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Effects of High Hydrostatic Pressure Processing on Hen Egg Compounds and Egg Products

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Abstract: High hydrostatic pressure (HHP), used alone or with other processes, is an emerging technology increasingly used in the food industry to improve microbial safety, and the functionality and bioactive properties of food products. HHP provides a way to reduce energy requirements for food processing and may contribute to improved energy efficiency in the food industry. Hen egg is used by the food industry to formulate many food products. To improve the microbiological safety of egg and egg-derived products, HHP processing is an attractive alternative to heat- pasteurization and a potential technology. However, HHP treatment induces structural modifications of egg components (such as proteins) which could positively or negatively affect the physicochemical and functional properties of egg-derived products. Improving our knowledge regarding the potential of HHP in the egg industry will add value to the final food products and increase profitability for egg producers and the food industry.

Keywords: egg composition, hen egg, high hydrostatic pressure processing

Introduction

Current food industry trends are towards less processed, natural foods without additives and use of eco-friendly preparation technologies. Therefore, innovative food processing techniques are required to guarantee safety and quality of food products. Physical treatment strategies such as pulsed electric fields, oscillating magnetic fields (OMF), ultraviolet (UV) radiation, and high hydrostatic pressure (HHP) processing provide possibilities for eliminating the probable risks for contamination of foods with foodborne pathogens without drastically changing the natural characteristic of foodstuffs (Smelt 1998). Applications of HHP technology has attracted the interest of food producers since the process allows microbial destruction at very low or moderate temperatures, preservation of bioactive nutrients, improvement of bioactive compound extraction and decreased food allergenicity (Koutchma 2014). Indeed, HHP technology uses isostatic pressures between 100 and 1000 MPa, with or without heat treatments, to eliminate different forms of spoilage and pathogenic microorganisms, viruses, molds, and yeasts to ensure the microbiological safety of final food products. The combined applications of heat and pressure in HHP treatment can cause different physical, chemical, or biological changes in food compounds (Balasubramaniam and others 2015). These changes can include changes in protein conformation, altered enzymatic activities and thermodynamic phase changes of materials, based on the method used to apply pressure or temperature and the length of the treatment (Messens and others 1997).

HHP technology was initiated in the 1890s with the work of Hite (1899). However, it was only available at the industrial-scale towards the end of the 20th century, with pressurized foods first commercialized in Japan in 1992 (Murchie and others 2005). Since then, HHP has been recognized as a clean-label technology by the food industry for manufacturing a high-quality food. Today, it is possible to purchase products derived from HHP in numerous countries. For instance, Meidi-ya Food Co. in Japan applies HHP to produce jams, jellies, and sauces. Some food producers in the United States use HHP in the production of guacamole (avocado paste or sauce). Recently, several food companies (such as Kraft, Hormel, Unilever, Basic American Foods, Stork Food and Dairy Systems, Washington Farms, ConAgra, and Fresherized Foods) have committed to collaborating as a syndicate in order to develop new pressurized food products (Bermúdez-Aguirre and Barbosa-Cánovas 2011). Market research has shown that the HHP equipment market was $350 million in 2013, and estimated to increase by an 11% compound annual growth rate (CAGR) until 2018 (Koutchma 2014). HHP technology was also recognized as having the highest potential for commercialization over the next 5 to 10 y. Commercial-scale HHP systems are available in both vertical and horizontal configurations, with internal single vessel volumes of 30 to 525 L. They cost approximately $300,000 to $3.0 million U.S., based on the capacity of the equipment and the volume of automation (Koutchma 2014).

The application of HHP became more interesting to the food industry due to several advantages over conventional processing techniques. In HHP, homogeneous and instant pressure can be transmitted to products, regardless of volume and shape of product and treatment, even at ambient temperature (Knorr 1999;

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Koutchma 2014). Compared to thermal processing, instant and homogeneous pressurization and decompression cycles can be achieved quickly, which results in shorter processing times (Knorr 1995). Additionally, HHP systems use less energy because when the system reaches the required pressure, it can sustain that pressure without any further input of energy (Farr 1990). It was shown that food products that have been produced through application of HHP maintained their appearance, flavor, texture, and nutritional qualities (Farkas and Hoover 2000). Despite all the advantages of the HHP technique, the high purchase price of final products and the batch processing technique may complicate some of its applications.

HHP processing can also be combined with other processing techniques. For example, pressure-assisted thermal sterilization or PATS, which is a FDA-approved food sterilization technique that uses combinations of pressure and temperature in the system (Bermúdez-Aguirre and Barbosa-Cánovas 2011). The HHP technique can also offer new opportunities for innovation and new product development by inhibiting and stabilizing microbial growth during subzero storage, with and without freezing (Koutchma 2014).

Hen egg is a food matrix that has recently been promoted as a functional food because of its diverse essential nutrients. Hen eggs and egg yolk are conventional functional foods that have been used in food industry formulations, largely as emulsifiers, for foam formation, and for thermal aggregation and gelation (Rossi and others 2010). World-wide, egg production increased between 2000 and 2014 by 36.5%, compared to a decade ago and China has traditionally been a leading country (Conway 2015a). Increasing protein consumption and rising incomes have been driving the increases in world-wide egg consumption (Conway 2015b). Of the primary macronutrients (proteins, lipids, and carbohydrates), eggs are largely composed of proteins and lipids. Eggs contain 75% water, 12% protein (of which 16% is in the yolk and 11% in the albumin or egg white), and 11% lipid, which is almost entirely in the egg yolk (Nys and Sauveur 2004; Pintea and others 2012). While serving as nutrient-dense food, eggs and egg-based foods can be vectors for food-borne illnesses, particularly those related to *Salmonella* (Rakonjac and others 2014). Therefore, food service establishments and commercial food producers have been interested in using pasteurized liquid egg products rather than fresh whole eggs (Garcia-Gonzalez and others 2009). Concurrently, the possibility of using a HHP process to develop a novel alternative to liquid whole egg heat pasteurization was evaluated (Monfort and others 2012). In 2015, Health Canada approved applications for HHP-treated egg products (such as egg salad, egg dips, and egg spreads) produced by Burnbrae Farms Ltd. The HHP treatment was designed to apply 600 MPa for a 2-min cycle on boiled eggs to extend the shelf-life of these products during refrigeration (Health Canada 2015). Precooked egg products (such as egg patties, omelets, scrambled eggs) are generally preserved and sold as frozen food, but the foremost issue is to guarantee the safety of product throughout the cooling, packaging, and post-packaging steps. Moreover, storage of egg products at temperatures higher than 70 °C can cause color degradation, changes in product texture, or even liquid separation (Juliano and others 2012). The postpackaging pasteurization of commercial egg patties has been shown to be possible through the use of HHP at chamber temperatures of 30 °C at 675 MPa (Juliano and others 2006a, 2006b).

