedad de ambos (47,3% y 39,35%, respectivamente) y la propia estabilidad de la masa del queso.

En el análisis de la textura de los quesos con 60 días, todos los parámetros se modificaron significativamente por la aplicación del tratamiento de APH. Los quesos tratados al inicio de la maduración presentaron los menores valores de dureza y los mayores de elasticidad. Los quesos con una mayor dureza fueron los tratados a 400 MPa a tiempos intermedios de maduración (30 días), que también presentaron el mayor contenido de extracto seco. De hecho, la dureza de los quesos correlacionó positivamente con el contenido en extracto seco ($r= +0,609$; $P<0,001$). Adicionalmente, todos los quesos tratados mostraron una reducción de la dureza proporcional a la intensidad de la presión aplicada. Los mayores valores de cohesividad correspondieron a los quesos tratados al inicio de la maduración a la mayor intensidad de presión. Por último, los valores más elevados de gomosidad y masticabilidad se hallaron en los quesos con mayor dureza y extracto seco.

iii. Principales rutas metabólicas del proceso de maduración

a. Fenómenos proteolíticos

Para evaluar el efecto de la APH sobre los procesos proteolíticos que tienen lugar durante la maduración del Queso Ibores, se calcularon las fracciones de nitrógeno y el contenido en AAL en quesos presurizados y control. En los quesos analizados al inicio de la maduración, el tratamiento de APH incrementó el ratio NS/NT, por lo que se podría pensar en una aceleración de la maduración en los quesos presurizados. Este incremento del índice NS/NT post-presurización al inicio de la maduración también ha sido encontrado en otros tipos de quesos, como por ejemplo en queso Cheddar (Rynne y cols., 2008). Sin embargo, en el Queso Ibores la APH indujo una reducción del contenido en AAL probablemente mediada por la inactivación de las peptidasas microbianas tras la presurización, responsables de la degradación de péptidos de pequeño tamaño hacia AAL (Sousa y cols., 2001). Este efecto de la APH sobre el contenido en AAL en quesos presurizados al inicio de la maduración ha sido previamente descrito en la literatura y correlacionado con una disminución de la actividad aminopeptidasa (Juan y cols., 2007b).

En contraste con los resultados descritos anteriormente, los quesos de Ibores tratados en períodos intermedios de la maduración (día 30) y analizados tras la presurización, mostraron un descenso de los valores de NS/NT, lo que se correspondería con una detención del proceso de maduración de los quesos presurizados. Sin embargo, el efecto sobre el contenido en AAL fue similar al descrito al inicio de la maduración.

Con el objetivo de dilucidar el efecto sobre la proteolisis del tratamiento por APH a lo largo de la maduración, los quesos tratados a día 1, 30 y 50 fueron analizados al final de la misma (día 60). Los niveles de NS/NT fueron significativamente mayores únicamente en aquellos quesos tratados a 600 MPa a día 50. Por lo tanto, durante el proceso de maduración se mitigó el efecto inducido por la APH sobre los quesos de Ibores presurizados al inicio y a mediados de su maduración. En sintonía con nuestros

resultados, O'Reilly y cols. (2000) describieron que el proceso de maduración reducía el incremento inmediato de los niveles de NS/NT mostrado en quesos presurizados tras dos días de elaboración. Por otra parte, la relación NNP/NT se incrementó significativamente en los quesos presurizados comparados con los quesos control, probablemente debido a las proteinasas/peptidasas liberadas tras la lisis celular inducida por la APH, que actuaron a lo largo de la maduración. Los quesos tratados a 600 MPa mostraron un menor contenido en AAL que los tratados a 400 MPa, pero no se encontraron diferencias significativas entre quesos tratados y control. Las diferencias entre intensidades de presión fueron probablemente debidas a una mayor inactivación enzimática a mayor intensidad de presurización (Reps y cols., 2003). El efecto de la APH sobre la enzimas proteolíticas depende en gran medida de la matriz y es complejo; por ejemplo, en función del tipo de aminopeptidasa (aminopeptidasa A, C y N) tratamientos a 400 MPa de presión durante 5 min incrementan su actividad (C), no afectan su actividad (A) o incluso la inactivan (N) (Malone y cols., 2003).

b. Cambios en ácidos grasos libres (AGL)

El tratamiento de APH sobre el Queso Ibores a día 1 dio lugar a un descenso del contenido de AGL al día siguiente de la presurización. Sin embargo, en los quesos presurizados y analizados a día 30, se observó un aumento significativo del contenido en AGL en los quesos tratados a 400 MPa. Este aumento a 400 MPa de presión puede atribuirse a una mejora de la interacción entre las lipasas microbianas y su substrato debido a la lisis celular producida por la APH (Juan y cols., 2008). En contraste, no se detectó un mayor incremento en el contenido de AGL en los quesos tratados a 600 MPa frente a los tratados a 400 Mpa, probablemente debido a un mayor grado de inactivación enzimática por un aumento de la intensidad de presión (Seyderhelm y cols., 1996). Se encontró un patrón opuesto al observado para el proceso proteolítico en los quesos tratados y analizados al inicio y a mediados de la maduración y que se resume en la Figura 1. Las diferencias en la matriz del queso en ambos momentos de maduración (ej., dureza, contenido en humedad), así como las distintas enzimas

lipolíticas (lipoproteína lipasa de la leche frente a lipasas microbianas) y proteolíticas (quimosina frente a proteinasas/peptidasas microbianas) predominantes en cada período, podrían explicar dichas diferencias.

