UNIVERSITAT POLITÈCNICA DE VALÈNCIA

MI QU ALI
Grupo de Investigación Microestructura y Química de los Alimentos

IMPACTO DE NUEVAS TECNOLOGÍAS DE CONSERVACIÓN SOBRE LA ESTRUCTURA Y LOS PRINCIPALES COMPONENTES QUÍMICOS DE ALIMENTOS FLUIDOS

TESIS DOCTORAL

Presentada por: **Raquel Marco Molés**

Dirigida por: **Dra. Isabel Hernando Hernando**

Valencia, diciembre de 2011

IMPACTO DE NUEVAS TECNOLOGÍAS DE CONSERVACIÓN SOBRE LA ESTRUCTURA Y LOS PRINCIPALES COMPONENTES QUÍMICOS DE ALIMENTOS FLUIDOS

Memoria presentada por RAQUEL MARCO MOLÉS, inscrita en el programa de doctorado de "Tecnología de Alimentos" del Departamento de Tecnología de Alimentos de la Universidad Politécnica de Valencia, para optar al grado de Doctor.

Trabajo realizado en el Departamento de Tecnología de Alimentos bajo la dirección de la Dra. Isabel Hernando Hernando.

Directora de Tesis **Autora**

Dra. Isabel Hernando Hernando Raquel Marco Molés

AGRADECIMIENTOS

Quisiera agradecer este trabajo a todas aquellas personas que de alguna manera han contribuido a que mi mayor pesadilla se convierta en un sueño hecho realidad:

En especial a ti Isa, porque para mí has sido mucho más que una directora de tesis. Sabes que sin ti, yo no hubiera podido. Gracias, de verdad, por confiar en mí y ayudarme a seguir adelante.

A Isabel, por darme la oportunidad de realizar esta tesis. Por todo su esfuerzo y dedicación en este trabajo y por el bien del grupo en general. Porque siempre estará con nosotros. Y porque nunca olvidaré todos los momentos que compartimos y sus sabios consejos.

A toda la gente del Lab 13 (Empar, Amparo, Ana, Virginia, Laura, Juanvi, José, Luis, María y Julia) por su ayuda constante y tiempo dedicado en el laboratorio, por los buenos momentos compartidos y sobre todo por su amistad.

A mis padres, porque a ellos se lo debo todo. A mi padre, por su gran apoyo y ayuda y por la confianza depositada en mí. A mi madre, por su incondicional cariño y compresión.

A mis hermanos, por estar siempre ahí, dispuestos a contar conmigo y a echarme una mano siempre que la necesito.

A mi hermana, porque a pesar de la distancia, siempre está a mi lado para escucharme y darme buenos consejos.

A mi abuela, por preocuparse por mí a lo largo de tantos años y porque sé que siempre estará ahí para cuidar de mí.

A mis sobrinas, por contagiarme con su alegría e ilusión en la vida.

A Bubu, por sacarme a pasear cada día y regalarme su compañía.

Y a ti Jesús, por estar siempre a mi lado y compartir tantos sueños juntos.

Por último, agradecer a todos los organismos que han contribuido a que la realización de esta tesis doctoral haya sido posible:

A la Comisión Europea por la financiación del proyecto "Innovative Non-Thermal Processing Technologies to Improve the Quality and Safety of Ready-to-Eat (RTE) Meals (FP6-2004-FOOD-23140 HIGHQ RTE)" en el cual se enmarca el presente trabajo.

Al Departamento de Tecnología de Alimentos de la Universidad Politécnica de Valencia, por formarme durante estos años y ofrecerme sus instalaciones para la realización de esta tesis.

Al Swedish Institute for Food and Biotechnology y a la Università di Bologna, por proporcionar las muestras analizadas en este trabajo.

Al Servicio de Microscopía Electrónica de la Universidad Politécnica de Valencia, y en especial a Manolo, José Luis y Merche, por su ayuda.

Al Ministerio de Educación y Ciencia (MEC) por concederme una beca, dentro del Programa de Formación del Profesorado Universitario (FPU), para la realización de esta tesis.

RESUMEN

La aplicación de nuevas tecnologías en el ámbito de la conservación de alimentos pretende dar respuesta al incremento de la demanda, por parte de los consumidores, de alimentos más parecidos a los frescos o naturales, más nutritivos y de fácil y rápida preparación (ready-to-eat meals). Las tecnologías más estudiadas en la actualidad se basan en el empleo de sistemas de destrucción o inactivación bacteriana sin necesidad de emplear un tratamiento térmico intenso. Se pretende así reducir los cambios perjudiciales en las propiedades físicas, químicas, nutricionales y sensoriales de los alimentos, pero manteniendo la vida útil.

El objetivo general de este trabajo ha sido el estudio del impacto de la aplicación de nuevas tecnologías de conservación, como los Campos Eléctricos Pulsados (PEF) y las Altas Presiones de Homogeneización (HPH), sobre la estructura y los principales componentes químicos de alimentos fluidos (huevo líquido y salsas). Para ello, se ha estudiado la microestructura y se ha realizado el análisis químico de la fracción proteica y lipídica de estos alimentos. Además, se han estudiado diferentes propiedades físicas del huevo líquido tratado por PEF que determinan su calidad, como son la viscosidad, el color, y la capacidad espumante. Los experimentos se han realizado con muestras procesadas por PEF en el SIK (the Swedish Institute for Food and Biotechnology) y por HPH en la Università di Bologna, y se han llevado a cabo también después de una semana de almacenamiento refrigerado a 4ºC.

Los resultados obtenidos en este estudio muestran que los tratamientos por PEF y HPH pueden ser una buena alternativa al tratamiento térmico convencional para la conservación de huevo líquido, por su menor impacto en la microestructura y en las propiedades químicas cuando se aplican a baja intensidad, si se compara con la pasteurización. Además, el tratamiento por PEF afecta mínimamente a las propiedades funcionales del huevo líquido. Por otro lado, la estabilidad fisicoquímica de las salsas tratadas por HPH está relacionada directamente con la presión aplicada durante el proceso. La pérdida de emulsionantes naturales cuando se aplican altas presiones, favorece la coalescencia de la fase grasa y por tanto la desestabilización. Sin embargo, la fracción lipídica de las salsas se mantiene químicamente estable tras el tratamiento.

RESUM

L'aplicació de noves tecnologies en l'àmbit de la conservació d'aliments pretén donar resposta a l'increment de la demanda, per part dels consumidors, d'aliments més pareguts als frescos o naturals, més nutritius i de fàcil i ràpida preparació (ready-to-eat meals). Les tecnologies més estudiades en l'actualitat es basen en la utilització de sistemes de destrucció o inactivació bacteriana sense necessitat d'emprar un tractament tèrmic intens. Es pretén així reduir els canvis perjudicials en les propietats físiques, químiques, nutricionals i sensorials dels aliments, però mantenint la vida útil.

L'objectiu general d'este treball ha sigut l'estudi de l'impacte de l'aplicació de noves tecnologies de conservació, com els Campos Elèctrics Polsats (PEF) i les Altes Pressions d'Homogeneïtzació (HPH), sobre l'estructura i els principals components químics d'aliments fluids (ou líquid i salses). Per a això, s'ha estudiat la microestructura i s'ha realitzat l'anàlisi químic de la fracció proteica i lipídica d'estos aliments. A més, s'han estudiat diferents propietats físiques de l'ou líquid tractat per PEF que determinen la seua qualitat, com són la viscositat, el color, i la capacitat espumant. Els experiments s'han realitzat amb mostres processades per PEF en el SIK (the Swedish Institute for Food and Biotechnology) i per HPH en la Università di Bologna, i s'han dut a terme també després d'una setmana d'emmagatzemament refrigerat a 4ºC.

Els resultats obtinguts en este estudi mostren que els tractaments per PEF i HPH poden ser una bona alternativa al tractament tèrmic convencional per a la conservació d'ou líquid, pel seu menor impacte en la microestructura i en les propietats químiques quan s'apliquen a baixa intensitat, si es compara amb la pasteurització. A més, el tractament per PEF afecta mínimament les propietats funcionals de l'ou líquid. D'altra banda, l'estabilitat fisicoquímica de les salses tractades per HPH està relacionada directament amb la pressió aplicada durant el procés. La pèrdua d'emulsionants naturals quan s'apliquen altes pressions, afavorix la coalescencia de la fase greix i per tant la desestabilització. No obstant això, la fracció lipídica de les salses es manté químicament estable després del tractament.

ABSTRACT

The application of new technologies for food processing and preservation aims to meet the growing consumer demand for minimally processed fresh-like foods, with high sensorial and nutritional quality and convenient to prepare (ready-to-eat meals). This has lead the food industry to develop novel non-thermal food preservation technologies that inactivate microorganisms without the need for intense heat treatment, thus obtaining products with similar properties to fresh foods, and preserving the nutritional and sensory properties, while also maintaining their shelf-life.

The general aim of this work was to study the impact of the application of new preservation technologies, such as Pulsed Electric Fields (PEF) and High Pressure Homogenisation (HPH), on the structure and the main chemical components of food fluids -liquid egg and dressings-. The microstructure and main chemical changes in the protein and lipid fraction of these foods were analyzed. In addition, several physical properties, such as viscosity, color, and foam capacity of liquid egg treated by PEF were studied. Studies were carried out with samples processed by PEF in the SIK (the Swedish Institute for Food and Biotechnology) and HPH at the Università di Bologna. The samples were also evaluated after a week of refrigerated storage at 4 ºC.

The results show that PEF and HPH treatments, when applied at low intensities, can be considered a potential alternative to conventional heat treatment for the preservation of liquid egg, because of its lower impact on the microstructure and chemical properties if compared with pasteurization. Moreover, PEF treatment has little effect on the functional properties of liquid egg. On the other hand, the physicochemical stability of sauces treated by HPH is directly related to the pressure applied during the process. The loss of the natural emulsifiers, when pressure increases, leads to the coalescence of the fat phase and consequently to the emulsion destabilization. However, the lipid fraction of sauces remains chemically stable after HPH treatment.

ÍNDICE

Introducción 1
Objetivos 49
Plan de trabajo 53
Capítulo 1:
"Effect of Pulsed Electric Fields on the main Chemical Components of Liquid Egg and Stability at 4ºC" 57
Capítulo 2:
"Changes on the Microstructure and Protein Fraction of Whole Liquid Egg treated by Pulsed Electric Fields (PEF)" 67
Capítulo 3:
"Physical and Structural Changes in Liquid Whole Egg treated with High-Intensity Pulsed Electric Fields" 83
Capítulo 4:
"Main Chemical Changes in Proteins and Structure of Egg treated with High Pressure Homogenisation" 107
Capítulo 5:
"Influence of High Pressure Homogenization (HPH) on the Structural Stability of an Egg/Dairy Emulsion" 115
Conclusiones 137
Anexos 141

Introducción

1. NUEVAS TECNOLOGÍAS DE CONSERVACIÓN DE ALIMENTOS

Desde que el hombre comenzó a cultivar plantas y domesticar animales, se encontró con el problema de conservar los alimentos de una estación a otra y conocer las operaciones para transformarlos. A partir de entonces, las necesidades de preservar los alimentos, y la industrialización de los mismos se ha hecho cada vez mayor, respondiendo a la necesidad de alimentos estables que se puedan almacenar y transformar, y se adecuen a las exigentes demandas de una sociedad que evoluciona continuamente (Morata, 2009).

Actualmente, y debido a la evolución y a las nuevas tendencias en los estilos de vida y los hábitos alimentarios, las expectativas del consumidor son cada vez más exigentes. Productos más saludables y con características sensoriales específicas, de alta calidad, nutritivos, naturales y fáciles y rápidos de preparar ("ready to eat"), pero a la vez microbiológicamente seguros y estables, se convierten en la prioridad de la demanda alimentaria.

La industria está desarrollando así nuevos ingredientes, mejorando de forma continua los procesos de elaboración e implementando nuevas tecnologías de procesado, alternativas a los tratamientos térmicos convencionales, que permitan conseguir alimentos seguros pero que conserven las propiedades nutritivas y organolépticas de los alimentos frescos (Gould 2001), adaptándose mejor así al tipo de alimentos demandados actualmente por el consumidor

Es por ello que en las últimas décadas, se ha despertado un creciente interés en las técnicas de preservación no térmicas capaces de inactivar microorganismos y enzimas (Mertens & Knorr, 1992; Barbosa-Cánovas *et al.*, 1998; Raso & Barbosa-Cánovas, 2003). En el procesado no térmico, la temperatura del alimento se mantiene por debajo de la temperatura normalmente utilizada en tratamientos térmicos, por lo que se espera una mínima degradación de su calidad. Sin embargo, estas tecnologías, además de mejorar la calidad de los alimentos, deben igualar o mejorar los niveles de seguridad de los tratamientos convencionales a los que quieren sustituir.

Los nuevos tratamientos no térmicos de conservación de alimentos incluyen técnicas como las Altas Presiones Hidrostáticas, Altas Presiones de

Homogeneización, los Campos Eléctricos Pulsados, Pulsos de Luz, Campos Magnéticos, Irradiación, Ultrasonidos, entre otras, así como tratamientos combinados y nuevas tendencias de envasado.

2. CAMPOS ELÉCTRICOS PULSADOS (PEF)

La primera aplicación de la inactivación de microorganismos mediante PEF data de principios de 1960 (Doevenspeck, 1961). En la actualidad es una de las tecnologías no térmicas de inactivación de microorganismos que está alcanzando más interés comercial y siendo estudiada por más laboratorios a nivel piloto y semiindustrial. Su elevado impacto se debe a que permite conseguir reducciones altas de la carga microbiana de un alimento trabajando a temperaturas bajas que no afectan a la calidad del producto (Morata, 2009).

2.1 LA TÉCNICA DE CAMPOS ELÉCTRICOS PULSADOS

Esta tecnología consiste en la aplicación de campos eléctricos de alta intensidad, generalmente en el intervalo de 20-80 kV/cm, aplicados en forma de pulso cortos (μs) (Jeantet *et al.*, 1999; Señorans *et al.*, 2003). El pulso eléctrico se aplica al producto mediante dos electrodos en la cámara de tratamiento del equipo PEF (Góngora-Nieto *et al.*, 2002). Los pulsos pueden ser de diferentes tipos, principalmente exponenciales decrecientes, ondas cuadradas y oscilantes o sinusoidales (Barbosa-Cánovas *et al.*, 1998 y 1999). Además pueden aplicarse de forma monopolar o bipolar (Figura 1).

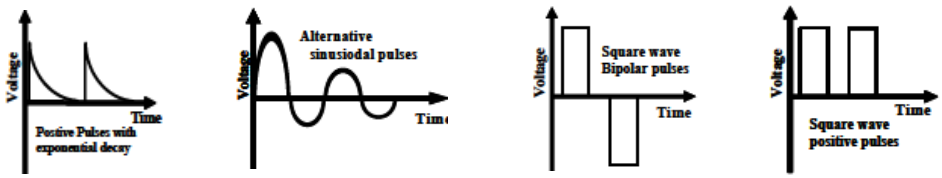


Figura 1. Tipos de pulso (Barbosa-Cábovas, *et al.*, 1998 y 1999).

Los elementos principales de un equipo de PEF son: fuente de potencia eléctrica de alto voltaje, generador de pulsos, cámara(s) de tratamiento, equipo de bombeo para llevar el alimento líquido a la zona de tratamiento, dispositivo de enfriamiento, dispositivo de medida y equipo de control informático (Figura 2) Tanto los diseños industriales como de laboratorio pueden adoptar múltiples configuraciones de flujo en continuo y en discontinuo (Barbosa-Cánovas *et. al.,* 1998 y 1999).

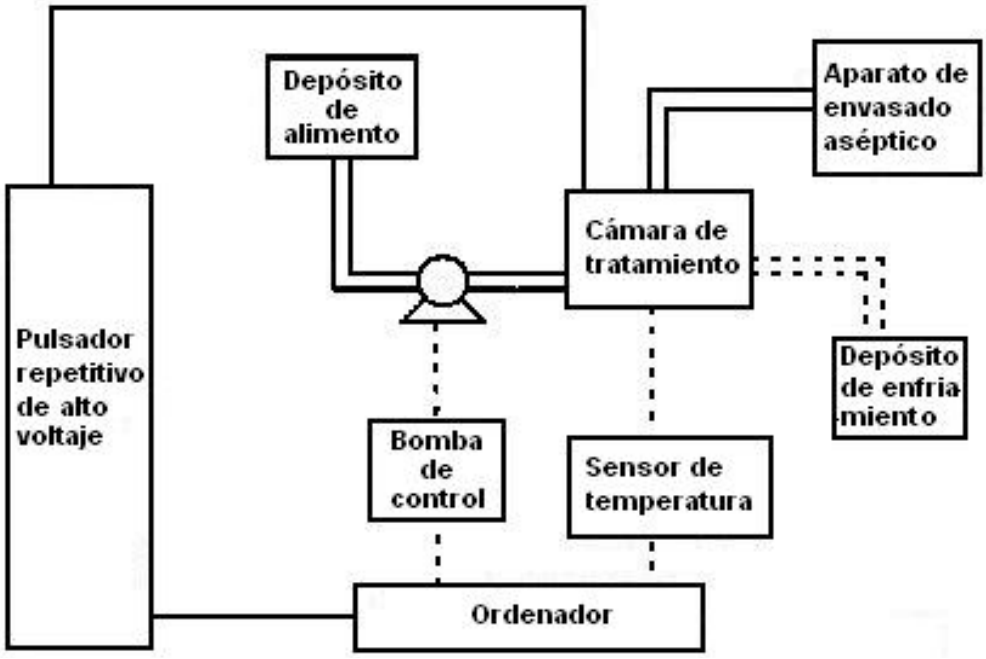


Figura 2. Diagrama esquemático de un proceso de PEF en continuo (Sosa, 2006).

La cámara de tratamiento consiste en al menos dos electrodos colocados en un aislante que forman la cavidad donde el alimento va a recibir los pulsos eléctricos. Los electrodos con los que se aplican los pulsos normalmente estan constituidos por metales inertes como el titanio. Además, esta cámara puede adoptar diferentes diseños y geometrías, tanto estáticas como continuas (Figura 3) (Barbosa-Cánovas *et al.*, 1998).

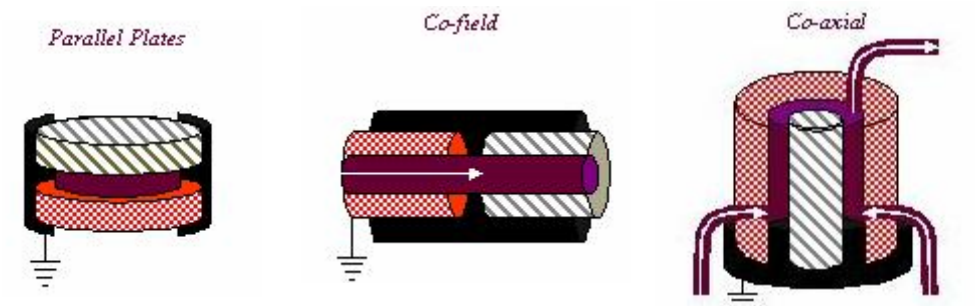


Figura 3. Diseño de cámaras estáticas y continuas de PEF (FDA, 2000).

Después de los tratamientos PEF, los alimentos deben ser envasados asépticamente y almacenarse refrigerados, para prevenir deterioros enzimáticos o germinación de esporas bacterianas.

En esta técnica, el campo eléctrico se utiliza para inactivar microorganismos patógenos y causantes del deterioro de los alimentos. El mecanismo de acción de los pulsos eléctricos es la destrucción de las membranas celulares y la migración del material intercelular. Se han descrito dos mecanismos de acción en la inactivación de microorganismos: la ruptura dieléctrica de la membrana y la electroporación. Los miroorganismos, cuando son sometidos a un campo eléctrico desarrollan poros en la membrana celurar, que pueden ser transitorios o permanentes, dependiendo de la intensidad de campo aplicada y de las condiciones del tratamiento. La formación de poros aumenta la permeabilidad de la membrana, lo que provoca transferencia de material intercelular (pérdida de contenido celular e intrusión del medio). Cuando se supera el potencial crítico transmembrana, se produce la perforación irreversible de la membrana celular, lo que da lugar a la muerte celular (Barbosa-Cánovas y Sepúlveda, 2005; Pagán *et al.*, 2005).

La efectividad del tratamiento PEF depende de los siguientes factores:

- Microorganismo: tipo, tamaño y forma, cantidad inicial y estado de crecimiento.
- Condiciones del tratamiento: intensidad del campo eléctrico, tiempo de tratamiento, energía aplicada, duración, número y forma del pulso, y configuración de la cámara de tratamiento (hueco entre electrodos y volumen).

- Alimento: composición, densidad, viscosidad, calor específico, conductividad eléctrica, fuerza iónica, pH y actividad de agua.

Hay que destacar que la presencia o la formación de burbujas en un alimento es un inconveniente si se quiere aplicar PEF, ya que si el campo eléctrico supera la resistencia dieléctrica de las burbujas de gas, esto puede dar lugar a que se produzcan descargas parciales en las burbujas y se volatilice el líquido aumentando la proporción de burbujas. Estas pueden llegar a ocupar el hueco entre los electrodos y dar lugar a la formación de chispas. También constituye una limitación el tamaño de partícula del alimento al que se aplique el tratamiento, el máximo admisible debe ser menor que el espacio entre electrodos de la cámara de tratamiento.

Por otra parte, son muy importantes la temperatura a la entrada y a la salida de la cámara de tratamiento. En el caso del procesado continuo, se debe considerar también el flujo (laminar o turbulento) y el tiempo de residencia en la cámara de tratamiento.

En cuanto a las características de la utilización de PEF como técnica de conservación de alimentos, en la tabla 1 se describen las principales ventajas e inconvenientes.

Tabla 1: Ventajas e inconvenientes de la aplicación de PEF como técnica de conservación de alimentos (Morata, 2009).

VENTAJAS	INCONVENIENTES
Tratamiento en continuo	Limitado a alimentos líquidos
Tiempos cortos de procesado	Resistentes a PEF
Alta eficiencia energética	Depende de la conductividad
Efectivo contra microorganismos	Baja concentración de sal
Mejora de la vida útil	Limitado tamaño de partícula
Alta calidad organoléptica y nutricional	Ausencia de burbujas de gas
	Esporas no sensibles
Útil para alimentos ácidos	Ineficaz para esterilización
Implementable en tratamientos combinados	Resultados contradictorios con enzimas
Sinergia con temperatura, antimicrobianos y pH	Equipamiento a escala industrial en desarrollo

Con respecto a la viabilidad económica, la tecnología PEF es un proceso más eficiente desde el punto de vista energético que la pasteurización térmica. Existen en la actualidad sistemas de procesado PEF a escala piloto y comercial que pueden procesar entre 1000 y 5000 l/h de alimentos líquidos. Además este equipamiento es facilmente escalable. El principal inconveniente en el procesado de grandes cantidades de alimento de forma económica es la generación de pulsos de alto voltaje que tengan suficiente pico de potencia. Sin embargo, se están desarrollando nuevas tecnologías que permitirán resolver este problema.

En la actualidad, se está investigando también la combinación de PEF con otras tecnologías (tratamientos combinados con antimicrobianos, pH, actividad de agua, temperatura suave…), para incrementar la efectividad de esta técnica no térmica de procesado (ej. para inactivar esporas) y permitir su aplicación a diferentes alimentos líquidos.

Actualmente la tecnología PEF no se utiliza habitualmente para la conservación de alimentos a nivel industrial. Únicamente Genesis Juice Corp (USA) comercializa diferentes zumos de fruta tratados con PEF. Sin embargo sus características la hacen adecuada para prolongar la vida útil de muchos más alimentos sin modificar aparentemente sus características sensoriales y nutricionales.

Las aplicaciones de esta técnica se reducen a alimentos líquidos que puedan ser tratados con campos eléctricos de alta intensidad y que posean una baja conductividad eléctrica, fuerza iónica, y viscosidad, una alta densidad (Ruhlman *et al.*, 2001) y un limitado tamaño de partícula. Los alimentos con una alta conductividad eléctrica reducen la resistencia de la cámara y requieren por tanto mayor energía para conseguir el campo eléctrico adecuado (Heinz *et al.*, 2002).

Esta nueva tecnología ha sido satisfactoriamente utilizada (a escala piloto o de laboratorio) en alimentos líquidos o semilíquidos como zumos de frutas y vegetales, leche y derivados, smoothies (bebida de leche y zumo), huevo líquido y derivados, vino, horchata, salsas y sopas (Butz y Tauscher, 2002; Knorr *et al.*, 2002; Raso y Barbosa-Cánovas, 2003; Devlieghere *et al.*, 2004; Min *et al.*, 2007; Morata, 2009), alterando mínimamente sus propiedades organolépticas.

2.2 EFECTO DE PEF EN LOS ALIMENTOS

2.2.1 Aplicación al procesado de alimentos

Como se ha comentado anteriormente, la tecnología PEF ha sido satisfactoriamente empleada en una gran variedad de alimentos líquidos, principalmente en zumos, leche y huevo líquido. La mayoría de los estudios han demostrado ampliamente la efectividad del tratamiento para inactivar microorganismos patógenos y alterantes en estos alimentos y ciertas enzimas, aumentando su vida útil, y afectando mínimamente a las propiedades fisicoquímicas y sensoriales (Castro *et al.*, 1993; Yeom *et al.*, 2000; Dunn, 2001; Bendicho *et al.*, 2002; Hodgins *et al.*, 2002; Knorr *et al.*, 2002; Ross *et al.*, 2003; Mañas y Pagán 2005; Odriozola-Serrano *et al.*, 2006).

Sin embargo, existen pocos estudios acerca del efecto que ejerce el PEF sobre la composición química, las propiedades físicas y la microestructura de los alimentos.

En cuanto a la sensibilidad de los microorganismos a la técnica de PEF, se ha demostrado que en general las células vegetativas de bacterias Gram+ son más resistentes que las bacterias Gram-, y que las levaduras suelen mostrar una mayor sensibilidad a este tratamiento que las bacterias mientras que parece no ser efectivo contra esporas (Raso y Barbosa-Cánovas, 2003, Devlieghere *et al.*, 2004; Barbosa-Cánovas y Sepúlveda, 2005; Pagán *et al.*, 2005).

El efecto del PEF en la actividad de las enzimas no está tan claro y se han publicado resultados contradictorios (Van Loey *et al.*, 2001; Raso y Barbosa-Cánovas, 2003; Min *et al.*, 2007). Además, la diversidad de equipos de PEF empleados, así como el tipo de enzima y el medio en que se encuentre y las condiciones del tratamiento, limita la comparación entre los diferentes estudios.

Vida útil, Propiedades Físicas y Sensoriales

En general, el tratamiento de alimentos por PEF implica un incremento de temperatura, siempre por debajo del tratamiento térmico convencional, durante un periodo de tiempo muy corto, por lo que no se esperan

modificaciones importantes en las propiedades sensoriales de los mismos.

La viabilidad de PEF para alargar vida útil de alimentos, sin provocar cambios aparentes en sus propiedades o reduciendo los cambios indeseados ocasionados por el calor, ha sido demostrada principalmente en diversos tipos de zumos y leche y sus derivados (Quin *et al.*, 1995a; FDA, 2000; Min *et al.*, 2007; Mosqueda-Melgar *et al.*, 2008).

La aplicación de PEF ha sido ampliamente estudiada en una gran variedad de zumos, principalmente de naranja (Sitzmann, 1995; Zhang *et al.*, 1997), manzana (Simpson *et al.*, 1995; Vega-Mercado *et al.*, 1997; Charles-Rodríguez *et al.*, 2007; Noci *et al.*, 2008), y tomate (Odriozola-Serrano *et al.*, 2009), así como en zumos de arándanos, mandarina, limón y pomelo (Cserhalmi *et al.*, 2006) y mezclas de naranja y zanahoria (Rivas *et al.*, 2006). Los resultados obtenidos indican, en general, una mejora en la calidad del zumo con respecto al tratamiento térmico convencional (Min *et al.*, 2007), ofreciendo productos con una aceptable vida útil, características sensoriales más parecidas al fresco, sin afectar aparentemente, o en menor medida que el tratamiento térmico, a las propiedades fisicoquímicas o atributos de calidad, principalmente color, pH, ºBrix, viscosidad y turbidez.

Con respecto a la calidad y la vida útil de la leche y sus derivados (yogur) tratados por PEF, distintos autores no observaron cambios importantes en sus propiedades fisicoquímicas (pH, color, ºBrix, humedad, tamaño de partícula) o sensoriales (apariencia, color, textura, flavor, aceptabilidad) (Sampedro *et al.*, 2005). Dunn (1995) observó un bajo deterioro organoléptico y ausencia de cambios fisicoquímicos en leche tratada por PEF, y sugirió que el queso, la mantequilla y el helado elaborados a partir de esta leche tenían similares características organolépticas que los elaborados con leche fresca. Quin *et al.* (1995b) trataron leche con PEF sin detectar cambios importantes en sus propiedades fisicoquímicas; el estudio sensorial no mostró diferencias entre la leche tratada por PEF y la pasteurizada. Evrendilek *et al.* (2004) y Yeom *et al.* (2004) evaluaron la calidad y seguridad de una bebida a base de yogur tratada por PEF y no detectaron diferencias significativas en los parámetros de calidad estudiados (color, ºBrix y pH), ni tampoco en los atributos sensoriales. Sampedro *et al.* (2005) realizaron una revisión bibliográfica acerca del efecto del PEF en la leche y sus derivados, concluyendo que era posible obtener alimentos microbiológicamente seguros, con una larga vida útil en

refrigeración, y con un mínimo impacto en la calidad y en los aspectos nutricionales.

Composición y Propiedades Químicas

Actualmente, se conoce poco acerca de los efectos de PEF en los componentes químicos de los alimentos (De Azerêdo *et al.*, 2008). La mayoría de estudios se han llevado a cabo en zumos de fruta y en leche, tratando principalmente el efecto de PEF en las vitaminas y componentes responsables de su aroma y sabor. También existen estudios en proteínas, pero mayoritariamente de forma aislada (Barsotti *et al.*, 2001; Knorr *et al.*, 2011).

En general, los resultados en zumos y leche son prometedores, ya que la mayoría de estudios demuestran la efectividad de PEF en reducir los cambios indeseados en las propiedades nutricionales generados por el tratamiento térmico.

Estudios realizados por diversos autores indican la presencia de mayores contenidos en Vitamina C en zumos tratados por PEF con respecto a los pasteurizados (Sharma *et al.*, 1998; Evrendilek *et al.*, 2000; Min *et al.*, 2003a; Min *et al.*, 2003b; Morata, 2008). Además, otros autores indican que la Vitamina C se retiene en mayor cantidad durante el almacenamiento, debido a una menor velocidad de degradación que en el zumo pasteurizado (Qiu *et al.*, 1998; Yeom *et al.*, 2000; Sánchez-Moreno *et al.*, 2004; Torregrosa *et al.*, 2006; Elez-Martínez *et al.*, 2006; Plaza *et al.*, 2006; Elez-Martínez y Martín-Belloso, 2007; Odriozola-Serrano *et al.*, 2008; Cortés *et al.*, 2008; Qitao-Teixeira *et al.*, 2009).

Resultados similares se obtienen cuando se llevan a cabo estudios en relación al contenido en vitaminas de la leche tratada por PEF. Bendicho *et al.* (2002) no observaron cambios en diferentes vitaminas hidrosolubles (Vitamina B1 y B2 y C) y liposolubles (Vitamina D y E) estudiadas, a excepción de la Vitamina C, aunque su retención fue mayor que en la leche pasteurizada (2002). Grahl y Märkl (1996) describieron que el tratamiento PEF reducía considerablemente el contenido en vitamina C de la leche, mientras que el contenido en vitamina A no se vio afectado. Riener *et al.* (2008) tampoco observaron cambios en los niveles de vitaminas (tiamina, riboflavina, retinol, α-tocoferol) de leche tratada por PEF. Por otra parte,

un estudio con una mezcla de zumo de naranja y leche enriquecida con vitaminas (biotina, acido fólico, pantoténico y riboflavina), confirmó la estabilidad de las vitaminas, después del tratamiento con PEF y durante el almacenamiento (Rivas *et al.*, 2007).

Con respecto al estudio de los principales componentes químicos responsables del color, se ha demostrado que en general, la tecnología estudiada ejerce un efecto mínimo en el contenido de carotenoides o antocianinas de los zumos. Esteve *et al.* (2001) observaron que las pérdidas de carotenos (β-caroteno, α-caroteno, β-criptoxantina y 13-cis-β-caroteno) en zumo de naranja y zanahoria tratado por PEF fueron más bajas que en la pasteurización y su contenido más estable durante el almacenamiento. Resultados similares se obtuvieron en zumo de tomate (licopeno, β-caroteno, fitoflueno) (Odriozola-Serrano *et al.*, 2007, 2008a y 2009a), zanahoria (Qitao-Teixeira *et al.*, 2009) y naranja (Cortés *et al.*, 2006). En cuanto al contenido en antocianos, el zumo de arándanos no se vio afectado por el tratamiento (Jin y Zhang 1999), y la retención de antocianos en zumo de fresa fue mayor que en la pasteurización (Odriozola-Serrano *et al.*, 2008b). Este efecto fue comprobado también en vinos, lo que produjo una mayor intensidad de color (López *et al.*, 2009).

Estudios realizados en los componentes responsables del aroma y sabor de los alimentos, también describen una mejora con respecto al uso de PEF frente a la pasteurización en zumos y leches. Jia *et al.* (1996) observaron que la destrucción de dos de los componentes aromáticos más importantes en zumo de naranja (limoneno y etil butirato) fue menor que durante pasteurización. Otros autores mostraron resultados similares en zumo de naranja (Qiu *et al.*, 1998) y en otros componentes responsables del aroma y el sabor en zumos (etil butirato, α-pineno, mirceno, limoneno, linalol, octanal, decanal y valenceno) (Jia *et al.*, 1999; Yeom *et al.*, 2000). Por otra parte, Sampedro *et al.* (2009) observaron que una bebida a base de zumo y leche conservaba mejor el aroma natural al preservar el contenido en componentes volátiles.

Con respecto al efecto del PEF sobre la capacidad antioxidante de los alimentos, diversos estudios realizados en zumos de fruta y verdura y extractos de té verde demuestran que el tratamiento con PEF mantiene el contenido natural de polifenoles en estos productos sin alterar su capacidad antioxidante (Elez-Martínez y Martín-Belloso, 2007; Schilling *et*

al., 2007; Odriozola-Serrano *et al.*, 2007, 2008a y 2009a; Zhao *et al.*, 2008). Sin embargo, otros autores describen cambios en el contenido fenólico de los zumos tratados por PEF, aunque en menor medida que en el tratamiento térmico, pero con la misma capacidad antioxidante (Plaza *et al.*, 2006; Aguilar-Rosas *et al.*, 2007; Odriozola-Serrano *et al.*, 2008b; Qitao-Teixeira *et al.*, 2009). Es por ello que algunos autores han realizado una evaluación de la influencia de los parámetro críticos del proceso, como frecuencia de pulso, duración y polaridad en estos componentes y en su capacidad antioxidante, para lograr las condiciones de tratamiento óptimo, que permita obtener zumos con un alto valor nutricional (componentes bioactivos) y alto poder antioxidante, limitando el pardeamiento no enzimático y la formación de hidroximetilfurfural (Odriozola-Serrano *et al.*, 2008c y 2009b; Aguiló-Aguayo *et al.*, 2009).

Con respecto a las proteínas, componentes importantes de los alimentos por su alto valor nutricional y propiedades funcionales, existen pocos artículos relacionados con el efecto que el PEF ejerce en estos constituyentes de los alimentos. La mayoría de ellos tratan sobre proteínas en disolución, y no como parte constituyente de los alimentos (Fernández-Díaz *et al.*, 2000; Barsotti *et al.*, 2002; Li *et al.*, 2005; Mañas y Vercet, 2006).

En cuanto a las proteínas del suero de leche, Barsotti *et al.* (2002) no observaron desdoblamiento ni agregación significativa en disoluciones de β-lactoglobulina tratadas por PEF, concluyendo que esta tecnología podría ser utilizada en la pasteurización de preparados industriales de proteína sin alterar sus propiedades funcionales. Sin embargo, Perez y Pilosof (2004) observaron una modificación parcial de la estructura nativa de la β-lactoglobulina (10%) que modificaría alguna de sus propiedades funcionales como la temperatura de desnaturalización térmica y la velocidad de gelificación.

Floury *et al.* (2006) estudiaron el efecto de PEF en las propiedades funcionales de las proteínas de leche desnatada y observaron que las condiciones del proceso afectaban a las micelas de caseína, provocando cambios en la viscosidad y en las propiedades de coagulación de la leche. En este sentido, Yu *et al.* (2009) observaron un aumento en las propiedades de coagulación por cuajo, al comparar el PEF con respecto al tratamiento térmico.

Hasta el momento, hay muy pocos estudios realizados acerca del efecto del PEF en la fracción lipídica de alimentos ricos en grasa. Zeng *et al.* 2010 estudiaron el efecto de PEF sobre la composición en ácidos grasos, la acidez y el índice de peróxidos del aceite de cacahuete y observaron un ligero cambio en la composición del aceite y en el índice de acidez, pero un aumento considerable del índice de peróxidos en el aceite tratado por PEF. Los autores sugirieron que la oxidación lipídica observada podría deberse a reacciones electroquímicas provocadas por el tratamiento con PEF, que influyeron en los componentes estructurales y funcionales. Sin embargo, durante el almacenamiento (100 días a 40 ºC), las muestras tratadas por PEF presentaron una mayor estabilidad química, por lo que se concluyó que el tratamiento permitía controlar la velocidad de oxidación lipídica, extendiendo la vida útil de productos ricos en grasas.

Microestructura

La mayor parte de estudios acerca de la influencia de PEF en los alimentos se centra en alimentos sólidos. En este sentido, existen trabajos que estudian los cambios ocurridos a nivel celular por el tratamiento con PEF en vegetales, que pueden afectar sobre todo a la textura (Knorr *et al.*, 2011). Además, Gudmundsson y Hafsteinsson (2001) estudiaron el efecto de PEF en la textura y microestructura de músculo de salmón, pollo y huevas de pescado, y observaron que incluso bajas intensidades de campo, el tratamiento con PEF afectaba a la microestructura de los mismos, siendo el músculo de salmón el más sensible al tratamiento. Por otra parte, Zhong *et al.* (2009) estudiaron el efecto de PEF en las propiedades fisicoquímicas de suspensiones de almidón de patata. A partir del análisis por Microscopía Electrónica de Barrido (SEM), concluyeron que se producía disociación, desnaturalización y daño de los gránulos de almidón, por reorganizaciones a nivel intragranular, que afectaban a las propiedades fisicoquímicas. Sin embargo, no existen estudios en microestructura de alimentos fluidos.

Sería interesante estudiar el efecto de los tratamientos de conservación no térmicos como PEF sobre la microestructura y la composición química en los sistemas alimenticios, para entender mejor los cambios que se producen en sus propiedades fisicoquímicas, así como su efecto en las propiedades funcionales.

2.2.2 Efecto del PEF en el huevo

El huevo es uno de los alimentos más completo y relativamente económico que tiene disponible el ser humano. Su procesado industrial como huevo líquido ofrece una serie de ventajas, tanto para las empresas de catering como para uso doméstico, como son la conveniencia, ahorro de tiempo, disminución de las necesidades de almacenamiento y evacuación de desechos, la facilidad de dosificación, así como la calidad, estabilidad y uniformidad del producto.

El huevo líquido, y en particular la clara de huevo, son materias primas importantes en la industria alimentaria, por sus propiedades tecnológicas, como su poder espumante y gelificante, así como por el contenido en proteínas de alto valor biológico y nutricional. Es por ello que su producción a escala industrial está aumentando constantemente en los últimos tiempos.

En la actualidad, la pasteurización del huevo procesado industrialmente es obligada para garantizar la destrucción de *Salmonella spp*, minimizar la presencia de microorganismos alterantes (ej: *Pseudomonas flurorescens*) y reunir todos los requerimientos de tipo bacteriológicos. Por lo tanto, los productores de huevo líquido deben proporcionar a los consumidores un producto seguro, que sea comparable en sabor, valor nutritivo y propiedades funcionales al huevo fresco natural (Góngora-Nieto *et al.*, 2001; Ma *et al.*, 2001).

Actualmente, el método de conservación del huevo líquido es la pasteurización (60-64 ºC, 2-5 min) sin embargo, ésta produce efectos indeseados que afectan tanto a sus atributos de calidad (sabor, color, aroma, etc…), como a sus propiedades funcionales (reducción en la capacidad espumante, disminución de la viscosidad, coagulación de las proteínas más termolábiles…). Por ello, las tecnologías no térmicas de conservación, como el PEF, son consideradas prometedoras alternativas a los procesos de pasteurización de huevo líquido y sus derivados (Sampedro *et al.*, 2006). En septiembre de 1996, la Food and Drug Administration (FDA, U.S.A) emitió una “carta de no objeción” para el uso de PEF en huevo líquido.

Existen varios autores que tratan huevo o sus derivados (huevo líquido, clara, yema, o disoluciones modelo de las principales proteínas de la clara)

mediante PEF (Sampedro *et al.*, 2006). También existen trabajos acerca del procesado de este producto con tratamientos combinados de PEF con otras tecnologías de conservación (tratamiento térmico moderado, antimicrobianos, pH…) (Calderón-Miranda *et al.*, 1999; Hermawan *et al.*, 2004; Jeantet *et al.*, 2004; Bazhal *et al.*, 2006; Huang *et al.*, 2006; Amiali *et al.*, 2006 y 2007; Zhao *et al.* 2007; Jin *et al.*, 2009; Pina-Pérez *et al.*, 2009).