It was established that HHP may cause protein denaturation, lipid crystallization, and destabilization of biomembranes. Therefore, it is necessary to study the effect of pressure treatment on egg components and the resulting possible changes in composition, taste, and aroma of final egg-derived food products. The application of HHP by the egg industry is an innovative approach to secure the quality and assure the acceptability of products, while introducing a new way to develop egg-derived products with a modified nutritional composition, which may be appropriate for use by food companies. In this article we review and discuss the scientific challenges and advances in the application of high-pressure technology for hen egg and egg products.

Fundamental Effects of HHP on Food Components

Technical background behind high hydrostatic pressure

HHP mainly uses elevated pressure between 100 and 1000 MPa with or without the application of thermal treatment. The HHP process is operated in a batch system, commonly using water as the pressure-transmitting medium. The food items are packed, loaded into the pressure vessel, and then pressurized by water. Laboratory-scale and commercial applications of HHP are both being used in food processing systems. In laboratory-scale HHP, an indirect compression system is used to pump a pressure-transmitting liquid into the vessel in order to reach a desired pressure. Under commercial conditions, HHP systems use the indirect method of pressurization (Koutchma 2014).

In HHP processing, an isostatic pressure is applied to the entire mass of food molecules, which means that a same pressure is instantly and equally applied to a food product (Patterson and others 2006). The HHP processing approach is based on Le Chatelier's principle, which refers to any phenomenon in which a reduction in volume is enhanced by boosting pressure, and vice versa. In this case, under pressure, reaction equilibria are shifted toward the most compact condition, and the reaction rate constant is increased or decreased, depending on whether the activation volume of the reaction is negative or positive (Cheftel and Culioli 1997). HHP mainly uses batch equipment; however, semi-continuous systems are available. The HHP apparatus is usually made of high-strength steel alloys with high oxidization resistance and breakage toughness. Generally, a batch HHP apparatus is composed of a pressure vessel which is a thick-wall cylinder, 2 end closures that cover the pressure vessel, a yoke which controls the end closures under the pressure condition, a pump and intensifier for creating high pressure, and a process control system for loading and unloading the product (Ting 2011). A batch HHP system can be used for liquid and solid foods, whereas a semi-continuous HHP process can be used for only pumpable foods. During a HHP treatment, the food product is packaged, sealed, and loaded into a sample-loading basket. The packaging materials should be flexible in order to resist the pressurization. The loading baskets then enter the pressure vessel, which generally contains pressure-transmitting fluid. The end closures will close the pressure vessel and the yoke structure moves around the closed vessel to control the 2 closures under high pressure. Water is commonly used as the pressure-transmitting fluid in industrial-scale HHP equipment. The pump and the intensifier deliver a desired pressure through compression of the pressure-transmitting fluid. Then the product is maintained under the preferred time and pressure to achieve the desired treatment. At the end of the treatment time, the vessel is depressurized and the product is unloaded from the sample-loading basket (Ting 2011; Balasubramaniam and others 2015).

For most food products, a pressure of 600 MPa is generally applied. During the HHP treatment the packaged size volume of food decreases in proportion to the increased pressure, but it still preserves its initial form after decompression. HHP has different

effects on the characteristics of food components, food poisoning microorganisms, and also enzymes (Patterson and others 2006). Japan was a pioneer in using and commercializing the application of high pressure in the range of 400 to 900 MPa in the food industry, and later the application of high pressure was used in the U.S. (during the 1990s). Over the past 50 years, high pressure technology was recognized and studied as a major and significant innovation in the area of food processing. In 2009, the U.S. Food and Drug Administration (FDA) approved the application of HHP for commercial sterilization of low-acid foods (Juliano and others 2012). HHP will continue to expand because it potentially reduces energy demands for food processing, which can effectively improve energy efficiency in the food industry (Toepfl and others 2006). HHP treatment can be combined with other treatments such as thermal processing. Hydrostatic pressure changes the interatomic distances and, consequently, influences the interactions where distance affects bonding energy (Martínez-Monteagudo and others 2012). For example, the strength of electrostatic interactions is inversely related to the distance between charged particles, so applying high pressure will influence the bonding strength (Balasubramaniam and others 2015). Pressure can significantly affect van der Waals interactions and hydrogen bonding since these molecular forces are distance-dependent (Balasubramaniam and others 2015). However, under pressure, covalent bonds can be only minimally compressed, so they cannot be changed by pressure (Balasubramaniam and others 2015).

Global structural changes in proteins and lipids under hydrostatic pressure

In HHP, an isostatic and instantaneous pressure is applied at each point of the sample or solution within a specific time, which is independent of the sample size (Cheftel and Culioli 1997). When the temperature is constant, increases in pressure will change the arrangement of molecules in certain substances. Thus, the combination of pressure and temperature in HHP applies antagonistic forces on chemical reactions and molecular structure (Balny and Masson 1993). The application of pressure may induce hydrogen bond formation, while some of the other weak linkages (such as in the structure of proteins) can be destabilized (Patterson and others 2006).