Sin embargo, en los quesos de Ibores maduros (60 días) la variación del contenido en AGL entre los quesos tratados al inicio y final de la maduración y control fue mínima. Por lo tanto, al igual que sucedió con la proteolisis, el efecto inducido por la APH en quesos con 1 y 30 días, fue reducido a lo largo del proceso de maduración. Con estos resultados, se puede afirmar que los cambios causados sobre los procesos proteolíticos y lipolíticos mediante la presurización del Queso Ibores al comienzo y a mediados de la maduración se minimizaron durante el transcurso de la misma.

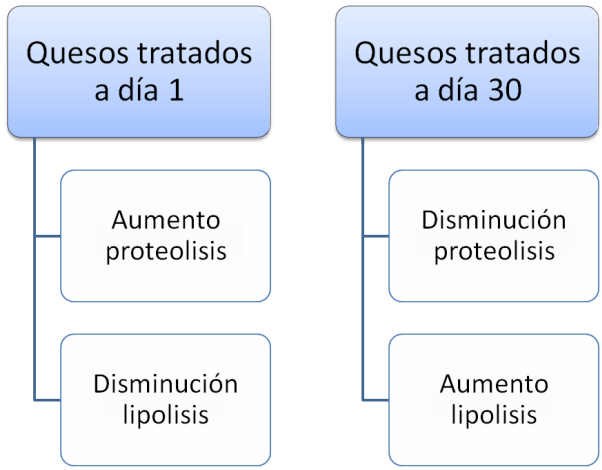

Figura 1. Resumen del efecto del tratamiento de APH sobre la proteolisis y la lipolisis de los quesos de Ibores tratados y analizados al inicio y mitad de la maduración.

c. Oxidación de lípidos y proteínas

En lo referente a los procesos oxidativos, cabe destacar el aumento en la oxidación de lípidos encontrado en los quesos presurizados al inicio y la final de la maduración, y analizados a los 60 días. Este resultado podría estar relacionado con el mayor contenido en ácido linoleico libre, no ligado al glicerol, en los quesos sometidos a APH.

De hecho, el análisis de correlaciones reveló la existencia de una correlación positiva significativa entre la oxidación de lípidos y el contenido en ácidos grasos libres insaturados de los quesos, altamente susceptibles a la oxidación (r= +0,407; *P*<0,05). A pesar de todo, los valores de oxidación lipídica fueron inferiores a los detectados en otros tipos de quesos (Balestrieri y cols., 2002; Delgado y cols., 2009) y éstos no perjudicaron a los quesos a nivel sensorial como se observará con posterioridad.

iv. Perfil de compuestos volátiles en el espacio de cabeza del queso

En cuanto al perfil volátil del Queso Ibores maduro (60 días), el tratamiento de APH disminuyó la abundancia relativa (UA) de la mayoría de compuestos volátiles (excepto cetonas) en los quesos presurizados al inicio de la maduración. También se observó una mayor modificación de la fracción volátil aislada en los quesos tratados a mayor intensidad de presión. Por otro lado, en los quesos presurizados se identificaron cuatro compuestos que no fueron aislados en los quesos control: 4-heptanona, 2-metil ciclohexanona, dimetil trisulfuro (DMTS) y etil benceno. Sin embargo, ninguno de estos compuestos proporcionó un aroma o sabor extraño en los quesos presurizados en comparación con los quesos control, como se comprobó posteriormente mediante el análisis sensorial.

Los cambios detectados en el perfil de compuestos volátiles de los quesos sometidos a alta presión fueron más intensos que los encontrados en el pefil de ácidos grasos libres. Es posible que el tratamiento de alta presión produzca importantes modificaciones en la estructura de la matriz del queso o en las rutas bioquímicas responsables de la producción de compouestos aromáticos. Estas modificaciones explicarían las diferencias entre la composicíón de ácidos grasos libres y la fracción volátil del Queso Ibores.

Tal y como se describe en el apartado 6.1.3, los ácidos constituyen un grupo de compuestos de gran importancia en el perfil aromático del Queso Ibores. En concreto, los niveles de algunos ácidos de cadena lineal relacionados con el aroma a cabra (ej.,

los ácidos hexanoico y octanoico) aumentaron en los quesos tratados a 400 MPa a día 50 de maduración. Asimismo, los niveles de los ácidos 2-metil propanoico y 3-metil butanoico, también se incrementaron en los quesos tratados a 600 MPa a los 30 días. Ambos incrementos sugieren la posibilidad de un aumento de la intensidad del olor e incluso flavor en los citados quesos puesto que esos ácidos son importantes compuestos relacionados con el aroma en el queso (Curioni y Bosset, 2002). No obstante, el análisis sensorial demostró la ausencia de efecto de dicho aumento sobre las características sensoriales del Queso Ibores.

v. Análisis sensorial

Un panel de cata entrenado realizó una evaluación sensorial de los quesos presurizados y control, a los 60 días de maduración. Los catadores percibieron diferencias en la apariencia, el olor y la textura en los quesos analizados; sin embargo, no se hallaron diferencias en el flavor y sabor de los quesos presurizados con respecto a los control.