La mayoría de trabajos tratan acerca de la inactivación de microorganismos o enzimas y del estudio del efecto del PEF sobre las propiedades físicas y sensoriales del huevo. Sin embargo existen muy pocos trabajos que estudien el efecto del PEF en las propiedades químicas del huevo. Tampoco se conocen estudios del efecto que el PEF tiene sobre la microestructura de este alimento.

Con respecto al efecto del tratamiento PEF sobre la calidad microbiológica el huevo, se ha estudiado principalmente la inactivación de microorganismos tales como *S. enteritidis, L. innocua, E. Coli, P. Fluorescens, A. Lwoffi, C. Freundii, S. liquefaciens, P. Aeruginosa, S. Gallinarum, S. Xylosus y Oligella sp.* (Sampedro *et al.*, 2006; Wesierska y Trziszka, 2007). Los altos niveles de inactivación alcanzados para algunos de los microorganismos patógenos y alterantes, sugiere la posibilidad de utilizar esta técnica para obtener huevo líquido de alta calidad y microbiológicamente seguro, con una larga vida útil en refrigeración.

Por otra parte, también existen estudios acerca del efecto del PEF en la vida útil del huevo y sus derivados, en los cuales se muestra que el PEF, junto con el uso de antimicrobianos y temperaturas moderadas, prolonga la vida útil de estos productos, con respecto a productos no tratados (Dunn *et al.*, 1989; Góngora-Nieto *et al.*, 2003).

En cuanto a la calidad del huevo y sus derivados, varios autores estudian el efecto del PEF en las propiedades físico-químicas como el color, la viscosidad y las propiedades sensoriales (Quin *et al.*, 1995a; Góngora-Nieto *et al.*, 1999; Hermawan *et al.*, 2004). Wesierska y Trziszka (2007) y Zhao *et al.* (2007) evaluaron algunas propiedades funcionales de la clara de huevo tratada por PEF, como la capacidad y estabilidad espumante, no observando diferencias con las muestras no tratadas.

Con respecto al efecto de PEF sobre las proteínas del huevo, existen varios estudios en sistemas modelo (disoluciones de β-lactoglobulina y ovoalbúmina) y en clara de huevo (Jeantet *et al.*, 1999; Fernández-Díaz *et*

al., 2000; Pérez y Pilosof, 2004; Barsotti *et al.*, 2002). Además, estos estudios están centrados principalmente, en los cambios estructurales de las proteínas y sus propiedades gelificantes (Sampedro *et al.*, 2006). La mayoría de los autores coninciden en que la aplicación de PEF no provoca modificaciones estructurales significativas o desnaturalización de proteínas, ni cambios notables en las propiedades funcionales (Jeantet *et al.*, 1999; Fernández-Díaz *et al.*, 2000; Ma *et al.*, 2001; Barsotti *et al.*, 2002). Sin embargo, Pérez y Pilosof (2004) y Zhao *et al.* (2009) observaron por electroforesis una desnaturalización parcial y agregación de las proteínas de la clara del huevo, afectando a sus propiedades funcionales. Por otra parte, Pérez (2002) describió cambios en la textura de geles obtenidos a partir de clara de huevo tratada por PEF.

En resumen, el tratamiento PEF no parece causar cambios notables en las proteínas, pero sí que provoca algunos cambios estructurales y modificaciones en las propiedades funcionales de la clara, afectando a la textura y microestructura de geles, por lo que hay que rediseñar las condiciones del tratamiento para minimizar estos posibles cambios (Sampedro *et al.*, 2006).

En vista de la escasez de estudios sobre el efecto del PEF sobre la microestructura de huevo líquido y su compoción química (sobre todo fracción lipídica), sería interesante estudiar como afectan las condiciones del tratamiento a la microestructura y química de los componentes del huevo líquido, ya que existe una relación entre ésta y su funcionalidad.

3. ALTAS PRESIONES DE HOMOGENEIZACIÓN (HPH)

La técnica de Altas Presiones de Homogeneización (HPH) es considerada una de las alternativas más recientes al tratamiento térmico convencional para la conservación de alimentos fluidos (Guerzoni *et al.* 2002; Guamis *et al.* 2006).

3.1 LA TÉCNICA DE ALTAS PRESIONES DE HOMOGENEIZACIÓN

La HPH se basa en los mismos principios de diseño que la homogeneización convencional (20-50 MPa), utilizada en la industria láctea para reducir el tamaño del glóbulo de grasa (1 a 10 µm), y a la vez, prevenir

el desnatado y la coalescencia de la grasa, incrementando la estabilidad de la emulsión durante el almacenamiento (Paquin, 1999; Vachon *et al.*, 2002; Hayes y Kelly, 2003b; Thiebaud *et al.*, 2003). Sin embargo, la HPH permite alcanzar presiones muy superiores de hasta 350-400 MPa, gracias a los avances tecnológicos obtenidos en el diseño de válvulas y a la utilización de nuevos materiales.

Básicamente, un homogeneizador de HPH consiste en un generador de alta presión, ensamblado a una válvula diseñada especialmente para resistir la aplicación de presiones muy altas. En cualquier tipo de válvula de homogeneización, el fluido procesado pasa a través de una sección convergente llamada espacio de válvula, que es el espacio comprendido entre el cabezal y el asiento de la válvula (Figura 4). Este espacio puede reducirse aplicando más fuerza al cabezal, para aproximarlo al asiento de válvula, lo que incrementa el nivel de presión de tratamiento (Floury *et al.*, 2004a). El fluido es bombeado por un estrecho espacio a través del asiento contra el cabezal de la válvula, produciéndose un choque contra éste y el anillo de impacto, con lo que primero se produce una rápida aceleración, y luego un brusco descenso de presión. A esta caída de presión del líquido en la válvula se le llama presión de homogeneización. Durante el tratamiento de homogeneización por alta presión el fluido se expone a altas presiones durante un corto periodo de tiempo (1-10s).

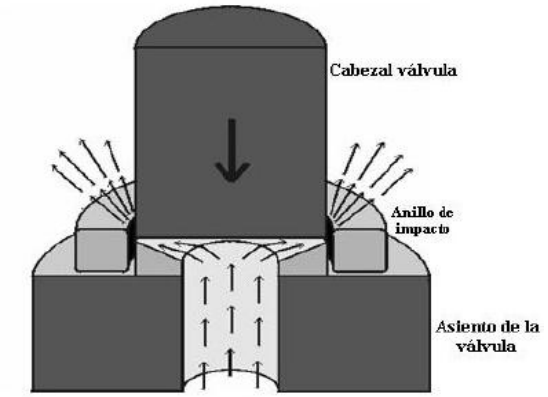


Figura 4. Sección de la válvula de un homogeneizador APV-Gaulin.

Consecuentemente, se produce un fenómeno de cavitación que provoca una gran turbulencia y esfuerzos cortantes (torsión y cizalla) en el seno del producto, lo que supone una reducción en el tamaño de partícula, así como la posible destrucción de tejidos celulares y microorganismos (Popper y Knorr, 1990; Lanciotti *et al*., 1994; Guerzoni *et al*., 1999; Vachon *et al*., 2002; Thiebaud *et al*., 2003; Hayes *et al*., 2005).

Los efectos de HPH en las células bacterianas no se conocen muy bien. La pared celular parece ser el objetivo principal de los daños ocasionados por la aplicación de este tipo de tratamientos, aunque también se produce la inactivación de ciertas enzimas intracelulares (Kheadr *et al*., 2002; Vachon *et al*., 2002). Durante la aplicación de la HPH se produce un incremento de la permeabilidad celular o una ruptura de la membrana, lo que ocasiona una lesión irreversible y la muerte celular. Este mecanismo de inactivación basado en la destrucción mecánica de la integridad de la célula parece estar causado principalmente por la presión, los gradientes de velocidad, turbulencia, choque y cavitación al que están sometidos los líquidos durante las HPH (Guerzoni *et al*., 1999b; Diels *et al*., 2003; Fluory *et al*., 2004b).

Dentro de los factores que afectan la eficacia de los tratamientos por HPH hay que destacar, la temperatura de entrada de la muestra, el tipo de matriz, el nivel de presión y el número de ciclos empleados en el tratamiento.

Además, junto a la válvula principal puede colocarse una válvula de homogeneización secundaria, con una caída de presión significativamente inferior, para romper los aglomerados que pueden formarse en la primera etapa, y que también influye en la eficacia antimicrobiana del tratamiento (Briñez *et al.,* 2006; Pereda *et al.,* 2006).

A pesar de que las HPH son considerada una tecnología no térmica, durante el proceso de alta presión homogeneización se produce un marcado incremento de la temperatura del producto debido: (1) al incremento de presión que ocurre en el intensificador y en la tubería situada antes de entrar a la válvula que genera una compresión del fluido (calentamiento adiabático) y (2) a la energía cinética de las fuerzas de cizalla, cavitación, esfuerzo de corte y turbulencia que sufre el fluido al pasar por la válvula, parte de la cual se convierte en energía térmica.

La rápida presurización del fluido (1) provoca un aumento de temperatura del orden de 3°C/100 MPa, mientras que la caída de presión instantánea en la válvula de homogeneización (2) provoca un aumento de temperatura aún más importante (15-20°C/100 MPa) (Donsì *et al.,* 2009).

Debido al incremento de temperatura durante el tratamiento de HPH, el grado de destrucción microbiana dependerá, por tanto, no solo de la presión aplicada sino también de la temperatura alcanzada. Además, el tratamiento puede aplicarse de forma continua en un solo paso o incluso permitiendo repetir el tratamiento en forma de ciclos hasta alcanzar un nivel de higienización adecuado.

Puesto que la temperatura final del producto tratado puede ser alta, dependiendo de la temperatura de entrada y de la presión de tratamiento, es importante poner un sistema de refrigeración a la salida del proceso para preservar los componentes termolábiles del producto (Figura 5).

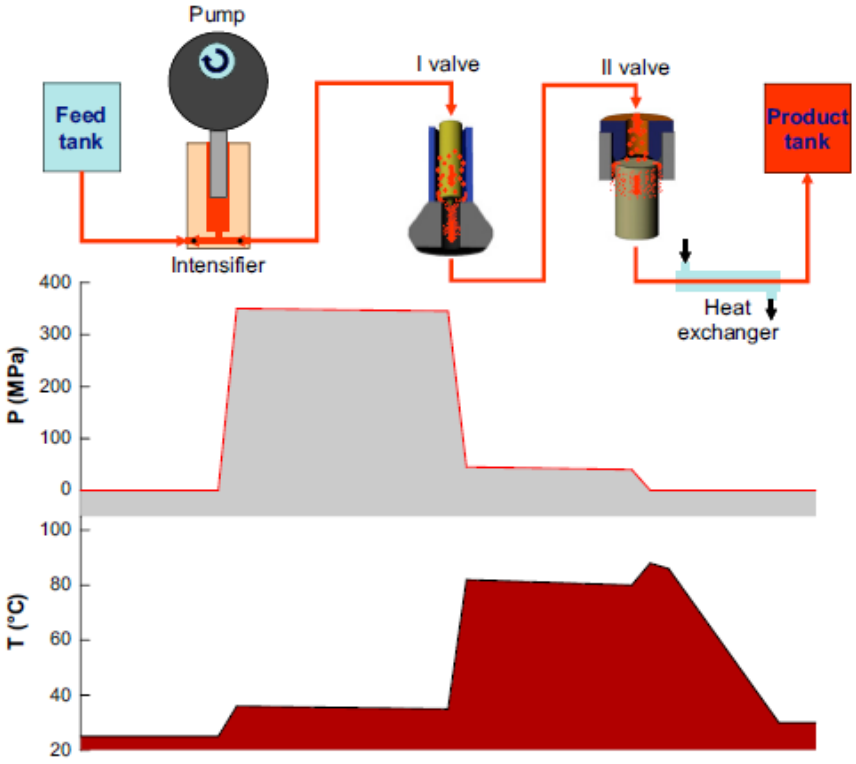


Figura 5. Representación esquemática de un homogeneizador de alta presión de Stansted con una doble válvula de homogeneización (Donsì *et al.,* 2009).

En la actualidad existen diversos fabricantes de equipos de alta presión de homogeneización, entre los que se encuentran: Stansted Fluid Power Ltd (Essex, UK), Avestin (Ottawa, Canadá), Microfluidics International Corporation (Newton, USA), Gea Niro Soavi S.p.A (Parma, Italia), Nanojet-Haskel (Burbank, USA), APV Systems (Unna, Alemania), IKA (Staufen, Alemania), Bee International (South Easton, USA) y FBF Italia (Parma, Italia).

Recientemente, con los últimos avances, se han diseñado equipos capaces de alcanzar presiones de hasta 400 MPa, con volúmenes máximos de 120 l/h, que están siendo utilizados en fase experimental, como el de Stansted y el homogeneizador UHP 4000 bar (Gea Niro Soavi S.p.A). El homogeneizador de Stansted incorpora material cerámico en su válvula, que lo hace capaz de soportar presiones de hasta 400 MPa. Por otra parte, Niro Soavi ha introducido el primer Homogeneizador de Ultra Alta Presión a escala piloto o prototipo piloto capaz de alcanzar una presión máxima de 400 MPa. Está diseñado para procesar productos a alta presión y en condiciones de flujo continuo, permite la reducción del tamaño de partícula a menos de 100 nanómetros en un solo paso.

Tradicionalmente y desde mediados del siglo pasado, la homogeneización ha sido ampliamente utilizada por la industria láctea y alimentaria, especialmente para la estabilización de emulsiones y rotura de los glóbulos de grasa en alimentos líquidos (Desrumaux y Marcand, 2002).

Desde principios de los 90, con los nuevos equipos y diseños que permiten alcanzar presiones muy superiores a los homogeneizadores convencionales, de hasta 300-400 MPa, se están abriendo nuevas áreas de aplicación de gran interés, como la utilización de los procesos de homogeneización para inactivar microorganismos (endógenos) patógenos y causantes del deterioro en alimentos líquidos.

Sin embargo, el interés de esta tecnología emergente no solo reside en su capacidad para inactivar microorganismos patógenos, reteniendo las características nutricionales y sensoriales de los alimentos y manteniendo la vida útil (Kheadr *et al*., 2002; Bríñez, 2008), sino en su potencial para desarrollar nuevos productos con alto valor añadido o con propiedades funcionales interesantes (Tewari y Juneja, 2007). Actualmente se está estudiando su aplicación en alimentos como la leche y derivados, leche de soja, zumos de frutas y otros alimentos líquidos (Corbo *et al*., 2009).

3.2 EFECTO DE HPH EN LOS ALIMENTOS

Como se ha comentado anteriormente, la utilización de las HPH para el tratamiento de alimentos líquidos está ganando importancia durante la última década, no sólo por su capacidad para preparar y estabilizar emulsiones o provocar cambios en las propiedades físicas de los productos, como la viscosidad, sino porque permite inactivar microorganismos y aumentar la vida útil de los alimentos tratados (Knorr *et al.*, 2011).

3.2.1 Aplicación de HPH a algunos alimentos

En la actualidad, el número de trabajos disponibles referentes a la inactivación de microorganismos y a los efectos sobre los componentes y las propiedades de los alimentos como consecuencia de la aplicación de HPH es limitado, sobre todo por ser una tecnología novedosa. Además, la mayoría de ellos se basan principalmente en alimentos líquidos como la leche y los zumos de fruta y verdura.

Debido a la principal aplicación de la homogeneización convencional en la industria alimentaria, es de entender que la mayor parte de la investigación en HPH se haya centrado principalmente en la reducción tanto del tamaño de los glóbulos de grasa como de las micelas de caseína, para prevenir el desnatado y la coalescencia de la leche durante el almacenamiento, así como en la texturización de sus derivados (yogur y queso). Más recientemente, y debido a la aplicación de presiones superiores, se está estudiando su aplicación en la higienización no térmica de la leche para extender su vida útil, preservando los compuestos termolábiles, y evitando así los efectos indeseados, tales como la generación los sabores extraños, el pardeamiento no enzimático y la desnaturalización de ciertas vitaminas y proteínas (Pereda *et al.*, 2009). La técnica de HPH en semicontinuo ha sido propuesta como técnica no térmica de descontaminación de alimentos desde 1990. Su efectividad en microorganismos patógenos y causantes del deterioro (entre ellos, *Listeria monocytogenes, Yersinia enterocolitica, Salmonella spp., Staphylococcus aureus, Escherichia coli,* y muchos más) en sistemas modelo y alimentos reales, como leche, zumos y huevo, ha sido estudiado por varios autores (Lanciotti *et al.*, 1994 y 1996; Fantin *et al.*, 1996; Guerzoni *et al.*, 1997,

1999a y 2002; Vachon *et al.*, 2002; Diels *et al.*, 2003; Thiebaud *et al.*, 2003; Smiddy *et al.*, 2007; Corbo *et al.*, 2009).

Existen varios estudios que indican que las bacterias Gram- son más sensibles a las HPH que las Gram+ (Popper y Knorr, 1990; (Wuytack *et al.*, 2002). Las levaduras y los mohos, presentan una resistencia intermedia entre las bacterias Gram+ y las Gram-, mientras que las esporas parecen ser las más resistentes a las HPH (Bevilacqua *et al.*, 2007; Chavez-López *et al.*, 2009).

La presión y la temperatura del proceso son los principales parámetros que afectan a la eficacia del tratamiento, siendo ésta mayor al aumentar la presión, el número de ciclos, y la temperatura de entrada del producto (Vachon *et al*., 2002; Diels *et al.*, 2003; Thiebaud *et al*., 2003; Briñez *et al.*, 2006; Picart *et al.*, 2006; Pereda *et al.*, 2007). Además, el incremento de temperatura alcanzado por el producto después de pasar por la válvula también puede tener un efecto destructivo sobre la inactivación microbiana (Hayes et al., 2005).

La composición del medio afecta a la eficacia del tratamiento HPH principalmente debido a las interacciones de los constituyentes del medio con los microorganismos. Por ejemplo, algunos estudios en leche sugieren que la grasa puede ejercer cierta protección frente al tratamiento HPH, mientras que otros (Briñez *et al.*, 2006; Roig-Sagués *et al.*, 2009) deducen que el contenido en grasa aumenta la eficacia del tratamiento y durante el almacenamiento. Otro parámetro importante a tener en consideración que depende de la matriz, es la viscosidad. Varios autores también muestran que la inactivación de microorganismos se ve inversamente afectada por la viscosidad del producto, lo que podría explicarse por una menor cavitación y turbulencia en fluidos viscosos. (Diels *et al.*, 2003, 2004 y 2005; Picart *et al.*, 2006).

Con respecto al efecto de HPH sobre las enzimas de alimentos, los estudios son limitados. Hasta el momento únicamente se ha estudiado el efecto (térmico y presión) de las HPH sobre las enzimas de la leche y la pectinmetilesterasa del zumo de naranja (Hayes y Kelly, 2003a; Datta *et al.*, 2005; Lacroix *et al.*, 2005; Lanciotti *et al.*, 2007a).

Mientras que algunos autores han centrado sus estudios en la inactivación de microorganismos y enzimas, otros han llevado a cabo trabajos de investigación acerca del efecto de HPH sobre los lípidos, las

proteínas de la leche y polisacáridos, así como sus propiedades fisicoquímicas y funcionales en algunos alimentos (Paquin, 1999). Sin embargo, la mayoría de trabajos se centran en la leche y sus derivados.

Efectos físicos y químicos en la fracción lipídica

Con respecto al efecto de HPH en los glóbulos de grasa de la leche, existen muchos estudios que demuestran, en general, una reducción en el diámetro del glóbulo de grasa, así como una distribución de tamaños más homogénea, a medida que aumenta la presión aplicada (hasta 200 MPa), limitando así la velocidad de separación de fases o desnatado (Kietczewska *et al.,* 2003; Hayes y Kelly, 2003b y 2005; Picart *et al.*, 2006). Además, esta disminución de tamaño se ve afectada por la temperatura de entrada (Datta *et al.,* 2005) y la aplicación de un segundo ciclo de homogeneización (Thiebaud *et al.*, 2003; Hayes y Kelly, 2003b). Por otra parte, durante la homogeneización, se altera también la composición de la membrana nativa de los glóbulos de grasa por adsorción principalmente de caseínas y proteínas del suero en la interfase (Cano-Ruiz y Richter, 1997; Michalski y Januel, 2006).

Sin embargo, se ha observado que a partir de una determinada presión (>250 MPa, 300 MPa), se produce la coalescencia de los glóbulos de grasa, posiblemente debida a la mayor superficie de exposición grasa e insuficiente cantidad de proteína (principalmente caseínas) para estabilizarla, a que se comparten constituyentes proteicos entre distintos glóbulos lo que provoca la aglomeración de los mismos o al efecto de HPH sobre las proteínas que perjudica sus propiedades emulsionantes (Thiebaud *et al.*, 2003; Hayes *et al.*, 2005; Pereda *et al.*, 2007).

Por otra parte, al reducirse el tamaño del glóbulo de grasa y aumentar su área superficial, junto con el aumento de temperatura asociado al tratamiento, podría incrementarse la susceptibilidad de la grasa de la leche a la lipólisis y la oxidación lipídica, por acción de las enzimas de la leche (Hayes y Kelly, 2003b y 2005; Pereda *et al.*, 2008a y 2008b), con la consecuente disminución de pH del medio. Pereda *et al.* (2008a) observaron una mayor lipólisis en leche tratada a presiones más bajas (200 MPa) por la actividad lipolítica residual, mientras que la oxidación lipídica fue mayor a 300 MPa, posiblemente debido a la temperatura alcanzada

(100ºC). Sin embargo, la formación de compuestos volátiles indeseados es menor que en la leche comercial (Pereda *et al.*, 2008b).

Este efecto en el aumento de la actividad lipolítica también ha sido observado en quesos, yogures y leche fermentada con propiedades probióticas, elaborados con leche tratada por HPH (Guerzoni *et al.*,1999b; Lanciotti *et al.*, 2004a, 2006 y 2007b; Vannini *et al.*, 2008; Serra *et al.*, 2008a), modificando el perfil de volátiles y sus propiedades sensoriales, lo que puede afectar a las características organolépticas del producto final.

Efectos físicos y químicos en proteínas

Con respecto al efecto de HPH en las proteínas de alimentos, se ha estudiado principalmente la proteína de la leche. También existen algunos estudios en proteína de leche de soja.

En general, la técnica de HPH es capaz de modificar las propiedades estructurales de la micela de caseína, disminuyendo el tamaño de la micela de caseína sobre todo a altas presiones e incrementando el número de ciclos (Hayes y Kelly, 2003b; Sandra y Dalgleish, 2005; Roach y Harte 2008). La reducción del tamaño en las micelas de caseína también se ha observado en queso y en yogur elaborados con leche tratada por HPH (Kheadr *et al.*, 2002; Lanciotti *et al.*, 2004b). Sin embargo, a presiones superiores a los 300 MPa, las micelas de caseína forman grandes agregados, que posteriormente precipitan (Roach y Harte, 2008).

Además, el tratamiento con HPH también genera un aumento en la susceptibilidad de las caseínas a la proteólisis, debido a cambios conformacionales en su estructura secundaria inducidos por la presión y a la diferente exposición de las macromoléculas a la actividad enzimática principalmente de la plasmina, así como a una mejora de la actividad proteolítica de determinadas cepas debido al tratamiento, lo que se atribuye a un aumento de la liberación de proteasas intracelulares o de la pared celular y/o a una mejora de sus actividades por HPH (Lanciotti *et al.,* 2007a). Estos resultados han sido demostrados también en quesos y yogures elaborados con leche tratada por HPH (Guerzoni *et al.,* 1999b; Lanciotti *et al.,* 2006 y 2007b; Vannini *et al.,* 2008; Burns *et al.,* 2008; Serra *et al.,* 2009a). Sin embargo, el grado de proteólisis disminuye al aumentar la presión de tratamiento, debido a una mayor inactivación de las enzimas

nativas y microbianas presentes en la leche (Lanciotti *et al.,* 2004a; Pereda *et al.,* 2008c).

Por otra parte, la aplicación de HPH a presiones iguales o inferiores a 200 MPa, no parece desnaturalizar las proteínas del suero (Hayes y Kelly, 2003b; Paquin *et al.,* 2003; Sandra y Dalgleish, 2005). Sin embargo, otros estudios han descrito la desnaturalización de las proteínas del suero de la leche, a presiones superiores, siendo mayor en β-lactoglobulina que en α-lactoalbúmina, y aumentando con la presión aplicada (Hayes *et al.,* 2005; Datta *et al.,* 2005). Además, Grácia-Juliá *et al.* (2008) han observado que las proteínas del suero forman agregados a presiones superiores a 250 MPa, mediante interacciones hidrofóbicas.

En cuanto a las proteínas de la soja, Floury *et al.* (2002a) observaron la desnaturalización y agregación de la globulina 11S, a presiones superiores a 150 MPa. La estructura de la proteína se modificó por exposición de los grupos hidrofóbicos y creación de nuevos grupos sulfhidrilo y/o formación o intercambio de puentes disulfuro, debido a las fuerzas mecánicas y a las altas temperaturas alcanzadas durante el tratamiento. Cruz *et al.* (2007) obtuvieron resultados similares con respecto a la desnaturalización y agregación de las proteínas de leche de soja, pero a presiones superiores.

Efecto en polisacáridos

Estudios recientes han demostrado que las HPH pueden romper enlaces covalentes (disrupción irreversible del polímero), disminuyendo el peso molecular medio de polisacáridos como la pectina (Lacroix *et al.,* 2005), xantana y metilcelulosa (Lagoueyete y Paquin, 1998; Fluory *et al.,* 2002b), afectando a sus propiedades funcionales.

Efecto en propiedades fisicoquímicas y funcionales

La aplicación de HPH, debido a su efecto en los glóbulos de grasa y las proteínas, puede modificar las propiedades físicas, reológicas, emulsionantes y de coagulación de la leche (Popper y Knorr, 1990; Thiebaud *et al*., 2003). La técnica de HPH puede utilizarse como una herramienta para modificar aspectos funcionales de las proteínas en leche,

debido a cambios en la conformación tridimensional definida por su estructura terciaria y cuaternaria (Roach y Harte, 2008).

Las HPH pueden ocasionar cambios en los parámetros de color de la leche sobretodo en la luminosidad como consecuencia de los efectos producidos sobre los glóbulos de grasa y las caseínas (Hayes y Kelly, 2003b; Hayes *et al.*, 2005; Pereda *et al.,* 2007).

Algunos autores han observado que los tratamientos de leche por HPH causan una disminución de la viscosidad con respecto a la leche no tratada, pero que ésta aumenta con la presión de tratamiento. Los agregados de las micelas de caseína y glóbulos de grasa, y la desnaturalización de las proteínas del suero ocasionados por el tratamiento a 300 MPa, pueden ser los causantes del aumento de la viscosidad con respecto al tratamiento a 200 MPa (Pereda *et al.,* 2007).

Por otra parte, Bouaouina *et al.* (2006) observaron una mejora en la capacidad y estabilidad espumante de las proteínas del suero tratadas por HPH debido al aumento de la hidrofobicidad superficial de las proteínas.

Otros autores evalúan el efecto de HPH en las propiedades de la leche para la elaboración de queso y yogur (Lanciotti *et al.,* 2004a y 2004b) encontrando resultados interesantes en cuanto a la velocidad de coagulación, capacidad de retención de agua, firmeza del gel y textura (Guerzoni *et al.*, 1999b; Kheadr *et al.*, 2002; Lanciotti *et al.*, 2004b, 2006 y 2007b; Zamora *et al.,* 2007; Burns *et al.,* 2008; Hernández y Harte, 2008; Vannini *et al.*, 2008; Serra *et al.*, 2007, 2008b y 2009b).

Además, Patrignani *et al.* (2007 y 2009a) demostraron la idoneidad de las HPH para la producción de leche fermentada probiótica con *Lactobacillus paracasei* y *acidophilus*, con mejores características de textura, consistencia, cohesividad y viscosidad, y una alta valoración sensorial. También se ha estudiado el tratamiento de leche con HPH para la elaboración de helado, observándose una mejora de las propiedades viscoelásticas y la viscosidad aparente, lo que permite obtener helados de bajo contenido en grasa con características similares a los de mayor contenido en grasa (Innocente *et al.*, 2009).

Por todo esto, el tratamiento de leche mediante HPH puede tener un gran potencial como técnica para la obtención y diferenciación de nuevos tipos de queso, yogur y leches fermentadas probióticas, con diferentes características sensoriales, reológicas y nutricionales, y diferentes

propiedades funcionales, con respecto a los productos obtenidos tradicionalmente, sin afectar a su seguridad ni vida útil (Kheadr *et al.*, 2002).

También se ha observado un aumento en la estabilidad de leche de soja tratada por HPH, y una mayor viscosidad comparada con las muestras tratadas térmicamente (Cruz *et al.*, 2007). Además, se ha evaluado también su aplicación a la elaboración de yogur de soja, obteniéndose yogures con una mayor firmeza y capacidad de retención de agua, que los elaborados con soja tratada térmicamente (Cruz *et al.*, 2009; Ferragut *et al.*, 2009).

Por otra parte, Floury *et al.* (2002a) han demostrado una mejora en la capacidad emulsionante de la proteína de soja globulina 11S a presiones por encima de 200 MPa, debido a las modificaciones en la estructura macromolecular de la proteína (desdoblamiento y agregación), aumentando las interacciones hidrofóbicas proteína-proteína.

Microestructura

Dado que la técnica de HPH puede afectar a los constituyentes de los alimentos, principalmente proteínas, grasas y polisacáridos, varios estudios tienen en consideración, por tanto, la posible modificación de su microestructura, principalmente mediante el estudio con técnicas como Microscopía Óptica, Microscopía Electrónica de Barrido, Microscopía Electrónica de Transmisión y Microscopía Láser Confocal. Algunos estudios muestran el efecto de HPH en la homogeneidad de la estructura de quesos (Guerzoni *et al.*, 1999b; Lanciotti *et al.,* 2006) y geles de leche cuajada (Zamora *et al.*, 2007). Otros autores estudian el efecto de HPH sobre la matriz proteica y los glóbulos de grasa de yogur (Ferragut *et al.*, 2009; Serra *et al.*, 2009b). También existen estudios microestructurales acerca del efecto de HPH en el tamaño de los glóbulos de grasa y las micelas de caseína en leche (Sandra y Dalgleish, 2005; Cruz *et al.*, 2007).

En este sentido, Kheadr et al. (2002) estudiaron por Microscopía Electrónica de Transmisión (TEM) el efecto de las HPH en la microestructura de queso Cheddar, observando una matriz de caseínas más compacta y regular, en la que pequeños glóbulos de grasa se encontraban inmersos.

Por otra parte, Wang *et al.* (2008) estudiaron disoluciones de almidón de maíz por microscopía óptica y observaron un aumento en el tamaño de gránulo de almidón, atribuido a la gelatinización parcial y a la agregación de los gránulos tras el tratamiento por HPH.

Zumos

Aunque la mayoría de trabajos de HPH están centrados en leche y sus derivados, debido al potencial ya demostrado de esta técnica, se está estudiando recientemente su aplicación en zumos.

Existen varios estudios acerca de la inactivación de microorganismos y enzimas en zumos de fruta y verdura, como naranja, manzana, albaricoque, zanahoria (Campos y Cristianini, 2007; Patrignani *et al.*, 2009b; Welti-Chanes *et al.*, 2009; Suárez-Jacobo *et al.*, 2010). También se ha estudiado el efecto sinérgico del tratamiento con HPH junto con la adición de antimicrobianos, como nisina y quitosano (Pathanibul *et al.*, 2009; Kumar *et al.*, 2009). Además, otros autores (Lacroix *et al.*, 2005) han estudiado la estabilidad de la "nube" de zumo de naranja tratado por HPH, observando que no sólo depende de la actividad de la PME, sino también de la reducción del tamaño de partícula en suspensión y de los cambios estructurales de la pectina debido al tratamiento.

Los resultados obtenidos en estos estudios muestran que las HPH suponen una prometedora alternativa no térmica en la conservación de zumos, ya que permite reducir la carga microbiana y aumentar su vida útil, preservando los atributos de frescura y reduciendo así el daño térmico ocasionado en zumos por el tratamiento térmico de pasteurización convencional (Nielsen *et al.*, 2009; Suárez-Jacobo *et al.*, 2010). Esta técnica además, permite modificar las propiedades físicas, tales como la viscosidad. Patrignani *et al.* (2009b) observaron un aumento de viscosidad de zumo de albaricoque, atribuido al efecto de HPH en la conformación y agregación de proteínas, así como a su interacción con otros componentes, como grasa o polisacáridos.

3.2.2 Efecto de HPH en huevo líquido y en salsas

La eficacia de las HPH como método de conservación ha sido ampliamente estudiada en leche y zumos, sin embargo existen muy pocos estudios en otros alimentos como el huevo. La importancia del estudio de la aplicación de tratamientos no térmicos de conservación como HPH en huevo reside en la susceptibilidad de sus proteínas a la coagulación a altas temperaturas, y al interés industrial de este alimento como ingrediente multifuncional y de alto valor nutricional.

Algunos autores han estudiado la eficacia de las HPH para la inactivación de microorganismos, principalmente *Salmonella enteritidis y Listeria monocytogenes*, en huevo líquido y productos a base de huevo, observando un efecto sinérgico con las enzimas antimicrobianas presentes en el mismo (Guerzoni *et al.*, 2002; Lanciotti *et al.*, 2008). Por su parte, Velázquez-Estrada *et al.* (2008) concluyen que la aplicación de HPH puede ser una prometedora alternativa a la pasteurización térmica para garantizar la seguridad del huevo líquido, a falta de la evaluación de esta tecnología en sus propiedades tecnológicas y sensoriales. Sin embargo, no existen prácticamente estudios sobre el efecto de esta tecnología en la composición, propiedades fisicoquímicas y sensoriales del huevo, así como en sus propiedades tecnológicas, lo que hace necesario su estudio. Únicamente Sirvente *et al.* (2007) estudiaron el efecto de las HPH en la estructura, propiedades fisicoquímicas como la solubilidad y reología y las propiedades emulsionantes de dispersiones de yema de huevo, a presiones de homogeneización de hasta 20 MPa, observando un descenso en la solubilidad de proteínas y un aumento en la viscosidad, especialmente en la fracción del plasma. Además, el tratamiento a estas presiones rompió las partículas de lipoproteínas de baja densidad y formó agregados entre las proteínas liberadas y las livetinas del plasma, pero no afectó apenas a la microestructura de los gránulos. Sin embargo, estas modificaciones observadas no ocasionaron cambios importantes en las propiedades emulsionantes.

Por otra parte, el huevo líquido presenta un gran interés como ingrediente en gran variedad de emulsiones de alimentos, debido a las excelentes propiedades emulsionantes de la yema y a sus características organolépticas. Sin embargo, la aplicación de tratamiento térmico produce

desnaturalización de proteínas, y agregación de las gotas de grasa, por lo que se suelen realizar tratamientos térmicos suaves que no aseguran la total eliminación de la flora microbiana, lo que restringe su vida útil. Consecuentemente, estas emulsiones tienen que mantenerse en refrigeración a 4ºC. Por esto, sería interesante la aplicación de una técnica como HPH que permita en un solo paso la estabilización y conservación emulsiones a base de huevo líquido.

La homogeneización convencional se ha utilizado desde siempre como tecnología para la preparación y estabilización de emulsiones en la industria alimentaria, cosmética y farmacéutica, mediante la disminución del tamaño de gota. Sin embargo, la aplicación de presiones superiores mediante HPH permite crear emulsiones mucho más finas, modificando no solo los glóbulos de grasa sino también los otros constituyentes, como proteínas y polisacáridos (Paquin, 1999), así como sus propiedades reológicas y microestructurales (Guerzoni *et al.*, 1997; Floury *et al.*, 2000), y con el potencial añadido de poder mejorar la seguridad microbiológica y su vida útil (Cortés-Muñoz *et al.*, 2009).

Existen varios estudios acerca del efecto de la técnica de HPH en la estabilidad y comportamiento reológico de emulsiones modelo, preparadas principalmente a base de aceites vegetales y usando como emulsionantes proteínas del suero lácteo, proteína de soja o metilcelulosa. Sin embargo, el efecto de la presión de homogeneización es complicado (Desrumaux y Marcand, 2002), y los resultados no están del todo claros, ya que pueden variar además de con la presión y el número de ciclos de homogeneización, con la composición ó proporción de los diferentes componentes de la emulsión, así como con la temperatura de entrada y salida al tratamiento (Paquin *et al.*, 1999; Yuan, *et al.* 2008).

En general, el tamaño de gota disminuye al aumentar la presión, y suele ser estable hasta un determinado nivel de tratamiento, a partir del cual se produce coalescencia de las gotas de grasa, con la consecuente desestabilización de la emulsión (Agboola *et al.*, 1998). La mayoría de investigaciones señalan que la estabilidad de las emulsiones depende, además del tamaño de gota, de la cantidad y estado de las moléculas que ejercen de emulsionantes, principalmente proteínas, ya que también se ven afectadas por el tratamiento con HPH. A partir de una determinada presión, puede producirse una desnaturalización de parte de las proteínas,

lo que puede afectar a sus propiedades emulsionantes (Floury *et al.*, 2000, 2002a, 2202b y 2003; Desrumaux y Marcand, 2002).

Otros autores asocian los cambios observados en las propiedades reológicas y la estructura de emulsiones estabilizadas con proteína y preparadas por HPH a un fenómeno de floculación (Kim *et al.*, 2002; Roesch y Corredig, 2003). Floury *et al.* (2002a) observaron cambios en el comportamiento reológico de emulsiones estabilizadas con proteína de soja, al aumentar la presión de homogeneización, indicando un amplio fenómeno de floculación en la emulsión, pero manteniendo constante el índice de coalescencia en todo el intervalo de presiones estudiadas y disminuyendo la velocidad de descremado. Se ha descrito que este efecto de floculación se debe a la agregación de las proteínas de la fase acuosa desnaturalizadas por la presión y su interacción con las proteínas desnaturalizadas adsorbidas en la superficie de los glóbulos de grasa, o interacción entre las proteínas que rodean gotas contiguas, formando una emulsión con estructura tipo gel (Dickinson, 1997; Dickinson y James, 1998; Martin-González *et al.*, 2009).

Por otra parte, algunos estudios demuestran que la estabilidad de las emulsiones tratadas por HPH no sólo depende de su efecto sobre la disminución del tamaño de gota, sino también sobre la capa de proteínas interfacial que se forma alrededor de las mismas para estabilizarlas, que es más rígida y compacta debido a las interacciones proteína-proteína inducidas la alta presión (Lee *et al.*, 2007 y 2009).

En cuanto a emulsiones alimentarias reales, como las salsas, no existen apenas estudios acerca de la aplicación de HPH como técnica no térmica de conservación de alimentos. Únicamente Lanciotti *et al.* (1994) evaluaron la eficacia de la aplicación de HPH, en la viabilidad de microorganismos patógenos y alterantes como *Listeria monocytogenes, Yersinia enterocolitica, Bacillus subtilis y Yarrowia lipolitica* en salsas (25% agua, 53% aceite, 22% yema de huevo, 1% NaCl, 3% goma xantana, 0.1% ácido acético). Los resultados obtenidos mostraron un aumento en la seguridad microbiológica y la vida útil de las salsas debido al tratamiento. Por otra parte, Guerzoni et al (2002) estudiaron el efecto de las HPH (20-50 MPa) en *Salmonella enteritidis*, en sistemas reales tipo mayonesa a base de huevo (10% yema, 13% yogur y 60% aceite girasol), y observaron una pérdida de la viabilidad de las células que dependía del pH y la cantidad de NaCl, así

como de la actividad de las enzimas antimicrobianas presentes en el huevo, que puede verse aumentada con la presión del tratamiento.

Con respecto a la estabilidad de salsas, Christiansen *et al.* (2004) observaron que las propiedades reológicas y la microestructura de diversas salsas, estabilizadas con diferentes proteínas del suero lácteo, y preparadas por HPH (70 kPa), dependía de la temperatura y composición de las mismas (aceite, proteína, pH, NaCl, $CaCl_2$ y sacarosa), así como del tipo de proteína utilizada para estabilizarla. Sin embargo, a pesar de la gran variedad de microestructuras observadas, y por tanto de comportamientos reológicos, todas las emulsiones fueron estables y no se observó en ningún caso separación del aceite. Además, en un estudio posterior, observaron que la cantidad de aceite y el tipo de proteína fue lo que más afectó a las propiedades sensoriales (sabor, olor y textura), mientras que el tipo de proteína y la adición de NaCl afectó principalmente a la microestructura de estas salsas (Christiansen *et al.* 2006).

Por otra parte Martinet *et al.* (2005) observaron la preferencia de adsorción de proteínas de huevo, frente a las de la leche, en la interfase aceite/agua de emulsiones tipo mousse a base de leche, huevo entero, sacarosa, lactosa, almidón y estabilizantes preparadas por HPH.

Sin embargo, no existen estudios acerca del efecto de HPH sobre los componentes químicos de emulsiones estabilizadas con huevo líquido. Esto hace interesante su estudio de cara a futuras aplicaciones industriales.

4. BIBLIOGRAFÍA

FDA (2000) U.S. Food and Drug Administration.

Agboola, S. O., Singh, H., Munro, P. A., Dalgleish, D. G., & Singh, A. M. (1998). Destabilization of oil-in-water emulsions using highly hydrolized whey proteins. *Journal of Agricultural and Food Chemistry*, 46: 84-90.