Three factors primarily characterize the effects of pressure: (1) energy, (2) compression effect, and (3) chemical reactivity (Rivalain and others 2010). In biological systems, the application of pressure under ambient conditions provokes changes in volume and thermal energy at the same time. Consequently, high pressure seemed a suitable tool for distinguishing between thermal and volumetric effects. For the stability of any biological system, the arrangement of noncovalent interactions is important. Water is the most common solvent in biological systems. Due to the presence of hydrogen bonds between water molecules, the pressure-temperature diagram for water supports the liquid state down to –20 °C, if the pressure is higher than 200 MPa (Winter and Dzwolak 2005).

As mentioned earlier, Le Chatelier's principle, or the equilibrium law, can be used to predict the behavior of biosystems under high pressure. Furthermore, noncovalent interactions also play an important role in determining the molecular characteristics during pressurization (Mozhaev and others 1994). The solvation of charged groups is associated with volume reduction due to electrostriction, but the dehydration of charged atoms and the formation of coulombic interactions occurs due to increases in volume but not pressure (Mozhaev and others 1994). Increases in volume cause hydrophobic interactions between aliphatic groups and these interactions can be destabilized by increasing pressure. The hydrogen bonds are almost pressure-insensitive because they can be formed and destroyed with no changes in volume (Gross and Jaenicke 1994).

Effects on protein. Generally, proteins can be denatured due to heat, chemicals, and pressure. Denaturation of proteins caused by temperature and/or chemicals induce a total and generally irreversible unfolding resulting from covalent bond disruptions and aggregation of the protein molecule (Rivalain and others 2010). The effects of HHP on proteins, at lower pressures, are usually reversible and they are rarely accompanied by changes in the covalent structure or aggregation (Gross and Jaenicke 1994). During the high pressure process, however, protein denaturation generally occurs as the result of water entry into the protein molecule. Usually, the stability of proteins under high pressure conditions are governed by the size of the cavities in the protein structure where water molecules can enter, as well as the protein's conformational stability (Rivalain and others 2010).

Protein retains its native conformation through a limited range of pressure and temperature (Mozhaev and others 1994). Any changes in these 2 parameters induce protein denaturation. Figure 1 shows an elliptical phase diagram for the transition midpoint for protein unfolding over a range of pressure compared with temperature (Meersman and others 2006). The phase diagram of protein unfolding is elliptical and the boundary of the ellipse is presented by Gibbs free energy equal to zero ($\Delta G = 0$) (Meersman and others 2006). In Figure 1, when $\Delta G = 0$, the equilibrium concentrations of the native and denatured forms of a protein are equal. The zone below this curve explains the higher stability of native conformation of the protein compared to the denatured state when $\Delta G > 0$. The zone outside the curve explains that protein is completely unfolded ($\Delta G > 0$) (Mozhaev and others 1994).

Pressures higher than 200 MPa reduce the freezing point of water to –20 °C which makes it very interesting to perform cold unfolding experiments in the liquid state (Meersman and others 2006). This elliptical diagram is only observed for protein solutions

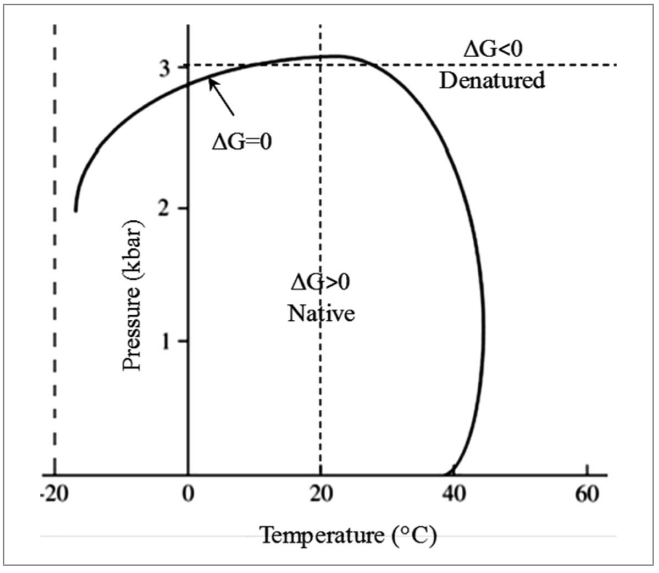

Figure 1–Pressure–temperature phase diagram for protein denaturation (modified from Mozhaev and others 1994).

where pressure causes protein denaturation. However, when under pressure in dry conditions, the proteins are very stable (Smeller 2002). Based on the phase diagram (Figure 1), which points inward at low temperatures, the cold unfolding of proteins is observed. At elevated pressures, cold and pressure unfolding are thermodynamically and mechanistically similar (Meersman and others 2006).

Effects on lipid. Lipid systems have large structural polymorphisms based on the structure of the molecule, moisture content, pH, ionic strength, temperature, and pressure. Lipids are considered to be a highly pressure-sensitive biological system (Rivalain and others 2010). The lamellar phospholipid bilayer matrix is the basic structural constituent of biological membranes. Due to lipid membrane compressibility, under high pressure conditions, the acyl chains in phospholipids straighten. This causes lateral shrinking, and it increases the concentration and transformation from the liquid–crystalline to the gel phase (Winter and Jeworrek 2009).

Therefore, pressure applications are effective for the destruction of microorganisms, since lipids are sensitive to pressure. The membranes of barophilic organisms are highly fluid, due to the higher ratio of unsaturated to saturated lipids (Winter and Jeworrek 2009).

Changes in temperature cause a thermotropic phase transition in phospholipid membranes and this strongly depends on the polarity of phospholipid head groups and the length of hydrophobic acyl chains. The phase transition of phospholipid membranes also depends on hydrostatic pressure. It was demonstrated that the transition temperature from gel phase to liquid-crystalline increases linearly at higher pressures (Kato and Hayashi 1999).

Impact of HHP on Egg Components

High pressure processing could be considered to have been introduced into the egg industry in 1914 when Bridgman (1914) observed the coagulation of egg white under pressure. However, after this discovery of pressure-induced denaturation of a monomeric protein, more efficient and methodical studies about the effects of pressure on the structure of proteins did not begin until 80 y later. The primary studies show that denaturation of single-chain proteins does not occur under pressures below 400 MPa at ambient temperature and neutral pH (Gross and Jaenicke 1994). Table 1 summarizes selected scientific highlights of high-pressure food processing applications related to hen egg and its components.