En el análisis del color instrumental descrito con anterioridad, se señaló el aumento del color amarillo (CIE*b) en los quesos tratados por APH en comparación con los quesos control; sin embargo, no hubo diferencias significativas en el color percibido por los catadores. Este hecho resulta importante para la aceptación de este producto por parte del consumidor, acostumbrado al típico color blanquecino de la masa del Queso Ibores. No obstante, los catadores apreciaron un mayor color amarillo en los quesos tratados al inicio de la maduración, corroborado por la correlación positiva entre el color instrumental y el color sensorial ($r= +0,502$; $P<0,05$).

Por otro lado, el Queso Ibores presenta ojos distribuidos irregularmente en su masa que, tras el tratamiento de APH, disminuyeron significativamente en todos los quesos tratados. Los quesos que tuvieron una menor cantidad de ojos fueron los presurizados al inicio de la maduración, seguidos de los tratados a mediados y al final de la misma. La intensidad de presión más elevada (600 MPa) también causó un mayor descenso del

contenido en ojos en los quesos sometidos a APH. Este hecho pudo deberse a la compresión del aire del interior del queso durante la presurización.

Los catadores detectaron una reducción significativa de la intensidad del olor de los quesos tratados al inicio de la maduración. También la dureza y la friabilidad presentaron un descenso significativo en los quesos tratados al inicio de la maduración en comparación con el resto, mientras que la elasticidad fue mayor. La dureza y la friabilidad correlacionaron significativamente con la dureza instrumental (r= +0,70; r= +0,64; *P*<0,001). De igual modo, Saldo y cols. (2000b) encontraron que la presurización (50, 400 ó 400+50 MPa) de quesos de cabra de leche pasteurizada al inicio de la maduración, dio lugar a quesos menos friables y más elásticos que los no tratados.

Los catadores no encontraron diferencias entre los quesos en cuanto al flavor, es decir, el conjunto de sensaciones olfativas y gustativas percibidas tras introducir un alimento en la boca. No obstante, como se señaló con anterioridad, se produjo una reducción en la intensidad del olor de los quesos tratados al comienzo de la maduración. Este resultado guarda relación con el menor contenido en compuestos volátiles encontrado en dichos quesos, existiendo una correlación positiva entre la intensidad de olor y el contenido total de compuestos volátiles aislados en el espacio de cabeza de estos quesos (r= +0,558; *P*<0,05).

En la figura 2 se muestra el resultado del análisis de componentes principales de las variables sensoriales analizadas en quesos maduros tratados a lo largo de la maduración. El componente principal 1 explicó el 48,3% de la variación mientras que el componente principal 2 explicó el 15,4 %. La friabilidad y la dureza, situadas en el extremo positivo del componente principal 1, junto con la elasticidad, localizada en el área negativa del mismo componente, explicaron en gran medida las diferencias encontradas entre quesos presurizados y control.

El análisis de componentes principales (Figura 3) de las variables sensoriales de los quesos analizados en función del día y tipo de tratamiento permitió establecer dos

grupos diferenciados de quesos, los tratados al inicio del proceso madurativo (día 1) situados en la zona negativa del componente principal 1, y los quesos control y tratados a los tiempos de 30 y 50 días localizados en la zona positiva del mismo componente, indicando un perfil sensorial más similar entre los quesos de este último grupo.

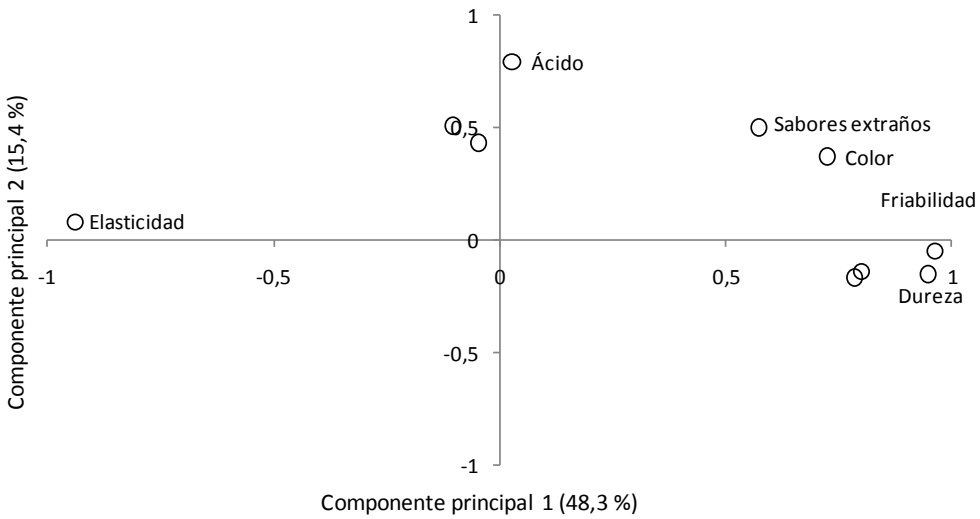

Figura 2. Análisis de componentes principales de las variables sensoriales de quesos maduros (60 días) sometidos a APH durante su maduración.

6.2.2. Quesos con recubrimiento en corteza (pimentón) tratados por APH al final de la maduración. Evolución de su almacenamiento posterior en refrigeración.