Aguilar-Rosas, S. F., Ballinas-Casarrubias, M. L., Nevarez-Moorillon, G. V., Martin-Belloso, O., & Ortega-Rivas, E. (2007). Thermal and pulsed electric fields pasteurization of apple juice: effects on physicochemical properties and flavour compounds. *Journal of Food Engineering*, 83: 41-46.

Aguiló-Aguayo, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Avoiding non-enzymatic browning by high-intensity pulsed electric fields in strawberry, tomato y watermelon juices. *Journal of Food Engineering*, 92: 37-43.

Amiali, M., Ngadi, M. O., Raghavan, V. G., & Smith, J. P. (2004). Inactivation of *Escherichia Coli O157:H7* in liquid dialyzed egg using pulsed electric fields. *Trans IChemE, Part C, Food and Bioproducts Porcessing*, 82(C2): 151-156.

Amiali, M., Ngadi, M. O., Smith, J. P., & Raghavan, V. G. (2006). Inactivation of *Escherichia coli O157:H7 and Salmonella enteritidis* in liquid egg white using pulsed electric field. *Food Microbiology and Safety*, 71(3): M88-M94.

Amiali, M., Ngadi, M. O., Smith, J. P., & Raghavan, G. S. (2007). Synergistic effect of temperature and pulsed electric field on inactivation of *Escherichia coli O157:H7 and Salmonella esteritidis* in liquid egg yolk. *Journal of Food Engineering*, 79: 689-694.

Barbosa-Cánovas, G. V., Pothakamury, U. R., Palou, E., & Swanson, B. G. (1998). Emerging Technologies in Food Preservation. En *Nonthermal Preservation of Foods.* Barbosa-Cánovas, G. V., Pothakamury, U. R., Palou, E., & Swanson, B. G., Ed. Marcel Dekker, Inc. Nueva York, 1-7; 53-71.

Barbosa-Cábovas, G. V., Góngora-Nieto, M. M., Pothakumary, U. R., & Swanson, B. G. (1999). *Preservation of foods with pulsed electric fields.* Food Science and Technology, International Series, Taylor S. L., Ed. Academic Press. San Diego, 1-46.

Barbosa-Cánovas, G. V., & Sepúlveda, D. (2005). Present status and the future of PEF technology. En *Novel Food Processing Technologies*. Barbosa-Cánovas, G. V., Tapia, M. S. & Cano, M. P., Ed. CRC Press. Florida, 1-44.

Barsotti, L., Dumay, E., Mu, T., Fernández-Díaz, M. D., & Cheftel, J. C. (2002). Effects of high voltage electric pulses on protein-based food constituents and structures. *Trends in Food Science & Technology*, 12: 136-144.

Bazhal, M. I., Ngadi, M. O., Raghavan, G. S., & Smith, J. P. (2006). Inactivation of *Escherichia coli O157:H7* in liquid whole egg using combined pulsed electric fields and thermal treatments. *LWT- Food Science and technology*, 39: 419-425.

Bendicho, S., Espachs, A., Arántegui, J., & Martín, O. (2002). Efect of high intesity pulsed electric field and heat treatments on vitamins of milk. *Journal of Dairy Research*, 69:113-123.

Bevilacqua, A., Cibelli, F., Corbo, M. R., & Sinigaglia, M. (2007). Effects of high-pressure homogenization on the survival of *Alicyclobacillus acidoterrestris* in a laboratory medium. *Letters in Applied Microbiology*, 45: 382-386.

Bouaouina, H., Desrumaux, A., Loisel, C., & Legrand, J. (2006). Functional properties of whey proteins as affected by dinamic high-pressure treatment. *International Dairy Journal*, 16: 275-284.

Briñez, W. J., Roig-Sagués, A. X., Hernández-Herrero, M. M., & Guamis-López, B. (2006). Inactivation of two strains of *Escherichia coli* inoculated into whole and skim milk by ultrahigh-pressure homogenisation. *Lait*, 241-249.

Bríñez, W. J. (2008). La ultra alta presión de homogeneización, una nueva tecnología para la conservación de productos lácteos fluidos. En *Desarrollo Sostenible de la Ganadería de Doble Propósito*. Pérez, P. & Díaz, P., Ed. Fundación Girarz,79: 930-941.

Burns, P., Patrignani, F., Serrazanetti, D., Vinderola, G. C., Reinheimer, J. A., Lanciotti, R., & Guerzoni, M. E. (2008). Probiotic Crescenza Cheese containing *Lactobacillus casei* and *Lactobacillus acidophilus* manufactured with high-pressure homogenized milk. *Journal of Dairy Science*, 91: 500-512.

Butz, P., & Tauscher, B. (2002). Emerging technologies: chemical aspects. *Food research international*, 35: 279-284.

Calderón-Miranda, M. L., Barbosa-Cánovas, G. V., & Swanson, B. G. (1999). Inactivation of *Listeria innocua* in liquid whole egg by pulsed electric fields and nisin. *International Journal of Food Microbiology*, 51: 7-17.

Campos, F. P., & Cristianini, M. (2007). Inactivation of *Sccharomyces cerevisiae* and *Lactobacillus plantarum* in orange juice using ultra high-pressure homogenisation. *Innovative Food Science and Emerging Technologies*, 8: 226-229.

Cano-Ruiz, M. E., & Richter, R. L. (1997). Effect of homogenization pressure on the milk fat globule membrane proteins. *Journal of Dairy Science*, 80: 2732-2739.

Castro, A., Barbosa-Cánovas, G., & Swanson, B. (1993). Microbial inactivation of foods by pulsed electric fields. *Journal of Food Processing and Preservation*, 17: 47-73.

Charles-Rodríguez, A. V., Nevárez-Moorillón, G. V., Zhang, Q. H., & Ortega-Rivas, E. (2007). Comparison of thermal processing and pulsed electric fields treatment in pasteurization of apple juice. *Trans IChemE, Part C, Food and Bioproducts Processing*, 85 (C2): 93-97.

Chavez-López, C., Lanciotti, R., Serio, A., Paparella, A., Guerzoni, E., & Suzzi, G. (2009). Effects of high pressure homogenizaion applied individually or in combination with other mild physical or chemical stresses on *Bacillus cereus* and *Bacillus subtilis* spore viability. *Food Control*, 20: 691-695.

Christiansen, K. F., Vegarud, G., Langsrud, T., Ellekjaer, M. R., & Egelandsdal, B. (2004). Hydrolyzed whey proteins as emulsifiers and stabilizers in high pressure processed dressings. *Food Hydrocolloids*, 18: 757-767.

Christiansen, K. F., Krekling, T., Kohler, A., Vegarud, G., Langsrud, T., & Egelandsdal, B. (2006). Microstructure and sensory properties of high pressure processed dressings stabilized by different whey proteins. *Food Hydrocolloids*, 20: 650-662.

Corbo, M. R., Bevilacqua, A., Campaniello, D., D'Amato, D., Speranza, B., & Sinigaglia, M. (2009). Prolonging microbial shelf life of foods through the use of natural compounds

and non-thermal approaches-a review. *International Journal of Food Science and Technology*, 44: 223-241.

Cortés, C., Esteve, M. J., Rodrigo, D., Torregrosa, F., & Frígola, A. (2006). Changes of colour and carotenoids contents during high intensity pulsed electric field treatment in orange juices. *Food and Chemical Toxicology*, 44 (11): 1932-1939.

Cortés, C., Esteve, M. J., & Frígola, A. (2008). Effect of refrigerated storage on ascorbic acid of orange juice treated by pulsed electric fields and thermal pasteurization. *European Food Research and Technology*, 227: 629-635.

Cortés-Muñoz, M., Chevalier, L., & Dumay, E. (2009). Characteristics of submicron emulsions prepared by ultra-high pressure homogenisation: efect of chilled or frozen storage. *Food Hydrocolloids*, 23: 640-654.

Cruz, N., Capellas, M., Hernández, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2007). Ultra high homogenization of soymilk: microbiological, physicohemical and microestructural characteristics. *Food Research International*, 40: 725-732.

Cruz, N. S., Capellas, M., Jaramillo, D. P., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009). Soymilk treated by ultra high-pressure homogeniztaion: acid coagulation properties and characteristics of a soy-yogurt product. *Food Hydrocolloids*, 23: 490-496.

Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. (2006). Study of pulsed electric fields treated citrus juices. *Innovative Food Science and Technology*, 7: 49-54.

Datta, N., Hayes, M. G., Deeth, H. C., & Kelly, A. L. (2005). Significance of frictional heating for effects of high pressure homogenisation on milk. *Journal of Dairy Research*, 72: 1-7.

De Azerêdo, G. A., De Oliveira, F. L., & De faro, Z. P. (2008). Pulsos elétricos na preservaçao de alimentos: fatores críticos na inactivaçao microbiana e efeitos sobre os constituintes alimentares. *B. CEPPA, Curitiba*, 26 (2): 171-178.

Desrumaux, A., & Marcand, J. (2002). Formation of sunflower oil emulsions stabilized by whey proteins with high pressure homogenization (up to 350 MPa): effect of pressure on emulsion characteristics. *International Journal of Food Science and Technology*, 37: 263-269.

Devlieghere, F., Vermeiren, L., & Debevere, J. (2004). New preservation technologies: possibilities and limitations. *International Dairy Journal*, 14: 273-285.

Dickinson, E. (1997). Controlling the structure and rheology of protein-stabilized emulsions and emulsion gels. *2nd World Congress on Emulsion*. EDS, Ed. Bordeaux, 4: 81-92.

Dickinson, E., & James, J. D. (1998). Rheology and flocculation of high-pressure-treated β-lactoglobulin-stabilized emulsions: comparison with thermal treatment. *Journal of Agricultural and Food Chemistry*, 46: 2565-2571.

Diels, A. M., Wuytack, E. Y., & Michiels, C. W. (2003). Modelling inactivation of *Staphylococus aureus* and *Yersinia enterocolítica* by high-pressure homogenisation at different temperatures. *International Journal of Food Microbiology*, 87: 55-62.

Diels, A. M., Callewaert, L., Wuytack, E. Y., Masschalck, B., & Michiels, C. W. (2004). Moderate temperatures affect Escherichia coli inactivation by high-pressure homogenization only through fluid viscosity. *Biotechnology Progress*, 20: 1512-1517.

Diels, A. M., Callewaert, L., Wuytack, E. Y., Masschalck, B., & Michiels, C. W. (2005). Inactivation of *Escherichia coli* by high-pressure homogenisation is influenced by fluid

viscosity but not by water activity and product composition. *Interntational Journal of Food Microbiology*, 101: 281-291.

Doevenspeck, H. (1961). Influencing cells and cells walls by electrostatic impulses. *Fleischwistschaaft*, 13: 986-987.

Donsì, F., Ferrari, G., & Maresca, P. (2009). High pressure homogenization for food sanitization. En *Global Issues in Food Science and Technology*. Barbosa-Cánovas, G.V., Mortimer, A., Lineback, D., Spiess, W., Buckle, K., & Colonna, P., Ed. Academic Press Elsevier, Inc. USA, 19: 309-352.

Dunn, J. E., Pearlman, J. S., & La Costa, R. (1989). *U.S. Patent 4,838,154.*

Dunn, J. E. (1995). Pulsed light and pulsed electric fields for foods and eggs. *Poultry Science*, 75: 1133-1136.

Dunn, J. E. (2001). Pulsed electric field processing: an overview. En *Pulsed electric fields in food processing: Fundamental Aspects and Applications*. Barbosa-Cánovas, G. V. & Zhang, Q. H., Ed. Technomic Publishing Company, Inc. Lancaster, 1-30.

Elez-Martínez, P., Soliva-Fortuny, R., & Martín-Belloso, O. (2006). Comparative study on shelf life of orange juice processed by high intensity pulsed electric fields or heat treatment. *European Food Research and Technology*, 222: 321-329.

Elez-Martínez, P., & Martín-Belloso, O. (2007). Effects of high intensity pulsed electric field processing conditions on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Food Chemistry*, 102 (1): 201-209.

Esteve, M. J., Frígola, A., Rodrigo, D., Rodrigo, M., & Torregrosa, F. (2001). Pulsed electric fields inactivation kinetics of carotenes in mixed orange-carrot juice. *EUROCAFT.* Berlin, Germany.

Esteve, M. J., Frígola, A., Rodrigo, C., & Rodrigo, D. (2005). Effect of storage period under variable conditions on the chemical and physical composition and colour of Spanish refrigerated orange juices. *Food and Chemical Toxicology*, 43: 1413-1422.

Evrendilek, G. A., Jin, Z. T., Ruhlman, K. T., Qiu, X., Zhang, Q. H., & Richter, E. R. (2000). Microbial safety and shelf life of apple juice processed by bench and pilot scale PEF systems. *Innovative Food Science and Emerging technologies*, 1: 77-86.

Evrendilek, G. A., Yeom, H. W., Jin, Z. T., & Zhang, Q. H. (2004). Safety and quality evaluation of a yogurt-based drink processed by a pilot plant PEF system. *Journal of Food Process Engineering*, 274: 197-212.

Fantin, G., Fogagnolo, M., Guerzoni, M. E., Lanciotti, R., Medici, A., Pedrini, P.,& Rossi, D. (1996). Effect of high hydrostatic pressure and high pressure homogenization on the enantioselectivity of microbial reductions. *Tetrahedron: Asymetry*, 7(10): 2879-2887.

Fernández-Díaz, M. D., Barsotti, L., Dumay, E., & Cheftel, J. C. (2000). Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. *Journal of Agricultural and Food Chemistry*, 48 (6): 2332-2339.

Ferragut, V., Cruz, N. S., Trujillo, A., Guamis, B., & Capellas, M. (2009). Physical characteristics during storage of soy yogurt made from ultra-high pressure homogenized soymilk. *Journal of Food Engineering*, 92: 63-69.

Floury, J., Desrumaux, A., & Lardières, J. (2000). Effect of high pressure homogenization on droplet size distribution and rheological properties of model oil-in-water emulsions. *Innovative Food Science and Emerging Technologies*, 1: 127-134.

Floury, J., Desrumaux, A., & Legrand, J. (2002a). Effect of ultra-high-pressure homogenization on structure and on rheological properties of soy protein-stabilized emulsions. *Journal of Food Science*, 67(9): 3388-3395.

Fluory, J., Desrumaux, A., Axelos, M. A., & Legrand, J. (2002b). Degradation of methylcellulose during ultra-high pressure homogenisation. *Food Hydrocolloids*, 16: 47-53.

Floury, J., Desrumaux, A., Axelos, M. A., & Legrand, J. (2003). Effect of high-pressure homogenization on methylcellulose as food emulsifier. *Journal of Food Engineering*, 58(3): 227-238.

Fluory, J., Bellettre, J., Legrand, J., & Desrumaux, A. (2004a). Analysis of a new type of high pressure homogeniser. A study of the flow pattern. *Chemical Engineering Science* , 59: 843-853.

Fluory, J., Legrand, J., & Desrumaux, A. (2004b). Analysis of a new type of high pressure homogeniser. Part B. Study of droplet break-up and recoalescence phenomena. *Chemical Engineering Science*, 59: 1285-1294.

Floury, J., Grosset, N., Leconte, N., Pasco, M., Madec, M., & Jeantet, R. (2006). Continuous raw skim milk prrocessing by pulsed electric field at non-lethal temperature: effect on microbial inactivation and functional properties. *Lait*, 86: 43-57.

Góngora-Nieto, M. M., Seignour, L., Riquet, P., Davidson, P. M., Barbosa-Cánovas, G. V., & Swanson, B. G. (1999). Hurdle aproach for the inactivation of *Pseudomonas fluorescens* in liquid whole egg. En *Food Engineering: Nonthermal Processing*. IFT Annual Meeting. Chicago, 83A-2.

Góngora-Nieto, M. M., Seignour, L., Riquet, P., Davidson, P. M., Barbosa-Cánovas, G. V., & Swanson, B. G. (2001). Nonthermal inactivation of *Pseudomonas fluorescens* in liquid whole egg. En *Pulsed Electric Fields in Food Processing: Fundamental Aspects and Aplications*. Barbosa-Cánovas, G. V., & Zhang, Q. H., Ed. Technomic Publishing Company, Inc. Lancaster, 193-212.

Góngora-Nieto, M. M., Sepúlveda, D. R., Pedrow, P., Barbosa-Cánovas, G. V., & Swanson, B. G. (2002). Food processing by pulsed electric fields tretament delivery, inactivation level, and regulatory aspects. *Lebensmittel Wissenschaft und-Technologie*, 35: 375-388.

Góngora-Nieto, M. M., Pedrow, P. D., Swanson, B., & Barbosa-Cánovas, G. V. (2003). Energy analysis of liquid whole egg pasteurized by pulsed electric fields. *Journal of Food Engineering*, 57: 209-216.

Gould, G. W. (2001). New processing technologies: an overview. *Proceedings of the Nutrition Society*, *60* (463-474).

Grácia-Juliá, A., René, M., Cortés-Muñoz, M., Picart, L., López-Pedemonte, T., ChevalierD., y otros. (2008). Effect of dynamic high pressure on whey protein aggregation: a comparison with the effect of continuous short-time thermal treatments. *Food Hydrocolloids*, 22: 1014-1032.

Grahl, T., & Märkl, H. (1996). Killing of microorganisms by pulsed electric fields. *Applied Microbiology and Biotechnology*, 45: 148-157.

Guamis, B., Trujillo, A., Ferragut, V., & Daoudy, L. (2006). Aplicaciones de las altas presiones en la industria alimentaria. En *Técnicas avanzadas de procesado y conservación de alimentos*. Blanco, C. A., Gomez, M., Ronda, F., & Caballero, P.A., Ed. Universidad de Valladolid, 61-76.

Gudmundsson, M., & Hafsteinsson, H. (2001). Effect of electric field pulses on microestructure of muscle foods and roes. *Trends in Food Science and Technology*, 12 (3-4): 122-128.

Guerzoni, M. E., Lanciotti, R., Westall, F., & Pittia, P. (1997). Interrelation between chemico-physical variables, microestructure and growth of *Listeria monocytogenes* and *Yarrowia lipolytica* in model food systems. *Sciences des les Aliments*, 17: 507-522.

Guerzoni, M. E., Ferruzzi, M., Gardini, F., & Lanciotti, R. (1999a). Combined effects of ethanol, high homogenization pressure, and temperature on cell faty acid composition in *Sacharomyces cerevisiae*. *Canadian Journal of Microbiology*, 45: 805-810.

Guerzoni, M. E., Vannini, L., Chavez-Lopez, C., Lanciotti, R., Suzzi, G., & Gianotti, A. (1999b). Effect of high pressure homogenization on microbial and chemico-physical characteristics of goat cheeses. *Journal of Dairy Science*, 82: 851-862.

Guerzoni, M. E., Vaninni, L., Lanciotti, R., & Gardini, F. (2002). Optimisation of the formulation and of the technological process of egg-based products for the prevention of *Salmonella enteritidis* survival and growth. *International Journal of Food Microbiology*, 73: 367-374.

Hayes, M. G., & Kelly, A. L. (2003a). High pressure homogenisation of milk (b) effects on indigenous enzymatic activity. *Journal of Dairy Research*, 70: 307-313.

Hayes, M. G., & Kelly, A. L. (2003b). High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. *Journal of Dairy Research*, 70: 297-305.

Hayes, M. G., Fox, P. F., & Kelly, A. L. (2005). Potential aplications of high pressure homogenisation in processing of liquid milk. *Journal od Dairy Research*, 72: 28-33.

Heinz, V., Alvarez, I., Angersbach, A., & Knorr, D. (2002). Preservation of liquid foods by high intensity pulsed electric fields-basic concepts for food processing dessing. *Trends in Food Science and Technology*, 12: 103-111.

Hermawan, N., Evrendiek, G. A., Dantzer, W. R., Zhang, Q. H., & Richter, E. R. (2004). Pulsed electric field treatment of liquid whole egg inoculated with *Salmonella Enteritidis*. *Journal of Food Safety*, 24: 71-85.

Hernández, A., & Harte, F. M. (2008). Manufacture of acid gels from skim milk using high-pressure homogenization. *Journal of Dairy Science*, 91: 3761-3767.

Hodgins, A. M., Mittal, G. S., & Griffiths, M. W. (2002). Pasteurization of fresh orange juice using low-energy pulsed electrical field. *Journal of Food Science*, 67: 2294-2299.

Huang, E., Mittal, G. S., & Griffiths, M. W. (2006). Inactivation of *Salmonella enteritidis* in liquid whole egg using combination treatments of pulsed electric field, high pressure and ultrasound. *Biosystems Engineering*, 94(3): 403-413.

Innocente, N., Biasutti, M., Venir, E., Spaziani, M., & Marchesini, G. (2009). Effect of high-pressure homogenization on droplet size distribution and rheological properties of ice cream mixes. *Journal of Dairy Science*, 92: 1864-1875.

Iordache, M., & Jelen, P. (2003). High pressure microfluidization treatment of heat denatured whey proteins for improved functionality. *Innovative Food Science and Emerging Technologies*, 4: 367-376.

Jeantet, R., Baron, F., Nau, F., Roignant, M., & Brulé, G. (1999). High intensity pulsed electric fields applied to egg white: Effect on *Salmonella enteritidi*s inactivation and protein denaturation. *Journal of Food Protection*, 62: 1381-1386.

Jeantet, R., Mc Keag, J. R., Carballeira-Fernández, J., Grosset, N., Baron, F., & Korolczuk, J. (2004). Traitement d'ovoproduits en continu par champs électriques pulsés. *Sciences des Aliments*, 24: 137-158.

Jia, M., Zhang, Q. H., & Min, D. B. (1996). Effect of pulsed electric field processing on orange juice flavor analyzed by dynamic headspace gas chromatography. *IFT Annual Meeting.* Louisiana, New Orleans.

Jia, M., Zhang, Q. H., & Min, D. B. (1999). Pulsed electric field processing effects on flavor compounds and microorganisms of orange juice. *Food Chemistry*, 65: 445-451.

Jin, Z. T., & Zhang, Q. H. (1999). Pulsed electric field inactivation of microorganisms and preservation of quality of cranberry juice. *Journal of Food Processing and Preservation*, 23(6): 481-497.

Jin, T., Zhang, H., Hermawan, N., & Dantzer, W. (2009). Effects of pH and temperature on inactivation of *Salmonella typhimurium DT104* in liquid whole egg by pulsed electric fields. *International Journal of Food Science and Technology*, 44: 367-372.

Kheadr, E. E., Vachon, J. F., Paquin, P., & Fliss, I. (2002). Efefct of dynamic high pressure on microbiological, rheological and microestructural quality of Cheddar cheese. *International Dairy Journal*, 12: 435-446.

Kietczewska, K., Kruk, A., Czerniewicz, M., Warmińska, M., & Haponiuk, E. (2003). The effect of high-pressure homogenization in changes in milk colloidal and emulsifying systems. *Polish Journal of Food and Nutrition Sciences*, 12/53(1): 43-46.

Kim, H. J., Decker, E. A., & McClements, D. J. (2002). Impact on protein surface denaturation on droplet floculation in hexadecane oil-in-water emulsions stabilized by β-lactoglobulin. *Journal of Agricultural and Food Chemistry*, 50: 7131-7137.

Knorr, D., Ade-Omawaye, B. I., & Heinz, V. (2002). Nutritional improvement of plant foods by non-thermal processing. *Proceedings of the Nutrition Society*, 61: 311-318.

Knorr, D., Froehling, A., Jaeger, H., Reineke, K., Schlueter, O., & Schoessler, K. (2011). Emerging technologies in food processing. *Annual Review Food Science and Technology*, 2: 203-235.

Kumar, S., Thippareddi, H., Subbiah, J., Zivanovic, S., Davidson, P. M., & Harte, F. (2009). Inactivation of *Escherichia coli K-12* in apple juice using combination of high-pressure homogenization and chitosan. *Journal of Food Science*, 74(1): M8-M14.

Lacroix, N., Fliss, I., & Makhlouf, J. (2005). Inactivation of pectin methylesterase and stabilization of opalescence in orange juice by dinamic high pressure. *Food Research International*, 38: 569-576.

Lagoueyete, N., & Paquin, P. (1998). Effects of microfluidization on the functional properties of xanthan gum. *Food Hydrocolloids*, 12: 365-371.

Lanciotti, R., Sinigaglia, M., Angelini, P., & Guerzoni, M. E. (1994). Effects of homogenization pressure on the survival and growth of some food spoilage and pathogenic microorganisms. *Letters in Applied Microbiology*, 18: 319-322.

Lanciotti, R., Gardini, F., Sinigaglia, M., & Guerzoni, M. E. (1996). Effects of growth conditions on the resistance of some pathogenic and spoilage species to high pressure homogenization. *Letters in Applied Microbiology*, 22: 165-168.

Lanciotti, R., Chavez-López, C., Patrignani, F., Paparella, A., Guerzoni, M. E., Serio, A., & Succi, G. (2004a). Effects of milk treatment with dynamic high pressure on microbial populations, and lipolytic and proteolytic profiles of Creszenza cheeses. *International Journal of Dairy Technology*, 57(1): 19-25.

Lanciotti, R., Vannini, L., Pittia, P., & Guerzoni, M. E. (2004b). Suitability of high-dynamic-pressure-treated milk for the production of yoghurt. *Food Microbiology*, 21: 753-760.

Lanciotti, R., Vannini, L., Pratignani, F., Iucci, L., Vallicelli, M., Ndagijimana, M., & Guerzoni, M. E. (2006). Effect of high pressure homogenisation of milk on cheese yield and microbiology, lypolysis and proteolysis, during ripening of Caciotta cheese. *Journal of Dairy Research*, 73: 216-226.

Lanciotti, R., Patrignani, F., Iucci, L., Saracino, P., & Guerzoni, M. E. (2007a). Potential of high pressure homogenization in the control and enhancement of proteolytic and fermentative activities of some *Lactobacillus* species. *Food Chemistry*, 102: 542-550.

Lanciotti, R., Patrignani, F., Iucci, L., Guerzoni, M. E., Suzzi, G., Belletti, N., & Gardini, F. (2007b). Effects of milk high pressure homogenization on biogenic amine accumulation during ripening of ovine and bovine Italian cheeses. *Food Chemistry*, 104: 693-701.

Lanciotti, R., Vannini, L., Patrignani, F., & Guerzoni, M. E. (2008). High pressure homogenization as alternative to thermal treatment to improve the microbial safety, shelf life and functionality of fluid foods and ingredients. *Evolving Microbial Food Safety and Quality. Foodmicro 2008. The 21st International ICFHM Symposium.* Aberdeen, 112-113.

Lee, S. H., Lefèvre, T., Subirade, M., & Paquin, P. (2007). Changes and roles of secundary structures of whey protein for the formation of protein membrane at soy oil/water interface under high pressure homogenization. *Journal of Agricultural and Food Chemistry*, 55: 10924-10931.

Lee, S. H., Lefèvre, T., Subirade, M., & Paquin, P. (2009). Effects of ultra-high pressure homogenization on the properties and structure of interfacial protein layer in whey protein-stabilized emulsion. *Food Chemistry*, 113: 191-195.

Li, S. Q., Bomser, A., & Zhang, Q. H. (2005). Effects of pulsed electric fields and heat treatment on stability and secondary structure of bovine inmmunoglobulin G. *Journal of Agricultural and Food Chemistry*, 53: 663-670.

López, N., Puértolas, E., Hernández-Orte, P., Álvarez, I., & Raso, J. (2009). Effect of pulsed electric field treatment on the anthocyanins composition and other quality parameters of Cabernet Sauvignon freshly fermented model wine obtained after different maceration times. *Food Science and Technology*, 42: 1225-1231.

Ma, L., Chang, F. J., Góngora-Nieto, M. M., Barbosa-Cánovas, G. V., & Swanson, B. G. (2001). Comparison study of pulsed electric fields, high hydrostatic presssure, and thermal processing on the electroforetic patterns of liquid whole eggs. En *Pulsed Electric Fields in Food Processing: Fundamental Aspects and Applications*. Barbosa-Cánovas, G. V., & Zhang, Q. H., Ed. Technomic Publishing Company, Inc. Lancaster, 225-239.

Mañas, P., & Pagán, R. (2005). Microbial inactivation by new technologies of food preservation. *Journal of Applied Microbiology*, 98: 1387-1399.

Mañas, P., & Vercet, A. (2006). Effect of pulsed electric fields on enzymes and food constituents. En *Pulsed electric fields Technology for the Food Industry: Fundamentals and Applications* (págs. 131-152). Raso, J., & Heinz, V., Ed. Springer. New York, 131-152.

Martinet, V., Valentini, C., Casalinho, J., Schorsch, C., Vaslin, S., & Courthaudon, J. L. (2005). Composition of interfacial layers in complex food emulsions before and after aeration: effect of egg to milk protein ratio. *Journal of Dairy Science*, 88: 30-39.

Martin-González, M. F., Roach, A., & Harte, F. (2009). Rheological properties of corn oil emulsions stabilized by commercial micellar caseins and high pressure homogenization. *LWT-Food Science and Technology*, 42: 307-311.

Mertens, B., & Knorr, D. (1992). Development of nonthermal processes for food preservation. *Food Technology*, 42: 124-133.

Michalski, M. C., & Januel, C. (2006). Does homogenization affect the human health properties of cow's milk? *Trends in Food Science and Technology*, 17: 423-437.

Min, S., Jin, Z. T., Min, S. K., Yeom, H., & Zhang, Q. H. (2003a). Comercial-scale pulsed electric field processing of orange juice. *Journal of Food Science*, 68: 1265-1271.

Min, S., Jin, Z. T., & Zhang, Q. H. (2003b). Comercial scale pulse electric field processing of tomato juice. *Journal of Agricultural and Food Chemistry*, 51: 3338-3344.

Min, S., Evrendilek, G. A., & Zhang, H. Q. (2007). Pulsed electric fields: processing system, microbial and enzyme inhibition, and shelf life extension of foods. *IEEE Transactions on Plasma Science*, 35 (1): 59-73.

Morata Barrado, A. (2009). *Nuevas Tecnologías de Conservación de Alimentos.* Madrid: A. Madrid Vicente Ediciones.

Mosqueda-Melgar, J., Elez-Martínez, P., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2008). Effects of pulsed electric fields on pathogenic microorganisms of major concern in fluid foods: a review. *Critical Reviews in Food Science and Nutrition*, 48: 747-759.

Nielsen, H. B., Sonne, A. M., Grunert, K. G., Banati, D., Pollák-Tóth, A., Lakner, Z., Olsen, N. V., Zontar, T. P., & Peterman, M. (2009). Consumer perception of the use of high-pressure processing and pulsed electric field technologies in food production. *Appetite*, 52: 115-126.

Noci, F., Riener, J., Walkling-Ribeiro, M., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2008). Ultravioles irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple juice. *Journal of Food Engineering*, 85: 141-146.

Odriozola-Serrano, I., Bendichio-Porta, S., & Martín-Belloso, O. (2006). Comparative study on shelf-life of whole milk processed by high intensity pulsed electric field or heat treatment. *Journal of Dairy Science*, 89: 905-911.

Odriozola-Serrano, I., Aguiló-Aguayo, I., Soliva-Fortuny, R., Gimeno-Añó, V., & Martín-Belloso, O. (2007). Lycopene, vitamin C, and antioxidant capacity of tomato juice as affected by high-intenisty pulsed electric fields critical parameters. *Journal of Agricultural and Food Chemistry*, 55: 9036-9042.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008a). Changes of health related compounds throughout cold storage of tomato juice stabilized by thermal or high intensity pulsed electric field treatments. *Innovative Food Science and Emerging Technologies*, 9: 272-279.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2008b). Phenolic acids, flavonoids, vitamin C and antioxidant capacity os strawberry juices by high-intensity pulsed electric fields or heat treatments. *European Food Research and Technology*, 228: 239-248.

Odriozola-Serrano, I., Soliva-Fortuny, R., Gimeno-Añó, V., & Martín-Belloso, O. (2008c). Modeling changes in health related compounds of tomato juice treated by high-intensity pulse electric fields. *Journal of Food Engineering*, 89: 210-216.

Odriozola-Serrano, I., Soliva-Fortuny, R., Hernández-Jover, T., & Martín-Belloso, O. (2009a). Carotenoid and phenolic profile of tomato juices processsed by high intensity pulsed electric fields compared with conventional thermal treatments. *Food Chemistry*, 112: 258-266.

Odriozola-Serrano, I., Soliva-Fortuny, R., & Martin-Belloso, O. (2009b). Impact of high-intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant capacity of strawberry juice. *LWT-Food Science and Technology*, 42: 93-100.

Pagán, R., Condón, S., & Raso, J. (2005). Microbial inactivation by pulsed electric fields. En *Novel Food Processing Technologies*. Barbosa-Cánovas, G. V., Tapia, M. S., & Cano, M. P., Ed. CRC Press. Florida, 45-68.

Paquin, P. (1999). Technological properties of high pressure homogenisers: the effect of fat globules, milk proteins and polysacharides. *International Dairy Journal*, 9: 329-335.

Paquin, P., Lacasse, J., Subirade, M., & Turgeon, S. (2003). Continuous process of dynamic high pressure homogenization for the denaturation of proteins. *US Patent 6,511,695 B1* .

Pathanibul, P., Taylor, M., Davidson, P. M., & Harte, F. (2009). Inactivation of *Escherichia coli* and *Listeria innocua* in apple and carrot juices using high pressure homogenization and nisin. *International Journal of Food Microbiology*, 129: 316-320.

Patrignani, F., Iucci, L., Lanciotti, R., Vallicelli, M., Mathara, J. M., Holzapfel, H., & Guerzoni, M. E. (2007). Effect of high-pressure homogenization, nonfat milk solids, and milkfat on the technological performance of functional strain for the production of probiotic fermented milks. *Journal of Dairy Science*, 90: 4513-4523.

Patrignani, F., Burns, P., Serrazanetti, D., Vinderola, G., Reibheimer, J., Lanciotti, R., & Guerzoni, M. E. (2009a). Suitability of high pressure-homogenized milk for the production of probiotic fermented milk containing *Lactobacillus paracasei* and *Lactobacillus acidophilus*. *Journal of Dairy Research* 76: 74-82.

Patrignani, F., Vannini, L., Kamdem, S. L., Lanciotti, R., & Guerzoni, M. E. (2009b). Effect of high pressure homogenization on *Saccharomyces cerevisiae* inactivation and physico-

chemical features in apricot and carrot juices. *International Journal of Food Microbiology*, 135: 26-31.

Pereda, J., Ferragut, V., Guamis, B., & Trujillo, A. J. (2006). Effect of ultra high pressure homogenisation on natural occurring microorganisms in bovine milk. *Milchwissenchaft*, 61(3): 246-248.

Pereda, J., Ferragut, V., Quevedo, J. M., Guamis, B., & Trujillo, A. (2007). Effects of ultra-high pressure homogenization on microbial and physicochemical shelf life of milk. *Journal of Dairy Science*, 90: 1081-1093.

Pereda, J., Ferragut, V., Quevedo, J. M., Guamis, B., & Trujillo, A. J. (2008a). Effects of ultra-high-pressure homogenization treatment on the lipolysis and lipid oxidation of milk during refrigerated storage. *Journal of Agricultural and Food Chemistry*, 56: 7125-7130.

Pereda, J., Jaramillo, D. P., Quevedo, J. M., Ferragut, V., Guamis, B., & Trujillo, A. J. (2008b). Characterization of volatile compounds in ultra-high-pressure homogenized milk. *International Dairy Journal*, 18: 826-834.

Pereda, J., Ferragut, V., Buffa, M., Guamis, B., & Trujillo, A. J. (2008c). Proteolysis of ultra-high pressure homogenised treated milk during refrigerated storage. *Food Chemistry*, 111: 696-702.

Pereda, J., Ferragut, V., Quevedo, J. M., Guamis, B., & Trujillo, A. J. (2009). Heat damage evaluation in ultra-high pressure homogenized milk. *Food Hydrocolloids*, 23: 1974-1979.

Pérez, O. E. (2002). Effects of pulsed electric fields on the gelation and microestruture of egg white. *Nonthermal Processing: Nonthermal Processing of Foods*. IFT Annual Meeting. Anaheim, 91E-25.

Pérez, O. E., & Pilosof, A. M. (2004). Pulsed electric fields effects on the molecular structure and gelation of -lactoglobulin concentrate and egg white. *Food Research International*, 37(1): 102-110.

Picart, L., Thiebaud, M., René, M., Guiraud, J. P., Cheftel, J. C., & Dumay, E. (2006). Effects of high pressure homogenisation of raw bovine milk on alkaline phosphatase and microbial inactivation. A comparison with continuous short-time thermal treatments. *Journal of Dairy Research*, 73: 454-463.

Pina-Pérez, M. C., Silva-Angulo, A. B., Rodrigo, D., & Martínez-López, A. (2009). Synergistic effect of pulsed electric fields and CocoanOX 12% on the inactivation kinetics of *Bacillus cereus* in a mixed beverage of liquid whole egg and skim milk. *International Journal of Food Microbiology*, 130: 196-204.

Plaza, L., Sánchez-Moreno, C., de Ancos, B., Martín-Belloso, O., & Cano, P. (2006). Effect of refrigerated storage on vitamin C and antioxidant activity of orange juice processed by high-pressure or pulsed electric fields with regard to low pasteurization. *European Food Research and Technology*, 223: 487-493.

Popper, L., & Knorr, D. (1990). Applications of high pressure homogenization for food preservation. *Food Technology*, 44: 84-89.

Qitao-Teixeira, L., Odriozola-Serrano, I., Soliva-Fortuny, R., Mota-Ramos, A., & Martín-Belloso, O. (2009). Comparative study on antioxidant properties of carrot juice stabilised by high-intensity pulsed electric fields or heat treatments. *Journal of the Science of Food and Agriculture*, 89: 2636-2642.

Qiu, X., Sharma, S., Tuhela, L., Jia, M., & Zhang, Q. H. (1998). An integrated PEF pilot plant for continuous nonthermal pasteurization of fresh orange juice. *Trans ASAE*, 41: 1069-1074.

Quin, B. L., Pothakamury, U. R., Vega, H., Martín, O., Barbosa-Cánovas, G. V., & Swanson, B. G. (1995a). Food pasteurization using high-intensity pulsed electric fields. *Journal of Food Technology*, 12: 55-60.

Quin, B. L., Vega-Mercado, H., Potakamury, U. R., & Barbosa-Cánovas, G. V. (1995b). Application of pulsed electric fields for the inactivation of bacteria and enzymes. *Journal of the Franklin Institute*, 332: 209-220.

Rampon, V., Riaublanc, A., Anton, M., Genot, C., & McClements, D. J. (2003). Evidence that homogenization of BSA-stabilized hexadecane-in-water emulsions induce structure modification of the nonadsorbed protein. *Journal of Agricultural and Food Chemistry*, 51: 5900-5950.

Raso, J., & Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. *Critical Reviews in Food Science and Nutrition* , 43 (3): 265-285.

Riener, J., Noci, F., Cronin, D. A., Morgan, D. J., & Lyng, J. G. (2008). Effect of high intensity pulsed electric fields on enzymes and vitamins in bovine raw milk. *International Journal of Dairy Technology*, 62: 1-6.

Rivas, A., Rodrigo, D., Martínez, A., Barbosa-Cánovas, G. V., & Rodrigo, M. (2006). Effect of PEF and heat pasteurization on the physical-chemical characteristics of blended orange and carrot juice. *LWT Food Science and Technology*, 39: 1163-1170.

Rivas, A., Rodrigo, D., Company, B., Sampedro, F., & Rodrigo, M. (2007). Effects of pulsed electric fields on water-soluble vitamins and ACE inhibitory peptides added to a mixed oranje juice and milk beverage. *Food Chemistry*, 104(4): 1550-1559.

Roach, A., & Harte, F. (2008). Disruption and sedimentation of caseins and casein micelle isolates under high-pressure homogenization. *Innovative Food Science and Technologies*, 9: 1-8.

Roesch, R. R., & Corredig, M. (2003). Texture and microestructure of emulsions prepared with soy protein concentrate by high-pressure homogenization. *Lebensmittel Wissenschaft und Technologie*, 36: 113-124.

Roig-Sagués, A. X., Velázquez, R. M., Montealegre-Agramont, P., López-Pedemonte, T. J., Briñez-Zambrano, W. J., Guamis-López, B., & Hernandez-Herrero, M. M. (2009). Fat content increases the lethality of ultra-high-pressure homogenization on *Listeria monocytogenes* in milk. *Journal of Dairy Science*, 92: 5396-5402.

Ross, A. I., Griffiths, M. W., Mittal, G. S., & Deeth, H. C. (2003). Combining nonthermal technologies to control foodborne microorganisms. *International Journal of Food Microbiology*, 89: 125-138.

Ruhlman, K. T., Jin, Z. T., & Zhang, Q. H. (2001). Physical properties of liquids foods for pulsed electric field treatment. En *Pulsed electric fields in Food Processing*. Barbosa-Cánovas, G. V., & Zhang, Q. H., Ed. Technomic Publishing Company, Inc. Lancaster, 45-55.

Sampedro, F., Rodrigo, M., Martínez, A., Rodrigo, D., & Barbosa-Cánovas, G. V. (2005). Quality and safety aspects of PEF aplication in milk and milk products. *Critical Reviews in Food Science and Nutrition*, 45: 25-47.

Sampedro, F., Rodrigo, D., Martínez, A., Barbosa-Cánovas, G. V., & Rodrigo, M. (2006). Review: application of pulsed electric fields in egg and egg derivates. *Food Science and Technology International*, 12(5): 397-405.