Egg is mainly composed of proteins and lipids and, although atomic bonds are minimally affected by pressure processing, the modification of proteins and/or lipids can be detected. Many researchers have been conducting studies to determine the effects of different pressure/timing/temperature conditions on different egg products and compounds. Many attempts have been made to verify whether the HHP technique can be used as a substitute for thermal pasteurization, while concurrently determining the structural changes in egg components as the result of high pressure.

Impact on egg proteins

Egg proteins are present in both albumin and yolk. Egg white proteins include lysozyme, ovotransferin, ovalbumin, ovomucin, riboflavin-binding protein, and avidin (Huopalahti and others 2007). Egg white is an excellent source of the basic protein lysozyme (Lesnierowski and Kijowski 2007). Egg yolk is composed of granular particles in soluble plasma. These 2 fractions can be separated by dilution and subsequent centrifugation of the egg yolk. The plasma fraction remains in the supernatant, while the granules precipitate on centrifugation (Bee and others 1979). Egg yolk proteins are distributed within both the plasma (liquid and water-soluble phase) and granules (particles). Proteins in plasma are low-density lipoproteins (LDLs) and livetin, while proteins in granules are high-density lipoproteins (HDLs) and phosvitin (Guerin-Dubiard and others 2010).

Systematic studies of high-pressure biochemistry started during the 1960s and 1970s, with research mainly concentrated on the effects of pressure on the structure of proteins, membranes, and nucleoproteins (Jaenicke and others 1981; Heremans 1982; Balny and others 1989). Biotechnologists started to explore the HHP process during the last decade, with pioneering food science applications carried out in Japan (Hayashi and others 1989). In 1992, a mutual conference of Japanese and European researchers working in high-pressure food technology published their interest in industrial applications of high-pressure biochemistry (Balny 1992). Later in 1994, high-pressure biochemistry was reviewed for the first time and the dynamic compressibility of proteins was compared with the effects of static pressure on structure (Gross and Jaenicke 1994).

Different classical techniques have been used to study the structure of proteins under pressure, including SDS-, native- and 2-dimensional polyacrylamide gel electrophoresis, ultraviolet and visible spectroscopy, fluorimetry, and tube gel electrophoresis at high pressure (Schade and other 1980; Heremans 1982; Gross and Jaenicke 1994). More advanced techniques, including NMR (nuclear magnetic resonance) spectroscopy, 2D NMR at hydrostatic pressures >500 MPa, and X-ray crystallography also became feasible (Gross and Jaenicke 1994). Furthermore, the first X-ray crystallographic studies conducted under pressure described the structure of lysozyme (Kundrot and Richards 1987). These systematic studies on the structural changes of egg proteins can all be traced back to the pioneering work of Bridgman (1914).

Egg white proteins. In 1914, Bridgman observed coagulation of egg white subjected to hydrostatic pressure (490 to 700 MPa) at room temperature (Bridgman 1914). The author observed that pressure caused coagulation of egg white with an appearance similar to hardboiled egg white. However, the author did not determine whether coagulation produced by pressure or by heat were due to the same mechanisms. Later on, Grant and others (1941) observed that protein denaturation under high pressure was due to the exposure of -SH linkages. The structural changes in proteins caused by high pressure are completely different from those caused by thermal or chemical treatments. Nevertheless, the mechanism of protein-unfolding as the result of pressure is not yet fully understood. Hoppe and others (2013) described the principal mechanism of pressure-induced protein denaturation as the result of water entrance into cavities of the protein molecule. For example, pressures above 450 MPa result in loss of the secondary structure of albumin (Hayakawa and others 1996) and exposure of buried hydrophobic and SH groups (Iametti and others 1998). Pressures between 400 and 700 MPa can greatly increase the surface hydrophobicity and exposed SH groups in egg white proteins (van der Plancken and others 2007; Lai and others 2010). Studies involving hen egg white lysozyme showed that HHP treatment significantly increased the number of partially unfolded forms of the protein (Nash and Jonas 1997). The HHP-assisted unfolding of lysozyme was also observed at lower temperatures (–13 °C). In this case, minor denaturation of lysozyme occurs and the protein structure contains large unaffected fractions (Vogtt and Winter 2005).

Different investigations on the structure of HHP-treated egg white proteins have shown effects other than thermal denaturation. The denaturation of ovalbumin as the result of high pressure

Table 1–Selected scientific highlights for HHP application to eggs compounds, and egg products.

Year	Application	Reference
1914	Coagulation of albumin (egg white).	(Bridgman 1914)
1989	Denaturation and coagulation of egg white and yolk, and enhancement of their protease susceptibility.	(Hayashi and others 1989)
1991	HHP-CO_2 treatment for controlling pathogenic microorganisms in egg yolk and whole egg.	(Wei and others 1991)
1994	Stability of egg yolk antibody (Ig Y) under HHP treatment (500 MPa, 60 °C).	(Shimizu and others 1994)
1999	Evaluation of HHP processing criteria in the treatment of liquid whole egg.	(Lee and others 1999)
1999	Destruction of *Salmonella enteritidis* in liquid whole egg.	(Ponce and others 1999)
1999	Characterization of HHP-treated egg albumin.	(Iametti and others 1999)
2003	Inactivation of *Listeria seelige* and *E. coli* in whole egg using combinations of nisin with HHP.	(Lee and others 2003)
2006	Pressure-assisted thermal processing application for inactivating bacterial spores of *Bacillus stearothermophilus* in egg patties.	(Rajan and others 2006)
2007	Foaming property of egg white protein affected by HHP.	(van der Plancken and others 2007)
2016	Effect of HHP (600 MPa) at 20 °C on a range of ovalbumin gels from low→intermediate→high levels of solids.	(Savadkoohi and others 2016)
2016	Production of anti-microbial agent from enzyme-treated phosvitin-phosphopeptides using HHP combined with enzymatic hydrolysis.	(Yoo 2016)