El Queso Ibores se suele presentar con la corteza cubierta con pimentón y aceite de oliva para su comercialización; este recubrimiento se aplica una vez que el queso ha madurado. Para evaluar el posible efecto de la APH (400 ó 600 MPa, 7 min) sobre las distintas características del Queso Ibores con madurez comercial almacenado a refrigeración, se procesaron quesos pimentonados maduros (60 días) y se estudió su evolución durante un período de almacenamiento de 30 días. Posteriormente, se analizaron distintos parámetros microbiológicos, físico-químicos e instrumentales,

proteolíticos, relacionados con el aroma y sensoriales.

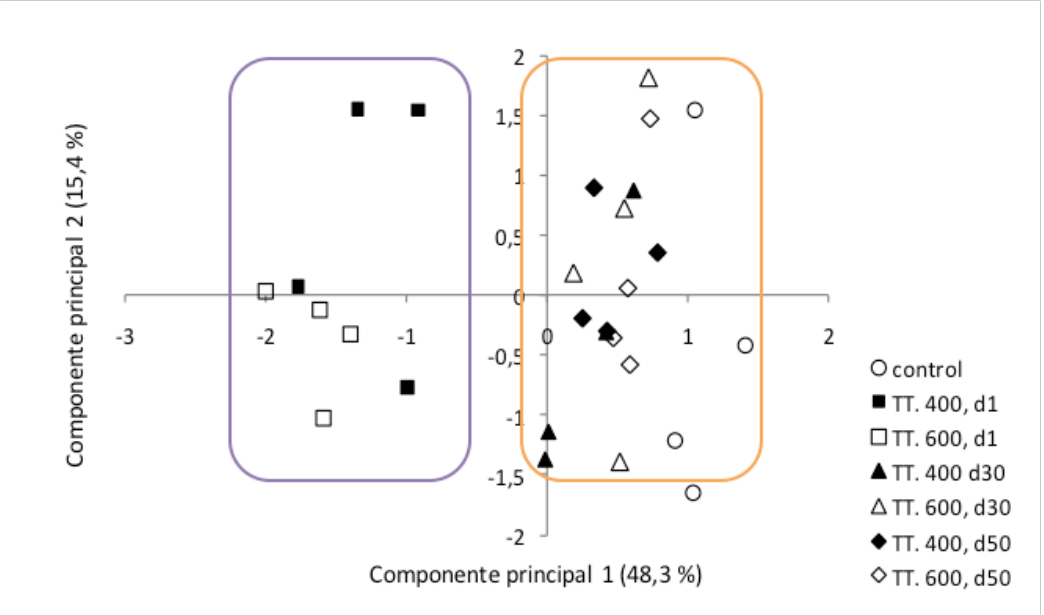

Figura 3. Análisis de componentes principales de los datos sensoriales de los quesos de Ibores maduros en función del día y tipo de tratamiento. TT.= tratamiento; d= día.

i. Microbiología

En los quesos maduros presurizados a 600 MPa y analizados después del tratamiento, todos los grupos microbianos sufrieron una reducción significativa tras el tratamiento de APH. Por el contrario, cuando el tratamiento se aplicó a 400 MPa, los recuentos de psicrótrofos y micrococos no descendieron significativamente. Por tanto, la reducción en los recuentos microbianos fue proporcional a la intensidad de presión aplicada. Las dos intensidades de presión ejercidas (400 y 600 MPa) provocaron una reducción significativa del contenido de enterobacterias y *Listeria spp*., que pueden incluir microorganismos patógenos (ej., *E. coli*, *L. monocytogenes*), por tanto a esas presiones de procesado, se mejora la seguridad alimentaria de los quesos.

Después de 30 días de almacenamiento de los quesos en condiciones de refrigeración, aún se encontraron diferencias en los niveles de microorganismos entre los quesos tratados y control, excepto para los recuentos de enterobacterias, que estuvieron por debajo del límite de detección del método de recuento utilizado en todos los grupos de quesos, y *Listeria spp*., cuyos niveles fueron inferiores a 1 log ufc/g en quesos presurizados. Por lo tanto, a día 90, el tratamiento por APH realizado a día 60 reduce el riesgo microbiológico en los quesos al disminuir los recuentos de *Listeria spp*. Sin embargo, al igual que cuando se evaluó el efecto de la APH en quesos en diferente estado de maduración, ninguna de las colonias identificadas pertenecieron a la especie *L. monocytogenes*. De igual manera, como se observó en los quesos tratados al final de la maduración (día 50), la aplicación de APH sobre quesos de Ibores con 40–50 días de maduración también sería un tratamiento eficaz para reducir el riesgo por *L. monocytogenes*, incrementando su seguridad alimentaria y con mínimas modificaciones de su calidad. En este sentido, los productores podrían reducir el tiempo necesario para la comercialización (por debajo de los 60 días) de sus productos en función de las necesidades del mercado, siempre y cuando sus características sensoriales no se alteren por una disminución del tiempo de maduración.

ii. Parámetros físico-químicos y color y textura instrumental

Los valores de pH y color siguieron la misma tendencia a la descrita en los quesos tratados a 50 días y analizados al final de la maduración (día 60). El pH no sufrió modificaciones significativas ni tras la presurización ni después de 30 días de almacenamiento de los quesos pimentonados. Este hecho es muy positivo dada la importancia de este parámetro sobre los procesos de maduración, tal y como se ha explicado en los apartados anteriores.