Sampedro, F., Geveke, D. J., Fan, X., & Zhang, H. Q. (2009). Effect of PEF, HHP and thermal treatment on PME inactivation and volatile compounds concentration of an orange-milk based beverage. *Innovative Food Science and Emerging Technologies*, 10: 463-469.

Sánchez-Moreno, C., Cano, M. P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., y otros. (2004). Pulsed electric fields-processed orange juice consumption increases plasma vitamin C and decreases F2-isoprostanes in healthy humans. *Journal of Nutrition Biochemistry*, 15: 601-607.

Sandra, S., & Dalgleish, D. G. (2005). Effecys of ultra-high-pressure homogenization and heating on structural properties of casein micelles in reconsituted skim milk powder. *International Dairy Journal*, 15: 1095-1104.

Schilling, S., Alber, T., Toepfl, S., Neidhart, S., Knorr, D., Schieber, A., & Carle, R. (2007). Effects of pulsed electric field treatment of apple mash on juice yield and quality atributes of apple juices. *Innovative Food Science and Emerging Technologies*, 8: 127-134.

Señorans, F. J., Ibáñez, E., & Cifuentes, A. (2003). New trends in food processing. *Critical Reviews in Food Science and Nutrition*, 43 (5): 507-526.

Serra, M., Trujillo, A. J., Quevedo, J. M., Guamis, B., & Ferragut, V. (2007). Acid coagulation properties and suitability for yogurt production of cow's milk treated by high-pressure homogenisation. *International Dairy Journal*, 17: 782-790.

Serra, M., Trujillo, A. J., Pereda, J., Guamis, B., & Ferragut, V. (2008a). 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.

Serra, M., Trujillo, A. J., Jaramillo, P. D., Guamis, B., & Ferragut, V. (2008b). Ultra-high pressure homogenization-induced changes in skim milk: impact on acid coagulation properties. *Journal of Dairy Research*, 75: 69-75.

Serra, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009a). Proteolysis of yogurts made from ultra-high-pressure homogenized milk during cold storage. *Journal of Dairy Science*, 92: 71-78.

Serra, M., Trujillo, B., Guamis, B., & Ferragut, V. (2009b). Evaluation of physical properties during storage of set and stirred yogurts made from unltra-high pressure homogenization-treated milk. *Food Hydrocolloids*, 23: 82-91.

Sharma, S. K., Zhang, Q. H., & Chism, G. W. (1998). Development of a protein fortified fruit beverage and its quality when processed wuth pulsed electric field treatment. *Journal of Food Quality*, 21: 459-473.

Simpson, M. V., Barbosa-Cánovas, G. V., & Swanson, B. G. (1995). The combined inhibitory effect of lysozyme and high voltaje pulsed electric fields on the growth of *Bacillus subtillis* spores. *IFT Annual Meeting.* Anaheim, 267.

Sirvente, H., Beaumal, V., Gaillard, C., Bialek, L., Hamm, D., & Anton, M. (2007). Structuring and functionalization of dispersions containing egg yolk, plasma and granules induced by mechanical treatments. *Journal of Agricultural and Food Chemistry*, 55: 9537-9544.

Sitzmann, V. (1995). High voltage pulse techniques for food preservation. En *New Methods for Food Preservation*. Gould, G. W., Ed. Blackie Academic Professional. London, 236-252

Smiddy, M. A., Martin, J. E., Huppertz, T., & Kelly, A. L. (2007). Microbial shelf-life of high-pressure homogenised milk. *International Dairy Journal*, 17: 29-32.

Sosa, D. (2006). Pulsos eléctricos de alta tensión para conservación de alimentos y esterilización médica. *XIV Seminario de Ingeniría Biomédica*. Montevideo, 1-6.

Suárez-Jacobo, Á., Gervilla, R., Guamis, B., Roig-Sagués, A. X., & Saldo, J. (2010). Effect of UHPH on indigenous microbiota of apple juice: a preliminary study of microbial shelf-life. *International Journal of Food Microbiology*, 136(3): 261-267.

Tewari, G., & Juneja, V. (2007). *Advances in Thermal and Non-Thermal Food Preservation.* Tewari, G., & Juneja, V. Ed. *Blackwell* Publishing. Iowa, 203-239.

Thiebaud, M., Dumay, E., Picart, L., Guiraud, J. P., & Cheftel, J. C. (2003). High-pressure homogenisation of raw bovine milk: effects on fat globule size distribution and microbial inactivation. *International Dairy Journal*, 13: 427-439.

Torregrosa, F., Esteve, M. J., Frígola, A., & Cortés, C. (2006). Ascorbic acid stability during refrigerated storage of orange-carrot juice treated by high pulsed electric field and comparison with pasteurized juice. *Journal of Food Engineering*, 73: 339-345.

Vachon, J. F., Kheadr, E. E., Giasson, J., Paquin, P., & Fliss, I. (2002). Inactivation of foodborne pathogens in milk using dynamic hygh pressure. *Journal of Food Protection*, 65: 345-352.

Van Loey, A., Verachtert, B., & hendrickx, M. (2001). Effects of high electric field pulses on enzymes. *Trends in Food Science & Technology*, 12 (3-4): 94-102.

Vannini, L., Patrignani, F., Iucci, L., Ndagijimana, M., Vallicelli, M., Lanciotti, R., & Guerzoni, M. E. (2008). Effect of a pre-treatment of milk with high pressure homogenization on yield as well as on microbiological, lipolytic and proteolytic patterns of "Pecorino" cheese. *International Journal of Food Microbiology*, 128: 329-335.

Vega-Mercado, H., Martín-Belloso, O., Quin, B. L., Chang, F. J., Góngora-Nieto, M. M., Barbosa-Cánovas, G. V., & Swanson, B. G. (1997). Non-thermal food preservation: pulsed electric fields. *Trends in Food Science and Technology*, 8: 151-157.

Velázquez-Estrada, R. M., Hernández-Herrero, M. M., López-Pedemonte, T., Guamis-López, B., & Roig-Sagués, A. X. (2008). Inactivation of *Salmonella enterica serovar senftenberg 775W* in liquid whole egg by ultrahigh pressure homogenization. *Journal of Food Protection*, 71(11): 2283-2288.

Wang, B., Li, D., Wang, L. J., Chiu, Y. L., Chen, X. D., & Mao, Z. H. (2008). Effect of high-pressure homogenization on the structure and thermal properties of maize starch. *Journal of Food Engineering*, 87: 436-444.

Welti-Chanes, J., Ochoa-Velasco, C. E., & Guerrero-Beltrán, J. Á. (2009). High-pressure homogenization of orange juice to inactivate pectinmethylesterase. *Innovative Food Science and Emerging Technologies*, 10: 457-462.

Wesierska, E., & Trziszka, T. (2007). Evaluation of the use of pulsed electrical field as a factor with antimicrobial activity. *Journal of Food Engineering*, 78: 1320-1325.

Wuytack, E. Y., Diels, A. M., & Michiels, C. W. (2002). Bacterial inactivation by high-pressure homogenisation and high hydrostatic pressure. *International Journal of Food Microbiology*, 77: 205-212.

Yeom, H. W., Streaker, C. B., Zhang, Q. H., & Min, D. B. (2000). Effects of pulsed electric fields on the quality of orange juice and comparison with heat pasteurization. *Journal of Agricultural and Food Chemistry*, 48: 4597-4605.

Yeom, H. W., Evrendilek, G. A., Jin, Z. T., & Zhang, Q. H. (2004). Processing of yogurt-based products with pulsed electric fields: microbial, sensory and physical evaluations. *Journal of Food Processing Preservation*, 28: 161-178.

Yu, L. J., Ngadi, M., & Raghavan, G. S. (2009). Effect of temperature and pulsed electric field treatment on rennet coagulation properties of milk. *Journal of Food Engineering*, 95: 115-118.

Yuan, Y., Gao, Y., Zhao, J., & Mao, L. (2008). Characterization and stability of β-carotene nanoemulsions preprared by high pressure homogenization under various emulsifying conditions. *Food Research International*, 41: 61-68.

Zamora, A., Ferragut, V., Jaramillo, P. D., Guamis, B., & Trujillo, A. J. (2007). Effects of ultra-high pressure homogenization on the cheese-making properties of milk. *Journal of Dairy Science*, 90: 13-23.

Zeng, X.-a., Han, Z., & Zi, Z.-h. (2010). Effects of pulsed electric field treatments on quality of peanut oil. *Food Control*, 21: 611-614.

Zhang, Q. H., Qiu, X., & Sharma, S. K. (1997). Recent development in pulsed electric field processing. *National Food Processors Association. New Technologies Yearbook*. Washington 31-42.

Zhao, W., Yang, R., Tang, Y., & Lu, R. (2007). Combined effects of heat and PEF on microbial inactivation and quality of liquid egg whites. *International Journal of Food Engineering*, 3(4): 1-20.

Zhao, W., Yang, R., Lu, R., Wang, M., Quian, P., & Yang, W. (2008). Effect of PEF on microbial inactivation and physical-chemical properties of green tea extracts. *LWT Food Science and Technology*, 41: 425-431.

Zhao, W., Yang, R., Tang, Y., Zhang, W., & Hua, X. (2009). Investigation of the protein-protein aggregation of egg white proteins under pulsed electric fields. *Journal of Agricultural and Food Chemistry*, 57: 3571-3577.

Zhong, H., Zeng, X. A., Yu, S. J., Zhang, B. S., & Chen, X. D. (2009). Effects of pulsed electric fields (PEF) treatment on physicochemical properties of potato starch. *Innovative Food Science and Emerging Technologies*, 10: 481-485.

Objetivos

El objetivo general de esta Tesis Doctoral es el estudio del impacto de nuevas tecnologías de conservación, como la aplicación de Campos Eléctricos Pulsados (PEF) y Altas Presiones de Homogeneización (HPH), sobre la estructura y los principales componentes químicos de alimentos fluidos.

Este objetivo general enmarca los siguientes objetivos particulares:

1. Estudio del efecto de la aplicación de Campos Eléctricos Pulsados (PEF) sobre la microestructura y principales componentes químicos de huevo líquido. Estabilidad durante el almacenamiento en refrigeración.

2. Caracterización de las propiedades físicas (capacidad espumante, capacidad de retención de agua, color, viscosidad, etc.) de huevo líquido tratado por PEF.

3. Comparación del efecto de PEF con el tratamiento térmico convencional de pasteurización sobre la estructura, principales componentes químicos y propiedades físicas de huevo líquido.

4. Estudio del efecto de las Altas Presiones de Homogeneización (HPH) sobre la microestructura y principales componentes químicos de alimentos fluidos (huevo líquido y salsas). Estabilidad durante el almacenamiento en refrigeración.

Plan de Trabajo

Para conseguir los objetivos propuestos, se plantea el siguiente plan de trabajo:

1. Búsqueda y estudio bibliográfico de forma continuada durante la realización de la tesis. Estudio de la normativa y protocolos a seguir en la parte experimental.

2. Diseño de experimentos.

3. Puesta a punto de los protocolos a seguir en el estudio químico, físico y microestructural de las muestras objeto de estudio.

4. Recepción, estabilización y almacenamiento de las muestras tratadas por PEF en the Swedish Institute for Food and Biotehcnology (SIK) y de las muestras tratadas por HPH en la Università di Bologna (Dipartimento di *Scienze degli Alimenti).*

5. Caracterización y estudio de la microestructura de alimentos fluidos tratados por Campos Eléctricos Pulsados, Altas Presiones de Homogeneización o pasteurización mediante Microscopía Electrónica de Barrido a Bajas Temperaturas (Cryo-SEM), Microscopía Electrónica de Transmisión (TEM) y Microscopía Láser Confocal (CLSM).

6. Análisis químico de la fracción proteica de alimentos fluidos tratados por Campos Eléctricos Pulsados, Altas Presiones de Homogeneización o pasteurización. Extracción y cuantificación de la fracción de proteínas totales y solubles (N-Kjeldahl) y estudio mediante electroforesis en geles de poliacrilamida con dodecil sulfato sódico (SDS-PAGE).

7. Análisis químico de la fracción lipídica de alimentos fluidos tratados por Campos Eléctricos Pulsados, Altas Presiones de Homogeneización o pasteurización. Extracción y estudio de la lipólisis de la grasa mediante el análisis del índice de acidez y del grado de oxidación mediante espectrofotometría.

8. Estudio de diferentes propiedades físicas: viscosidad aparente (viscosímetro Brookfield), color (coordenadas CIELab), capacidad espumante, textura y capacidad de retención de agua en huevo líquido tratado por Campos Eléctricos Pulsados y pasteurización.

9. Análisis comparativo de los resultados obtenidos. Tratamiento estadístico de los datos e interpretación de resultados.

10. Redacción de informes y divulgación científica.

11. Redacción del documento final de la Tesis.

Capítulo 1

Czech Journal of Food Sciences *Vol.27, 2009, Special Issue*

Effect of Pulsed Electric Fields on the main Chemical Components of Liquid Egg and Stability at 4ºC

R. Marco-Molés, I. Pérez-Munuera, A. Quiles and I. Hernando*

Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia, Valencia, Spain
**E-mail: iperez@tal.upv.es, web: http://www.upv.es*

Abstract: The effect of PEF on the main components of whole liquid egg, proteins and lipids, and the microstructure was studied and compared with pasteurisation. The effect of the refrigerated storage one week after the treatments was too studied. Only pasteurised samples showed water-soluble protein values significantly lower than the non-treated and PEF treated samples, even after the refrigerated storage. This could be related to the reinforcement of protein-protein interactions generated by the partial denaturalisation of proteins after heating, observed by Cryo-SEM. Moreover, a water-soluble protein decrease was detected in the non-treated and PEF treated samples after refrigeration, probably due to the aggregation of the egg lipoproteins during the storage. Furthermore, a slight lipolysis was observed in the non-treated and PEF treated samples after refrigeration; but this effect was lower as higher was the PEF treatment. The study of the oxidation parameters showed an intermediate degradation of the lipids in treated samples, compared to the pasteurised eggs. These would reflect a higher microbiological stability of the PEF treated samples compared to the non-treated liquid egg.

Keywords: *Pulsed Electric Fields; Egg; Structure; Proteins; Lipids*

INTRODUCTION

Nowadays, consumers are more and more demanding for minimally processed foods. This has led to the food industry to develop new technologies as Pulsed Electric Fields (PEF) that allows obtaining products with similar properties to fresh foods (Mañas and Pagán, 2005). Specially, this technology is considered a very promising alternative to pasteurisation processes when processing high thermal sensitive liquid foods such as whole liquid egg (Sampedro *et al*., 2006). However, the main studies in egg treated by PEF are focused on inactivation of different target microorganisms. For that reason, the aim of this work has been to study the effect of PEF on proteins and lipids of whole liquid egg, and the microstructure of these components by Low Temperature Scanning Electron Microscopy. The effect of PEF treatment on whole liquid egg was studied and compared with pasteurisation, immediately after the treatment and after one week of refrigerated storage at 4 ºC.

MATERIALS AND METHODS

Materials

Whole liquid egg (WLE) samples: non-treated, pasteurised (66 ºC, 4.5 min) or treated by Pulsed Electric Fields (PEF) at 19 and 32 kV (5 pulses, 6 μs, 250 Hz), were supplied by the Swedish Institute for Food and Biotechnology (Göteborg, Sweden). All the samples were vacuum-packed in 250 mL plastic bags and kept under refrigerated storage at 4 ºC.

Low Temperature Scanning Electron Microscopy (Cryo-SEM)

A JSM-5410 SEM microscope (Jeol, Tokyo, Japan) coupled to a Cryo CT-1500C unit (Oxford Instruments, Witney, UK) was used. The sample was placed in the holder, fixed with slush nitrogen ($T \leq$ -210ºC), transferred frozen to the Cryo unit, fractured, etched (-90 ºC), and gold-coated (2 mbar, 2 mA). The sample was then transferred onto the microscope and examined at -130 ºC, 15 kV.

Extraction and quantification of the water-soluble proteins

Water-soluble nitrogen was extracted according to AOAC 932.08 (AOAC, 2000) and quantified by Kjeldahl, using a mixture of K_2SO_4, $CuSO_4$ and Se (10:1:0.1) as catalyser. All the results were expressed as protein using a conversion factor of 6.25.

Analysis of lipids

Lipids were extracted from the whole liquid egg samples according to Boselli *et al*. (2001). The acidity grade was determined in accordance with AOAC (1990) regulations. Hydroperoxides and secondary oxidation products were detected by spectrophotometric methods (UNE 55-047-73).

Statistical Analysis

Statistical analysis of the results was performed using a one-way analysis of variance (ANOVA); the least significant differences (LSD) were calculated at the $\leq$ 0.05 significance level. The Statgraphics Plus 5.1 computer-assisted statistics program was employed.

RESULTS AND DISCUSSION

Table 1 shows the protein values obtained by N-Kjeldahl. After the treatments or one week of storage, pasteurised samples showed water-soluble protein values significantly lower than the non-treated and PEF treated samples. This could be attributed to the partial insolubilisation of the proteins caused by the high temperatures reached during the thermal treatment. No changes were observed in the pasteurised samples after one week of storage, although an N-soluble decrease was observed in the non-treated and PEF treated samples. The egg natural colloidal emulsion was destabilised during the refrigerated storage, and a partial insolubilisation of proteins was produced in the non-treated and in the PEF treated samples.

Table 1. Water-soluble proteins analysed by N-Kjeldahl, in just treated samples (A) and after one week of refrigerated storage (B): non-treated WLE (C), treated by PEF at 19 kV and 32 kV, and pasteurised (P).

	A	B
C	8.72 ± 0.06^{a}	$8.11 \pm 0.06^{ab*}$
19 kV	8.46 ± 0.15^{a}	$7.96 \pm 0.15^{a*}$
32 kV	8.69 ± 0.33^{a}	$8.15 \pm 0.09^{ab*}$
P	7.02 ± 0.19^{b}	6.80 ± 0.25^{c}

Different letters in the same column indicate significant differences at $p<0.05$ according to the LSD multiple range test; *Means that N-soluble values of the just treated samples differ from those after one week at 4ºC ($p<0.05$) according to the LSD multiple range test.

The lipidic fraction was analysed to determine chemical changes due to a possible lipolytic activity (Table 2). After one week of storage, a slight lipolysis occurred in all the samples, except in the pasteurised ones. This was attributed to the growing of microorganisms, but this effect was lower as higher was the PEF treatment applied (32 kV).

Table 2. Acidity grade of the control WLE (C), treated by PEF at 19 kV and 32 kV, and pasteurised (P), in just treated samples (A) and after one week of refrigerated storage (B).

	A	B
C	1.21 ± 0.06^{c}	$1.42 \pm 0.02^{c*}$
19 kV	1.06 ± 0.04^{a}	$1.33 \pm 0.03^{b*}$
32 kV	1.12 ± 0.05^{b}	$1.29 \pm 0.04^{b*}$
P	1.10 ± 0.04^{ab}	1.09 ± 0.02^{a}

Different letters in the same column indicate significant differences at $p<0.05$ according to the LSD multiple range test; *Means that acidity grade values of the just treated samples differ from those after one week at 4ºC ($p<0.05$) according to the LSD multiple range test.

Furthermore, an increase in the K_{232} parameter was observed in all the samples, after one week of storage, although the highest effect was produced in the pasteurised samples. After one week of refrigerated storage, the K_{270} parameter decreased in all the samples (Table 3).

Table 3. Oxidation spectrophotometric parameters (K_{232} and K_{270}) of the control WLE (C), treated by PEF at 19 kV and 32 kV, and pasteurised (P), in just treated samples (A) and after one week of refrigerated storage.

	A		B	
	K_{232}	**K_{270}**	**K_{232}**	**K_{270}**
C	2.05 ± 0.09^{b}	3.19 ± 0.31^{b}	$2.52 \pm 0.17^{b*}$	$2.27 \pm 0.27^{b*}$
19 kV	2.70 ± 0.19^{d}	2.02 ± 0.07^{a}	2.90 ± 0.23^{c}	1.66 ± 0.23^{a}
32 kV	1.61 ± 0.07^{a}	3.46 ± 0.08^{b}	1.80 ± 0.06^{a}	2.72 ± 0.23^{c}
P	2.12 ± 0.09^{bc}	2.63 ± 0.33^{c}	$3.49 \pm 0.79^{d*}$	$1.71 \pm 0.54^{a*}$

Different letters in the same column indicate significant differences at $p<0.05$ according to the LSD multiple range test; *Means that K_{232} and K_{270} values of the samples at week 0 differ from those at week one ($p<0.05$) according to the LSD multiple range test.

The values of K_{232} and K_{270} oxidation parameters after the treatments and one week of storage showed an intermediate degradation effect of the lipidic fraction in PEF treated samples compared to the pasteurised ones. It would be related to a higher microbiological stability of these samples, compared to the non-treated whole liquid egg. The thermal treatment during the pasteurisation affected in a greater level the chemical stability of the lipidic fraction during the storage.

Figure 1 shows the Cryo-SEM micrographs of the non-treated, pasteurised and treated by PEF samples, respectively. In the non-treated egg, the lipoprotein matrix is observed as a continuous network in which the protein granules are immersed and closely interacting with the other components of the matrix (Fig. 1A). The empty areas would be mainly occupied by water in the original sample. Pasteurisation (Fig. 1B) produces a thickening of the lipoprotein matrix, which is observed closer in these

samples; this is probably due to the intensification of the protein-protein interactions, caused by the partial denaturalization or insolubilization of the proteins during the thermal treatment. In the PEF treated samples, some discontinuities could be observed in the lipoprotein matrix and it was even broken in some areas (Fig. 1C and 1D). This effect could be related to a weakening of the interactions among the liquid egg components.

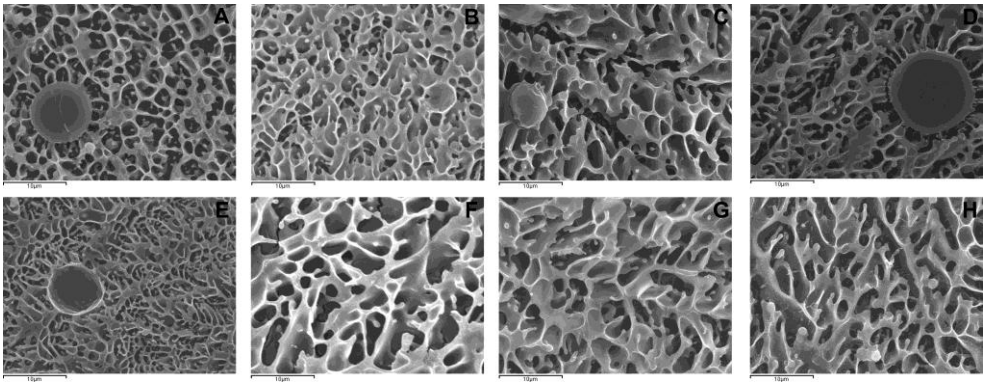


Figure 1 Cryo-SEM. **A**: control WLE; **B**: pasteurised WLE; **C**: WLE treated by PEF at 19 kV; **D**: WLE treated by PEF at 32 kV; **E**: control WLE, after one week of refrigerated storage; **F**: pasteurised WLE, after one week of refrigerated storage; **G**: WLE treated by PEF at 19 kV, after one week of refrigerated storage; **H**: WLE treated by PEF at 32 kV, after one week of refrigerated storage. 3500x

After one week of refrigerated storage, an aggregation effect can be observed in all the samples (Fig. 1E, F, G and H). However, this effect was greater in the non-treated egg (Fig. 1E), in which the areas originally occupied with water had been highly reduced. These observations could explain the N-soluble decrease observed in the water-soluble protein analysis, due to an aggregation effect during the pasteurisation and the PEF treatments.

REFERENCES

AOAC (1990): Official methods of analysis of AOAC International. 15th Ed. Association of Official Analytical Chemist, Washington.

AOAC (2000): Official methods of analysis of AOAC International. 17th Ed. Association of Official Analytical Chemist, Gaithersburg.

BOSELLI E.; VELAZCO, V.; FIORENZA CABONI, M.; LERCKER, G. (2001). Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food. Journal of Chromatography A, **917**: 239-244.

MAÑAS P., PAGÁN R. (2005). Microbial inactivation by new technologies of food preservation. Journal of Applied Microbiology, **98**: 1387-1399.

SAMPEDRO F., RODRIGO D., MARTÍNEZ A., BARBOSA-CÁNOVAS G.V., RODRIGO M. (2006). Review: Application of Pulsed Electric Fields in eggs and eggs derivates. Food Science and Technology International, **12** (5): 397-406.

UNE 55-047-73 (1973). Materias grasas. Medida espectrofotométrica de la absorción en la región ultravioleta. Instituto Nacional de Racionalización y Normalización (IRANOR). Patronato Juan de la Cierva de Investigación Técnica: Consejo Superior de Investigaciones Científicas (España).

Capítulo 2

International Journal of Food Science & Technology *Enviado*

Changes in the Microstructure and Protein Fraction of Whole Liquid Egg treated by Pulsed Electric Fields (PEF)

R. Marco-Molés, I. Hernando, A. Quiles, E. Llorca, and I. Pérez-Munuera*

Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de Alimentos. Universitat Politècnica de València. P.O. Box 22012, 46071 Valencia, Spain
*To whom correspondence should be addressed. e-mail: emllomar@tal.upv.es.
Phone: 963877363 Fax: 963877363

Abstract: This study examines the main changes that take place in the ultrastructure and protein fraction of whole liquid egg (WLE) treated by Pulsed Electric Fields (PEF), compared with the pasteurised egg. The effect of field strength (19 and 32 kV) is studied by Transmission Electron Microscopy (TEM) and SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE). The results show that the application of PEF produces discontinuities in the liquid egg lipoprotein matrix and degradation of the protein granules structure, which increases at higher electrical field strengths. These changes can modify the functionality of WLE when it is used as raw material in the elaboration of food products, and it could be related to the changes observed by other authors in gelling, flow or sensory properties after PEF treatment.

Keywords: *Pulsed Electric Fields; Egg; Ultrastructure; Protein; SDS-PAGE*

INTRODUCTION

Research into "non thermal" food conservation techniques has come about in partial response to the increased consumer demand for fresh, premium quality "ready to eat" (RTE) foods. This has lead to the development of minimally processed products which have high quality and freshness with high sensorial and nutritional properties while also guaranteeing safe foods (Gould, 2001).

The application of Pulsed Electric Fields (PEF) is one of the non-thermal food preservation technologies which have received the most interest and attention in the last years. It is less damaging to the physical and organoleptic properties of food and offers high quality food with excellent flavour, nutritional value and improved shelf-life (Knorr *et al.*, 2002; Ross *et al.*, 2003; Mañas and Pagán, 2005).

PEF treatment uses strong electric fields, generally greater than 20 kV/cm applied as short pulses (μs) (Señorans *et al.*, 2003). This technique has been successfully used for the pasteurisation of liquid or semi-liquid foods such as juices, milk, yoghurt, liquid egg, sauces and soups; this processing method may offer to the consumer safe, fresh-life, and nutritious food products (Knorr *et al.*, 2002; Raso *et al.*, 2003; Devlieghere *et al.*, 2004; Sobrino-López *et al.*, 2006; Mosqueda-Melgar *et al.*, 2008).

Liquid egg, and particularly egg white, is an important raw material for the food industry due to its foaming and gelling properties, and also its protein content of high biological and nutritional value. Up to now, pasteurisation has been used as a preservation method for liquid egg but this has undesirable effects such as reducing viscosity, altering functional properties, reducing foaming capacity and the possible coagulation of the more thermo labile proteins.

Studies on the effect of PEF on the shelf-life of egg and its derivatives have shown that PEF combined with antimicrobial agents and moderate temperatures lengthens the shelf-life of these products compared with control samples (Martín-Belloso *et al.*, 1997, Góngora-Nieto *et al.*, 2003). Studies on the effect of PEF on egg physicochemical properties such as colour, viscosity, and sensorial properties (Qin *et al.*, 1995) showed that following PEF treatment the viscosity and colour of liquid egg are retained, whilst no differences are observed in sensorial test acceptance.

As regards the effect of PEF on proteins, studies have only been carried out on model systems and on egg white focusing principally on the gelling properties. In this respect Jeanet *et al.* (1999) verified that the surface hydrophobic of egg white proteins did not increase following PEF treatments and suggested that the proteins were not denatured. Fernández-Díaz *et al.* (2000) did not observe any protein coagulation problems when they studied the effects of PEF on solutions of ovalbumin and egg white, nor any notable changes in their protein functionality properties such as solubility and gelling power. Barsotti *et al.* (2001) applied PEF to solutions of ovalbumin and dialysed egg white: the PEF treatment did not induce unfolding or significant aggregation in the ovalbumin, nor did it produce significant changes in the gelling properties of dialysed egg white. Using electrophoresis, Pérez and Pilosof (2004) observed partial denaturation of egg white proteins, in addition to a reduction in gelling rate.

The analysis of microstructure in food systems treated with non-thermal preservation technologies such as PEF is a useful tool for understanding the functional properties of the processed products. No studies have been found on the effect of PEF on the microstructure of liquid whole egg. So, it would be useful to establish how the field strength affects the microstructure of its components, since there is a link between this and their functionality.

The aim of this study is to examine the principal changes that take place in the ultrastructure of homogenised liquid egg treated with PEF, and the effect on protein fraction, compared with pasteurisation, a conventional thermal treatment.

MATERIALS AND METHODS

Materials

Whole liquid egg subjected to PEF treatment and pasteurised liquid egg (66 ºC, 45 min) were used. Table 1 shows the PEF treatment conditions for each sample.

Table 1. Technological characteristics of the pulsed electric fields applied. U: electric field strength; t: pulse width; N: number of applied pulses; prf: pulse repetition frequency; R: internal resistance.

	Pulse generator parameters					Temperature (ºC)	
Sample	**U (kV)**	**t (μs)**	**N**	**prf (Hz)**	**R (Ω)**	**In**	**Out**
HighPEF	32	6	5	250	1000	3.8	33.5
LowPEF	19	6	5	250	1000	5.4	16.0

Transmission Electron Microscopy (TEM)

The ultrastructure of the liquid egg samples was characterised by means of Transmission Electron Microscopy (TEM).

For observation, the samples were stabilised by mixing with a low gelling temperature agarose solution (3%) at 30 ºC, which facilitates fixation and embedding prior to TEM observation (Sharma *et al.*, 1996). Next, samples were cut into cubes (1 mm^3), fixed (primary fixation with 2.5% glutaraldehyde and secondary fixation with 2% osmium tetroxide), dehydrated with 30, 50 and 70% ethanol, contrasted with uranyl acetate (2%) and embedded in epoxy resin (Durcupan ACM, FLUKA). The blocks thus obtained were cut using a Reichter-Jung ULTRACUT ultramicrotome (Leila, Barcelona, Spain). The ultrathin sections obtained (=100Å) were collected in copper grids and stained with 4% lead citrate to be observed in a Philips EM 400 Transmission Electronic Microscope (Eindhoven, Holland) at 80 kV.

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE study of the total protein fraction was carried out using an aliquot of whole liquid egg. The water-soluble protein fraction was extracted, prior to SDS-PAGE, as follows. First, samples were freeze-dried (TELSTAR Lioalfa-6, Terrassa, Spain) for 24 h at 10^3 Pa and –45ºC. After freeze-drying, they were defatted in a continuous extraction method (Soxhlet) for 16 h with n-hexane-isopropanol (77:23) as solvent. 2.5 g of defatted samples were mixed with 15 mL of distilled water. These mixtures were centrifuged at 3500 rpm for 20 min in a Sorvall Super T_{21} centrifuge

(KENDRO Laboratory Products, Newtown, CT). Protein concentration of these water-soluble extracts was determined by the Bradford method (Bradford, 1976) using standard BSA for the preparation of the standard curve.

The protein concentration of the samples was adjusted to 1.25 mg/mL with Laemmli buffer. Electrophoresis was performed using the method of Laemmli (Laemli, 1970) on a Multiphor II Electrophoresis System (Pharmacia Biotech, Piscataway, New Jersey, USA), using 12.5% polyacrylamide gels ExcelGel SDS Homogeneous (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at 600 V, 38 mA, 23 W and 15 ºC for 1h 30 min. 8 μl of each sample were loaded in the gel in duplicate.

The standard was an Amersham low molecular weight calibration kit (GE Healthcare, UK) consisting of: phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 Da).

Protein bands were stained with Coomassie Brilliant Blue tablets (PhastGel Blue R., GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Destaining was performed in an aqueous solution of 25% ethanol and 8% acetic acid. Samples were conserved in a solution of 10% glycerol and 7.2% acetic acid. The molecular weight of each band was determined using a densitometer Intelligent Dark Box II Fujifilm LAS 1000 (Fujifilm, Tokyo, Japan) with the software Image Gauge (Fujifilm USA, Valhalla, NY, USA).

RESULTS AND DISCUSSION

Transmission Electron Microscopy (TEM)

90% of egg white is water, and proteins represent 90% dry matter of the remaining; the main protein in egg white is ovalbumin (58%), which gels on heating. The yolk is oil in water emulsion with about 50% dry matter content (64% lipids and 33% proteins). The lipids are mainly low density lipoproteins (20-40 μm diameter) dissolved in continuous plasma. In the plasma, there are also numerous spherical particles of different sizes called protein granules (1-1.3 μm) which also contain lipids (Belitz and Grosch, 1997).

When samples are observed using TEM (Fig. 1A), a continuous matrix mainly composed of protein (the majority albumins), and fat from the yolk that have been incorporated into this matrix during beating can be observed. In this lipoprotein matrix, round-shaped compact protein granules with a diameter of up to 1.5 μm are immersed. The protein granules are interacting with the lipoprotein matrix in such a way that there is continuity between the surface of the granules and the chemical components of the matrix.

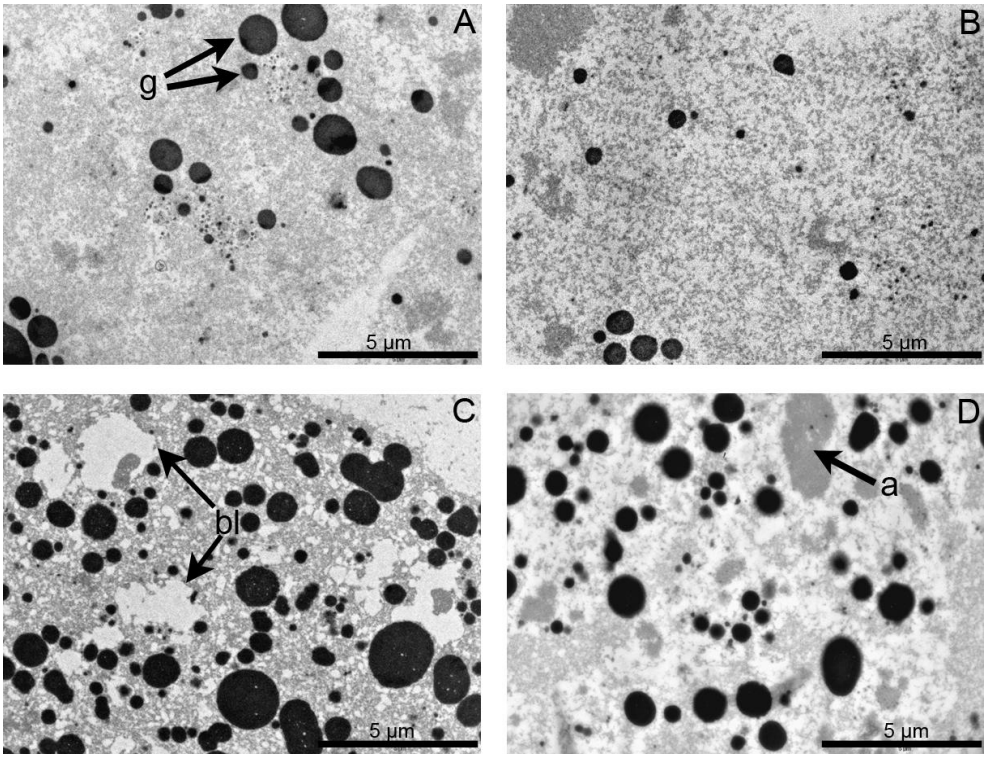


Figure 1. TEM. **A**: Control WLE; **B**: WLE treated by PEF at 19 kV; **C**: WLE treated by PEF at 32 kV; **D**: pasteurised WLE. 1650x (g: protein granules; a: aggregated compounds; bl: broken lipoprotein matrix)

When samples are treated by PEF (19kV), the lipoprotein matrix is observed to be more dense (Fig. 1B) and slightly altered if compared to the control one. The application of high intensity pulses (32kV) has a greater effect on these samples (Fig. 1C); the lipoprotein matrix appears

aggregated and broken in some areas. The discontinuity among the components of the lipoprotein matrix that has been generated by the PEF treatment could affect the stability of the egg natural emulsion. In figure 1D showing pasteurised whole liquid egg samples, areas where the lipoprotein matrix appears highly aggregated can be observed. It could be due to the denaturing or coagulation of the proteins during the thermal treatment (66 ºC). Guilmineau *et al.* (2005) demonstrated by SDS-PAGE, that plasma proteins are more sensitive to heat than protein granules. The lipoprotein matrix of the pasteurised samples is clearly more affected than the control samples or treated with low field strength (figures 1A and 1B).

When whole liquid egg (WLE) treated by PEF (32 kV) and pasteurised WLE are observed using a higher magnification (Fig. 2), details of the ultrastructural differences between the two treatments can be studied. Figure 2B shows WLE treated by PEF (32kV), interaction between the protein granules and the lipoprotein matrix is weakened if it is compared with the control sample (Fig. 2A). This is also observed in the pasteurised samples (Fig. 2C), where the effect of protein aggregation appears to be greater. In both, high intensity PEF treated and pasteurised samples, some protein granules altered by the treatments can be seen.

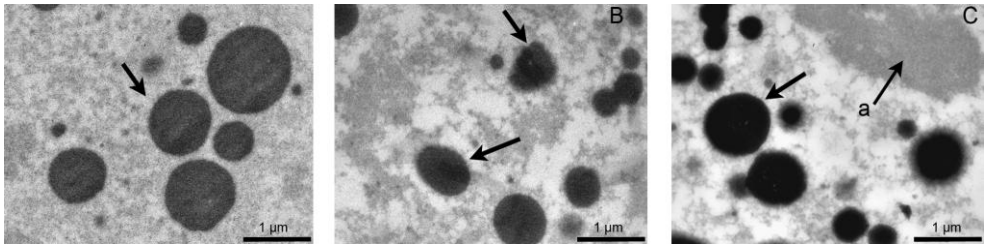


Figure 2. TEM. **A**: Control WLE; **B**: WLE treated by PEF (32 kV); **C**: pasteurised WLE. 5200x (arrow: protein granules; a: aggregated compounds)

The observations at ultrastructural level could be linked to a liquid egg instability following the application of these treatments; it has been seen that PEF treated samples are not stable as there is a phase separation 48 h after treatment (images not shown). The pulsed electric fields break the continuity of the protein network, destabilising the colloidal emulsion;

however, pasteurisation produces a greater protein aggregation and not phase separation.

SDS-PAGE

The electrophoregram obtained for the total protein fraction is shown in figure 3. The bands in the control whole liquid egg (C) are taken as reference to study the effect of the different treatments.

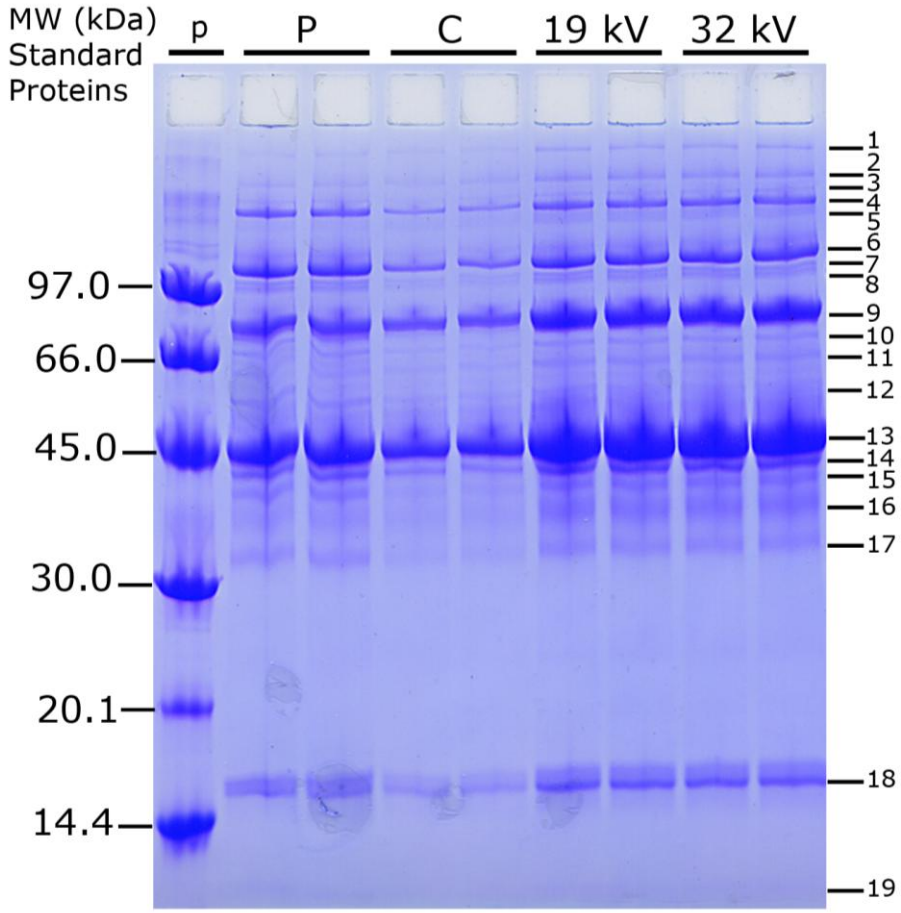


Figure 3. Total protein electrophoregram. **P**: pasteurized whole liquid egg; **C**: control whole liquid egg; **19 kV**: low intensity PEF (19 kV) treated whole liquid egg; **32 kV**: high intensity PEF (32 kV) treated whole liquid egg; **p**: standard protein. The MW of the studied bands are: 1: 186-200 kDa; 2: 166-169 kDa; 3: 151-155 kDa; 4: 142-146 kDa; 5: 132-139

kDa; 6: 105-107 kDa; 7: 99-102 kDa; 8: 97-98 kDa; 9: 79-81 kDa; 10: 69-72 kDa; 11: 63-65 kDa; 12: 55-57 kDa; 13: 43-44 kDa; 14: 40-41 kDa; 15: 37-38 kDa; 16: 33-35 kDa; 17: 29-30 kDa; 18: 16-17 kDa; 19: 11 kDa.