treatment was observed to be due to the exposure of aromatic residues of solvent to the hydrophobic regions of protein (Smith and others 2000). Ovalbumin (54% of egg white) is a monomeric phosphoglycoprotein which contains 4 sulfhydryl groups and 1 disulfide bond buried in the protein core (Mine 1995). The molecular weight of ovalbumin has been reported as 45 kDa with an isoelectric point of 4.5. The 3-dimensional structure of ovalbumin includes an α -helical reactive loop, β-sheet, β-turns, and random coils which are comprised of 41%, 34%, 12%, and 13%, respectively, of the ovalbumin molecule (Ngarize and others 2004). The native conformation of ovalbumin remained mainly stable below 400 MPa pressure when low-protein concentrations were used; however, 12% of the α-helix structure of ovalbumin was diminished (Hayakawa and others 1996). The FTIR analysis also indicated the formation of intermolecular hydrogen bonds in pressurized ovalbumin due to rearrangements in the secondary structure of the protein (Mine 1995). The combined effects of high pressure (600 MPa, 20 °C, 15 min) and medium concentration (dilute, semi-dilute, and high-solid) on the structure of ovalbumin revealed that this protein maintains its native conformation in a high-solid medium (80% w/w solid). The structure of ovalbumin is irreversibly changed in an aqueous medium (20% w/w solid) and only moderately altered in a semi-dilute medium (30% to 60% w/w solid). This phenomenon was explained by the fact that ovalbumin is highly hydrophobic and high pressure treatment causes rearrangements between sulfhydryl and disulfide bonds (Savadkoohi and others 2016). The effects of nonthermal processing treatments on structural changes of proteins cannot be described as adverse effects. The structural changes in proteins are sometimes beneficial. For instance, egg is considered to be an allergenic food item and its proteins are major allergens (Benede and others 2015). The allergy-causing compound has been recognized in egg yolk and egg white; however, egg white has more potential for allergenicity. Ovomucoid, ovalbumin, lysozyme, and ovotransferrin are allergy-causing proteins in egg white (Benede and others 2015). Different disulfide bonds in the molecular structures of ovomucoid and lysozyme make the proteins very stable, which possibly contributes to their resistance to digestion and their allergenicity (Hazebrouck and others 2012). Unfolding of these structures via the disruption of intramolecular disulfide bonds usually reduces the allergenicity of proteins. However, the majority of allergy-causing proteins generally remain unaffected by thermal and protease treatments. The majority of food processing techniques in industry are also ineffective at destroying the allergens in egg. The main challenge in the food industry is to preserve the taste, flavor, and nutritional and functional properties of the native food protein (Hildebrandt and others 2008). Therefore, enzymatic hydrolysis techniques are not good options for destroying the allergenicity of egg proteins (Benede and others 2015). In this case, using high pressure to minimize and destroy allergy-causing compounds in food processing could be an interesting strategy. Pressure can be used to unfold the protein structure and, subsequently, decrease polymerization of the allergen compound. Ma and others (2015) treated ovalbumin and egg white with a combined enzymatic and high pressure technique. The transglutaminase (TG) unfolded the pressurized egg proteins and increased their susceptibility to TG-induced cross-linking.

Using pepsin to hydrolyze egg white can also lower the allergenicity of protein (Yoshino and others 2004). Hoppe and others (2013) examined the effect of pressure treatment combined with pepsin hydrolysis on egg white. Prior to *in vitro* pepsin digestion, the egg white was treated with high pressure (400, 600, and 800 MPa) and heated (65, 85, and 95 °C). Higher pressure (800 MPa) increased susceptibility of egg white to pepsin hydrolysis compared to heating at 95 °C (Hoppe and others 2013). Pressure-treated egg white was incubated with pepsin, which subsequently enhanced extensive hydrolysis of most proteins and separation of various peptides (Hoppe and others 2013). The combined effect of HHP treatment and pepsin digestion of egg white preserved the nutritional quality of egg white since digestibility of the protein was improved.

Differential scanning calorimetry (DSC) analysis was used to study HHP-treated egg white and it was observed that 2 major peaks, the first at 65 °C and the second at 78 °C, corresponded with primary denaturation of conalbumin followed by ovalbumin (Andrassy and others 2006). Pressures between 250 and 400 MPa increased denaturation of liquid egg white proteins, which gradually resulted in the loss of their solubility due to formation of aggregates (Andrassy and others 2006).

Lysozyme (3.5% of egg white) is the main protein of egg white which has long been used as a food preservative (Abeyrathne and others 2013). Following on research about the application of high pressure on protein structure, Groß and Jaenicke (1991) evaluated the crystallization phenomenon of lysozyme under 100 MPa pressure. Some experiments have revealed that protein crystallization can be inhibited by high pressure. For example, Groß and Jaenicke (1991) showed that lysozyme crystallization can be prevented by hydrostatic pressure, however, the experiment was

carried out under standard conditions of pH 4.7 and 0.8 M NaCl. The application of hydrostatic pressure might cause conformational or hydration changes which can affect the crystallization. These findings further provided a working model of how pressure can change protein self-assembly (Gross and Jaenicke 1994). Different pressures have been used to study the compressible regions in the structure of lysozyme, with the aid of crystallography. The crystallographic method applied by Kundrot and Richards (1987; 1988) was based on a comparison of crystal structures of lysozyme obtained at pressures of 0.1 MPa and at 100 MPa. The crystal structures of lysozyme contained 2 protein domains which were called the α- and β-domains. The crystallographic observations revealed that the β-domain in the lysozyme structure was effectively incompressible under pressure, while pressure altered the α-domain (Gross and Jaenicke 1994). However, in discussing such a phenomenon, the effect of water at high hydrostatic pressure should not be considered. Kharakoz and Sarvazyan (1993) believed that the effect of water molecules compressed into the protein structure would enhance further relaxational contributions in the adiabatic compressibility. This would cause overestimation of the internal compressibility and enlargement of the total protein volume which, therefore, would underrate the fundamental role of the crystallographic approach. The NMR measurement method of chemical alterations has revealed the structural changes in lysozyme (a globular protein) under pressure (3 to 200 MPa) (Refaee and others 2003). The interdomain and α-helical domain were compressed under pressure, but the β-sheet domain presented less compression with more structural deformation (Refaee and others 2003). The isothermal compressibility associated with variations in volume and, thereby, structural changes, occurs mainly around the region of hydrated cavities (Refaee and others 2003). Under normal pressure, water molecules inside the protein molecules have significant impact on conformational fluctuation and structural changes in nucleation sites, which leads to pressure denaturation or channel opening (Refaee and others 2003). Lysozyme was added in some food items as an antimicrobial agent, and the effect of this protein, under application of HHP, was investigated for inactivation of microorganisms. Different mechanisms of inactivation of microorganisms under HHP appear to be pH-dependent (Nakimbugwe and others 2006).