En cuanto al color instrumental, la APH provocó un aumento de la luminosidad y un descenso de CIE a*, efectos que se mantuvieron tras 30 días de almacenaje. Sin embargo, al contrario que los quesos tratados al inicio de la maduración, no se

encontraron cambios en el parámetro CIE b*, probablemente porque cuando se trataron estos quesos, el color de los mismos ya se había formado y en este punto ya no se modifica con tanta facilidad la estructura proteica del queso, que es el factor que podría guardar más relación con la coloración del queso.

Por otro lado, en lo que se refiere al análisis instrumental de la textura, el tratamiento de APH aplicado al final de la maduración (60 días) causó un incremento significativo en la adhesividad, gomosidad y masticabilidad de los quesos presurizados. Transcurridos los 30 días de almacenamiento refrigerado, únicamente en los quesos tratados a 600 MPa se mantuvieron dichas diferencias. Por el contrario, en el estudio de APH realizado a lo largo de la maduración del Queso Ibores, el tratamiento de presurización únicamente modificó la textura de los quesos tratados al inicio de la maduración, como denotaron los análisis realizados sobre los quesos maduros.

iii. Fenómenos proteolíticos

Al analizar los procesos proteolíticos, se hallaron incrementos significativos en las fracciones de nitrógeno obtenidas en los quesos pimentonados presurizados a día 60 a 600 MPa y analizados tras el tratamiento, que desaparecieron transcurridos los 30 días de almacenamiento, por lo que el efecto fue reversible. Lo mismo sucedió con el efecto de la APH sobre la proteolisis de quesos tratados al inicio (aumento) y mediados (disminución) de la maduración, que también se diluyó con el transcurso de la maduración. Igualmente, Voigt y cols. (2010) encontraron un incremento significativo en la relación NS/NT en queso azul irlandés maduro (42 días) sometido a 600 MPa de presión durante 10 min, que no se mantuvo tras 28 días de almacenamiento a 4 ºC. Por tanto, podemos afirmar que los cambios proteolíticos inducidos por la presión desaparecieron durante el almacenamiento.

iv. Perfil de compuestos volátiles en el espacio de cabeza del queso

En lo referente al efecto del tratamiento de APH sobre el perfil volátil de los quesos pimentonados, no se observaron diferencias significativas entre los quesos tratados y

control para el contenido de la mayoría de compuestos volátiles. Estos resultados están en consonancia con los obtenidos en el análisis sensorial de los quesos pimentonados donde, como se verá posteriormente, ni un panel de catadores entrenados ni un panel de consumidores detectaron diferencias en el olor y el flavor.

Los terpenos son compuestos volátiles de origen vegetal importantes para el aroma global del queso debido a sus bajos umbrales de percepción. En el queso pimentonado se identificaron una gran variedad de terpenos, mientras que en los quesos sin recubrimiento sólo se aisló el limoneno. Por lo tanto, esos terpenos proceden del pimentón que recubre al queso, debido a que se han aislado una gran variedad de terpenos y sus derivados en pimentón (Kocsis y cols., 2002). El tratamiento de APH sobre quesos con 60 días a 400 MPa, causó un aumento significativo del contenido relativo de algunos terpenos. Sin embargo, ese efecto desapareció tras 30 días de almacenamiento refrigerado (día 90). Por lo que la posibilidad de aumentar el aroma proporcionado por el pimentón al queso mediante la presurización queda en principio descartada.

El análisis global del perfil de compuestos volátiles de los quesos evidenció la falta de efecto de la presión sobre el aroma de los quesos, ya que sólo se hallaron diferencias entre grupos debidas al diferente estado de maduración de los quesos. Por tanto, la aplicación de tratamientos de APH en quesos maduros pimentonados, no afectaría al aroma final del queso durante el periodo de comercialización estudiado (30 días).

v. Análisis sensorial

Un aspecto relevante de la calidad es el análisis sensorial de los quesos pimentonados tratados mediante APH. Aunque se detectaron leves diferencias en el color, la textura instrumental y en la proteolisis (a día 60) los jueces del panel sensorial no encontraron diferencias significativas para los atributos evaluados. En comparación con estos resultados y como se indicó con anterioridad en el análisis sensorial de los quesos presurizados a lo largo de la maduración, los quesos tratados al inicio de la maduración

presentaron una menor cantidad de ojos, intensidad de olor y dureza, y una mayor elasticidad que los quesos control. Por otro lado, ni la intensidad de olor ni de flavor se modificaron tras la aplicación de tratamientos de APH en los quesos pimentonados, lo que resulta positivo dado la importancia de estos descriptores para la aceptación de un producto a nivel comercial (Plutowska y Wardencki, 2007).

Asimismo, el test de consumidores realizado para evaluar si los cambios sensoriales en el queso procesado eran detectados por consumidores no entrenados, mostró que los quesos pimentonados presurizados fueron indistinguibles de los quesos control, obteniendo todos una elevada puntuación media (superior a 7 puntos). En las catas realizadas a día 60, los consumidores prefirieron los quesos control (42%), seguidos de cerca por los tratados a 600 MPa (34%). Tras un mes de almacenamiento en refrigeración, un 45% de los consumidores eligieron los quesos tratados a 600 MPa. Esto supone que la presurización no afecta (o incluso mejora) a la calidad de los quesos y por tanto, el tratamiento de APH se presenta como una alternativa real para mejorar la seguridad microbiológica de los quesos de leche cruda sin efecto sobre las características tradicionales del producto.