Using densitometric analysis, 19 bands are identified for the control liquid egg. Bands 4, 6, 9, 13, 14 and 18, of (142-146), (105-107), (79-81), (43-44), (40-41) and (16-17) kDa, respectively, stand out for their greater intensity. Band 4 (142-146 kDa) would be LDL apoproteins (low density lipoproteins in plasma) according to Raikos *et al.* (Raikos *et al.*, 2006, Jolivet et el., 2006); and band 6 (105-107 kDa) would be HDL apoproteins (high density lipoproteins) according to Guilmineau *et al.* (2005) or even γ-livetin (104 kDa). The ovotransferrin or conalbumin (band 9) is identified by estimated molecular weight (79-81 kDa) and because it is the second most accentuated band, fitting its relative proportion in egg white (13%) (Belitz and Grosch, 1997). The ovalbumin (band 13) is identified through comparison with mobility of the standards (45 kDa) and also because it is the main band in the gel as is to be expected, since it is the most widespread protein found in egg white (58%) (Belitz and Grosch, 1997). Similarly, band 14 (40-41 kDa) would correspond to LDL, HDL or ovoglobulins, as described by Guilmineau *et al.* (2005); Raikos *et al.* (2006) and Belitz and Grosch (1997). Band 18 is identified as lysozyme.

Other bands can also be noticed in figure 4, although they are not heavily stained. The bands 1 and 2 of molecular weights between 166 and 200 kDa could correspond to the α sub-units of ovomucin (170 kDa) in reducing conditions (Desert *et al.*, (2001) and the ovostatin dissociated into 4 monomers of 195 kDa (Nagase *et al.*, 1983) or 165 kDa (Desert *et al.* (2001). It could also contain LDL apoproteins of 170 and 203 kDa, respectively.

Band 10 (69-72 kDa) corresponds to avidin, whose theoretical molecular weight is 68 kDa (Belitz and Grosch, (1997), but could also contain LDL apoproteins from the yolk. Band 12 (55-57 kDa) could correspond to α-livetin and phosphovitin (55 and 59 kDa, respectively, Guilmineau *et al.* (2005), clusterin (51 kDa, Guérin-Dubiard *et al.* (2006) and even ovoinhibitor (49 kDa, Belitz and Grosch, (1997) or LDL apoproteins. Band 15 (37-38 kDa) could be identified as ovoglobulin G2 and G3 (30-45kDa), phosphovitin (36-40 kDa) and flavoprotein (35-36 kDa) according to

Allerton and Perlmann (1995) and Belitz and Grosch (1997). Band 16 (33-35 kDa) could be phosphovitin (Allerton and Perlmann, (1965), corresponding to 35-36 kDa, due to its high degree of glycosylation, approximately 25%, according to Miguel *et al.*, (2005). Band 16 could also contain LDL apoprotein, β-livetin (33 kDa, Guilmineau *et al.*, (2005), ovoglobulin G2 and G3 and even clusterin (35 kDa, Guérin-Dubiard *et al.*, 2006). Lastly, ovomucoid protein (28 kDa), with a relative proportion of 11% in egg white, could correspond to band 17 (29-30 kDa) along with others such as HDL apoprotein (31 kDa, Guilmineau *et al.* (2005), ovoglobulin G2 and G3 and flavoprotein.

The densitometric analysis of the main changes on the total proteins (Fig. 3) of the samples subjected to the different treatments (pasteurisation and PEF), compared to the control whole liquid egg, shows that there are not significant changes on the characteristic bands. Martínez *et al.* (1994) observed changes in the electrophoregrams of ultrapasteurised whole liquid egg using native electrophoresis. Ma *et al.* (2001) did not observe changes in the native electrophoregram of the liquid egg total proteins treated by 48kV PEF for 2 μs.

Figure 4 shows the electrophoregram obtained for the water-soluble protein fraction. The bands 9 and 13, corresponding to ovotransferrin and ovoalbumin, are the main water-soluble proteins in egg white. Pérez and Pilosof (2004) observed that egg white proteins were denatured when millisecond pulsed electric fields (12.5 kV) was applied. They also observed the formation of aggregates with covalent bonds, but not significant changes in the amount of native proteins were detected.

In the water-soluble protein fraction (Fig. 4), band 14 of the control sample corresponds to LDL, HDL or ovoglobulins, and has a higher concentration than that of the treated samples, which could be due to insolubilisation following the different treatments. Guilmineau *et al.* (2005) observed that egg yolk proteins changed their solubility during heating (74 ºC), and that some of the HDL apoproteins were partially denatured, whilst the LDL apoproteins and livetins were more thermolabile. The effect of protein aggregation observed in the microstructural study of the PEF-treated and pasteurised samples (Fig. 1 and 2) would indicate general changes on protein solubility not linked to significant chemical changes on their electrophoretic pattern.

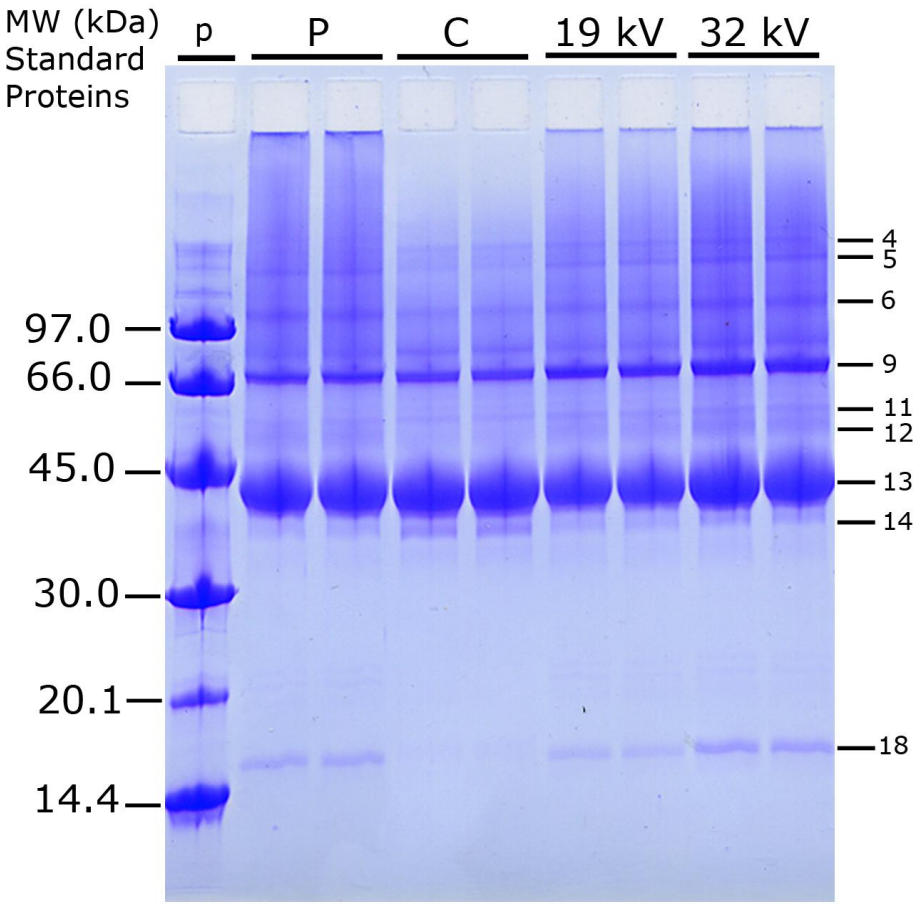


Figure 4. Water soluble protein electrophoregram. **P**: pasteurized whole liquid egg; **C**: whole liquid egg control; **19 kV**: low intensity PEF (19 kV) treated whole liquid egg; **32 kV**: high intensity PEF (32 kV) treated whole liquid egg; **p**: standard protein.

CONCLUSIONS

The application of pulsed electric fields (19kV and 32 kV) to whole liquid egg produces breakdown of the lipoprotein matrix and the alteration of the protein granule structure. These structural changes might explain the destabilisation of the colloidal system following this treatment. However, PEF treatments (19 and 32 kV) and pasteurisation do not cause proteolysis of the egg proteins. A clear effect of aggregation in the lipoprotein matrix components is observed when samples are pasteurised.

ACKNOWLEDGEMENTS

This research has been supported by the European Comission (project FP6-FOOD-023140). The authors are indebted to Swedish Institute for Food and Biotechnology (Göteborg, Suecia) for supplying the samples analyzed in this work and to the Ministerio de Educación y Ciencia (Spain) for the grant awarded to the author R. Marco-Molés. Finally, we would like to thank the R&D&I Linguistic Assistance Office, Universidad Politécnica de Valencia (Spain), for translating this paper.

REFERENCES

Allerton, S. E., & Perlmann, G. E. (1965). Chemical characterization of the phosphoprotein, phosphovitin. Journal of Biological Chemestry, **240**: 3892-3898.

Barsotti, L., Dumay, E., Mu, T. H., Fernández-Díaz, M. D. & Cheftel, J. C. (2001). Effects of high voltage electric pulses on protein-based food constituents and structures. Trends in Food Science & Technology, **12** (3-4): 136-144.

Belitz, H. D. & Grosch, W. (1997). Química de los alimentos. Acribia, Zaragoza España, pp 587-602.

Boselli, E., Velazco, V., Caboni, M. F. & Lercker, G. (2001). Pressurized liquid extraction of lipids for the determination of oxysterols in egg-containing food, Journal of Chromatography A, **917**: 239-244.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding, Analytical Biochemistry, **72**: 248–254.

Chung, L. S. & Ferrier, L. K. (1991). Partial lipid extraction of egg yolk powder: effects on emulsifying properties and soluble protein fraction. Journal of Food Science, **56** (5): 1255-1258.

Desert, C., Guérin-Dubiard, C., Nau, F., Jan, G., Val, F. & Mallard, J. (2001). Comparison of different electrophoretic separations of hen egg white proteins. Journal of Agricultural and Food Chemistry, **49** (10): 4553-4561.

Devlieghere, F., Vermeiren, L. & Debevere, J. (2004) New preservation technologies: possibilities and limitations. International Dairy Journal, **14**: 273-285.

Fernández-Díaz, M. D., Barsotti, L., Dumay, E. & Cheftel J. C. (2000). Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. Journal of Agricultural and Food Chemistry, **48** (6): 2332-2339.

Góngora-Nieto, M. M., Pedrow, P. D., Swanson, B. & Barbosa-Cánovas, G. V, (2003). Energy analysis of liquid whole egg pasteruized with of pulsed electric fields. Journal of Agricultural and Food Chemistry, **48**: 2332-2339.

Gould, G. W. (2001) New processing technologies: an overview. Proceedings of the Nutrition Society, **60**: 463-474.

Guérin-Dubiard, C., Pasco, M., Mollé, D., Désert, C., Croguennec, T. & Nau, F. (2006). Proteomic analysis of hen egg white. Journal of Agricultural and Food Chemestry, **54**: 3901-3910.

Guilmineau, F., Krause, I. & Kulozik, U. (2005). Efficient analysis of egg yolk proteins and their thermal sensitivity using sodium dodecyl sulphate polyacrylamide gel electrophoresis under reducing and nonreducing conditions. Journal of Agricultural and Food Chemistry, **53**: 9329-9336.

Jeantet, R., Baron, F., Nau, F., Roignant, M. & Brulé, G. (1999). High intensity pulsed electric fields applied to egg white: effect on Salmonella enteriditis inactivation and protein denaturation. Journal of Food Protection, **62** (12): 1381-1386.

Jolivet, P., Boulard, C., Beaumal, V., Chardot, T. & Anton, M. (2006). Protein components of low-density lipoproteins purified from hen egg yolk. Journal of Agricultural and Food Chemistry, **54**: 4424-4429

Knorr, D., Ade-Omawaye, B. I. O. & Heinz, V. (2002). Nutritional improvement of plant foods by non-thermal processing. Proceedings of the Nutrition Society, **61**: 311-318.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, **227**: 680–685.

Ma, L., Chang, F. J., Góngora-Nieto, M. M., Barbosa-Cánovas, G. V. & Swanson, B. G. (2001). Comparison study of pulsed electric fields, high hydrostatic pressure and thermal processing on the electrophoretic patterns of liquid whole eggs. In G. V. Barbosa-Cánovas & Q. H. Zhang (Eds.), Pulsed electric fields in food processing: 65-82. Lancaster, PA: Technomic Publishing.

Mañas, P & Pagán, R. (2005). Microbial inactivation by new technologies of food preservation. Journal of applied microbiology, **98**: 1387-1399.

Martín-Belloso, O., Vega-Mercado, H., Qin, B. L., Chang, F. J., Barbosa-Cánovas, G. V. & Swanson, B. G. (1997). Journal of Food Processing and Preservation, **21**: 193-208

Martínez, R. M., Ball, H. R. Jr. & Dawson, P. L. (1994). Effect of ultrapasteurization with and without homogenization on the electrophoretic patterns of aseptically processed liquid whole egg. Poultry Science, **73**: 317-321.

Miguel, M., Manso, M. A., López-Fandiño, R. & Ramos, M. (2005). Comparative study of egg white proteins from different species by chromatographic and electrophoretic methods. European Food Research and Technology, **221** (3-4): 542-546.

Mosqueda-Melgar, J., Elez-Martínez, P., Raybaudi-Massilia, R. M. & Martín-Belloso, O. (2008). Effects of Pulsed Electric Fields on Pathogenic Microorganisms of Major Concern in Fluid Foods: A Review. Critical reviews in food science and nutrition, **48**: 747-759

Nagase, H., Harris, E. D., Woessner, J. F. & Brew, K. (1983). Ovostatin: a novel proteinase inhibitor from chicken egg white. Purification, physicochemical properties, and tissue distribution of ovostatin. Journal of Biological Chemestry, **258** (12): 7481-7489.

Pérez, O. E. & Pilosof, A. M. R. (2004). Pulsed electric fields effects on the molecular structure and gelation of beta-lactoglobulin concentrate and egg white. Food Research International, **37** (1): 102-110.

Qin, B. L., Pothakamury, U. R., Vega-Mercado, H., Martín-Belloso, O. M., Barbosa-Cánovas, G. V. & Swanson, B. G. (1995). Food Technology, **12**: 55–60.

Raikos, V., Hasen, R., Campbell, L. & Euston, S. R. (2006). Separaion and identification of hen egg protein isoforms using SDS-PAGE and 2D gel electrophoresis with MALDO-TOF mass spectrometry. Food Science, **99**: 702-710.

Raso, J. & Barbosa-Cánovas, G. V. (2003). Nonthermal preservation of foods using combined processing techniques. Critical Reviews in Food Science and Nutrition, **43** (3): 265-285.

Ross, A. I. V., Griffiths, M. W., Mittal, G. S. & Deeth, H. C. (2003). Combining nonthermal technologies to control foodborne microorganisms. International Journal of Food Microbiology, **89**: 125-138.

Señorans, F. J., Ibáñes, E. & Cifuentes, A. (2003). New trends in food processing. Critical Reviews in Food Science and Nutrition, **43** (5): 507-526.

Sharma, R., Singh, H. & Taylor, M. W. (1996). Composition and structure of fat globule surface layers in recombined milk. Journal of Food Science, **61** (1): 28-32.

Sobrino-López A. & Martín-Belloso, O. (2006). Enhancing inactivation of Staphylococcus aureus in skim milk by combining high-intensity pulsed electric fields and nisin. Journal of Food Protection, **69**: 345-353.

Capítulo 3

Journal of Food Science, C: Food Chemistry *Vol.76, Nr 2, 2011*

Physical and Structural Changes in Liquid Whole Egg treated with High-Intensity Pulsed Electric Fields

*Raquel Marco-Molés[2], María A. Rojas-Graü[1], Isabel Hernando[2], Isabel Pérez-Munuera[2], Robert Soliva-Fortuny[1], and Olga Martín-Belloso[1]**

[1]Department of Food Technology TPV-XaRTA, University of Lleida, Rovira Roure 191, 25198 Lleida, Spain. Phone: +34 973 702593 Fax: + 34 973 702596

[2]Department of Food Technology, Universidad Politécnica de Valencia, Camino de Vera, s/n, 46022 Valencia, Spain. Phone: +34 963879360 Fax: + 34 963879360

**Direct inquires to the author Martín-Belloso (E-mail: amartin@tecal.udl.cat).*

Abstract: Liquid whole egg (LWE) is currently pasteurized through the application of heat; however, this treatment entails deleterious effects against some of the functional and technological properties of the product. In this study, the effect of high-intensity pulsed electric fields (HIPEF) processing (field strength: 19, 32, 37 kV/cm) was compared to the traditional heat pasteurization (66 ºC for 4.5 min). Different physical and structural characteristics of LWE, subjected or not to homogenization, were evaluated and compared, having the untreated LWE as a reference. Thermal treatment caused an increase in the viscosity of LWE, especially in nonhomogenized samples. HIPEF treatments did not modify the original color of LWE, whereas thermally treated samples developed an opaque appearance. LWE treated at 19 and 32 kV/cm exhibited a similar foaming capacity as fresh untreated egg, whereas treatments of 37 kV/cm caused a decrease in the foaming capacity that was substantially lower than in the fresh untreated egg. Regarding the microstructure, the lipoprotein matrix appeared to be less affected by the HIPEF than by heat treatment if compared to the control. In addition, heat pasteurization had a significant impact on both the water-soluble protein content of the LWE samples (19.5-23.6% decrease) and the mechanical properties of the egg gels (up to 21.3% and 14.5% increase in hardness and cohesiveness, respectively). On

the other hand, these parameters were not substantially affected in the HIPEF-treated samples. Similarly to heat-treated samples, heat-induced gels obtained from HIPEF-treated samples did not exhibit remarkable changes in the water-holding capacity (WHC).

Keywords: *High Intensity Pulsed Electric Fields; Liquid Whole Egg; Physical Properties; Microstructure*

INTRODUCTION

Research on nonthermal food preservation technologies is currently in an exciting phase, in the quest for processes that can be an alternative or a complement to those traditionally applied (Góngora-Nieto *et al.,* 2001). Nonthermal technologies have a mild impact on the sensory profile and the quality attributes of the treated foods (such as taste, color, aroma, nutrients, and functional properties) offering to food processors the opportunity to produce safe products of high quality.

Among these technologies, high-intensity pulsed electric fields (HIPEF) may constitute one of the most suitable methods for processing fluid foods. During the last years, it has received considerable attention from scientists, governments, and industrial stakeholders as a potential technique to be fully scaled-up in the near future. HIPEF technology is claimed to be able to kill microorganisms and inactivate enzymes in liquid foods without significant losses of aroma, color, taste, or nutrients.

HIPEF processing involves the application of an electric field, usually ranging from 20 to 60 kV/cm (Barbosa-Cánovas and Sepúlveda, 2004), in the form of short pulses (about 1-5 μs), to a fluid food confined in or flowing through a pair of high-voltage electrodes. Continuous HIPEF treatment systems have been successfully used to pasteurize foods, such as milk and dairy products, a variety of fruit juices, beaten eggs, and cream soups (Palomeque *et al.,* 2001). In such studies, no significant changes have been detected in either chemical or physical parameters; furthermore, differences between HIPEF-treated and fresh untreated products have been claimed to be unnoticeable by sensory panels (Barbosa-Cánovas *et al.,* 1999; Vega-Mercado *et al.,* 2007).

HIPEF processing is of particular interest for products containing heat-sensitive components such as whey, immunoglobulins, and liquid egg (Fernández-Díaz *et al.,* 2000; Pérez and Pilosof 2004; Sampedro *et al.,* 2006; Deeth *et al.,* 2007).

Liquid whole egg (LWE) is widely used by the foodservice industry and other commercial food manufacturers because of convenience, ease in handling, and long storing as compared to shell eggs (Góngora-Nieto *et al.,* 2001). Egg is a polyfunctional ingredient because of its thickening, gelling, emulsifying, foaming, coloring, and flavoring characteristics, which can be

used to modify the organoleptic and technological properties of many other food products (Zhao *et al.,* 2007). In addition, liquid egg products are also valuable due to their high-quality protein content and their low cost (Mine 1995).

The microstructural analysis of foodstuffs is a useful tool to study their functional properties as well as possible changes occurring during processing. However, no literature is available regarding the effect of HIPEF treatments on the microstructure of liquid whole egg.

The objective of this work was to evaluate and compare the effects of HIPEF processing and heat pasteurization on the physical properties and microstructure of liquid whole egg. The effect of homogenization before treatments was also studied.

MATERIALS AND METHODS

Sample processing

Liquid whole egg samples (untreated, HIPEF-treated, or thermally treated) were supplied by SIK, the Swedish Institute for Food and Biotechnology (Göteborg, Sweden). Treatments of different intensity were compared (19 kV/cm for 30 µs, 32 kV/cm for 30 µs, and 37 kV/cm for 18 µs) in order to study the effect of HIPEF conditions on the main physicochemical properties of the LWE matrix. Additional information, critical to HIPEF processing, is displayed in Table 1. A pilot-scale system was used. A pulse generator (Scandinova, Uppsala, Sweden) was attached to a PEF treatment chamber (0.78 cm^3) developed by SIK. Before treatment the samples were kept at 5 ºC using a cooling medium containing water and ethanol. The temperature was registered before and after the treatment using a thermocouple (PClogger-3100i; Intab, Stenkullen, Sweden).

Table 1. Selected values for critical processing parameters in the HIPEF treatments.

Sample	E (kV/cm)	t (µs)	N	prf (Hz)	R (Ω)	Inlet T (ºC)	Outlet T (ºC)
Mild Treatment	19	6	5	250	700	4.1	13.4
Medium Treatment	32	6	5	250	700	5.6	31.4
Intense Treatment	37	6	3	250	700	5.5	31.3

E: electric field strength; t: pulse width; N: number of applied pulses; prf: pulse repetition frequency; R: internal resistance, T: temperature.

The heat-pasteurized samples were treated at 66 ºC for 4.5 min. The homogenization treatment was carried out by pumping and filtering the samples through a 0.4-mm-diameter hole. All the samples were vacuum-packed in 250-mL plastic bags and kept in refrigerated storage at 4 ºC.

Viscosity

The viscosity of liquid whole eggs was measured in triplicate using a Brookfield rotational viscometer (Brookfield Engineering Labs, Inc., Milddleboro, USA) equipped with a cylindrical spindle (LV-61). The rotational speed of the spindle was 60 rpm. An aliquot of 50 mL was added to the 80 mL flask and left to equilibrate at 20 ºC prior to testing.

Color

The liquid egg color was measured using a Minolta Chroma Meter CR-400 (Minolta Co. Ltd., Osaka, Japan). The samples were placed into an optical glass cuvette and CIELAB parameters corresponding to lightness, redness, and yellowness (L*, a*, and b*) of the product were measured.

Foaming capacity

Foam-forming potential (FC) was evaluated according to the method proposed by Ferreira *et al.* (1995). The volume of foam and liquid phase

were measured in stoppered graduated cylinders. For the determination of FC the following formula was used:

$$FC\ (\%) = (FV/ILV) \times 100$$

where:

FV: volume of foam

ILV: volume of the initial liquid phase

Low temperature scanning electron microscopy (Cryo-SEM)

A JSM-5410 SEM microscope (Jeol, Tokyo, Japan) coupled to a Cryo CT-1500C unit (Oxford Instruments, Witney, UK) was used. The sample was placed in the holder, fixed with slush nitrogen ($T \leq -210$ ºC), transferred frozen to the Cryo unit, fractured, etched (-90 ºC), and gold-coated (2 mbar, 2 mA). The sample was then transferred onto the microscope and examined at 15 kV and -130 ºC.

Image analysis

Image analysis was performed with analySIS AUTO software (Soft Imaging Systems GmbH, Münster, Germany). Diameters of the protein granules were calculated from at least 20 protein granules in micrographs obtained by Cryo-SEM.

Extraction and quantification of the water-soluble nitrogen fractions

Water-soluble nitrogen fractions were extracted according to AOAC 932.08 (AOAC 2000) and quantified by Kjeldahl, using a mixture of K_2SO_4, $CuSO_4$, and Se (10:1:0.1) as catalyst. All the results were expressed as protein using a conversion factor of 6.25.

Preparation of egg gels

Egg gels were prepared according to the method described by Handa *et al.* (1998) with some modifications. Egg solutions were poured into polypropylene recipients and heated at 80 ºC for 40 min in a water bath.

After heating, samples were immediately cooled to room temperature (24 ± 1 ºC) and kept refrigerated before analysis.

Texture profile analysis

Egg gels were cut into cylindrical samples (20 mm dia x 20 mm ht). Texture profile analysis (TPA) was carried out with a TA-XT2 texture analyzer. The cylinders were compressed twice to 50% of their original height with a 2.54-cm-diameter rod. Textural variables obtained from force and area measurements were: hardness, elasticity, cohesiveness, and chewiness.

Water-holding capacity

The water-holding capacity (WHC) of gels was carried out using the method described by Hammershoj *et al.* (2006) with some modifications. Centrifuge tubes filled with liquid egg (1.5 mL) were placed in a water bath at 80 ºC for 30 min for coagulation, and then cooled at 20 ºC for 1 h. The tubes were then removed and centrifuged at 10,000 × *g* for 30 min. The WHC was calculated from the following equation:

$$WHC\ (\%) = (WGAC / WGBC) \times 100$$

where:

WGAC: weight of gel after centrifugation
WGBC: weight of gel before centrifugation

Statistical analysis

Results were analyzed by means of ANOVA, using the LSD test with a 95% confidence interval for the comparison of the test means.

RESULTS AND DISCUSSION

Viscosity

As it can be observed in Table 2, viscosity of LWE was influenced by the treatment applied (HIPEF or heat pasteurization) as well as by sample

homogenization. LWE viscosity was increased when the thermal treatment was applied, regardless of the degree of homogenization. Hamid-Samimi and Swartzel (1984) reported that egg functionality is impaired when heated for several minutes above 57 ºC. Hence, the increase in viscosity in thermally treated samples can probably be attributed to the incipient unfolding, insolubilization, and entangling, via intermolecular bonds, of heat-sensitive proteins at 66 ºC. Denaturation of ovotransferrin, a glycoprotein found in egg white that exhibits the greatest temperature sensitivity, starts to take place at 62-65 ºC (Watanabe *et al.,* 1985). Similar results have been reported by Herald *et al.* (1989) who observed a viscosity increase in pasteurized liquid whole eggs. Atilgan and Unluturk (2008) also observed a similar effect of heat on the apparent viscosity of liquid egg through disruption of its chemical structure by protein coagulation.

Table 2. Effect of HIPEF treatments (19, 32 kV/cm and 37 kV/cm) and heat-pasteurization on color and viscosity of liquid whole egg, subjected or not to previous homogenization.

Treatments	Viscosity (mPa.s)	L*	a*	b*
Homogenized				
Untreated	9.72±0.25^{aA}	68.87±0.51^{aA}	5.43±0.10^{aA}	41.16±1.66^{aA}
19 kV/cm	10.05±0.21^{aA}	68.61±0.14^{aA}	5.55±0.01^{aA}	41.93±1.61^{aA}
32 kV/cm	10.35±0.49^{aA}	68.07±0.34^{aA}	5.93±0.04^{bA}	43.41±0.30^{aA}
37 kV/cm	10.40±0.42^{aA}	68.22±0.52^{aA}	5.73±0.07^{cA}	43.01±0.23^{aA}
Heat-pasteurized	11.40±0.28^{bA}	72.53±0.97^{bA}	2.79±0.16^{dA}	32.15±0.56^{bA}
Non-homogenized				
Untreated	16.40±0.28^{aB}	64.86±0.31^{aB}	4.30±0.23^{aB}	24.46±0.70^{aB}
19 kV/cm	17.90±1.13^{aB}	64.92±0.91^{aB}	3.60±0.26b^{cB}	23.11±0.97^{aB}
32 kV/cm	18.40±1.27^{bB}	64.40±0.55^{aB}	3.96±0.42^{abB}	24.17±0.46^{aB}
37 kV/cm	19.97±0.89^{bB}	63.89±0.62^{aB}	3.71±0.01b^{cB}	26.17±0.04^{aB}
Heat-pasteurized	25.75±0.49^{cB}	72.47±0.25^{bA}	3.29±0.10^{cB}	30.72±0.26^{bB}

Data are expressed as mean ± standard deviation. Different lowercase letters within the same column indicate significant differences between mean values ($p \leq 0.05$). Uppercase letters indicate significant differences between homogenized and non homogenized samples within the same treatment conditions ($p \leq 0.05$).

On the other hand, almost no increase in viscosity was observed in HIPEF-treated LWE. Similar results were reported by Hermawan *et al.* (2004) who did not observe significant changes in viscosity of untreated samples and HIPEF-treated samples. No statistical differences ($p>0.05$) could be established between untreated and HIPEF-treated samples when the product was previously homogenized. Nevertheless, HIPEF processing applied to nonhomogenized samples induced an increase in viscosity that could be directly related to the treatment intensity (Table 2). However, this increase in viscosity was less pronounced than that of heat-treated LWE. Our results are not in agreement with those published by Qin *et al.* (1995) who reported that a HIPEF treatment decreased the viscosity of LWE, which, in that case, could be attributed to a certain homogenizing effect of the applied treatment. This would involve several aspects related to the flow, properties, characteristics of the pumping system and treatment chamber, among others.

Color

HIPEF treatments did not appear to cause major changes in the color of LWE, either in homogenized or in nonhomogenized samples. Oppositely, when the thermal treatment was applied, the color of LWE underwent substantial changes (Table 2). Hence, untreated and HIPEF-treated samples exhibited a translucent appearance, whereas heat-treated LWE was turned to a more opaque fluid.

Thermally treated samples exhibited higher L* values, regardless of previous homogenization. However, previously homogenized HIPEF-treated samples exhibited higher L*values than those unhomogenized. A similar effect was observed for the green-red (a*) and blue-yellow (b*) chromatisms. Values for both parameters increased significantly due to homogenization. In contrast, heating treatments caused a depletion of b* and a* values, in this order, thus leading to a product with decreased yellow tonality. According to Hutchings (1999), a* and b* coordinates have been described as the most sensitive to structural changes in the food matrix. In fact, many food processes, such as those entailing a thermal processing, may cause denaturation of certain proteins, thus resulting into the development of translucency and/or opacity (Su and Lin, 1993;

Hutchings, 1999). Thermal coagulation of egg proteins would explain the main color changes occurred in treated LWE samples.

Foaming capacity

Foam-forming ability values of LWE as affected by the assayed treatments are shown in Figure 1. HIPEF (19, 32 and 37 kV/cm) or thermally treated samples generally exhibited significantly lower foaming capacity than untreated samples. Regarding HIPEF processing, the effect of HIPEF on the foaming ability of LWE was strongly dependent on the intensity of the applied treatment. Thus, the higher the applied electric field strength, the poorer the foaming capacity. Nevertheless, HIPEF treatments did not so much affect the foaming properties of egg proteins. Foaming capacity values of samples treated at 19 kV/cm were similar to those of the untreated product, whereas at the highest electric field strength applied, the reduction of the foaming capacity was much less pronounced than that caused by the thermal treatment. In fact, the foaming ability of LWE was dramatically reduced when it was exposed to heat-pasteurizing conditions, which was probably caused by protein aggregation and denaturation.

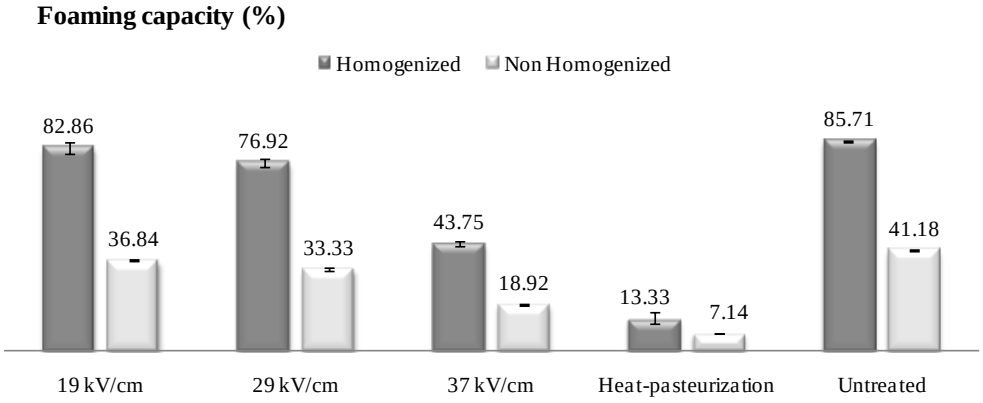


Figure 1. Effect of HIPEF treatments (19, 32 and 37 kV/cm) and heat pasteurization on the foaming capacity of liquid whole egg, subjected or not to previous homogenization.

On the other hand, the foaming capacities of homogenized samples were significantly ($p<0.05$) higher than those of unhomogenized samples, regardless of the applied treatment (Figure 1). During foaming, proteins form a monolayer at the interface through hydrophobic bonds or hydrogen bonds, which presumably determines the stability of the foams. Proteins such as ovomucin may stabilize foams due to its long protein strands linked with oligosaccharide chains, which may support water retention in the foam (Watanabe *et al.,* 1998). In addition, ovomucin can be solubilized by mechanical treatments such as homogenization. Forsythe and Bergquist (1951) indicated that a previous homogenization of LWE could have an effect on the physical state of ovomucin, slightly reducing the fiber length and improving the foaming properties.

Microstructure

The Cryo-SEM technique was used to study the interactions among the main structural components of liquid whole egg (proteins and lipids) and its native water.

The network observed in the micrographs (Figure 2) is due to the eutectic artefact or solute aggregation phenomenon generated during the etching of the sample for its observation. This phenomenon is inherent to the Cryo-SEM technique and it has been previously observed in foodstuffs of animal origin such as ham (Larrea *et al.,* 2007) or vegetables like apple (Quiles *et al.,* 2007).

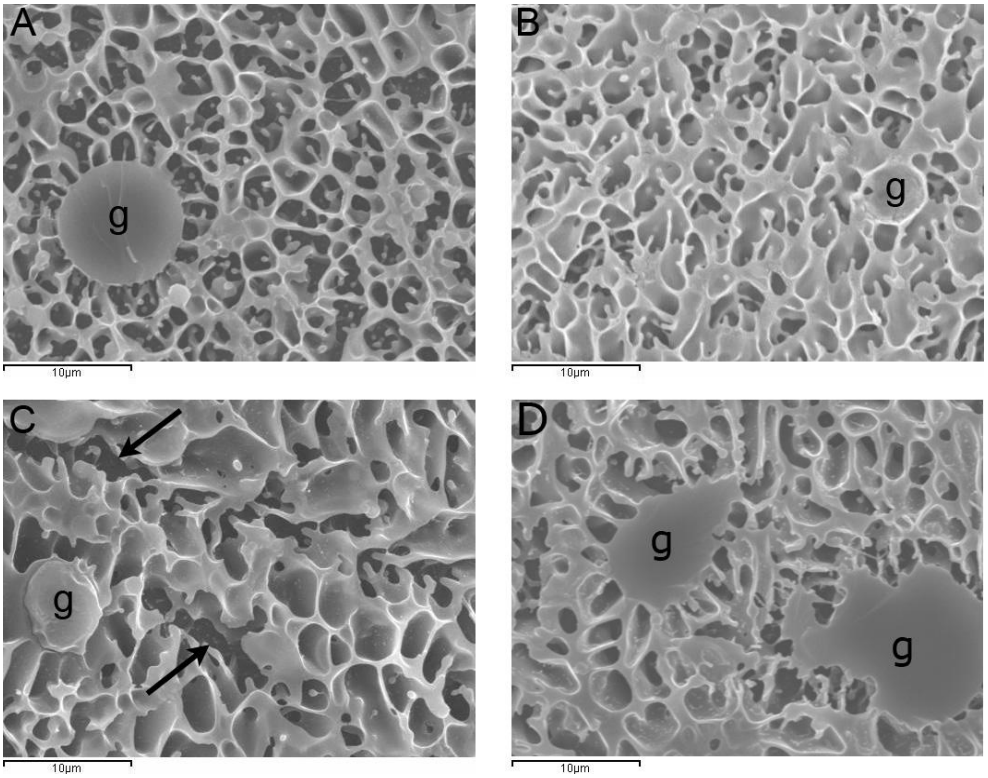


Figure 2. Cryo-SEM. **A**: untreated homogenized LWE; **B**: thermally-treated LWE; **C**: HIPEF-treated (19 kV/cm) LWE; **D**: HIPEF-treated (37 kV/cm) LWE. (arrow: broken lipoprotein matrix g: protein granules) 3500x

Figure 2 shows the microstructure of untreated homogenized LWE by Cryo-SEM. A continuous network mainly composed of egg white proteins like ovalbumin (58%) and ovomucoid (2%), both proteins with gelification properties, is observed. The lipoproteins and protein granules from the yolk are incorporated into the protein network during beating, and a close interaction between proteins and lipids is established, constituting a lipoprotein matrix.

Heat pasteurization (Figure 2B) produces an increase in the lipoprotein matrix density; this is probably due to the intensification of the protein-protein interactions, caused by the partial denaturation or insolubilization of the proteins during the heat treatment. This observation could be linked to the increase in the viscosity values detected in heat pasteurized

samples. However, in the HIPEF-treated samples (Figures 2C and 2D), the lipoprotein matrix appears to be less affected by the treatment if compared to the control. It could be related to a weakness of the interactions between the liquid egg components, which produces the breakdown of the lipoprotein network in some areas. Furthermore, when high-intensity pulses (37 kV/cm) are applied (Figure 2D), the protein granules appear to be more deformed and degraded.

These observations at the microstructural level seem to be related to a loss of stability of the egg samples after processing, since phase separation occurred in HIPEF-treated samples 48 h after treatment (Figure 3). Pulsed electric fields affected the lipoprotein network, destabilizing the colloidal emulsion; however, the heat treatment produced a greater protein aggregation effect, thus inducing global coagulation. It can be observed at a macroscopic level in Figure 3.

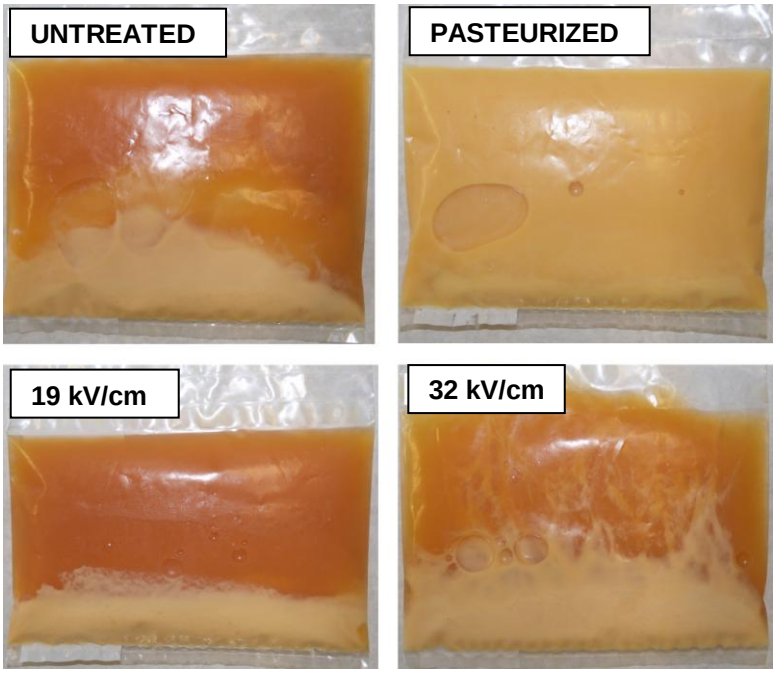


Figure 3. Homogenized LWE samples 48 h after treatment.

Previously homogenized liquid egg samples exhibited a decreased and more uniform size of the protein granules. Figure 4 shows, as an example, the structure of control and HIPEF-treated samples (19 kV/cm and 37 kV/cm), either previously homogenized or not. The diameters of the protein granules for the nonhomogenized samples were 4.9±3.7 μm, while the diameters of the protein granules for the homogenized samples were 2.6±1.9 μm.