Some research indicates that HHP treatment provokes protein denaturation with partial coagulation of both liquid constituents of the eggs, which is linked to the severity of the pressure treatment (Andrassy and others 2006). Andrassy and others (2006) provided evidence of the pressure-dependent formation of insoluble aggregates in egg white at 250 MPa, with aggregation increasing gradually as pressure increased. The formation of protein aggregates or the denaturation of food proteins under pressure is not always a drawback. As discussed above, egg is a food matrix that can cause allergic reactions in children, and ovalbumin is a major protein in egg white which can cause allergic reactions (Matsuda and Nakamura 1993). The effect of processing techniques such as HHP on the structural modification of this protein and other allergens in egg may provide a solution for reducing the allergenicity of these types of components and improve security of the final product for consumption (Andrassy and others 2006).

Egg yolk proteins. The effect of HHP on purified egg proteins demonstrated different results based on the structure and composition of the proteins. Egg yolk proteins can be categorized into plasma (LDL, livetin) and granule (HDL, phosvitin) proteins, and the impact of HHP may be fraction-dependent. For example, Naderi and others (2017) showed that a 5 min, 600 MPa HHP treatment of granule from egg yolk caused disintegration of HDL-phosvitin linkages and separation of phosvitin.

Egg yolk plasma proteins. Low-density lipoproteins (LDLs) are specific proteins in the plasma fraction of egg yolk. These LDL proteins are the key contributors to the emulsification properties of yolk, and they form a film at the interface of oil and water. The heat sensitivity of LDL and the demand for microbial safety for egg yolk products has spurred investigations into denaturing treatment techniques other than heat (Speroni and others 2005). In this regard, HHP may prove to be a promising technology for eliminating microorganisms in yolk that also has less harmful physicochemical effects on LDLs (Speroni and others 2005). High-pressure techniques can modify the functionality and physicochemical characteristics of LDL dispersions. Different pressures (200, 400, and 600 MPa) were applied to LDLs at pH 3 and 8 at 20 °C. The solubility of LDLs was not altered by HHP treatment irrespective of the pH, whereas aggregation and protein denaturation were greatly increased at pH 8, indicating HHP combined with an alkaline pH decreased droplet flocculation of LDL dispersions (Speroni and others 2005).

Hen egg yolk is an excellent source of antibodies, including IgG-type immunoglobulin which is well-known as IgY. IgY is the main constituent of the livetin fraction of egg yolk plasma (Schade and others 2005). These antibodies can be separated from egg yolk using precipitation techniques (Shimizu and others 1994). Attempts have been made to use IgY antibodies as dietary supplements, but assessing the stability of IgY under processing and preservation conditions is a critical issue. Shimizu and others (1994) investigated the stability of IgY subjected to different HHP treatments (0 to700 MPa at 15, 40, 50, or 60 °C for 30 min). Pressures up to 300 MPa at 15 °C for 30 min did not adversely affect the antibody activity of IgY. However, higher temperatures and pressures (>500 MPa) reduced its antibody activity. In fact, IgY antibody showed good stability against pressure treatment (Shimizu and others 1994). These findings are of interest when considering using HHP for industrial-scale manufacturing of pharmaceutical-grade IgY from egg yolk. Using HHP to produce IgY from egg proteins would be a novel approach; therefore, it would be important to establish specific parameters for efficient industrial-scale production.

Egg yolk granule proteins. Phosvitin is a heavily phosphorylated glycoprotein found in yolk granules that has a specific amino acid composition (50% serine) with high iron-binding capacity (Castellani and others 2004). These characteristics make phosvitin a strong metal chelator with potential antioxidant properties. Changes in the iron-binding capacity of phosvitin were studied by applying 300 and 600 MPa pressure for 10 min. Even under extreme pressure conditions (600 MPa, 10 min), phosvitin did not aggregate and it retained its high iron-binding capacity. Castellani and others (2004) stated that the resistance of the phosvitin structure under pressure may be related to its unordered primary structure and high negative molecular charge, which prevent modifications to its potential for binding to iron.

The stability of phosvitin under high-pressure conditions makes it an interesting choice as an oxidation inhibitor in high-pressurized meat products (Jung and others 2013). Addition of phosvitin to cooked and raw ground beef delayed lipid and protein oxidation during HHP treatments (up to 600 MPa) and the color of the product also remained stable (Jung and others 2013). The combined application of HHP at 600 MPa and phosvitin addition improved the oxidation stability induced by HHP in minced chicken leg meat (Jung and others 2012).

Changes in egg enzymes due to the effects of HHP

It is evident now that high pressure causes structural rearrangements in proteins, and this is the main reason why enzymes can be inactivated under high-pressure conditions (Hendrickx and others 1998). High pressure also causes hydration changes in proteins, along with other noncovalent intramolecular interactions (Balny and others 1997). Covalent bonds remain mostly unaffected during pressure treatments, so proteins generally maintain their specific primary structure. To begin to design, optimize, and reproduce optimal preservation processes it is necessary to compile information about the kinetics of enzyme inactivation (Mújica-Paz and others 2011).