Por otra parte, cabe señalar la importancia de realizar estudios de almacenamiento en los alimentos presurizados, y no sólo evaluar el producto después del tratamiento, ya que muchos de los cambios inducidos en los alimentos son reversibles o se diluyen durante el periodo posterior a la aplicación del procesado.

Referencias

Adda, J., Czulak, J., Mocquot, G., & Vassal, L. (1998). In Cross H.R., Overby A.J. (Eds.), Cheese, Meat Science, Milk Science and Technology (pp. 373–392). The Netherlands: Elsevier Science Publications.

Atasoy, A.F., & Türkoglu, H. (2009). Lipolysis in Urfa cheese produced from raw and pasteurized goats' and cows' milk with mesophilic or thermophilic cultures during ripening. Food Chemistry, 115, 71–78.

Balestrieri, M., Spagnuolo, M.S., Cigliano, L., Storti, G., Ferrara, L., Abrescia, P., & Fedele E. (2002). Evaluation of oxidative damage in mozzarella cheese produced from bovine or water buffalo milk. Food Chemistry, 77, 293–299.

Buffa, M.N., Guamis, B., Pavia, M., & Trujillo, A.J. (2001). Lipolysis in cheese made from raw, pasteurized or high-pressure-treated goats' milk. International Dairy Journal, 11, 175–179.

Capellas, M., Mor-Mur, M., Sendra, E., Pla, R., & Guamis, B. (1996). Populations of Aerobic Mesophiles and Inoculated E. coli During Storage of Fresh Goat's Milk' Cheese Treated with High Pressure. Journal of Food Protection, 59, 582–587.

Carbonell, M., Núñez, M., & Fernández-García, E. (2002). Evolution of the volatile components of ewes raw milk La Serena cheese during ripening. Correlation with flavour characteristics. Lait, 82, 683–698.

Castillo, I., Calvo, M. V., Alonso, L., Juárez, M., & Fontecha, J. (2007). Changes in lipolysis and volatile fraction of a goat cheese manufactured employing a hygienized rennet paste and a defined strain starter. Food Chemistry, 100, 590–598.

Collins, Y.F., McSweeney, P.L.H., & Wilkinson, M.G. (2003). Lipolysis and free fatty acid catyabolism in cheese: A review of current knowledge. International Dairy Journal, 13, 841–866.

Creamer, L. K., & Olson, N. F. (1982). Rheological evaluation of maturing Cheddar cheese. Journal of Food Science, 47, 631–647.

Curioni, P. M. G., & Bosset, J. O. (2002). Key odorants in various cheese types as determined by gas chromatography–olfactometry. International Dairy Journal, 12, 959–984.

Delgado, F.J., González-Crespo, J., Ladero, L., Cava, R., & Ramírez, R. (2009). Free fatty acids and oxidative changes of a Spanish soft cheese (PDO "Torta del Casar") during ripening. International Journal of Food Science & Technology, 44, 1721–1728.

Delgado, F. J., Rodríguez-Pinilla, J., González-Crespo, J., Ramírez, R., & Roa, I. (2010a).

Proteolysis and texture changes of a Spanish soft cheese (“Torta del Casar”) manufactured with raw ewe milk and vegetable rennet during ripening. International Journal of Food Science & Technology, 45, 512–519.

Delgado, F.J., González-Crespo, J., Cava, R., García-Parra, J. & Ramírez, R. (2010b). Characterization of the volatile profile of a Spanish ewe raw milk soft cheese P.D.O. Torta del Casar during ripening by SPME-GC-MS. Food Chemistry, 118, 182–189.

Fedele, E., & Bergamo, P. (2001). Protein and Lipid Oxidative Stresses During Cheese Manufacture. Journal of Food Science, 66, 932–935.

Ferrandini, E., López, M.P., Castillo, M., & Laencina, J. (2011). Influence of an artisanal rennet paste on proteolysis and textural properties of Murcia al Vino cheese. Food Chemistry, 124, 583–588.

Franco, I., Prieto, B., Bernardo, A., González, J., & Carballo, J. (2003). Biochemical changes throughout the ripening of a tradicional Spanish goat cheese variety (Babia-Laciana). International Dairy Journal, 13, 221–230.

Freitas, A.C., & Malcata, F.X. (1998). Lipolysis in Picante cheese: influence of milk type and ripening time on free fatty acid profile. Lait, 78, 251–258.

Fresno, J. M., Tornadijo, M. E., Carballo, J., González-Prieto, J., & Bernardo, A. (1996). Characterization and biochemical changes during the ripening of a Spanish craft goat’s milk cheese (Armada variety). Food Chemistry, 55, 225–230.

Juan, B., Ferragut, V., Guamis, B., Buffa, M., & Trujillo, A. J. (2004). Proteolysis of a high pressure-treated ewe’s milk cheese. Milchwissenschaft, 59, 616–619.

Juan, B., Trujillo, A.J., Guamis, V., Buffa, M., & Ferragut, V. (2007a). Rheological, textural and sensory characteristic of high-pressure treated semi-hard ewes’ milk cheese. International Dairy Journal, 17, 248–254.