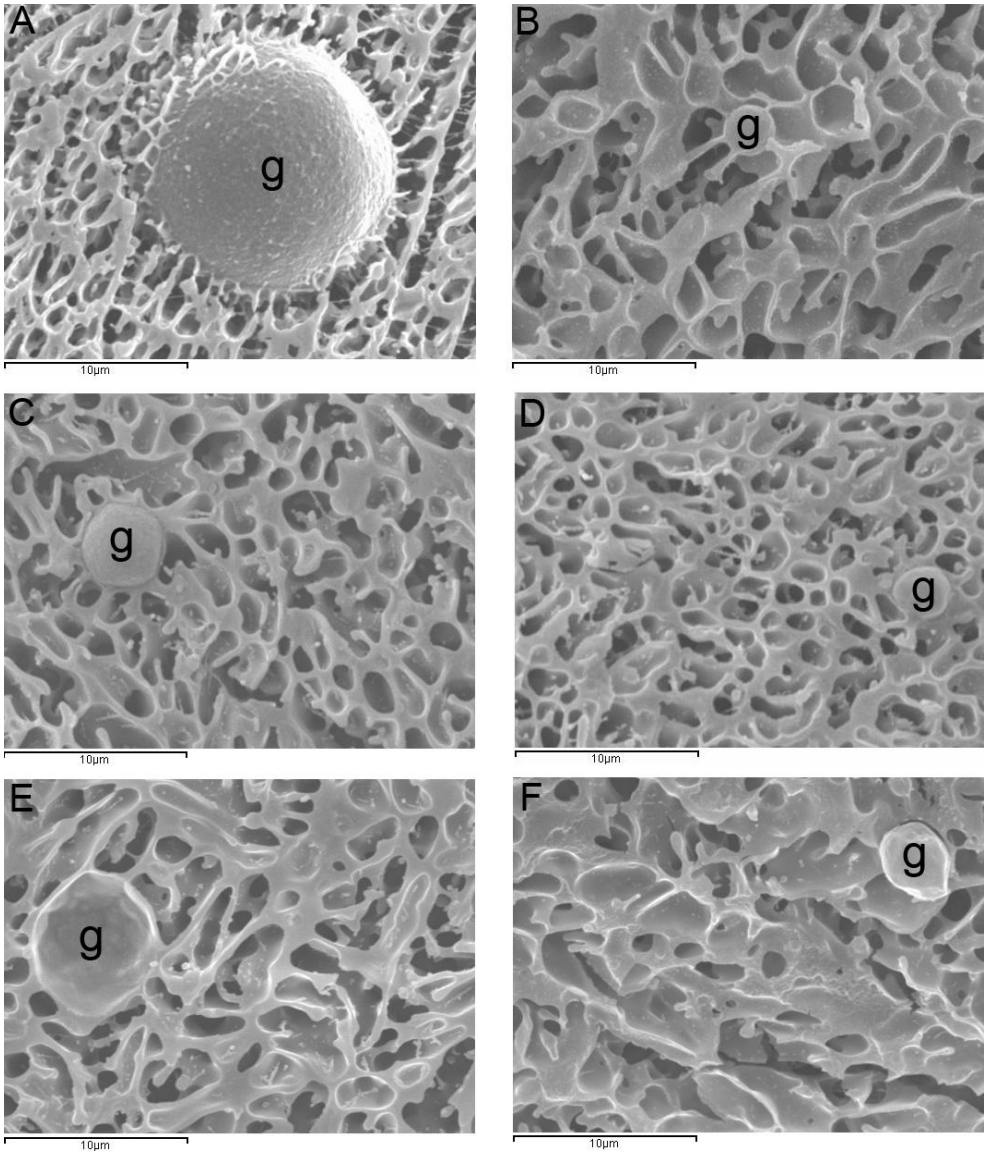


Figure 4. Cryo-SEM. **A**: untreated LWE; **B**: previously homogenized untreated LWE; **C:** unhomogenized HIPEF-treated LWE (19 kV/cm); **D:** previously homogenized HIPEF-treated LWE (19 kV/cm); **E:** unhomogenized HIPEF-treated LWE (37 kV/cm); **F:** previously homogenized HIPEF-treated LWE (37 kV/cm). (g: protein granule) 5000x

Water-soluble protein fraction

The water-soluble protein content of LWE samples is shown in Table 3. Either homogenized or nonhomogenized, thermally treated samples exhibited significantly lower water-soluble protein content than other samples. This can probably be attributed to the partial insolubilization of the proteins caused by the high temperatures reached during the thermal treatment. These changes in proteins could be the cause of the decrease in the foaming capacity of heat treated samples (Figure 1). Moreover, the water-soluble protein content of homogenized HIPEF-treated samples (32 and 37 kV/cm) was significantly higher than that of nonhomogenized ones. This must be linked to the structural observations: the decrease in size of the protein granules due to the homogenization makes the protein extraction easier.

Table 3. Water-soluble protein fractions of untreated, HIPEF-treated (19, 32 and 37 kV/cm), and heat-pasteurized samples, subjected or not to previous homogenization.

	P-soluble fraction	
Treatments	**Homogenized**	**Non Homogenized**
Untreated	8.90 ± 0.22^{aA}	8.72 ± 0.06^{aA}
19 kV/cm	8.15 ± 0.11^{bA}	8.46 ± 0.15^{aA}
32 kV/cm	9.13 ± 0.16^{aB}	8.69 ± 0.33^{aA}
37kV/cm	9.13 ± 0.12^{aB}	8.50 ± 0.32^{aA}
Heat-pasteurized	6.80 ± 0.14^{cA}	7.02 ± 0.19^{bA}

Data are expressed as mean ± standard deviation. Different lowercase letters within the same column indicate significant differences between mean values ($p \leq 0.05$). Uppercase letters indicate significant differences between homogenized and non homogenized samples within the same treatment conditions ($p \leq 0.05$).

Texture profile analysis of heat-induced gels

As can be seen in Table 4, nonsignificant differences ($p > 0.05$) were observed in the textural properties of homogenized heat-induced gels, regardless of the applied treatments. On the contrary, significant differences could be noticed among gels obtained from nonhomogenized

LWE. Gels from unhomogenized thermally treated liquid egg were harder, more cohesive, and exhibited a higher chewiness than those obtained from HIPEF–treated egg (Table 4). Only a slight increase in hardness values was observed in the gels obtained from nonhomogenized HIPEF-treated LWE. The extent of this increase was tightly related to the treatment intensity, so that the highest electric field strengths led to gels with the greatest hardness values. High values of cohesiveness in egg gels indicate an ability to maintain an intact network structure, so that the gels will not break easily and will not exhibit resilient properties (Handa *et al.,* 1998; Fernández-López *et al.,* 2006).

Table 4. Effect of HIPEF treatments (19, 32 and 37 kV/cm) and heat-pasteurization on the texture profile of heat-induced gels from liquid whole egg, subjected or not to previous homogenization.

Treatments	Hardness (N)	Elasticity	Cohesiveness	Chewiness
Homogenized				
Untreated	26.93 ± 1.57^{aA}	0.85 ± 0.02^{aA}	0.44 ± 0.01^{aA}	10.16 ± 0.34^{aA}
19 kV/cm	26.95 ± 0.93^{aA}	0.83 ± 0.03^{aA}	0.44 ± 0.01^{aA}	9.70 ± 0.03^{aA}
32 kV/cm	26.51 ± 1.82^{aA}	0.82 ± 0.01^{aA}	0.45 ± 0.01^{aA}	9.89 ± 0.69^{aA}
37 kV/cm	27.94 ± 0.63^{aA}	0.84 ± 0.01^{aA}	0.46 ± 0.01^{aA}	9.87 ± 0.27^{aA}
Heat-pasteurized	27.21 ± 1.83^{aA}	0.79 ± 0.07^{aA}	0.46 ± 0.02^{aA}	11.13 ± 1.06^{aA}
Non-homogenized				
Untreated	23.54 ± 1.01^{aB}	0.80 ± 0.02^{aB}	0.62 ± 0.01^{aB}	13.20 ± 0.84^{aB}
19 kV/cm	25.29 ± 1.27^{bA}	0.80 ± 0.02^{aA}	0.65 ± 0.01^{aB}	13.27 ± 0.81^{aB}
32 kV/cm	24.64 ± 0.71^{abA}	0.83 ± 0.01^{aA}	0.65 ± 0.02^{aB}	13.43 ± 0.47^{aB}
37 kV/cm	26.35 ± 0.98^{bB}	0.78 ± 0.01^{aB}	0.65 ± 0.05^{aB}	13.40 ± 0.84^{aB}
Heat-pasteurized	28.55 ± 1.02^{cA}	0.83 ± 0.03^{aA}	0.71 ± 0.02^{bB}	16.94 ± 0.89^{bB}

Data are expressed as mean ± standard deviation. Different lowercase letters within the same column indicate significant differences between mean values ($p\leq0.05$). Uppercase letters indicate significant differences between homogenized and non homogenized samples within the same treatment conditions ($p\leq0.05$).

The increases in hardness of the gelled egg, as well as the increases in lipoprotein matrix density and the aggregation observed by Cryo-SEM, are a sign that protein-based conformational structures may be irreversibly impaired by high-temperature processes.

Water-holding capacity

The ability of egg gels to hold water is important because of both sensory properties and technological characteristics. As can be seen in Table 5, the water-holding capacity of LWE was influenced by the previous homogenizing treatment. In this way, no statistically significant differences in WHC could be found between previously homogenized HIPEF-treated, heat-pasteurized, and untreated samples. Oppositely, when LWE was not previously homogenized, WHC was greater in HIPEF-treated and thermally treated samples than in the untreated ones (Table 5). The significant increase in WHC could be related to an alteration of the gel structure. In fact, when nontreated samples are homogenized, the protein granule sizes decrease, as observed earlier in microstructure results. Hermansson (1994) indicated that the gel structure greatly determines WHC, as finer pore sizes are able to bind water more firmly than larger pores. Hammershoj *et al.,* (2006) also indicated that such changes in the gel network are assumed to reflect molecular differences in secondary structure, tertiary structure, and/or flexibility.

Table 5. Effect of HIPEF treatments (19, 32 and 37 kV/cm) and heat-pasteurization on water-holding capacity of heat-induced gels from liquid whole eggs, subjected or not to previous homogenization.

	WHC (%)	
Treatments	**Homogenized**	**Non-homogenized**
Untreated	98.99±0.09^{aA}	93.12±1.62^{aB}
19 kV/cm	98.78±0.68^{aA}	98.65±0.34^{bA}
32 kV/cm	98.53±0.81^{aA}	97.55±0.46^{bA}
37 kV/cm	98.79±0.19^{aA}	99.98±1.77^{bA}
Heat-pasteurized	99.09±0.85^{aA}	99.22±0.95^{bA}

Data are expressed as mean ± standard deviation. Different lowercase letters within the same column indicate significant differences between mean values ($p \leq 0.05$). Uppercase letters indicate significant differences between homogenized and non homogenized samples within the same treatment conditions ($p \leq 0.05$).

CONCLUSIONS

Although thermal treatments still represent the most available pasteurization methods for LWE, they may affect their functional properties and degrade the quality of egg products. In fact, both functional and structural properties of liquid whole egg have been shown to be affected by thermal processing. Because of this, high-intensity pulsed electric fields, as a nonthermal food processing technology, may be a good alternative to the conventionally applied heat preservation methods. HIPEF-treatments, combined with homogenization, show a great potential to preserve LWE with little modifications of its native color, viscosity, and foaming capacity. Thermal pasteurization significantly affects the viscosity, foaming capacity, and the water-soluble protein content of the LWE samples. At a microstructural level, pasteurization produces an intensification of the protein-protein interactions, which is connected to the decrease of the water-soluble protein content and the increase in viscosity. Moreover, the homogenization process produces a decrease in protein granule size. The textural parameters and water-holding capacity of heat-induced egg gels are not affected by the HIPEF treatments. Homogenization of the LWE produces an increase in the water-holding capacity of heat-induced egg gels that could be related to the microstructural changes.

ACKNOWLEDGMENTS

This research was supported by the European Comission (project FP6-FOOD-023140). The authors are indebted to SIK, the Swedish Institute for Food and Biotechnology (Göteborg) for supplying the samples analyzed in this work and to the Ministerio de Educación y Ciencia (Spain) for the grant awarded to author R. Marco-Molés.

REFERENCES

AOAC. 2000. Official methods of analysis. 17th ed., Arlington, Va.: AOAC International.

Atilgan MR, Unluturk S. 2008. Rheological properties of liquid egg products (LEPS). International Journal of Food Properties, 11: 296-309.

Barbosa-Cánovas GV, Góngora-Nieto MM, Pothakamury UR, Swanson BG. 1999. Preservation of foods by pulsed electric fields. San Diego, CA: Academic Press. p 197.

Barbosa-Cánovas GV, Sepúlveda D. 2004. Present status and the future of PEF technology in novel food processing technologies. In: Cano MP, Tapia MS, Barbosa-Cánovas GV, editors. Novel food processing techniques. Boca-Raton, Fl: CRC Press. p 1- 44.

Deeth HC, Datta N, Ross AIV, Dam XT. 2007. Pulsed electric field technology: effect on milk and fruit juices. In: Tewari G, Juneja VK, editors. Advances in thermal and nonthermal food preservation. Oxford: Blackwell Publishing. p 241-269.

Ferreira M, Benringer R, Jost R. 1995. Instrumental method for characterizing protein foams. Journal of Food Science, 60: 90-93.

Fernández-Díaz MD, Barsotti L, Dumay E, Cheftel JC. 2000. Effects of pulsed electric fields on ovalbumin solutions and dialyzed egg white. Journal of Agricultural and Food Chemistry, 48: 2332-2339.

Fernández-López J, Martínez A, Fernández-Ginés JM, Sayas-Barberá E, Sendra E, Pérez-Alvarez JA. 2006. Gelling and color properties of ostrich (*Struthio Camelus*) egg white. Journal of Food Quality, 29(2): 171-183.

Forsythe RH, Bergquist DH. 1951. The effect of physical treatment on some properties of egg white. Poultry Science, 60: 302-311.

Góngora-Nieto MM, Seignour L, Riquet P, Davidson PM, Barbosa-Cánovas GV, Swanson BG. 2001. Nonthermal inactivation of *Pseudomonas fluorescens* in liquid whole egg in pulsed electric fields in food processing. In: Barbosa_Cánovas GV, Zhang QH, editors. Pulsed electric fields in food processing. Lancaster: Technomic Publishing Company, Inc. p 193-211.

Hamid-Samimi MH, Swartzel KR. 1984. Pasteurization design criteria for production of extended shelf-life refrigerated liquid whole egg. Journal of Food Processing and Preservation, 8: 219-224.

Hammershoj M, Rasmussen HC, Carstens JH, Pedersen H. 2006. Dry-pasteurization of egg albumen powder in a fluidized bed. II. Effect on functional properties: gelation and foaming. International Journal of Food Science and Technology, 41: 263-274.

Handa A, Takahashi K, Kuroda N, Froning GW. 1998. Heat-induced egg white gels as affected by pH. Journal of Food Science, 63: 403-407.

Herald TJ, Osorio FA, Smith DM. 1989. Rheological properties of pasteurized liquid whole egg during frozen storage. Journal of Food Science, 54: 35-38.

Hermawan N, Evrendilek GA, Dantzer WR, Zhang QH, Richter ER. 2004. Pulsed electric field treatment of liquid whole egg inoculated with Salmonella enteritidis. Journal of Food Safety, 24: 71-85.

Hermansson AM. 1994. Microstructure of protein gels related to functionality. In: Yada RY, Jackman RL, Smith JL, editors. Protein structure-function relationships in foods. London: Blackie Academic and Professional. p 22-42.

Hutchings JB. 1999. Food color and appearance, 2nd ed. New York: Aspen Publishers. p 610.

Larrea V, Pérez-Munuera I, Hernando I, Quiles A, Llorca E, Lluch MA. 2007. Microstructural changes in Teruel dry-cured ham during processing. Meat Science 76: 574-582.

Mine Y. 1995. Recent advances in the understanding of egg white protein functionality. Trends in Food Science and Technology, 6: 225-231.

Palomeque LA, Góngora-Nieto MM, Bérmudez AS, Barbosa-Cánovas GV, Swanson BG. 2001. Nonthermal inactivation of endoproteases by pulsed electric fields technology in Pulsed Electric Fields in Food Processing. In: Barbosa_Cánovas GV, Zhang QH, editors. Pulsed electric fields in food processing, Lancaster, PA: Technomic Publishing Company, Inc. p 135-148.

Pérez OE, Pilosof AMR. 2004. Pulsed electric fields effects on the molecular structure and gelation of β-lactoglobulin concentrate and egg white. Food Research International, 37: 102-110.

Qin BL, Pothakamury UR, Vega-Mercado H, Martin O, Barbosa-Cánovas GV, Swanson BG. 1995. Food pasteurization using high-intensity pulsed electric fields. Food Technology, 49: 55-60.

Quiles A, Hernando I, Pérez-Munuera I, Lluch MA. 2007. Effect of calcium propionate on the microstructure and pectin methylesterase activity in the parenchyma of fresh-cut Fuji apples. Journal of the Science of Food and Agriculture, 87: 511-519.

Sampedro F, Rodrigo D, Martínez A, Barbosa-Cánovas GV, Rodrigo M. 2006. Application of pulsed electric fields in egg and egg derivates. Food Science and Technology International, 12(5): 397-405.

Su HP, Lin CW 1993. A new process for preparing transparent alkalised duck egg and its quality. Journal of the Scince of Food and Agriculture, 61: 117-120.

Vega-Mercado H, Góngora-Nieto MM, Barbosa-Cánovas GV, Swanson BG. 2007. Pulsed electric fields in food preservation. In: Shafiur RM, editor. Handbook of food preservation. 2nd ed. Boca Raton, Fl: CRC Press. p 783-813.

Watanabe K, Matsuda T, Nakamura R. 1985. Heat-induced aggregation and denaturation of egg white proteins in acid media. Journal Food Science 50: 507-510.

Watanabe K, Tsuge Y, Shimoyamada M. 1998. Binding activities of pronase-treated fragments from egg white ovomucin with anti-ovomucin antibodies and Newcastle disease virus. Journal of Agricultural and Food Chemistry 46: 4501-4506.

Zhao W, Yang R, Tang Y, Lu R. 2007. Combined effects of heat and PEF on microbial inactivation and quality of liquid egg whites. International Journal of Food Engineering 3: 1-20.

Capítulo 4

Czech Journal of Food Sciences *Vol.27, 2009, Special Issue*

Main Chemical Changes in Proteins and Structure of Egg treated with High Pressure Homogenisation

*R. Marco-Molés, I. Hernando, E. Llorca, and I. Pérez-Munuera**

Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain
**E-mail: iperez@tal.upv.es, web: http://www.upv.es*

Abstract: The aim of this work was to study the main chemical changes in the protein fraction (SDS -PAGE) and the microstructure (TEM) of whole liquid egg treated with HPH and stored one week at 4°C. In the electrophoretic study, no changes were observed after the treatments neither after one week of refrigerated storage in the water-soluble proteins of the samples, indicating that no proteolysis was produced. The typical structure of the egg lipoprotein matrix was maintained after the HPH treatments and one week of refrigerated storage. However, a progressive disaggregation of the protein granules was observed, especially when high intensity HPH treatment was applied. It was attributed to the HPH treatment and the microstructural changes observed could affect the functionality of the whole liquid egg when it is used as an ingredient in foodstuffs.

Keywords: *Pulsed Electric Fields; Egg; Structure; Proteins; Electrophoresis*

INTRODUCTION

During the last years, non-thermal food processing technologies are regarded with special interest by the food industry. Among them, High Pressure Homogenisation (HPH) is gaining popularity with food processors because it inactivates microorganisms, preserving the nutritional and sensory properties of foods, and maintaining their shelf life. Furthermore, it has the capacity to develop "value-added" food products or foods with interesting functional properties (Tewari and Juneja, 2007). The egg is an ingredient commonly used in the food industries because of their chemical components functionality. These properties have to be preserved when new technologies are applied. The aim of this work has been to study the main chemical changes in proteins and the microstructure of whole liquid egg treated with HPH at different pressure levels (0, 1000, 3000 and 5000 bar), and stored one week at 4 ºC. Egg soluble proteins were studied by Polyacrylamide Gel Electrophoresis with Sodium Dodecil Sulphate (SDS-PAGE) and the microstructure was observed by Transmission Electron Microscopy (TEM).

MATERIALS AND METHODS

Materials

The egg samples were treated with HPH at different pressure levels: 1 cycle (1000 bar), 3 cycles (1000 bar) and 5 cycles (1000 bar) by means of a continuous homogenizer equipped with a PNSA valve. All the samples were packed at sterilization conditions and kept under refrigerated storage (4 ºC). Non-treated egg samples were also studied and compared to those treated by HPH. All the samples were supplied by the Department of Food Science of the University of Bologna (Italy).

Transmission Electron Microscopy (TEM)

The liquid egg samples were stabilised by mixing with a low gelling temperature agarose solution (3%) at 30 ºC, which facilitates fixation and embedding prior to TEM observation (Sharma *et al.*, 1996). Next, samples were cut into cubes (1 mm^3), fixed (primary fixation with 2.5%

glutaldehyde and secondary fixation with 2% osmium tetroxide), dehydrated with 30, 50 and 70% ethanol, contrasted with uranyl acetate (2%) and embedded in epoxy resin (Durcupan ACM, FLUKA, Buchs, Switzerland). The blocks thus obtained were cut using a Reichter-Jung ULTRACUT ultramicrotome (Leica, Barcelona, Spain). The ultrathin sections obtained (≅100Å) were collected in copper grids and stained with 4% lead citrate to be observed in the Philips EM 400 Transmission Electronic Microscope (Eindhoven, Holland) at 80 kV.

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The water-soluble proteins were extracted as follows. First, samples were freeze-dried (TELSTAR Lioalfa-6, Terrassa, Spain) for 24 h at 10^3 Pa and –45ºC. After freeze-drying, they were defatted in a continuous extraction method with n-hexane-isopropanol, 77:23 (CHUNG *et al.*, 1991). The extraction of the water-soluble fraction was carried out with distilled water (15 mL) from the defatted samples (2.5 g). Next, these mixtures were centrifuged at 3500 rpm for 20 min in a Sorvall Super T_{21} centrifuge (KENDRO Laboratory Products, Hanau, Germany). Protein concentration of these water-soluble extracts was determined by the Bradford method (1976) using BSA for the preparation of the standard curve.

The preparation of the samples for the electrophoretic study was carried out adjusting the protein concentration to 1.25 mg/mL with Laemmli buffer.

Electrophoresis was performed using the method of LAEMLI (1970) on a Multiphor II Electrophoresis System (Pharmacia Biotech, Piscataway, USA), using 12.5% polyacrylamide gels ExcelGel SDS Homogeneous (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at 600 V, 38 mA, 23 W and 15 ºC for 1h 30 min, and 8 μl of each sample were loaded in the gel in duplicate.

The standard proteins were an Amersham low molecular weight calibration kit (GE Healthcare, UK) consisting of: phosphorylase b (97 kDa), bovine albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

Proteins were stained with Coomassie Brilliant Blue tablets (PhastGel Blue R., GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Destaining was

performed in an aqueous solution of 25% ethanol and 8% acetic acid. Gels were preserved in a solution of 10% glycerol and 7.2% acetic acid.

Gels were scanned with an ImageScanner III LabScan 6.0 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and analysed with the ImageQuant TL Image Analysis Software v7.0 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) to determine the molecular weight of each band.

RESULTS AND DISCUSSION

Figure 1 shows the electrophoregram obtained from the water-soluble proteins of the treated eggs and those after refrigeration during one week. 10 bands were identified in the non-treated samples; the majority bands were 5 and 8 corresponding to ovotransferrin and ovalbumin, respectively, main proteins in the egg white.

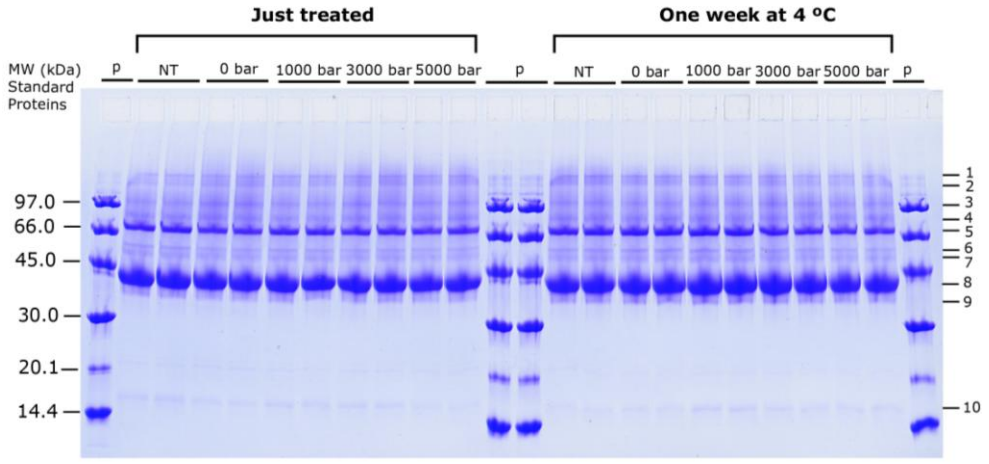


Figure 1. Water soluble protein electrophoregram of the whole liquid egg treated by HPH (0, 1000, 3000 and 5000 bar), and after one week of refrigerated storage. **NT**: non-treated samples; **p**: standard proteins.

Changes were observed neither after the treatments nor after one week of refrigerated storage in the water-soluble proteins. This would indicate that these treatments do not produce proteolysis in the egg proteins. Furthermore, endogenous proteolytic activity was not detected after one week at 4ºC, because no changes were observed in these electrophoregram after the refrigerated storage.

Figure 2 shows the TEM micrographs of the non-treated liquid egg and HPH treated samples (0, 1000, 3000 and 5000 bar). The characteristic structure of whole liquid egg consists of a continuous lipoprotein matrix in which protein granules are dispersed (Fig. 2A). These protein granules are strongly electrodense, round shaped and have different sizes (1-1.3 μm). The structure of the lipoprotein matrix was not affected by the HPH treatments and it was also maintained after one week of refrigerated storage. However, a progressive disaggregation of the protein granules was observed, especially when high pressure levels (Fig. 2C) were applied. This microstructure does not change after one week of refrigerated storage. This is in concordance with the results obtained in the electrophoretic study, in which no proteolysis was observed.

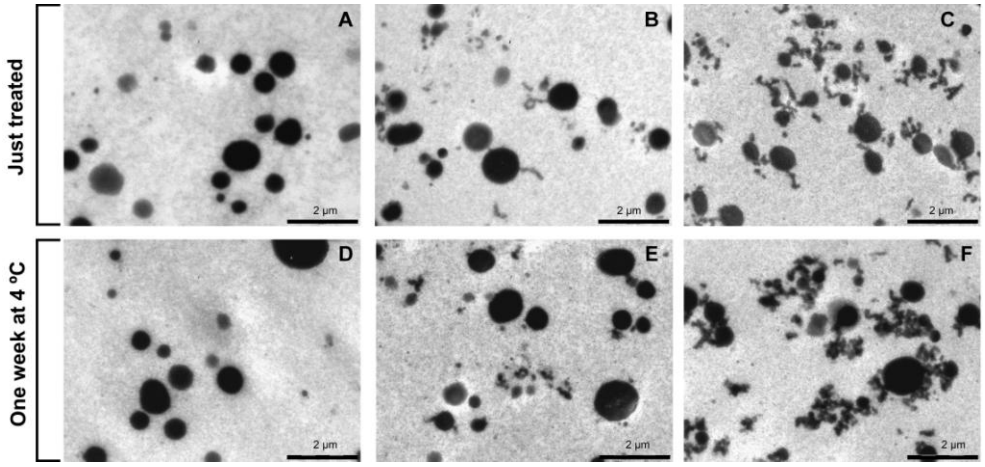


Figure 2. TEM. **A**: whole liquid egg (non-treated); **B**: HPH treated (1000 bar) whole liquid egg; **C**: HPH treated (5000 bar) whole liquid egg; **D**: whole liquid egg (non-treated), after one week of refrigerated storage; **E**: HPH treated (1000 bar) whole liquid egg, after one week of refrigerated storage; **F**: HPH treated (5000 bar) whole liquid egg, after one week of refrigerated storage. 2950x

It could be concluded that the structural changes observed in the protein granules were mainly due to the HPH treatment. These changes could affect the whole liquid egg functionality when it is used as a raw material in foodstuffs.

REFERENCES

BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Analytical Biochemistry **72**: 248–254.

CHUNG L.S., FERRIER L.K. (1991). Partial lipid extraction of egg yolk powder: effects on emulsifying properties and soluble protein fraction. Journal of Food Science, **56**: 1255-1258.

LAEMLI U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227**: 680–685.

SHARMA R., SINGH H., TAYLOR, M. W. (1996). Composition and structure of fat globule surface layers in recombined milk. Journal of Food Science, **61**: 28-32.

TEWARI G., JUNEJA V. K. (2007). Advances in Thermal and Non-Thermal Food Preservation. Blackwell Publishing, Iowa: 203-239.

Capítulo 5

Journal of Food Engineering *doi: 10.1016/j.jfoodeng.2011.11.031*

Influence of High Pressure Homogenization (HPH) on the Structural Stability of an Egg/Dairy Emulsion

Marco-Molés, R.; Hernando, I.; Llorca, E*; Pérez-Munuera, I.

Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de Alimentos. Universitat Politècnica de València. P.O. Box 22012, 46071 Valencia, Spain
*To whom correspondence should be addressed. E-mail: emllomar@tal.upv.es.
Phone: +34 963877363 Fax: +34 963877363

Abstract: High Pressure Homogenisation (HPH) is a novel non-thermal preservation technology, which can improve the microbiological quality of products without affecting their stability. The main objective of this paper is to study the influence of different HPH treatments on the structure of a sauce (an egg/dairy emulsion), in order to obtain the higher physicochemical stability. The oil-in-water emulsion was stable up to 100 MPa with the oil droplets surrounded by several layers of natural emulsifiers. Critical pressures, between 150 and 250 MPa, produced a destabilisation of the emulsion thus causing a separation of phases. A coalescence phenomenon progressively occurred when pressure increased. This phenomenon was due to the loss of the natural emulsifier barrier. Changes on the electrophoretic pattern were also observed when at high pressure levels, showing an insolubilisation of proteins. Lipid fraction was observed to be chemically stable after the HPH treatment.

Keywords: *High Pressure Homogenization, Emulsion, Microstructure, Proteins, Lipids*

INTRODUCTION

High Pressure Homogenization (HPH) is one of the most promising alternatives to traditional thermal treatment of food preservation and diversification (Burns *et al.*, 2008). This technology is based on the same principle as conventional homogenization, but working at significantly higher pressures (Serra *et al.*, 2009). In HPH the fluid is forced to pass through a narrow gap of the valve, after which it is subjected to an ultrarapid depression (Guerzoni *et al.*, 1999). Then, fluids are subjected to a wide range of forces, such as turbulence, shear, cavitation and large temperature increases (Hayes and Kelly, 2003; Floury *et al.*, 2000).

Homogenizers were developed for the stabilization of food and dairy emulsions. Over the years, the homogenization technology has evolved; the demand for longer shelf-life and products with better stability has led to new developments, based on a very high pressure capacity as well as on a new reaction chamber design. The development of this high pressure technology has influenced research work on emulsions stability, but it has also been observed that such high pressures can affect not only fat globules but also other food constituents such as macromolecules or colloids, and then their functional properties (Paquin, 1999).

The effects of high pressure on the gelation of proteins (Rastogi *et al.*, 1994) and on the creation or modification of functional properties of proteins (Rastogi *et al.*; 1994; San Martin *et al.*, 2002) have been investigated in the last years. In this context, the functional properties of proteins from different origin treated by high pressure homogenization (HPH) were analysed resulting in an increased foam ability of soybean and egg white proteins and an increased water binding capacity of faba bean protein (Heinzelmann *et al.*, 1994).

Regarding the structure of high pressure treated foods, emulsions and dairy products are the most studied ones. Floury *et al.* (2002) found that HPH caused denaturation of proteins and reduced droplet sizes in emulsions; they suggested that the gel-like network structure of some emulsions was caused by hydrophobic interactions between proteins. Significant modifications in the structure of emulsions regarding droplet size distribution were observed as the pressure was modified (Desrumaux and Marcand, 2002). Rosenberg and Lee (1993) observed by Scanning

Electron Microscopy the microstructure of emulsions consisting of whey protein and anhydrous milk fat. The emulsions prepared under HPH exhibited a bimodal particle-size distribution with clustering of the protein coated droplets. The effects of HPH on the microstructure of oil-in-water emulsions were evaluated by Roesch and Corredig (2003); heating before homogenization had no significant impact on microstructure. SEM analysis of goat cheeses revealed that cheeses made from HPH-treated milk had a more homogeneous microstructure than those made from untreated milk or pasteurised milk (Guerzoni *et al.*, 1999).

However research on the effect of HPH on the structure of emulsions is needed in order to optimise the conditions of the treatments preserving their physicochemical stability.

The main objective of this study was to investigate the influence of different HPH treatments on the structure and physicochemical stability of a sauce (an egg/dairy emulsion). The distribution of the main chemical components of the egg/dairy emulsion affected by the HPH treatments was studied by microscopic techniques (TEM and CLSM). Moreover, the effect of these treatments on the soluble protein and lipid fractions was also analysed. The results of this study could contribute to understand how the HPH treatment affects the functionality of the chemical components of the sauce, some of them acting as possible stabilizers in the system.

MATERIALS AND METHODS

Sauce-making process

In this study, sauce was prepared with sunflower oil (59%), full-fat yoghurt (13%), whole eggs (10%), water (17%) and salt (1%). The whole eggs and the salt were mixed at 700 rpm; then the sunflower oil was added slowly during mixing, and finally the yoghurt and the water were added. pH was adjusted to pH=5 with citric acid (5%).

High Pressure Homogenization (HPH)

The sauce underwent to the following single HPH treatments with industrial equipment: 0 MPa (control sauce), 70 MPa, 100 MPa, 150 MPa and 250 MPa. All the samples were analysed just after treatment and after storing them one week at 4ºC.

A continuous high pressure homogeniser PANDA (Niro Soavi, Parma, Italy) was used for all the homogenising treatments. The homogenizer was supplied with a homogenizing PS type valve; the valve assembly included a ball type impact head made of ceramics, a stainless steel large inner diameter impact ring and a tungsten carbide passage head. The inlet temperature of the samples was 2-4 ºC and the increase rate of temperature was 3ºC/10 MPa. The increase in temperature was controlled by a heat exchanger installed after the valve. Treatments of the samples were carried out in duplicate.

Confocal Laser Scanning Microscopy (CLSM)

A drop of the sample was put on a slide and stained with Rhodamine B solution (2 g/l) for protein observation. Then, a solution Nile Red (1 g/l) was used to stain lipids. After that, the mixture was covered with a cover glass. The samples were observed in a CSLM (Nikon confocal microscope C1 fitted to a Nikon Eclipse E800 microscope, Nikon Co., Ltd., Tokyo, Japan) in single photon mode equipped with an Ar-Kr laser. The excitation wavelength and emission maxima of the applied fluorescent dyes were 568/625 nm for Rhodamine B and 647/675 nm for Nile Red. Images were stored using EZ-C1 software (Nikon Co., Ltd., Tokyo, Japan).

Transmission Electron Microscopy (TEM)

The ultrastructure of the samples was characterised by means of TEM. The samples were stabilised by mixing them with a low gelling temperature agarose solution (3%) at 30ºC, which facilitates fixation and embedding prior to TEM observation (Sharma *et al.*, 1996). Next, samples were cut into cubes (1 mm^3), fixed (primary fixation with 2.5% glutaraldehyde and secondary fixation with 2% osmium tetroxide), dehydrated with 30, 50 and 70% ethanol, contrasted with uranyl acetate

(2%) and embedded in epoxy resin (Durcupan ACM, FLUKA). The blocks thus obtained were cut using a Reichter-Jung ULTRACUT ultramicrotome (Leila, Barcelona, Spain). The ultrathin sections obtained (100Å) were collected in copper grids and stained with 4% lead citrate to be observed in the Philips EM 400 Transmission Electronic Microscope (Eindhoven, Holland) at 80 kV.

Analysis of water soluble protein fraction

SDS Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The SDS-PAGE study of the water-soluble protein fraction was carried out using an aliquot of the sample. The water-soluble protein fraction was extracted, prior to SDS-PAGE, as follows. First, samples were freeze-dried (TELSTAR Lioalfa-6, Terrassa, Spain) for 24 h at 10^3 Pa and –45ºC. After freeze-drying, they were defatted in a continuous extraction method (Soxhlet) for 16 h with n-hexane-isopropanol (77:23) as solvent. 2.5 g of defatted samples were mixed with 15 mL of distilled water and centrifuged at 3500 rpm for 20 min in a Sorvall Super T_{21} centrifuge (KENDRO Laboratory Products, Newtown, CT). Protein concentration of these water-soluble extracts was determined by the Bradford method (1979) using standard BSA for the preparation of the standard curve.

The protein concentration of the samples was adjusted to 1.25 mg/mL with Laemmli buffer. Electrophoresis was performed on a Multiphor II Electrophoresis System (Pharmacia Biotech, Piscataway, New Jersey, USA), using 12.5% polyacrylamide gels ExcelGel SDS Homogeneous (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) at 600 V, 38 mA, 23 W and 15 ºC for 1h 30 min. 8 μl of each sample were loaded in the gel in duplicate.

The standard was an Amersham low molecular weight calibration kit (GE Healthcare, UK) consisting of: phosphorylase b (97 kDa), albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa).

Protein bands were stained with Coomassie Brilliant Blue tablets (PhastGel Blue R., GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Destaining was performed in an aqueous solution of 25% ethanol and 8%

acetic acid. Samples were conserved in a solution of 10% glycerol and 7.2% acetic acid. The molecular weight of each band was determined using a densitometer Intelligent Dark Box II Fujifilm LAS 1000 (Fujifilm, Tokyo, Japan) with the software Image Gauge (Fujifilm USA, Valhalla, NY, USA).

N-Kjeldahl

Water-soluble fraction was quantified by Kjeldahl, using a mixture of K_2SO_4, $CuSO_4$ and Se (10:1:0.1) as catalyser. All the measurements were carried in triplicate and the results were expressed as protein using a conversion factor of 6.25.

Analysis of lipid fraction

The lipid extracts were obtained by the Folch method (1957).

Acidity index

It was determined in accordance with regulations (AOAC, 1990). The measurements were carried out in triplicate.

Detection of hydroperoxides and secondary oxidation products

The lipid extracts (0.01g) were dissolved in cyclohexane (100 ml) and their absorbances were taken at 232 and 270 nm to measure hydroperoxides and secondary oxidation products, respectively in a spectrophotometer (Cecil Instruments Limited, Series 1020), using 1-cm quartz cuvettes. The measurements were carried out in triplicate and the results were expressed as:

$$K_\lambda = A_\lambda / c.\ e$$

where:

$A_\lambda \equiv$ Absorbance at 232 or 270 nm.
$c \equiv$ Concentration (g/100 ml).
$e \equiv$ Thickness of the cuvette (cm).

Statistical analysis

Statistical analysis of the results was performed using analysis of variance (ANOVA); the least significant differences (LSD) were calculated at the $p \leq 0.05$ significance level. The Statgraphics 5.1 computer-assisted statistics program was employed.

RESULTS AND DISCUSSION

The sauces were observed by Transmission Electron Microscopy (TEM) and Confocal Laser Scanning Microscopy (CLSM). The use of different microscopic techniques offers complementary information about the changes produced by HPH on the main components of the samples.

Microstructural analysis by CLSM

Figure 1A shows a continuous phase stained in red with Rodhamine B which corresponds to the water phase. This water phase is composed mainly by the soluble components of the sample and water (17%). Another continuous phase, slightly black, probably made up with proteins and fat from the yoghurt and egg (lipoprotein network), can also be observed. Oil droplets (1 to 15 µm) from the sunflower oil were observed closely interacting with the lipoprotein network whereas, they were not observed in the water phase.

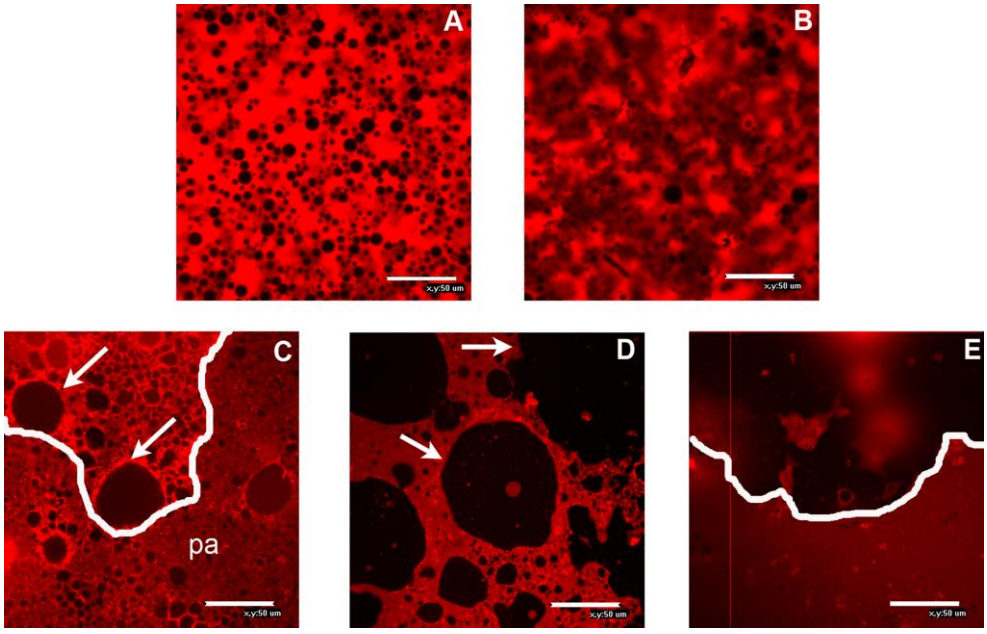


Figure 1. CLSM (red channel). Sauce treated by HPH at 0 MPa or control sauce (A); 70 MPa (B); 100 MPa (C); 150 MPa (D); and 250 MPa (E). (pa: protein aggregation area, arrows: oil droplets). 60x (bar=30 µm)

The structure of the samples treated at 70 MPa (Fig. 1B) was found to be similar to that of the control sample (0 MPa), although the lipoprotein network appeared to be more extended and the oil droplets were smaller. This was probably due to the homogenization effect during the treatment (Cortés-Muñoz *et al.*, 2009).