It is well documented that lysozyme (EC 3.2.1.17) in egg albumin hydrolyzes the β-1,4-glycosidic bond between the N-acetylmuramic acid and N-acetylglucosamine of peptidoglycan, which is the main constituent of Gram-positive bacterial cell walls (Tribst and others 2008). Lysozyme has been used as a generally recognized as safe (GRAS) antimicrobial agent that can be used as a food preservative due to its ability to inhibit spoilage and pathogenic bacteria (Gill and Holley 2000; Nattress and others 2001). Studies have shown that lysozyme activity is stable at pressures lower than 200 MPa, but higher pressures reduce its enzymatic activity, while its antimicrobial activity remains unaffected (Tribst and others 2008). It is still not clear why pressures above 200 MPa affect lysozyme activity (Eisenmenger and Reyes-De-Corcuera 2009). Applying lower pressure (100 MPa) can increase the antimicrobial activity of lysozyme. The improved hydrolyzing effect of lysozyme on the cell walls of microbes can subsequently facilitate the entry of enzymes through the damaged cell membrane (Iucci and others 2007). Pressures below 200 MPa, therefore, may facilitate the antimicrobial activity of lysozyme due to a synergistic or additive effect not attributable to improved enzymatic activity (Eisenmenger and Reyes-De-Corcuera 2009).

Degradation of egg vitamins under HHP

It is well documented in the literature that vitamins in food are irreversibly damaged during heat treatment (Noble and Gomez 1962; Sancho and others 1999). Water-soluble vitamins are particularly sensitive to physical factors such as temperature and light (Baldwin and others 1976; Jenkins and others 1989). Since high pressure treatment is a nonthermal process, it may be a better process for the preservation of sensitive nutrients such as vitamins (Rovere and others 1996).

Little work has been done to determine the effects of high pressure on the vitamins of egg/egg products. One study looked at the degradation of the water-soluble vitamins B1, B6, and C in a multivitamin model system made with egg yolk and strawberry *coulis* (sauce) during high-pressure and moderate-temperature processing (Sancho and others 1999). Sancho and others (1999) showed that none of the process parameters applied (200, 400, 600 MPa, 30 min) induced significant changes in vitamin retention in egg yolk after HHP treatment. The percentages of vitamin loss in egg yolk were small and never higher than 12%.

The effect of HHP processing on folate (vitamin B9) stability was examined in a buffer solution and it was reported that folate degradation occurred due to oxidation. At high temperatures, high pressure increases oxidation reactions (Oey and others 2006). Detailed kinetic studies on the stability of folate derivatives, such as 5-formyl and 5-methyltetrahydrofolic acid, under pressure and in buffer solution, have shown that different folate derivatives have different pressure and temperature stabilities. Folate degradation can be effectively enhanced by increasing pressure at constant temperature (> 40 °C) or by increasing the temperature at constant pressure (Indrawati and Hendrickx 2005; Oey and others 2006). The natural 5-methyltetrahydrofolic acid in egg yolk granule solutions was treated with HHP at 600 MPa for 5 min which revealed the stability of this folate form (Naderi and others 2017).

Applications of HHP in the Production of Bioactive Peptides from Hen Eggs

Various peptides with high biological activity can be released through enzymatic hydrolysis of food proteins. Bioactive peptides with angiotensin-converting enzyme (ACE)-inhibitory properties have received attention due to their potential to control blood pressure (Li and others 2004). The ACE-inhibitory peptides can be enzymatically liberated from some food proteins through gastrointestinal digestion or during food processing (Lopez-Fandino and others 2006). Peptides with ACE-inhibitory activity can be produced through hydrolysis of crude egg white with pepsin, trypsin, and chymotrypsin, and this activity derives mainly from the proteolysis of ovalbumin (Miguel and others 2004). Applications of HHP can improve the proteolytic digestibility of some proteins, due to conformational changes that make it more susceptible to proteolysis. Under high pressure conditions, the unfolded protein can expose novel cleavage sites for enzymatic hydrolysis (Bonomi and others 2003).

High pressure has also been used to liberate bioactive peptides from egg proteins. Quirós and others (2007) investigated the applicability of the HHP technique (100 to 400 MPa and 37 °C for 5 to 60 min) to change the proteolytic arrangement of ovalbumin from egg white and to determine if HHP processing had an impact on the liberation of peptides with ACE-inhibitory activity. Their results provided evidence that the pressurization of ovalbumin at pH 8.0 resulted in the development of dimerized protein aggregates, with an apparent MW of 90 kDa. Ovalbumin contains 4 free sulfhydryl groups with 1 disulfide bridge, all of which have increased reactivity as the result of denaturation. However, pressure-treated ovalbumin at lower pH (2.5) did not develop any protein polymers since sulfhydryl groups at acidic pH have low reactivity (Quirós and others 2007). The application of HHP in the range of 300 to 400 MPa for 60 min increased the susceptibility of ovalbumin to proteolysis by chymotrypsin and trypsin (Quirós and others 2007). Quirós and others showed that the proteolysis of ovalbumin under pressures of 200 to 400 MPa accelerated release of the peptides YAEERYPIL, FRADHPFL, and RADHPFL which have *in vivo* antihypertensive effects.

As stated earlier, ovalbumin in hen eggs can cause allergic reactions in infants. The application of HHP has been used as a novel technique to increase the enzymatic hydrolysis of ovalbumin and alter its immunoreactivity (López-Expósito and others 2008). Ovalbumin was proteolyzed with pepsin under high-pressure (400 MPa) and the resulting hydrolysates and peptides were identified. After treating ovalbumin with pepsin at 400 MPa, the entire protein was degraded within a few minutes to produce the hydrolysates. The pressure caused changes in the peptide structure, especially with pepsin at acidic pH. The researchers identified IgG- and IgE-binding properties in the resulting hydrolysates and they observed that the hydrolysates retained these binding properties due to aggregation of large hydrophobic peptides during the first steps of hydrolysis (López-Expósito and others 2008). The conformational changes to ovalbumin under pressure resulted in the production of new peptide targets, but only partially assisted the separation of allergenic epitopes (López-Expósito and others 2008).