Juan, B., Ferragut, V., Buffa, M., Guamis, B., & Trujillo, A. J. (2007b). Effects of high pressure on proteolytic enzymes in cheese: Relationship with the proteolysis of ewe milk cheese. Journal of Dairy Science, 90, 2113−2125.

Juan, B., Ferragut, V., Guamis, B., & Trujillo, A.J. (2008). The effect of high-pressure treatment at 300 MPa on ripening of ewes' milk cheese. International Dairy Journal, 18, 129−138.

Kocsis, N., Amtmann, M., Mednyanszky, Z., & Korany, K. (2002). GC–MS Investigation of the Aroma Compounds of Hungarian Red Paprika (Capsicum annuum) Cultivars. Journal of Food Composition and Analysis, 15, 195–203.

Kraggerud, H., Skeie, S., Høy, M., Røkke, L., & Abrahamsen, R. K. (2008). Season and ripening temperature influence fatty acid composition and sensory properties of semi-hard cheese during maturation. International Dairy Journal, 18, 801–810.

Le Querré, J.L., Pierre, A., Riaublanc, A., & Demaizières, D. (1998). Characterization of aroma compounds in the volatile fraction of soft goat cheese during ripening. Lait, 78, 279–290.

Lück, H., & Dunkeld, M. (1981). Enterobacteriaceae in cheese. South African Journal of Dariy Technology, 13, 9–14.

Malone, A.S., Wick, C., Shellhammer, T.H., & Courtny, P.D. (2003). High pressure effects on proteolytic an glycolytic enzymes involved in cheese manufacturing. Journal of Dairy Science, 86, 1139–1146.

Martín-Hernández, M. C., & Juárez, M. (1992). Biochemical characteristics of three types of goat cheese. Journal of Dairy Science, 75, 1747–1752.

Messens, W., Van de Walle, D., Arevalo, J., Dewettinck, K., & Huyghebaert, A. (2000). Rheological Properties of High-pressure-treated Gouda Cheese. International Dairy Jorunal, 10, 359–367.

Moio, L., & Addeo, F. (1998). Grana Padano cheese aroma. Journal of Dairy Research, 65, 317–333.

Nájera, A.I., Barron, L.J.R., & Barcina, Y. (1993). Review: Lipid fraction composition of cow's, sheep's and goat's cheese, and the influence on its quality. Science and Food Technology Spanish Journal, 33, 345–363.

O'Reilly, C.E., O'Connor, P.M., Kelly, A.L., Beresford, T.P., & Murphy, P.M. (2000). Use

of Hydrostatic Pressure for Inactivation of Microbial Contaminants in Cheese. Applied and Environmental Microbiology, 66, 4890–4896.

Pinho, O., Ferreira, I. M. P. L. V. O., & Ferreira, M. A. (2003). Quantification of shortchain free fatty acids in ‘‘Terrincho” ewe cheese: intravarietal comparison. Journal of Dairy Science, 86, 3102–3109.

Plutowska, B., & Wardencki, W. (2007). Aromagrams – Aromatic profiles in the appreciation of food quality. Food Chemistry, 101, 845-872.

Poveda, J.M., & Cabezas, L. (2006). Free fatty acid composition of regionally-produced Spanish goat cheese and relationship with sensory characteristics. Food Chemistry, 95, 307–311.

Poveda, J.M., Sánchez-Palomo, E., Pérez-Coello, M.S., & Cabezas, L. (2008). Volatile composition, olfactometry profile and sensory evaluation of semi-hard Spanish goat cheeses. Dairy Science & Technology, 88, 355–367.

Psoni, N. T., & Litopoulou-Tzanetaki, E. (2003). Microbiological characteristics of Batzos, a traditional Greek cheese from raw goat's milk. Food Microbiology, 20, 575–582.

Reps, A., Kolakowski, P., Wisniewska, K., & Krasowska, M. (2003). The effect of high pressures on proteolytic enzymes in ripened cheeses. Milchwissenschaft, 58, 138–140.

Rynne, N.M., Beresford, T.P., Guinee, T.P., Sheehan, E., Delahunty, C.M., & Kelly, A.L. (2008). Effect of high-pressure treatment of 1 day-old full-fat Cheddar cheese on subsequent quality and ripening. Innovative Food Science & Emerging Technologies, 9, 429−440.

Sabio, E., & Vidal-Aragon, M. C. (1996). Analysis of the volatile fraction of Ibores cheese. Alimentaria, 278, 101–103.

Saldo, J., McSweeney, P.L.H., Sendra, E., Kelly, A.L., & Guamis, B. (2000a). Changes in Curd Acidification Caused by High Pressure Treatment. Irish Journal of Agriculture Food Research, 39, 169.

Saldo, J., Sendra, E., & Guamis, B. (2000b). High hydrostatic pressure for accelerating

ripening of goat's milk cheese: Proteolysis and texture. Journal of Food Science, 65, 636–640.

Serhana, M., Lindera, M., Hosrib, C., & Fannia, J. (2010). Changes in proteolysis and volatile fraction during ripening of Darfiyeh, a Lebanese artisanal raw goat's milk cheese. Small Ruminant Research, 90, 75–82.

Serra, M., Trujillo, A.J., Pereda, J., Guamis, B., & Ferragut, V. (2008). Quantification of lipolysis and lipid oxidation during cold storage of yogurts produced from milk treated by ultra-high pressure homogenization. Journal of Food Engineering, 89, 99–104.