When the sample was treated at 100 MPa, the lipoprotein network could be observed denser which indicates aggregation. This could be related to the water-soluble protein fraction study, which showed a progressive decrease in the extraction of soluble proteins as the pressure increased. The oil droplets from the sunflower oil appeared to be deformed and a coalescence phenomenon could be observed (Fig. 1C). Other authors observed an increase of the apparent rate of coalescence with homogenization pressure, since the concentration of suitable proteins was assumed to be limited. This situation, as expected, got worse with increasing homogenization pressure and the attendant increase in surface areas being created (Agboola *et al.*, 1998).

When higher pressure was applied (150 MPa) very big oil droplets appeared (Fig. 1D). A new fat phase was formed and the other components of the emulsion, mainly proteins, were closely interacting with each other. Similar results were obtained by Floury *et al.* (2002) when treating soy protein-stabilized emulsions. HPH led to changes in macromolecular structure and interaction of the proteins, enhancing protein-protein interactions and showing emulsions strongly aggregated. They also observed that at pressures above 150 MPa, the droplet size strongly increased, indicating a lack of free protein to cover the newly created interface of the droplets. Moreover, proteins could be much too denatured and aggregated for an efficient adsorption at newly formed oil-water interfaces.

A progressive separation of phases took place as the pressure increased, with a completed disruption of the emulsion at 250 MPa (Fig. 1E). This effect can be also observed in figure 2, where samples stained with Nile Red show the fat phase in green. At 250 MPa, two phases were observed: a water phase composed mainly of proteins and lipoproteins from the egg and yogurt, and a fat phase with lipids mainly from the sunflower oil. Desrumaux and Marcand (2002) described an “overprocessing” phenomenon over 210 MPa, were changes in the conformation of proteins were confirmed, probably because of the combined effects of high pressure treatment and rise in temperature observed. This change probably modified the emulsifying properties of proteins.

Samples observed after one week of refrigerated storage (Fig. 3) showed a similar aspect to those observed just after treatment (Fig. 2). Only the fat phase showed greater deformation and appeared unevenly distributed.

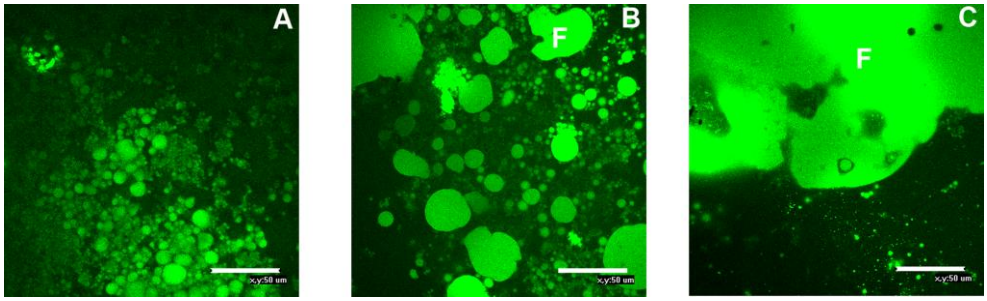


Figure 2. CLSM (green channel). Sauce treated by HPH at 100 MPa (A); 150 MPa (B); and 250 MPa (C) just treated samples. (F: fat). 60x (bar=30 µm)

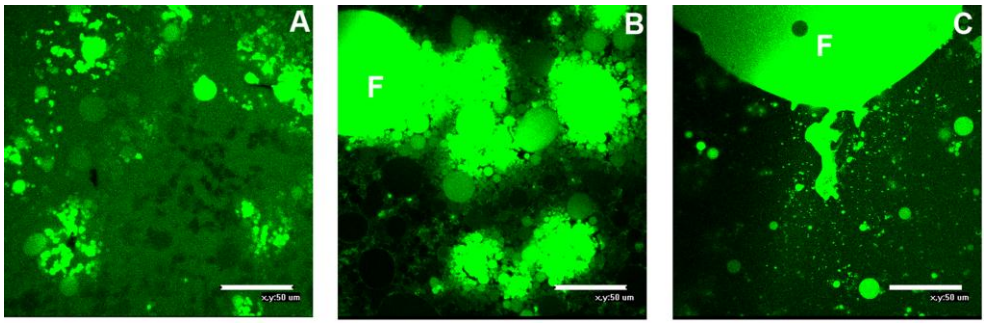


Figure 3. CLSM (green channel). Sauce treated by HPH at 70 MPa (A); 150 MPa (B); and 250 MPa (C) after one week of refrigerated storage. (F: fat). 60x (bar=30 µm)

The pressure produced microstructural changes in the protein phase, thus allowing a higher aggregation of these components. Critical pressures between 150 and 250 MPa produced a destabilisation of the emulsion thus causing a separation of phases.

Figure 4 shows the macroscopic appearance of the samples treated by HPH at different pressures, just treated and after 1 week at 4 ºC. The separation of phases was observed after the HPH treatment at 250 MPa. Furthermore, after 1 week at 4 ºC, a slight separation of phases was observed in the control samples but it was not observed in 70, 100 and 150 MPa treated samples. This could indicate that HPH treatment up to 150 MPa helps to stabilise the emulsion during storage.

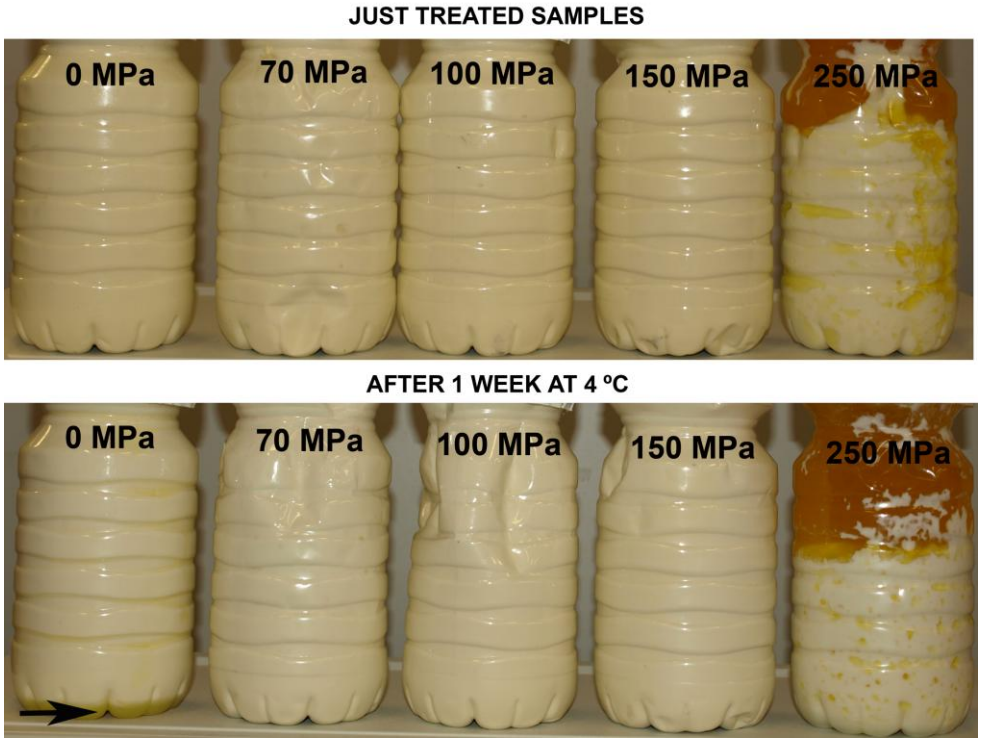


Figure 4. Macroscopic observations of the samples treated by HPH at different pressures, just treated and after 1 week at 4 ºC. (arrow: separation of phases)

Ultrastructural analysis by Transmission Electron Microscopy (TEM)

The oil droplets trapped in the protein matrix are observed at an ultrastructural level in figure 5A. The oil droplets were surrounded by a barrier of natural emulsifiers (caseins from the yoghurt and phospholipids from the egg) that stabilised the oil-in-water emulsion. Figure 5B shows a detail of the oil droplets surrounded by several layers of emulsifiers. In the samples treated by HPH (Fig. 5C, D, E and F), a coalescence phenomenon progressively occurred as pressure increased. This phenomenon is due to the loss of the emulsifier barrier that was observed surrounding the oil droplets. An accumulation of tensoactives was produced on the interface water-oil, which was separated due to the HPH effect.

After one week of refrigerated storage, the tensoactive barrier was still observed in the control sample. Furthermore, the samples treated by HPH

showed the same effects due to the treatment: loss of tensoactives, coalescence of the oil droplets and disruption of the oil-in-water emulsion. No significant differences were observed during the storage (images not shown).

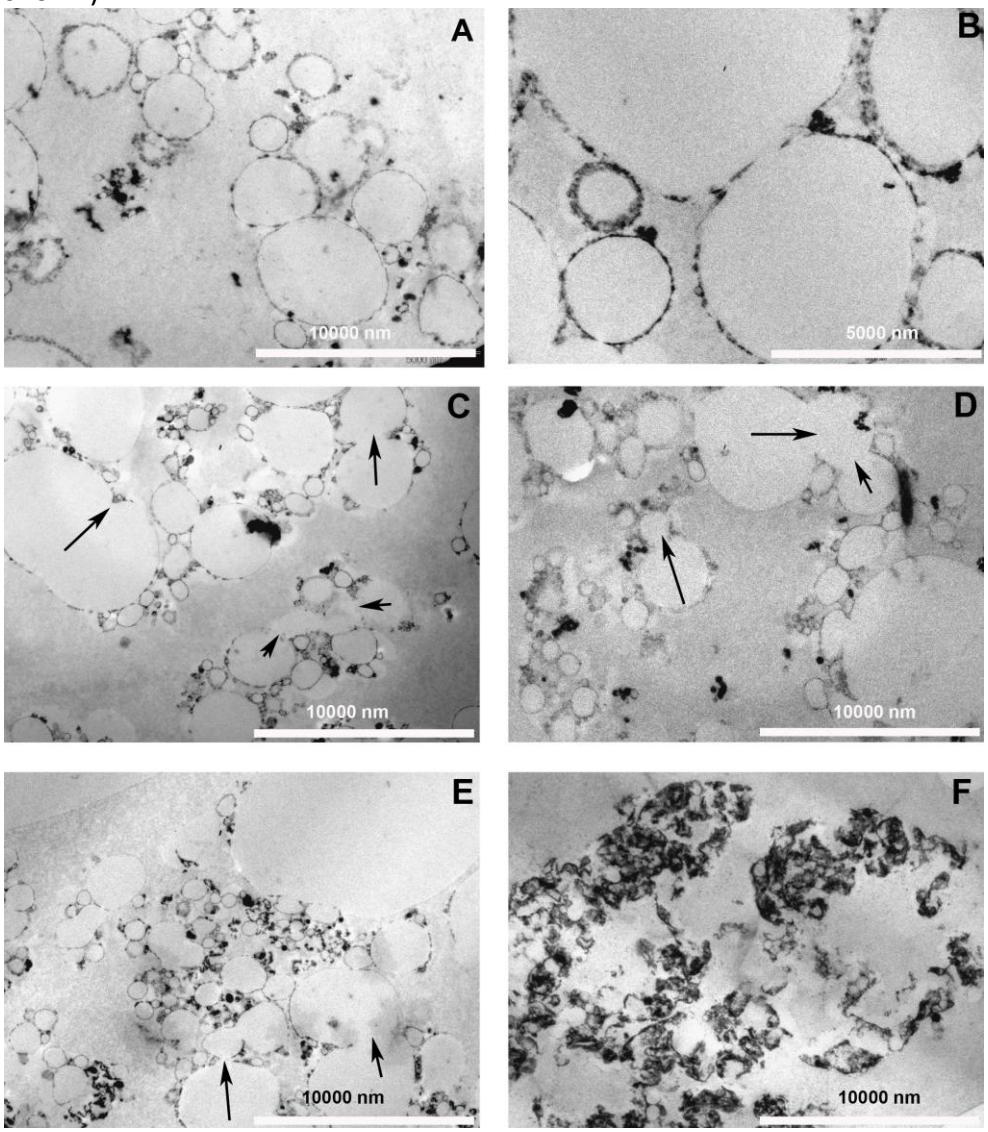


Figure 5. TEM. Control: Sauce treated by HPH at 0 MPa or control sauce (A); Detail of control sauce (B) 2200x (bar=5000nm); 70 MPa (C); 100 MPa (D); 150 MPa (E); 250 MPa (F). (arrows: coalescence) 1200x (bar=10000nm)

Analysis of protein fraction

The water-soluble protein fraction of the HPH treated samples was studied by SDS-PAGE and quantified by N-Kjeldahl. The results inform about the protein solubility, which can be related to their functionality.

Table 1. Percentages (g/100g sample w.m) of the water-soluble protein values (Kjeldahl) of the HPH treated samples at different pressures.

	SOLUBLE PROTEIN (KJELDAHL)			
HPH treatment	**Just treated samples**		**After 1 week at 4 ºC**	
	Mean	**SD**	**Mean**	**SD**
0 MPa	0.77^{a}	0.03	0.86^{a}	0.09
70 MPa	0.77^{a}	0.04	0.76^{ab}	0.26
100 MPa	0.71^{ab}	0.01	0.73^{ab}	0.15
150 MPa	0.57^{b}	0.03	0.61^{b}	0.02
250 MPa	0.25^{c}	0.01	0.26^{c}	0.01

a, b, ...z Values with different letters within the same columns are significantly different ($p<0.05$) according to the LSD multiple range test. The absence of * means that just treated sample does not differ ($p<0.05$) of the corresponding refrigerated sample.

After the HPH treatments, a progressive decrease in the percentages of water-soluble protein values was observed in just treated samples, as the pressure increased (Table 1), indicating protein insolubilisation. Significant differences were observed in the soluble protein values when 150 and 250 MPa were applied if compared to the values of samples treated at lower pressure. The factors involved in the protein insolubilisation can be pH and T. With the low pH of the system (pH=5), a relatively low temperature during the process at 150 MPa would explain the denaturation and aggregation of proteins. When high pressure was applied (250 MPa) the temperature rose to 75-80 ºC, which explains the protein denaturation. In these conditions the emulsion was easily destabilised. Moreover, some proteins, as albumins, are especially sensitive to temperature, and it increases during the HPH treatment. Some authors (Sirvente *et al.*, 2007) observed a protein solubility decrease of egg yolk proteins when the HPH

treatment was between 100 and 200 MPa. Other type of proteins as soy globulin has been observed to be affected by high pressure treatment above 150 MPa. Increasing the homogenization pressure above 200 MPa led to a quite strong decrease in the globulin solubility due to very strong forced induced phenomena of cavitation, high shear and turbulence, and the rise in temperature (Floury *et al.*, 2002).

After one week of refrigerated storage similar effects were observed. However, no significant changes were observed in the treated samples due to the storage. The highest value of the water-soluble protein values was observed in the non-treated sample, probably due to a lower microbiological stability after 1 week at 4 ºC.

Figure 6 shows the electrophoregram obtained for the water-soluble protein fraction of the HPH treated samples immediately after the treatment and after one week of refrigerated storage.

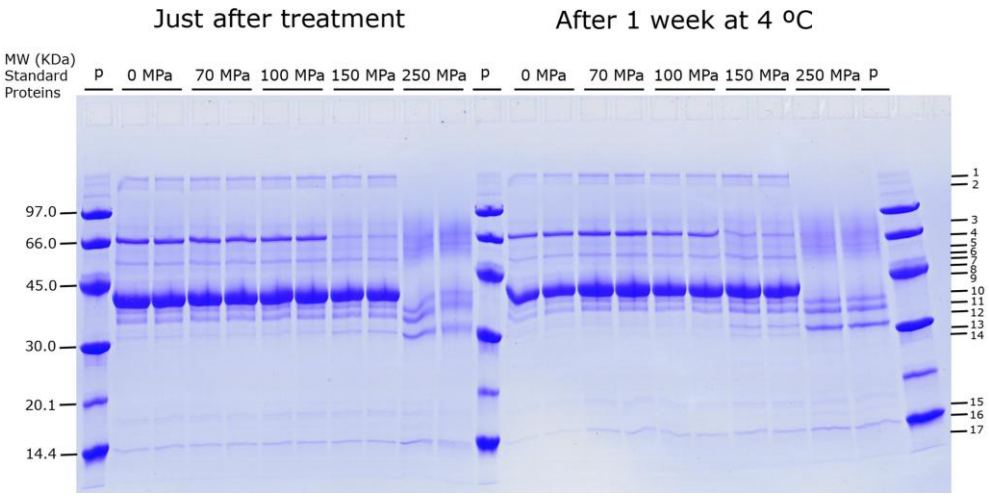


Figure 6. Water soluble protein electrophoregram of the sauces treated by HPH, just after the treatment and after one week of refrigerated storage. (p: standard proteins)

Treatment at 150 MPa and 250 MPa produced changes on the electrophoretic pattern of the samples, showing an insolubilisation of proteins as albumin (MW=66 kDa). Furthermore, an intensification of other proteins bands (30-45 kDa) is observed when the sample is treated at 250 MPa. Desrumaux et al. (2002) concluded using SDS-PAGE that HPH

treatment (from 20 up to 350 MPa) did not show any significant change in the molecular weight of whey proteins from sunflower oil emulsions.

After one week of refrigerated storage, similar effects were observed in the samples.

Analysis of lipid fraction

The fat was analysed to determine possible chemical changes in this fraction due to the lypolitic activity. The chemical analysis of the fat (Table 2) showed neither significant change in the acidity index as the pressure increased, nor during storage, which could indicate that lipolysis was not produced.

Table 2. Acidity grade of the lipid fraction of the HPH treated samples at different pressures.

HPH treatment	ACIDITY INDEX			
	Just treated samples		After 1 week at 4 ºC	
	Mean	SD	Mean	SD
0 MPa	0.15[a]	0.01	0.17[a]	0.02
70 MPa	0.16[a]	0.01	0.19[a]	0.02
100 MPa	0.17[a]	0.02	0.16[a]	0.01
150 MPa	0.17[a]	0.01	0.18[a]	0.05
250 MPa	0.18[a]	0.02	0.17[a]	0.02

a, b, ...z Values with different letters within the same columns are significantly different ($p<0.05$) according to the LSD multiple range test. The absence of * means that just treated sample does not differ ($p<0.05$) of the corresponding refrigerated sample.

Furthermore, as expected, no significant changes in the oxidation parameters (K_{232} and K_{270}) were observed due to the HPH treatment and after 1 week of storage at 4 º C (Table 3 and 4).

Table 3. Oxidation spectrophotometer parameter (K_{232}) of the lipid fraction of the HPH treated samples at different pressures.

	K_{232}			
HPH treatment	**Just treated samples**		**After 1 week at 4 ºC**	
	Mean	**SD**	**Mean**	**SD**
0 MPa	2.70[a]	0.14	2.71[a]	0.04
70 MPa	2.71[a]	0.30	2.73[a]	0.03
100 MPa	2.80[a]	0.32	2.84[a]	0.01
150 MPa	2.91[a]	0.04	2.88[a]	0.10
250 MPa	2.82[a]	0.07	2.81[a]	0.04

a, b, ...z Values with different letters within the same columns are significantly different ($p<0.05$) according to the LSD multiple range test. The absence of * means that just treated sample does not differ ($p<0.05$) of the corresponding refrigerated sample.

Table 4. Oxidation spectrophotometer parameters (K_{270}) of the lipid fraction of the HPH treated samples at different pressures.

	K_{270}			
HPH treatment	**Just treated samples**		**After 1 week at 4 ºC**	
	Mean	**SD**	**Mean**	**SD**
0 MPa	0.55[a]	0.03	0.54[a]	0.01
70 MPa	0.55[a]	0.05	0.54[a]	0.03
100 MPa	0.54[a]	0.06	0.53[a]	0.01
150 MPa	0.52[a]	0.02	0.54[a]	0.02
250 MPa	0.53[a]	0.01	0.54[a]	0.02

a, b, ...z Values with different letters within the same columns are significantly different ($p<0.05$) according to the LSD multiple range test. The absence of * means that just treated sample does not differ ($p<0.05$) of the corresponding refrigerated sample.

Therefore, according to the analysis, the lipid fraction showed to be chemically stable after HPH treatment and after one week of refrigerated storage.

CONCLUSION

The physicochemical stability of this emulsion is directly related to the pressure applied during the process. The oil-in-water emulsion is stable up to 100 MPa with the oil droplets surrounded by several layers of natural emulsifiers. Critical pressures, between 150 and 250 MPa, affect the stability of the emulsion thus causing a separation of phases. A coalescence phenomenon progressively occurs when pressure increases. This phenomenon is due to the loss of the natural emulsifier barrier. Changes on the electrophoretic pattern are also observed when the pressure was applied at 150 MPa and 250 MPa, showing protein insolubilisation while lipid fraction is chemically stable during the HPH treatment.

ACKNOWLEDGEMENTS

Authors want to thank to the European Comission for the financial support (Project FP6-FOOD-023140), to the “Ministerio de Educación y Ciencia” (Spain) for the grant awarded to the author R. Marco-Molés, and to the “Ministerio de Ciencia e Innovación” (Spain) for the financial support (AGL2007-30951-E). Finally, authors thank to the University of Bologna (Italy) for providing us the HPH treated samples.

REFERENCES

Agboola, S. O., Singh, H., Munro, P. A., Dalgleish, D. G., & Singh, A. M. (1998). Destabilization of oil-in-water emulsions using highly hydrolized whey proteins. Journal of Agricultural and Food Chemistry, 46: 84-90.

AOAC (1990). Official methods of analysis. (14th edition). Association of Official Analytical Chemist, Washington. D.C.

Bradford, M.M. (1976). A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. Analytical Biochemistry, 72: 248-254.

Burns, P., Patrignani, F., Serrazanetti, D., Vinderola, G. C., Reinheimer, J. A., Lanciotti, R., & Guerzoni, M. E. (2008). Probiotic Crescenza Cheese containing Lactobacillus casei and Lactobacillus acidophilus manufactured with high-pressure homogenized milk. Journal of Dairy Science, 91: 500-512.

Cortés-Muñoz, D., Chevalier-Lucia, D., & Dumay, E. (2009). Characteristic of submicron emulsions prepared by ultra-high pressure homogenization: Effect of chilled or frozen storage. Food Hydrocolloids, 23: 640-654.

Datta, N., Hayes, M. G., Deeth, H. C., & Kelly, A. L. (2005). Significance of frictional heating for effects of high pressure homogenisation on milk. Journal of Dairy Research, 72: 1-7.

Desrumaux, A., & Marcand, J. (2002). Formation of sunflower oil emulsions stabilized by whey proteins with high pressure homogenization (up to 350 MPa): effect of pressure on emulsion characteristics. International Journal of Food Science & Technology, 37(3): 263-269, 21.

Diels, A. M., Callewaert, L., Wuytack, E. Y., Masschalck, B., & Michiels, C. W. (2005). Inactivation of Escherichia coli by high-pressure homogenisation is influenced by fluid viscosity but not by water activity and product composition. Interntational Journal of Food Microbiology, 101: 281-291.

Floury, J., Desrumaux, A., & Lardières, J. (2000). Effect of high pressure homogenization on droplet size distribution and rheological properties of model oil-in-water emulsions. Innovative Food Science and Emerging Technologies, 1: 127-134.

Floury, J., Desrumaux, A., & Legrand, J. (2002). Effect of ultra high pressure homogenization on structure and on rheological properties of soy protein stabilized emulsions. Journal of Food Science, 67(9): 3388-3395, 32.

Fluory, J., Bellettre, J., Legrand, J., & Desrumaux, A. (2004a). Analysis of a new type of high pressure homogeniser. A study of the flow pattern. Chemical Engineering Science , 59: 843-853.

Folch, L., Lees, M., & Sloane-Stanley, G.H. (1957). Biological Chemistry, 226: 497-509.

Guerzoni, M. E., Ferruzzi, M., Gardini, F., & Lanciotti, R. (1999). Combined effects of ethanol, high homogenization pressure, and temperature on cell faty acid composition in Sacharomyces cerevisiae. Canadian Journal of Microbiology, 45: 805-810.

Guerzoni, M. E., Vannini, L., Chaves Lopez, C., Lanciotti, R., Suzzi, G., & Gianotti, A. (1999). Effect of high pressure homogenization on microbial and chemico-physical characteristics of goat cheeses. Journal of Dairy Science, 82(5): 851-862, 28.

Hayes, M. G., & Kelly, A. L. (2003). High pressure homogenisation of raw whole bovine milk (a) effects on fat globule size and other properties. Journal of Dairy Research, 70: 297-305.

Heinzelmann, K., Hoene, T., Muschiolik, G., & Rawel, H.M. (1994). Protein modification by high pressure homogenization. Nahrung, 38(5): 478-490.

Paquin, P. (1999). Technological properties of high pressure homogenisers: the effect of fat globules, milk proteins and polysacharides. International Dairy Journal, 9: 329-335.

Rastogi, N. K., Subraminian, R., & Raghavarao, K. S. M. S. (1994). Application of high pressure technology in food industry. Indian Food Industry, 13(1): 30-34, 17.

Roesch, R. R., & Corredig, M. (2003).Texture and microstructure of emulsions prepared with soy protein concentrate by high pressure homogenization. Lebensmittel Wissenschaft und Technologie, 36(1): 113-124, 31.

Rosenberg, M., & Lee, S. L. (1993). Microstructure of whey protein/anhydrous milk fat emulsions. Food Structure, 12(2): 267-274, 32.

San Martin, M. F., Barbosa Canovas, G. V., & Swanson, B. G. (2002). Food processing by high hydrostatic pressure. Critical Reviews in Food Science and Nutrition, 42(6): 627-645, 82.

Serra, M., Trujillo, A. J., Guamis, B., & Ferragut, V. (2009a). Proteolysis of yogurts made from ultra-high-pressure homogenized milk during cold storage. Journal of Dairy Science, 92: 71-78.

Serra, M., Trujillo, B., Guamis, B., & Ferragut, V. (2009b). Evaluation of physical properties during storage of set and stirred yogurts made from unltra-high pressure homogenization-treated milk. Food Hydrocolloids, 23: 82-91.

Sharma, R., Singh, H., & Taylor, M.W. (1996). Composition and structure of fat globule surface layers in recombined milk. Journal of Food Science, 61 (1): 28-32.

Sirvente, H., Beaumal, V., Gaillard, C., Bialek, L., Hamm, D., & Anton, M. (2007). Structuring and functionalization of dispersions containing egg yolk, plasma and granules induced by mechanical treatments. Journal of Agricultural and Food Chemistry, 55: 9537-9544.

Conclusiones

1. La matriz lipoproteica del huevo pareció estar menos afectada por el tratamiento de PEF que por la pasteurización, sobre todo a bajas intensidades de campo. Sin embargo, la aplicación de altas intensidades de campo produjo discontinuidades en la matriz lipoproteica y degradación de la estructura de los gránulos proteicos. Tras una semana de almacenamiento refrigerado a 4ºC, se produjo un fenómeno de agregación de la matriz en todas las muestras, siendo este efecto mayor en las muestras no tratadas.

2. El tratamiento de huevo líquido por PEF no afectó a la fracción de proteínas solubles, mientras que la pasteurización produjo una insolubilización de las mismas. No se observó proteólisis en ninguna de las muestras estudiadas. Durante el almacenamiento refrigerado a 4 ºC, la cantidad de proteína soluble disminuyó, debido al efecto de agregación que también fue observado a nivel microestructural.

3. En general la pasteurización produjo una mayor oxidación de la fracción lipídica que el tratamiento por PEF. Además, la fracción lipídica de las muestras tratadas por PEF se mantuvo más estable que en las muestras control a lo largo del almacenamiento a 4 ºC.

4. El tratamiento por PEF puede ser una buena alternativa al tratamiento convencional para la conservación de huevo líquido por su menor impacto en las propiedades funcionales: produjo pocas modificaciones en el color, viscosidad y capacidad espumante, mientras que la pasteurización sí que afectó significativamente a la viscosidad y capacidad espumante. En cuanto a las características de los geles obtenidos, el tratamiento por PEF no afectó a la textura ni a la capacidad de retención de agua del gel, siendo sin embargo estas características afectadas por la pasteurización.

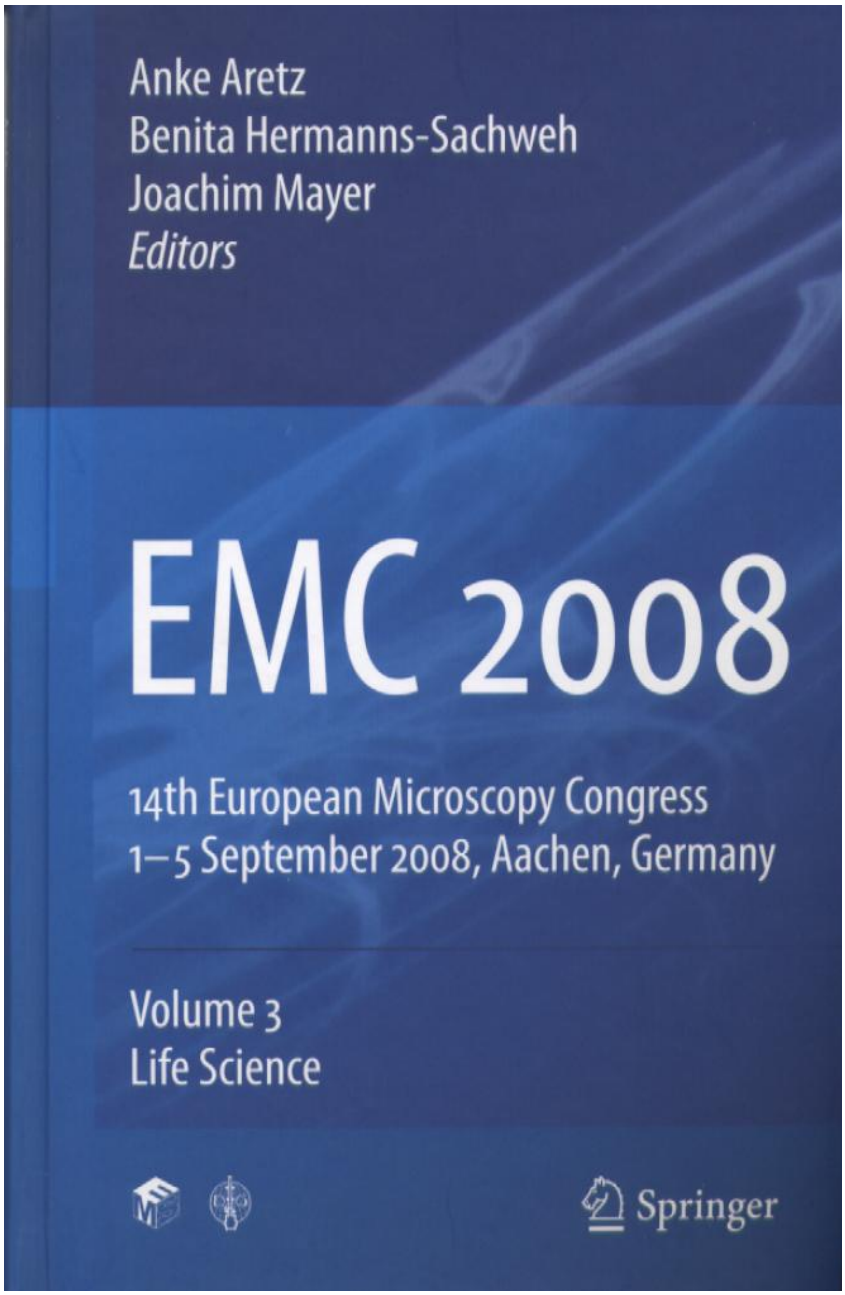
5. La estructura típica de la matriz lipoproteica del huevo no se vio afectada por el tratamiento por HPH y se mantuvo después de una semana en refrigeración a 4ºC. Sin embargo, se observó una desintegración progresiva de los gránulos proteicos, sobre todo a altos niveles de presión. Estos cambios observados por microscopía podrían afectar a la funcionalidad de huevo líquido cuando se utiliza como ingrediente alimentario.

6. Con respecto a la fracción proteica del huevo, no se observaron cambios en el estudio electroforético de las proteínas solubles ni debido a los tratamientos por HPH, ni tras una semana de almacenamiento refrigerado, lo que indicó ausencia de proteólisis.

7. La estabilidad fisicoquímica de la emulsión se relacionó directamente con la presión aplicada durante el proceso. La salsa tratada por HPH fue estable hasta los 100 MPa, sin embargo, presiones superiores a 150 MPa produjeron la desestabilización progresiva de la emulsión con la consecuente separación de fases. Esto se atribuyó al fenómeno de coalescencia, asociado a la pérdida de emulsionantes naturales de la interfase que también se observó a nivel microestructural.

8. El tratamiento por HPH a niveles de presión elevados produjo la insolubilización de algunas proteínas de la fracción soluble de la salsa. Sin embargo, la fracción lipídica de la salsa se mantuvo químicamente estable tras los distintos tratamientos de HPH y durante el almacenamiento.

Anexos


Anke Aretz
Benita Hermanns-Sachweh
Joachim Mayer
Editors
EMC 2008
14th European Microscopy Congress
1–5 September 2008, Aachen, Germany
Volume 3
Life Science
Springer

Anke Aretz
RWTH Aachen
Central Facility for Electron Microscopy
Ahornstr. 55
52074 Aachen
Germany
aretz@gfe.rwth-aachen.de

Benita Hermanns-Sachweh
RWTH Aachen
University Hospital
Department of Nuclear Medicine
Pauwelsstraße 30
52074 Aachen
Germany
bhermanns@ukaachen.de

Prof. Dr. Joachim Mayer
RWTH Aachen
Central Facility for Electron Microscopy
Ahornstr. 55
52074 Aachen
Germany
mayer@gfe.rwth-aachen.de
and
Ernst Ruska-Centre for Microscopy and
Spectroscopy with Electrons
Institute of Solid State Research
Research Centre Jülich
52425 Jülich
Germany

ISBN 978-3-540-85227-8 e-ISBN 978-3-540-85228-5

DOI 10.1007/978-3-540-85228-5

Library of Congress Control Number: 2008934066

Typesetting: digital data supplied by the authors
Production: le-tex publishing services oHG, Leipzig, Germany
Cover design: eStudioCalamar S.L., F. Steinen-Broo, Girona, Spain

Printed on acid-free paper

9 8 7 6 5 4 3 2 1

springer.com

Microstructural changes produced by Pulsed Electric Fields in liquid whole egg studied by Transmission Electron Microscopy (TEM)

I. Pérez-Munuera, R. Marco-Molés, I. Hernando, A. Quiles and A. Puig

Departamento de Tecnología de Alimentos, Universidad Politécnica de Valencia, Camino de Vera s/n 46022 Valencia, Spain.

iperez@tal.upv.es
Keywords: Pulsed Electric Fields, whole liquid egg, Transmission Electron Microscopy

The application of new technologies for food processing and preservation aims to meet to the increasing number of catering companies due to the growing demand for fresh foods, high quality and ready-to-eat meals. Among the non-thermal technologies for food preservation, the application of Pulsed Electric Fields (PEF) is receiving big attention over the past few years, as it reduces the detrimental changes on physical and sensorial properties of foods and offers fresh fluid foods of high quality, taste, nutritional value and improved shelf-life [1, 2].

The aim of this work is to study the microstructural changes that occur in liquid whole egg treated by PEF (Table I). The effect of the electric field strength and the homogenization, through a perforated surface (∅ ≅0.4 mm), are studied.

Samples are stabilized with agarose [3], fixed with glutaraldehyde and osmium tetroxide, dehydrated with ethanol, contrasted with uranyl acetate and embedded in epoxy resin to be observed in a Philips EM 400 (Philips, Eindhoven, Holland transmission electronic microscope at 80 kV.

Non-treated samples (Figure 1A) shows a continuous matrix mainly composed of a protein network, from the egg white, and lipids, from the yolk, incorporated into the protein network during beating, with small yolk protein granules (∅ ≅1.5 μm) inserted in the continuous protein matrix. LowPEF (19kV) produces a protein aggregation effect (Figure 1B) that is increased when high field strength is applied (32kV). The lipoporotein matrix is specially affected in HighPEF treated samples, the continuity of the protein network is broken and some protein granules from the yolk are degradated (Figure 1C).

When non-treated samples are homogenized (Figure 2A) the protein granules size increase. The samples homogenized and treated with HighPEF (Figure 2B) shows the effect of both treatments with bigger protein granules (Figure 2A), although the lipoprotein matrix seems not to be so degradated than in the non-homogenized ones (Figure 1C). Homogenization produces a protein granules aggregation and a reinforcement of the protein-protein interactions.

1. D. Knorr, B.I.O. Ade-Omawaye and V.Heinz, Proceedings of the Nutrition Society 61 (2002), p. 311.
2. P. Mañas and R. Pagán, Journal of applied microbiology 98 (2005), p. 1387.

3. R. Sharma, H. Singh and M.W. Taylor, Journal of Food Science 61, 1 (1996), p.28.
4. The authors thank to the Swedish Institute for Food and Biotechnology (Göteborg, Sweden) for supplying the samples studied in this work, the European Commission for the financial support of the project FP6-FOOD-023140 and the "Ministerio de Educación y Ciencia" of Spain for the grant awarded to R. Marco-Molés.

Table I. Technological characteristics of the pulsed electric fields applied. U: electric field strength; t: pulse width; N: number of applied pulses; prf: pulse repetition frequency; R: internal resistance.

	Pulse generator parameters					Temperature (ºC)		
Sample	U (kV)	t (µs)	N	prf (Hz)	R (Ω)	In	Out	Increase
HighPEF	32	6	5	250	1000	3.8	32.5	28.7
LowPEF	19	6	5	250	1000	5.4	16.0	10.6

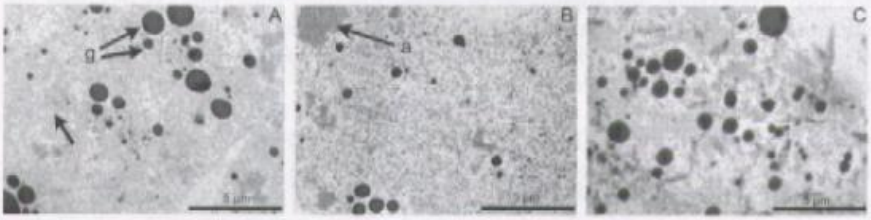


Figure 1. TEM. A: non-treated liquid whole egg. B: LowPEF (19 kV) treated liquid whole egg. C: HighPEF (32 kV) treated liquid whole egg. 1650x. (arrow: lipoprotein matrix; g: protein granules; a: aggregated compounds).

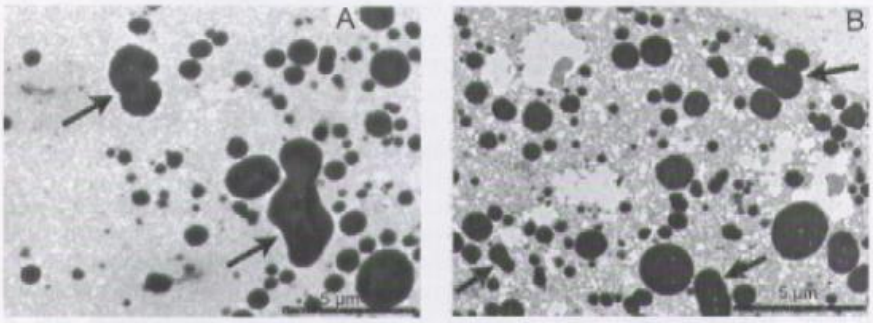


Figure 2. TEM. A: non-treated homogenized liquid whole egg. B: HighPEF (32 kV) treated and homogenized liquid whole egg. 1650x. (arrow: aggregated protein granules).

MICROSTRUCTURAL CHANGES PRODUCED BY PULSED ELECTRIC FIELDS IN LIQUID EGG STUDIED BY TRANSMISSION ELECTRON MICROSCOPY

R. MARCO-MOLÉS, I. HERNANDO, A. QUILES, A. PUIG, I. PÉREZ-MUNUERA.

Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia. Camino de Vera s/n 46022 Valencia, Spain.
E-mail: iperez@tal.upv.es

INTRODUCTION

The application of new technologies for food processing and preservation aims to meet to the increasing number of catering companies due to the growing demand for fresh foods, high quality and ready-to-eat meals. Among the non-thermal technologies for food preservation, the application of Pulsed Electric Fields (PEF) is receiving big attention over the past few years, as it reduces the detrimental changes on physical and sensorial properties of food and offers fresh fluid foods of high quality, taste, nutritional value and improved shelf-life. The aim of this work is to study the microstructural changes that occur in liquid whole egg treated by PEF:. The effect of the electric field strength and the homogenization are studied.