Seyderhelm, I., Boguslawski, S., Michaelis, G., & Knorr, D. (1996). Pressure induced inactivation of selected food enzymes. Journal of Food Science, 61, 308–310.

Sousa, M. J., Ardö, Y., & McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. International Dairy Journal, 11, 327–345.

Tavaria, F. K., Silva Ferreira, A. C., & Malcata, F. X. (2004). Volatile free fatty acids as ripening indicators for Serra da Estrela cheese. Journal of Dairy Science, 87, 4064–4072.

Tejada, L., Abellán, A., Cayuela, J. M., Martínez-Cacha, A., & Fernández-Salguero, J. (2008). Proteolysis in goats' milk cheese made with calf rennet and plant coagulant. International Dairy Journal, 18, 139–146.

Van Hekken, D. L., Tunick, M.H., & Park, Y. W. (2004). Rheological and proteolytic properties of Monterey Jack goat milk cheese during 6 months aging. Journal of Agriculture and Food Chemistry, 52, 5372–5377.

Voigt, D.D., Chevalier, F., Qian, M.C., & Kelly, A.L. (2010). Effect of high-pressure treatment on microbiology, proteolysis, lipolysis and levels of flavour compounds in mature blue-veined cheese. Innovative Food Science and Emerging Technologies, 11, 68–77.

Wick, C., Nienabert, U., Anggraeni, O., Shellhammer, T.H., & Courtney, P.D. (2004). Texture, proteolysis and viable lactic acid bacteria in commercial Cheddar cheeses treated with high pressure. Journal of Dairy Research, 71, 107–115.

Woo, A. H., & Lindsay, R. C. (1982). Rapidmethod for quantitative analysis of individual free fatty acids in Cheddar cheese. Journal of Dairy Science, 65, 1102–1109.

Yokohama, H., Swamura, N., & Motobyashi, N. (1992). Method for accelerating cheese ripening. European Patent Application EP 0 469 857 A1.

Zerfiridis, G. K., Vafopoulou-Mastrogiannaki, A., & Lipoulou-Tzanetaki, E. (1984). Changes during ripening of commercial Gruyère cheese. Journal of Dairy Science, 67, 1397–1405.

7. CONCLUSIONES

1.- El perfil de compuestos volátiles del Queso Ibores muestra una gran complejidad por la variedad y abundancia de compuestos detectados. Cuando el queso alcanza su madurez comercial (60 días), el perfil de compuestos volátiles se encuentra completamente formado y éste apenas se modifica durante periodos de almacenamiento cortos.

2.- Las principales rutas metabólicas implicadas en la formación de compuestos responsables del aroma y del sabor durante la maduración del Queso Ibores son las relacionadas con los procesos lipolíticos. Por el contrario, la influencia de los procesos oxidativos y proteolíticos en la formación del aroma es limitada. Los procesos proteolíticos de las caseínas están más relacionados con la formación de la textura típica del Queso Ibores.

3.- El tratamiento del Queso Ibores con altas presiones hidrostáticas constituye un método eficaz para reducir el riesgo microbiológico que puede suponer el consumo de quesos de leche de cabra no pasteurizada. Su aplicación a la mitad y al final de la maduración del Queso Ibores, no afecta a los parámetros de calidad del queso, manteniéndose sus características propias y distintivas.

4.- La aplicación de altas presiones hidrostáticas al Queso Ibores modifica sustancialmente el perfil de compuestos volátiles, especialmente cuando el tratamiento se aplica al principio o a la mitad de la maduración; no así en el caso del perfil de ácidos grasos libres, en los que los cambios son de menor intensidad. Dichos cambios son dependientes del momento de aplicación del tratamiento, minimizándose cuando el tratamiento se realiza al final de la maduración.

5.- La aplicación de altas presiones hidrostáticas al Queso Ibores incrementa los procesos oxidativos de los lípidos, sin embargo la oxidación de proteínas no es modificada. Los cambios oxidativos de los lípidos son cuantitativamente bajos y no afectan a la calidad sensorial del queso.

6.- La aplicación de altas presiones al Quesos Ibores al inicio de la maduración provoca cambios en sus características sensoriales modificando su aspecto, olor y textura. Estos cambios son de mayor intensidad en los quesos tratados al inicio de su maduración.

7.- Los cambios inducidos en los procesos proteolíticos y lipolíticos debidos al tratamiento de alta presión en el Queso Ibores, se minimizan durante la maduración y el almacenamiento de los quesos. A la vista de estos cambios, cobran importancia los estudios a lo largo de la maduración/almacenamiento en los quesos presurizados, ya que algunas de las modificaciones inducidas inmediatamente después del tratamiento son reversibles o se diluyen durante el periodo posterior a su aplicación.

8.- Los tratamientos de altas presiones hidrostáticas del Queso Ibores pimentonado maduro (60 días), reducen el riesgo microbiológico aumentando su seguridad alimentaria, con un efecto mínimo sobre sus características sensoriales y de calidad.

INTAEX
Instituto Tecnológico Agroalimentario de Extremadura

JUNTA DE EXTREMADURA
Vicepresidencia Segunda y Consejería de Economía, Comercio e Innovación

UNIÓN EUROPEA
Fondo Europeo de Desarrollo Regional
Fondo Social Europeo
Una manera de hacer Europa