MATERIAL

Liquid eggs and the same samples homogenized and/or treated by PEFare studied. The technological characteristics of the pulsed electric fields applied (U: electric field strength; t: pulse width; N: number of applied pulses; prf: pulse repetition frequency; R: internal resistance) are the following:

	Pulse generator parameters					Temperature (°C)		
Sample	U (kV)	t (μs)	N	prf (Hz)	R (Ω)	In	Out	Increase
HighPEF	32	6	5	250	1000	3.8	32.5	28.7
LowPEF	19	6	5	250	1000	5.4	16.0	10.6

TRANSMISSION ELECTRON MICROSCOPY

Liquid Egg
↓
Stabilization: Agarose (3%)
↓
Sample: 1-2 mm³
↓
Fixation: Glutaraldehide (2.5%)
↓
Rinsing: Phosphate Buffer 0.025 M, pH 6.8
↓
Postfixation: OsO_4 (2%)
↓
Rinsing: distilled water
↓
Dehydration: Ethanol 30, 50 and 70 %
↓
Contrast: Uranyl Acetate (2 %)
↓
Dehydration: Ethanol 96 and 100 %
↓
Infiltration: Epoxy Resin
↓
Embedding: 65.5 °C / 3 days
↓
Ultramicrotomy: ultrathin sections
↓
Stainning: Lead Citrate (4%) /Uranyl Acetate (2%)
↓
Observation: TEM

RESULTS AND DISCUSSION

Non-treated samples (Figure 1A) shows a continuous matrix mainly composed of a protein network, from the egg white, and lipids, from the yolk, incorporated into the protein network during beating, with small yolk protein granules (1.5 μm) inserted in the continuous protein matrix. LowPEF (19kV) produces a protein aggregation effect (Figure 1B). The lipoporotein matrix is specially affected in HighPEF(32 kV) treated samples (Figure 1C).

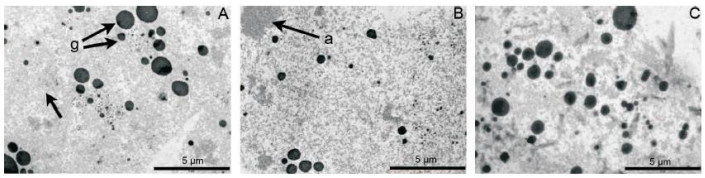


Figure 1. TEM. A: non-treated liquid egg. B: LowPEF (19 kV) treated liquid egg. C: HighPEF (32 kV) treated liquid egg. 1650x. (arrow: lipoprotein matrix; g: protein granules; a: aggregated compounds).

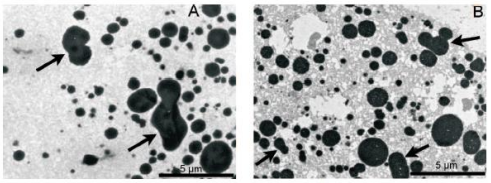


Figure 2. TEM. A: non-treated homogenized liquid egg. B: Homogenized liquid egg and treated by HighPEF (32 kV). 1650x. (arrow: aggregated protein granules).

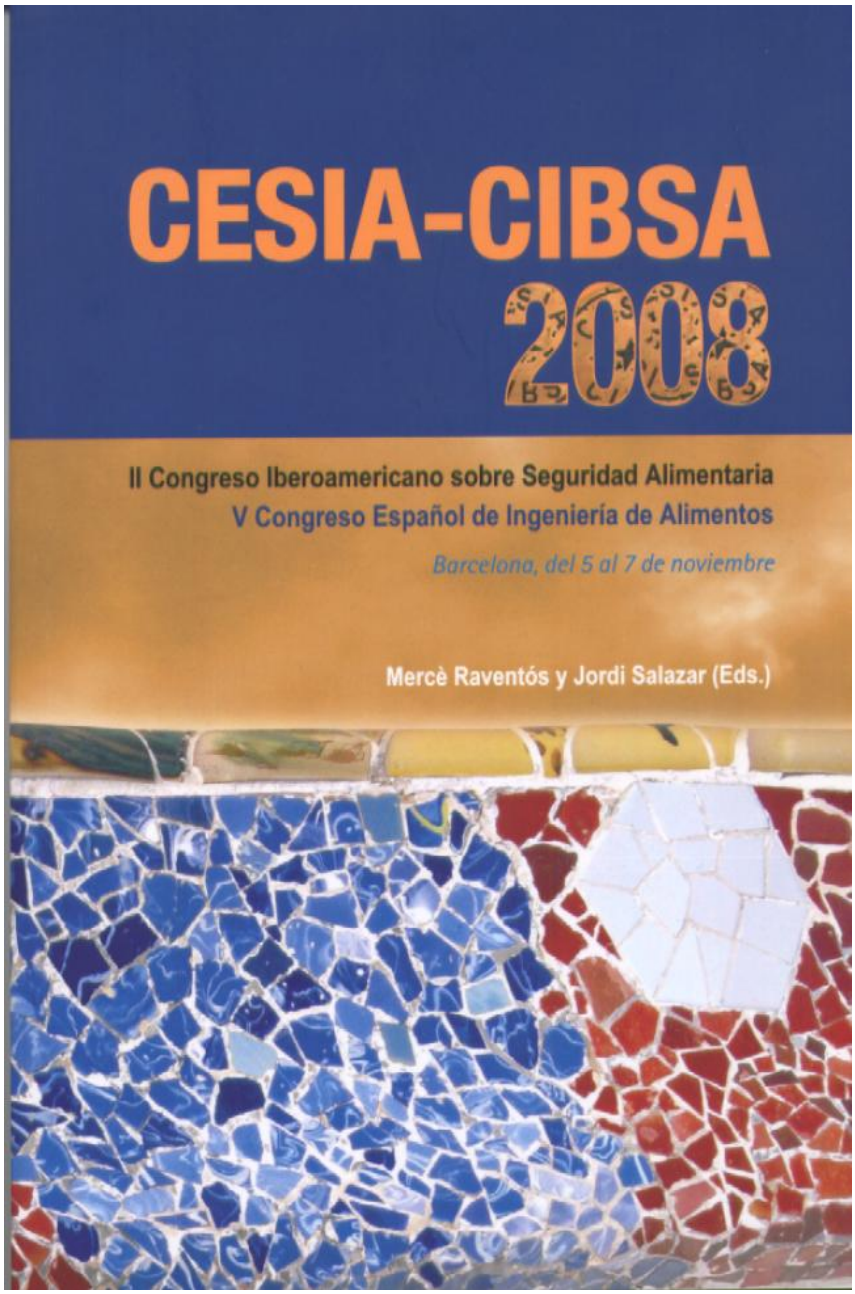
When non-treated samples are homogenized (Figure 2A) the protein granules size increases. The samples homogenized and treated with HighPEF (Figure 2B) shows the effect of both treatments with bigger protein granules, although the lipoprotein matrix seems not to be so degraded than in the non-homogenized ones (Figure 1C).

CONCLUSIONS

The application of pulsed electric fields to non-homogenized liquid egg produces protein aggregation and when the intensity of the pulses increases, the lipoprotein matrix appears more degraded. Homogenization produces a protein granules aggregation but the lipoprotein matrix in samples homogenized previously to a HighPEF treatment appears less degraded.

ACKNOWLEDGEMENTS

The authors thank to the Swedish Institute for Food and Biotechnology (Göteborg, Sweden) for supplying the samples studied in this work, the European Commission for the financial support of the project FP6-FOOD-023140 and the "Ministerio de Educación y Ciencia" of Spain for the grant awarded to R. Marco-Molés.


CESIA-CIBSA
2008
II Congreso Iberoamericano sobre Seguridad Alimentaria
V Congreso Español de Ingeniería de Alimentos
Barcelona, del 5 al 7 de noviembre
Mercè Raventós y Jordi Salazar (Eds.)

CESIA-CIBSA 2008
Mercè Raventós y Jordi Salazar (Eds.)

Primera edición: Octubre 2008

Edificio C1, Campus Norte UPC
Gran Capitán s/n, 08034 Barcelona, Spain
www.cimne.upc.es

Impreso por: Zasoram, Pamplona 96-104, local 14,
08018 Barcelona, Spain

Depósito legal: B-49702-2008

ISBN: 978-84-96736-57-3

IMPRESO EN ESPAÑA

EFECTO DE LA APLICACIÓN DE PULSOS ELÉCTRICOS (PEF) SOBRE LA FRACCIÓN PROTEICA DEL HUEVO, POR SDS-PAGE. CAMBIOS MICROESTRUCTURALES POR CRYO-SEM.

R. Marco-Molés, I. Hernando, A. Quiles, E. Llorca y I. Pérez-Munuera[1*]

1: Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia
Camino de Vera 14 46071 Valencia
e-mail: iperez@tal.upv.es, web: http://www.upv.es

RESUMEN

La aplicación de nuevas tecnologías en el ámbito de la conservación de alimentos pretende dar respuesta al incremento de las empresas de catering por aumento en la demanda de alimentos frescos, de primera calidad y de fácil y rápida preparación ó "ready to eat" (RTE). De entre las tecnologías no térmicas de conservación de alimentos, la aplicación de pulsos eléctricos (PEF) es de las que mayor interés y atención está recibiendo durante los últimos años [1]. Reduce los cambios perjudiciales en las propiedades físicas y sensoriales de los alimentos y ofrece alimentos fluidos frescos de alta calidad, con excelentes sabores, valores nutricionales y mejora de la vida útil [2]. El objetivo de este trabajo es el estudio de los principales cambios que tienen lugar en la microestructura de huevo líquido entero tratado por PEF, mediante Microscopía Electrónica de Barrido a Baja Temperatura (Cryo-SEM), así como evaluar el efecto de éstos sobre la fracción proteica total y soluble del huevo, por Electroforesis en Geles de Poliacrilamida con Dodecil Sulfato Sódico (SDS-PAGE). Se estudia el efecto de la intensidad de campo (19 y 32 kV) y se compara con la pasteurización. Los resultados obtenidos muestran que la pasteurización produce un aumento de la densidad de la matriz lipoproteica, lo que se relaciona con intensificación de las interacciones proteína-proteína, debido a la desnaturalización e insolubilización de proteínas producida por el tratamiento térmico. Sin embargo, en muestras tratadas por PEF (19 ó 32 kV) la matriz lipoproteica aparece más laxa si se compara con el control, lo que podría estar relacionado con un debilitamiento de las interacciones entre los componentes del huevo. Por otra parte, el estudio por SDS-PAGE muestra que los tratamientos de pasteurización y PEF no producen degradación de las proteínas del huevo, pero si influyen en su solubilidad y por tanto en su funcionalidad.

REFERENCIAS

[1] P. Butz and B. Tauscher, "Emerging technologies: chemical aspects", *Food Res. Int.* Vol. 35, pp. 279-284, (2002).

[2] P. Mañas and R. Pagán, "Microbial inactivation by new technologies of food preservation", *Journal of Applied Microbiology*. Vol. 98, pp. 1387-1399, (2005).

EFECTO DE LA APLICACIÓN DE PULSOS ELÉCTRICOS (PEF) SOBRE LA FRACCIÓN PROTEICA DEL HUEVO, POR SDS-PAGE. CAMBIOS MICROESTRUCTURALES POR CRYO-SEM.

Raquel Marco-Molés, Isabel Hernando, Amparo Quiles, Empar Llorca, Isabel Pérez-Munuera
Departamento de Tecnología de Alimentos. Escuela Superior de Ingenieros Agrónomos
Universidad Politécnica de Valencia. Camino de Vera 14, 46071 Valencia
e-mail: iperez@tal.upv.es, web: http://www.upv.es

INTRODUCCIÓN

La aplicación de pulsos eléctricos (PEF) es una nueva tecnología de conservación de alimentos de gran interés en los últimos años. Se usa satisfactoriamente en la pasteurización de alimentos líquidos o semilíquidos como zumos, leche, yogurt, huevo líquido, salsas y sopas, y reduce los cambios perjudiciales ocasionados por el tratamiento térmico convencional. El objetivo de este trabajo es el estudio de los principales cambios que tienen lugar en la microestructura de huevo líquido tratado por pulsos eléctricos, así como el efecto de éstos sobre la fracción proteica.

CRYO-SEM

MUESTRA
↓
CONGELACIÓN (T ≤ -210°C)
↓
FRACTURA
↓
SUBLIMACIÓN (-90°C)
↓
RECUBRIMIENTO CON ORO (2 mbar/2 mA)
↓
OBSERVACIÓN POR CRYO-SEM (15kV/-130°C)

EXTRACCIÓN DE PROTEÍNAS

Huevo líquido
↓
Liofilización: 10^3 Pa, - 45 °C, 24 h
↓
Eliminación grasa: *Método Soxhlet*
↓
Solubilización proteínas: *2.5 g extracto +15 mL H_2O*
↓
Centrifugación: *3500 rpm, 20 °C, 20 min*
→ Eliminación pellet
↓
Extracto proteínas solubles

SDS-PAGE

Proteína total: Huevo líquido
Proteína soluble: Extracto proteína soluble
↓
Disolución: 1.25 mg/ml en T. Laemmli
↓
Filtración: 0.45 µm diámetro
→ Proteínas Patrón
↓
Siembra: 8 µL/ pocillo ExcelGel SDS Homogeneous, 12.5 %
↓
Desarrollo electroforético: 600 V, 38 mA y 23 W; 1 h 30 min
↓
Revelado: *Tinción Coomassie*

RESULTADOS Y DISCUSIÓN

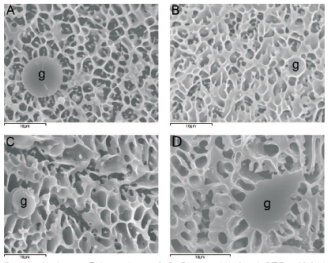


Figura 1. Cryo-SEM. A: Control; B: Pasteurizado; C: PEF a 19 kV; D: PEF a 32 kV. g: gránulos protéicos.

El estudio microestructural muestra que la pasteurización (Fig. 1B) produce un aumento de la densidad de la matriz lipoproteica del huevo, lo que se relaciona con intensificación de las interacciones proteína-proteína, debido a la desnaturalización e insolubilización de proteínas producida por el tratamiento térmico. Sin embargo, en muestras tratadas por PEF (Fig. 1C y 1D), aunque la matriz lipoproteica aparece más compacta se observa agrietada, si se compara con el control. Estos cambios podrían relacionarse con la desestabilización de la emulsión coloidal que constituyen las proteínas y lípidos en el huevo batido.

El análisis electroforético (Fig. 2) revela que no hay cambios significativos en las bandas características correspondientes a la fracción proteica total de las muestras estudiadas. En la fracción de proteínas solubles, todas las muestras, a excepción del control, presentan una marcada "cola" en la zona de Pm superiores a 41- 43 kDa. Además, se aprecia que la banda 12, que se correspondería con las LDL, HDL u ovoglobulinas, tiene mayor concentración en las muestras control que en las tratadas. Estos hechos podrían relacionarse con cambios en la extractabilidad de las proteínas debido al efecto de los distintos tratamientos sobre la estructura (Fig. 1C y 1D).

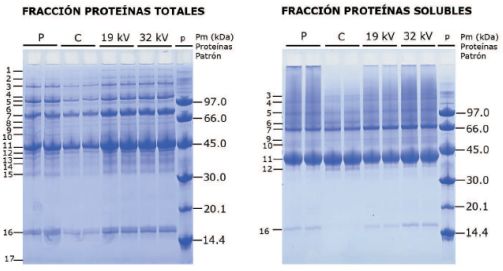


Figura 2. Electroforegrama correspondiente a la fracción proteica total y soluble de las muestras de huevo líquido. P: pasteurizado; C: control; 19 kV: PEF a 19 kV; 32 kV: PEF a 32 kV; p: patrón. Los pesos moleculares de las bandas identificadas son: 1: 230-280 kDa; 2: 200-230 kDa; 3: 160-170 kDa; 4: 125-128 kDa; 5: 110 kDa; 6: 100 kDa; 7: 79 kDa; 8: 67-69 kDa; 9: 62-64 kDa; 10: 54 kDa; 11: 44-46 kDa; 12: 41-43 kDa; 13: 38-41 kDa; 14: 35-38 kDa; 15: 31-34 kDa; 16: 16-17 kDa; 17: 11 kDa.

CONCLUSIONES

La aplicación de pulsos eléctricos a huevo líquido afecta la microestructura de la matriz lipoproteica, y la solubilidad de sus proteínas, lo que podría afectar a su funcionalidad. Sin embargo, ni los tratamientos con PEF ni la pasteurización producen hidrólisis de la fracción proteica del huevo.


Innovative Applications of Nonthermal Technologies in Foods: Technology, Safety, Health and Consumer Acceptability
Madrid, Spain · 19-22 November 2008
Program and Book of Abstracts
CSIC
Universitat de Lleida
EFFoST
IFT
INSTITUTE OF FOOD TECHNOLOGISTS

A29

Effect of high pressure homogenisation (HPH) on the protein functionality of whole liquid egg

R. Marco-Molés, I. Hernando, A. Quiles, E. Llorca, I. Pérez-Munuera*
Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia
Camino de Vera s/n, 46022 Valencia
e-mail: iperez@tal.upv.es

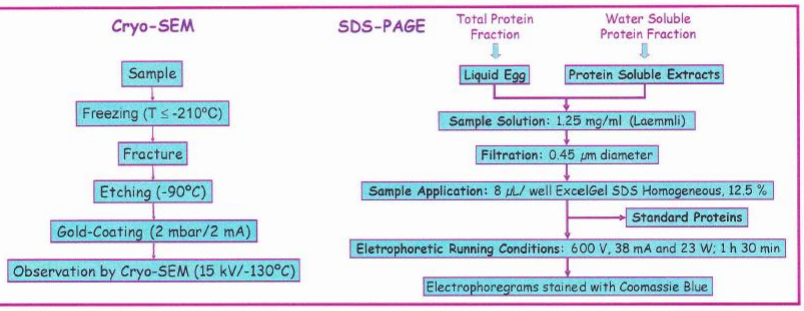
During the last years, non-thermal food processing technologies are regarded with special interest by the food industry. Among them, HPH is gaining popularity with food processors because it inactivates microorganisms, preserving the nutritional and sensory properties of foods, and maintaining their shelf life. Furthermore, it has the capacity to develop "value-added" food products or foods with interesting functional properties. The egg is an ingredient usually used in the food industries because of their chemical components functionality. These properties have to be preserved when new technologies are applied. The aim of this work is to study the main changes on the microstructure and proteins of whole liquid egg treated with HPH. Samples are treated at different intensities (0, 1000, 3000 and 5000 bar) and studied by Low Temperature Scanning Electron Microscopy (Cryo-SEM) and by Polyacrylamide Gel Electrophoresis with Sodium Dodecil Sulphate (SDS-PAGE). The results show that the treatment with HPH, regardless the pressure, affects the liproprotein matrix of the egg at a microstructural level, producing a weakening of the interactions between its chemical components. On the other hand, the study by SDS-PAGE does not show apparent changes in the electrophoretic profiles of the samples, and it could be concluded that the HPH treatments do not produce proteolisis or insolubilisation of whole liquid egg proteins, thus maintaining their functionality.

EFFECT OF HIGH PRESSURE HOMOGENISATION (HPH) ON THE PROTEIN FUNCTIONALITY OF WHOLE LIQUID EGG.

R. MARCO-MOLÉS, I. HERNANDO, A. QUILES, E. LLORCA AND I. PÉREZ-MUNUERA.

Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia. Camino de Vera s/n, 46022 Valencia, Spain.
e-mail: iperez@tal.upv.es; web: www.upv.es

During the last years, non-thermal food processing technologies are regarded with special interest by the food industry. Among them, HPH is gaining popularity with food processors because it inactivates microorganisms, preserving the nutritional and sensory properties of foods, and maintaining their shelf life. Furthermore, it has the capacity to develop "value-added" food products or foods with interesting functional properties. The egg is an ingredient usually used in the food industries because of their chemical components functionality. These properties have to be preserved when new technologies are applied. The aim of this work is to study the microstructural changes produced by HPH using Cryo-SEM and possible changes in the protein fraction, main structural and functional component of whole liquid egg, by SDS-PAGE.



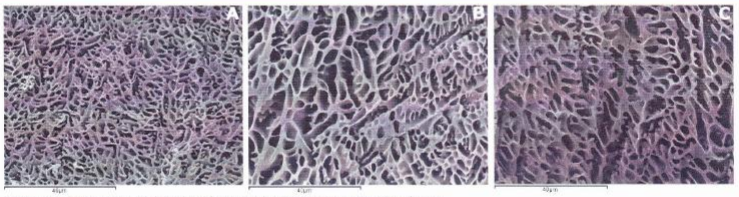


Figure 1. Cryo-SEM. Not treated whole liquid egg (A), and treated by HPH (B: 1000 bar and C: 5000 bar). 1500x.

The Cryo-SEM micrographs (Fig. 1) show that the treatment with HPH affects the liproprotein matrix of the whole liquid egg, producing changes in the interactions between its chemical components. The typical lipoprotein matrix of the whole liquid egg (Fig. 1A) is observed more open after applying the HPH treatment (Fig. 1B and 1C). The empty areas were occupied by water in the original sample; these areas are higher when HPH is applied, which could indicate a weakening of water-protein interactions. The study by SDS-PAGE (Fig. 2) does not show apparent changes in the electrophoretic profiles of the samples; the HPH treatments do not produce proteolysis of whole liquid egg proteins.

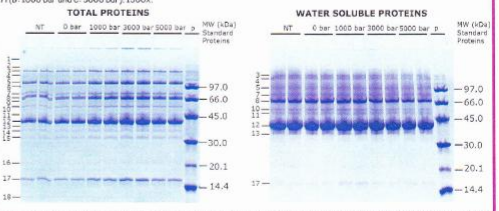


Figure 2. Electrophoregrams obtained by SDS-PAGE, of whole liquid egg samples treated by HPH, at different intensities: 0, 1000, 3000 and 5000 bar. (NT: non treated samples; p: standard proteins).

CONCLUSION

The HPH treatment does not produce degradation of the egg protein fraction, but protein-water interactions are weakened. This could destabilise the colloidal emulsion of the whole liquid egg during storage and it could change the functionality of this food ingredient.

B1

Changes on microstructure and proteins during the storage of whole liquid egg treated by pulsed electric fields (PEF)

R. Marco-Molés, I. Pérez-Munuera, A. Quiles, E. Llorca, I. Hernando*
Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia
Camino de Vera s/n, 46022 Valencia

e-mail: mihernan@tal.upv.es

Nowadays, consumers are more and more demanding for minimally processed foods. This has led to the food industry to develop new technologies as Pulsed Electric Fields (PEF) that allows obtaining products with similar properties to fresh foods. The aim of this work is to study the main changes on the microstructure of whole liquid egg treated by PEF by Transmission Electron Microscopy (TEM), and the proteolytic activity, by Polyacrylamide Gel Electrophoresis with Sodium Dodecil Sulphate (SDS-PAGE), after one week of refrigerated storage. The effect of the field strength (19 and 32 kV) and its comparison with non treated and pasteurised samples is studied. The results obtained by TEM show that one week after the refrigerated storage, the lipoprotein matrix has been degraded by different types of microorganisms in non treated and low PEF treated egg samples. Furthermore, a protein aggregation effect is observed in all the samples, although the protein granules remain quite unaltered. In the pasteurised samples, the whole lipoprotein matrix is affected by this aggregation phenomenon. When high field strength is applied, the lipoprotein matrix appears less affected, probably due to a lower microbiological activity than in non treated samples. The electrophoretic study of the protein soluble fraction shows that after one week of storage, a slight proteolysis occurs in the not treated and PEF treated samples. So, these PEF treatments produce changes on the lipoprotein matrix, which could affect the functionality of the chemical components in liquid whole egg.

CHANGES ON MICROSTRUCTURE AND PROTEINS DURING THE STORAGE OF WHOLE LIQUID EGG TREATED BY PULSED ELECTRIC FIELDS (PEF)

R. MARCO-MOLÉS, I. PÉREZ-MUNUERA, A. QUILES, E. LLORCA AND I. HERNANDO.

Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia. Camino de Vera s/n, 46022 Valencia, Spain.
e-mail: mihernan@tal.upv.es, web: www.upv.es

INTRODUCTION

Nowadays, consumers are more and more demanding for minimally processed foods. This has led to the food industry to develop new technologies as Pulsed Electric Fields (PEF) that allows obtaining products with similar properties to fresh foods. The aim of this work is to study the main changes on the microstructure of whole liquid egg treated by PEF using Transmission Electron Microscopy (TEM), and the proteolytic activity, using Polyacrylamide Gel Electrophoresis with Sodium Dodecil Sulphate (SDS-PAGE), after one week of refrigerated storage. The effect of the field strength (19 and 32 kV) and its comparison with non treated and pasteurised samples is studied.

METHODS

SDS-PAGE

- **Soluble Protein Extracts**
- **Sample Solution:** 1.25 mg/ml (Laemmli)
- **Filtration:** 0.45 μm diameter
- **Sample Application:** 8 μL/ well ExcelGel SDS Homogeneous, 12.5 %
  - **Standard Proteins**
- **Eletrophoretic Running Conditions:** 600 V, 38 mA and 23 W; 1 h 30 min
- **Detection:** *Coomassie Staining*

TRANSMISSION ELECTRON MICROSCOPY

- **Liquid Egg**
- **Stabilization:** Agarose (3%)
- **Sample:** 1-2 mm^3
- **Fixation:** Glutaraldehide (2.5%)
- **Rinsing:** Phosphate Buffer 0.025 M, pH 6.8
- **Postfixation:** OsO_4 (2%)
- **Rinsing:** Distilled Water
- **Dehydration:** Ethanol 30, 50 and 70%
- **Contrast:** Uranyl Acetate (2%)
- **Dehydration:** Ethanol 96 and 100%
- **Infiltration:** Epoxy Resin
- **Embedding:** 65.5 °C / 3 days
- **Ultramicrotomy:** Ultrathin Sections
- **Stainning:** Lead Citrate (4%) /Uranyl Acetate (2%)
- **Observation:** TEM

RESULTS AND DISCUSSION

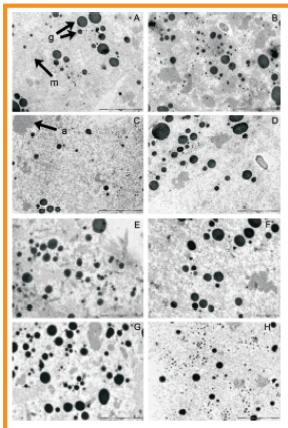


Figure 1. TEM. Samples imediately after treatments (first column) and 1 week after refrigerated storage (second column). A and B: non-treated liquid egg; C and D: LowPEF (19 kV) treated liquid egg; E and F: HighPEF (32 kV) treated liquid egg; G and H: pasteurized liquid egg. 1650x. (G: protein granules; a: aggregated compounds.)

Non-treated sample (Figure 1) shows a continuous matrix mainly composed of proteins and lipids, from the yolk, incorporated into this matrix during beating. In this lipoprotein matrix, round shaped protein granules (1.5 μm) are inserted. After one week of refrigerated storage, the lipoprotein matrix has been degraded by different types of microorganisms in non treated and low PEF treated egg samples. Furthermore, a protein aggregation effect is observed in all the samples, although the protein granules remain quite unaltered. When high field strength is applied, the lipoprotein matrix appears less affected, probably due to a lower microbiological activity than in non treated samples.

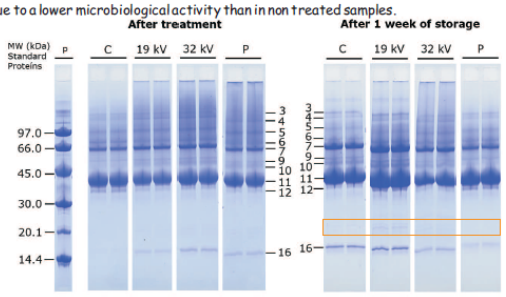


Figure 2. Water soluble protein electrophoregram of whole liquid egg, inmediately after treatment and 1 week after refrigerated storage. P: pasteurized whole liquid egg; C: whole liquid egg control; 19 kV: low intensity PEF (19 kV) treated whole liquid egg; 32 kV: high intensity PEF (32 kV) treated whole liquid egg; p: standard protein. The MW of the studied bands are: 3: 160-170 kDa; 4: 125-128 kDa; 5: 110 kDa; 6: 100 kDa; 7: 79 kDa; 9: 62-64 kDa; 10: 54 kDa; 11: 44-46 kDa; 12: 41-43 kDa; 16: 16-17 kDa.

The electrophoretic study of the protein soluble fraction (Fig. 2) shows that after one week of storage, a slight proteolysis occurs in the not treated and PEF treated samples.

CONCLUSIONS

These PEF treatments produce changes on the lipoprotein matrix, which could affect the functionality of the chemical components in liquid whole egg.


UNIVERSIDAD CATÓLICA SAN ANTONIO
UCAM
LIBRO DE COMUNICACIONES
V CONGRESO NACIONAL DE
CIENCIA Y TECNOLOGÍA
DE LOS ALIMENTOS
26, 27, 28 y 29 de mayo • Murcia
09
UNIVERSIDAD DE MURCIA

LIBRO DE COMUNICACIONES
V CONGRESO NACIONAL DE CIENCIA Y TECNOLOGÍA
DE LOS ALIMENTOS.
MURCIA. 26. 27. 28 Y 29 DE MAYO DE 2009

Diseño e impresión: GLG Creativos Asociados
ISBN: 978-84-933078-3-7
Depósito legal: MU-1585-2009
Mediprint Ediciones, S.L.
Plaza Condestable, 5 - 30009 Murcia

CAMBIOS EN LOS PRINCIPALES COMPONENTES QUÍMICOS DEL HUEVO LÍQUIDO TRATADO POR ALTAS PRESIONES DE HOMOGENEIZACIÓN. ESTABILIDAD DURANTE EL ALMACENAMIENTO.

Marco-Molés R., Pérez-Munuera I., Llorca E., Hernando I.

Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de Alimentos. Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia.
iperez@tal.upv.es

Introducción

La técnica de Altas Presiones de Homogeneización (HPH) es considerada una prometedora alternativa al tratamiento térmico convencional para la conservación de alimentos fluidos (Guerzoni et al., 2002). El objetivo de este trabajo es el estudio de los cambios químicos y de la microestructura de huevo líquido tratado por HPH.

Materiales y Métodos

Se trata huevo líquido por HPH a diferentes intensidades (0, 1000, 3000 y 5000 bar) y se almacena durante una semana en refrigeración. Se estudian los cambios químicos en sus proteínas por N-Kjeldahl (AOAC, 2000) y lípidos, mediante el análisis del índice de acidez (AOAC, 1990) y de las K232 y K270 (UNE 55-047-73). También se observa la microestructura por Microscopia Electrónica de Barrido a bajas Temperaturas (Cryo-SEM), utilizando un microscopio electrónico de barrido JSM-5410 (Jeol, Tokyo, Japan) acoplado a una unidad Cryo CT-1500C (Oxford Instruments, Witney, UK).

Resultados y Discusión

La tabla 1 muestra los valores de N-soluble, índice de acidez, K_{232} y K_{270} correspondientes a las fracciones proteicas y lipídicas de las muestras de huevo líquido.

Tabla 1. N-soluble, índice de acidez, K_{232} y K_{270} de las muestras de huevo líquido control y tratado por HPH a diferentes intensidades. Letras diferentes en una misma columna indican diferencias significativas ($P<0.05$) en la misma semana y * diferencias entre las muestras recién tratadas y tras una semana a 4 °C para un mismo parámetro. El N-soluble aumenta después de los distintos tratamientos; sin embargo, a medida que aumenta la presión aplicada, el N-soluble disminuye ligeramente lo que podría atribuirse al efecto de la temperatura durante el proceso. Tras una semana de almacenamiento, no hay diferencias significativas. El análisis de la fracción lipídica muestra que los valores de acidez de todas las muestras son relativamente bajos, por lo que se mantiene la calidad de la grasa, aunque se produce una ligera hidrólisis de los lípidos a medida que aumenta la presión aplicada. Después de una semana de almacenamiento, la acidez aumenta en todos los casos excepto para la muestra tratada a 5000 bar, lo que estaría relacionado con una mayor eficacia antimicrobiana del tratamiento. Esta muestra presenta también una mayor estabilidad a la oxidación durante el almacenamiento, evidenciado por las medidas de K_{232} y K_{270}

		N-Solub	IA	K_{232}	K_{270}
Recién tratadas	Control	8.03±0.10^a	2.04±0.12^a	2.84±0.23^{ab}	2.38±0.07^a
	0 bar	8.53±0.21^b	1.92±0.06^a	2.82±0.24^a	2.32±0.34^a
	1000 bar	10.82±0.49^c	2.25±0.10^b	3.04±0.15^b	2.10±0.14^b
	3000 bar	9.47±0.37^d	2.41±0.14^c	3.34±0.15^c	1.89±0.10^b
	5000 bar	9.37±0.35^d	2.53±0.10^c	3.44±0.14^c	2.01±0.13^b
1 semana a 4°C	Control	8.18±0.32^a	2.83±0.14^{a*}	2.51±0.15^{ab*}	2.61±0.20^a
	0 bar	8.49±0.28^a	2.75±0.07^{a*}	2.70±0.23^b	2.53±0.17^a
	1000 bar	10.82±0.39^b	2.82±0.09^{a*}	2.44±0.12^{a*}	2.58±0.28^{a*}
	3000 bar	9.46±0.33^c	2.87±0.16^{a*}	2.62±0.13^{ab*}	2.54±0.30^{a*}
	5000 bar	10.05±0.37^{d*}	2.17±0.09^{b*}	3.03±0.31^{a*}	1.98±0.30^{b*}

Figura 1. Cryo-SEM. A: huevo líquido control; B: huevo líquido tratado por HPH a 1000 bar; C: huevo líquido tratado por HPH a 5000 bar. 1500x.

El estudio microestructural muestra un debilitamiento de las interacciones entre los componentes de la matriz lipoproteica del huevo líquido tratado por HPH (Figura 1).

Agradecimientos

A la Comision Europea por la financiación del proyecto "Innovative non thermal processing technologies to improve the quality and safety of ready-to-eat (RTE) meals" (FP6-FOOD-023140).

Bibliografía

Guerzoni, M.E. et al. (2002). International Journal of Food Microbiology, 73, 367-374.

AOAC (1990). Official methods of analysis of AOAC International. (15th edition). Association of Official Analytical Chemist, Washington. D.C, U.S.A.

AOAC (2000). Official methods of analysis of AOAC International. (17th edition). Association of Official Analytical Chemist, Gaithersburg.

UNE 55-047-73. (1973) Materias grasas. Medida espectrofotométrica de la absorción en la region ultravioleta. Instituto Nacional de Racionalización y Normalización (IRANOR). Patronato Juan de la Cierva de Investigación Técnica: Consejo Superior de Investigaciones Científicas (España).

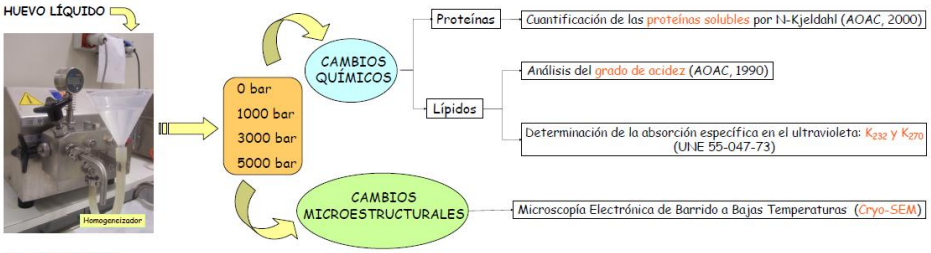
CAMBIOS EN LOS PRINCIPALES COMPONENTES QUÍMICOS DEL HUEVO LÍQUIDO TRATADO POR ALTAS PRESIONES DE HOMOGENEIZACIÓN. ESTABILIDAD DURANTE EL ALMACENAMIENTO.

Marco-Molés R., Pérez-Munuera I., Llorca E.; Hernando I.
Grupo de Microestructura y Química de Alimentos. Departamento de Tecnología de Alimentos.
Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia.
e-mail: iperez@tal.upv.es, web: http://www.upv.es

OBJETIVOS

La técnica de Altas Presiones de Homogeneización (HPH) es considerada una prometedora alternativa al tratamiento térmico convencional para la conservación de alimentos fluidos (Guerzoni et al., 2002). El objetivo de este trabajo es el estudio de los cambios químicos en las proteínas y los lípidos, así como en la microestructura de huevo líquido tratado por HPH a diferentes presiones. El efecto del tratamiento se estudia también tras una semana de almacenamiento refrigerado (4ºC).

MATERIAL Y MÉTODOS



RESULTADOS

El estudio microestructural muestra que en la matriz lipoproteica típica del huevo líquido (Fig. 1A), cuando las muestras son tratadas por HPH (Fig. 1B y 1C), se produce un desequilibrio en las interac-ciones entre los componentes de la matriz. Hay zonas en las que se acumula agua y otras en las que se observa un engrosamiento de la matriz lipoproteica que indicaría reforzamiento de las interac-ciones entre los componentes químicos en estas áreas.

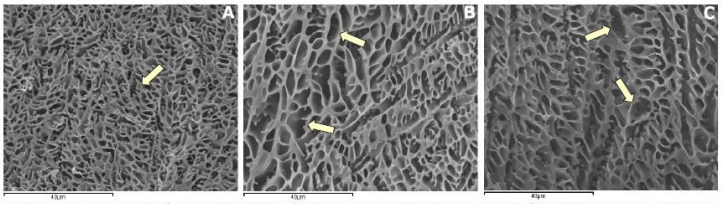


Figura 1. Cryo-SEM. A: huevo líquido control; B: huevo líquido tratado por HPH a 1000 bar; C: huevo líquido tratado por HPH a 5000 bar. 1500x. (Flecha: zonas ocupadas por agua en la muestra original)

		N-Soluble	IA	K_{232}	K_{270}
Recién tratadas	Control	8.03±0.10^{a}	2.04±0.12^{a}	2.84±0.23^{ab}	2.38±0.07^{a}
	0 bar	8.53±0.21^{b}	1.92±0.06^{a}	2.82±0.24^{a}	2.32±0.34^{a}
	1000 bar	10.82±0.49^{c}	2.25±0.10^{b}	3.04±0.15^{b}	2.10±0.14^{b}
	3000 bar	9.47±0.37^{d}	2.41±0.14^{c}	3.34±0.15^{c}	1.89±0.10^{b}
	5000 bar	9.37±0.35^{d}	2.53±0.10^{c}	3.44±0.14^{c}	2.01±0.13^{b}
1 semana a 4ºC	Control	8.18±0.32^{a}	2.83±0.14^{a*}	2.51±0.15^{ab*}	2.61±0.20^{a}
	0 bar	8.49±0.28^{a}	2.75±0.07^{a*}	2.70±0.23^{b}	2.53±0.17^{a}
	1000 bar	10.82±0.39^{b}	2.82±0.09^{a*}	2.44±0.12^{a*}	2.58±0.28^{a*}
	3000 bar	9.46±0.33^{c}	2.87±0.16^{a*}	2.62±0.13^{ab*}	2.54±0.30^{a*}
	5000 bar	10.05±0.37^{d*}	2.17±0.09^{b*}	3.03±0.31^{c*}	1.98±0.30^{b}

Tabla 1. N-soluble, índice de acidez (IA), K_{232} y K_{270} de las muestras de huevo líquido control y tratado por HPH a diferentes intensidades. Letras diferentes en una misma columna indican diferencias significativas ($P<0.05$) en la misma semana y * diferencias entre las muestras recién tratadas y tras una semana a 4 ºC para un mismo parámetro.

El N-soluble aumenta después de los distintos tratamientos; sin embargo, a medida que aumenta la presión aplicada, el N-soluble disminuye ligeramente lo que podría atribuirse a una insolubilización de las proteínas por efecto de la temperatura en el proceso. Tras una semana de almacenamiento, no hay diferencias significativas.
Los valores de acidez de todas las muestras son relativamente bajos, por lo que se mantiene la calidad de la grasa, aunque se produce una ligera hidrólisis de los lípidos a medida que aumenta la presión aplicada. Después de una semana de almacenamiento, la acidez aumenta en todos los casos excepto para la muestra tratada a 5000 bar, lo que estaría relacionado con una mayor eficacia antimicrobiana de este tratamiento. Esta muestra presenta también una mayor estabilidad a la oxidación durante el almacenamiento, evidenciado por las medidas de K_{232} y K_{270}.

CONCLUSIONES

La aplicación de Altas Presiones de Homogeneización a huevo líquido afecta a la estructura típica de su matriz lipoproteica desestabilizando las interacciones entre los principales componentes químicos y el agua. Además, a medida que aumenta la presión aplicada, se produce una ligera lipólisis de la fracción lipídica, así como un aumento de la solubilidad de las proteínas, lo que estaría relacionado con los cambios microestructurales observados, que podrían afectar la funcionalidad del huevo al ser utilizado como ingrediente en alimentos.

BIBLIOGRAFÍA

Guerzoni, M.E. et al. (2002). International Journal of Food Microbiology, 73, 367-374.
AOAC (1990). Official methods of analysis of AOAC International. (15th edition). Association of Official Analytical Chemist, Washington. D.C, U.S.A.
AOAC (2000). Official methods of analysis of AOAC International. (17th edition). Association of Official Analytical Chemist, Gaithersburg.
UNE 55-047-73. (1973) Materias grasas. Medida espectrofotométrica de la absorción en la región ultravioleta. Instituto Nacional de Racionalización y Normalización (IRANOR). Patronato Juan de la Cierva de Investigación Técnica: Consejo Superior de Investigaciones Científicas (España).

AGRADECIMIENTOS

A la Comision Europea por la financiación del proyecto "Innovative non thermal processing technologies to improve the quality and safety of ready-to-eat (RTE) meals" (FP6-FOOD-023140).

"La mejor forma de hacer tus sueños realidad es despertar"

- *Paul Valery*