In another study, researchers optimized the parameters of HHP combined with enzymatic hydrolysis (HHP-EH) processing (100 MPa, at 37 to 50 °C for 12 to 24 h) to increase the yield of phosvitin-phosphopeptides (PV-P). Phosvitin is the most phosphorylated protein in egg yolk, and it is primarily found in the granule fraction. The PV-P released from phosvitin is short phosphopeptides (<3 kDa) with high iron-chelating capacities that make them useful as antimicrobial agents. Recently, a possible correlation has been discovered between iron-binding capacity and antimicrobial activity of the PV-P fraction of phosvitin (<3 kDa) against bacteria causing tooth decay (Yoo 2016).

Challenges of Using HHP for Microbial Inactivation in Egg Products

Generally, HHP at room temperature inhibits the growth of vegetative cells as a function of the applied pressure and holding time of pressurization (Rendueles and others 2011). The resistance of microorganisms to HHP is extremely variable, depending mainly on the food matrix and the nature of organism (Gross and Jaenicke 1994). HHP usually has more adverse effects in organisms with increased structural complexity—prokaryotes tend to be more resistant compared to eukaryotes (Yuste and others 2001). Pressure damages the cytoplasmic membrane in vegetative bacterial cells, altering cell permeability and, in some cases, disrupting the membrane and cell wall. Elevated pressure can cause crystallization of membrane phospholipids, potentially leading to the destruction of numerous microorganisms (Cheftel 1995). Finally, inhibition of bacterial spores is quite low at pressures up to 1000 MPa, but pressurization induces spore germination (Gross and Jaenicke 1994; Cheftel 1995).

Egg product processors currently apply pasteurization techniques in order to eliminate the causes of food-borne illnesses associated with egg products. The required periods and temperatures for pasteurization of egg products are summarized in Table 2. Eggs or egg-containing foods have been identified as potential vehicles of transmission for food-borne illnesses. *Salmonella* spp., especially serovars Enteritidis and Typhimurium, cause most food-borne infections related to the use of eggs and egg products. To overcome this problem, researchers have introduced pasteurization techniques to destroy *Salmonella* in liquid egg products (Winter and others 1946; Swartzel and others 1990; Cunningham 1995).

Table 2–Required pasteurization conditions for egg products (provided by USDA).

Liquid egg products	Minimum temperature (°C)	Minimum holding time (min)
White (albumen)	56.7	3.5
	55.6	6.2
Whole egg	60	3.5
Blended whole egg (less than 2% added nonegg ingredient)	61.1	3.5
	60	6.2
Fortified whole egg and blends (24% to 38% solids, 2% to 12% added ingredients)	62.2	3.5
	61.1	6.2
Salted whole egg (2% or more salt)	63.3	3.5
	62.2	6.2
Sugared whole egg (2% or more sugar)	61.1	3.5
	60	6.2
Plain yolk	61.1	3.5
	60	6.2
Sugared yolk (2%)	63.3	3.5
	62.2	6.2
Salted yolk (2% to 12%)	63.3	3.5
	62.2	6.2

Common thermal treatments used by food processors to pasteurize liquid whole egg (such as 60 °C for 3.5 min in the U.S., or 64 °C for 2.5 min in the United Kingdom) guarantee the microbial safety of these food products (Monfort and others 2012). These pasteurization conditions have no significant effect on nutritional value, but they may significantly alter the functional properties (Punidadas and McKellar 1999). For this reason, egg product processors have restricted the pasteurization thermal treatment for liquid egg yolk to 60 to 68 °C for 3.5 to 4.5 min (Powrie and Nakai 1986; Kobayashi and others 1997; Anton and others 2001a, 2001b). These pasteurization conditions do not absolutely guarantee complete eradication of the microbial flora and thereby yolk and yolk containing products (such as emulsions made with egg yolk) have limited shelf-life. After pasteurization these products must be kept at 4 °C. Since egg yolk solutions are sensitive to thermal treatments, high pressure treatment is an approved and effective alternative to eliminate micro-organisms in food products (Anton and other 2001a, 2001b). To circumvent the limitation of conventional pasteurization, HHP has been applied as a nonthermal technique for egg pasteurization (Anton and others 2001a, 2001b; Pina-Pérez and others 2009; Monfort and others 2012). The application of N_2 gas at a pressure of 13.7 MPa and 35 °C for 2 h has also been studied; however, it was shown that the combined application of HHP and N_2 was not effective in killing bacteria added to egg yolk (Wei and others 1991).

Pressurization restricts the pH for the growth of microorganisms and has a restrictive effect on membrane ATP-ase, one of the central enzymes in the acid–base physiology of cells (Hoover and others 1989; Ross and others 2003). Monfor and others (2012) combined HHP (20 °C, 300 MPa/3 min) processing with thermal treatment (52 °C/3.5 min or 55 °C/2 min) and added 2% triethyl citrate to whole egg samples. The researchers found that the microbial safety of liquid whole egg was equal to the industrially treated (71°C/1.5 min) product, but its quality was comparable to fresh liquid egg. The addition of 2% triethyl citrate to whole egg yolk, combined with HHP processing at 20 or 4 °C, had a synergistic lethal effect and reduced the amounts of *E. coli* and of *L. innocua* in fresh liquid yolk (Monfort and others 2012). Monfort and others (2012) designed a combined application of HHP (300 MPa/3 min/20 °C) and heat treatment (52 °C/3.5 min or 55 °C/2 min) with the addition of 2% triethyl citrate in order to reach approximately 5 $\log_{10}$ reductions in the population of *E. coli* and *L. innocua* in liquid whole egg.

The destruction of *Salmonella enteritidis* inoculated into liquid whole egg (10^7 to 10^8cfu mL^{-1}) was investigated by using pressure treatments (350 and 450 MPa) combined with temperature (50, 20, 2, and −15 °C) and time (5, 10, 15 min and cycles of 5+5 min and 5+5+5 min). The inactivation rate increased with increasing pressure and time, and it was negligible at 350 MPa and −15 °C for 5 min (over 1 $\log_{10}$ of reduction). Complete inactivation (8 $\log_{10}$ of reduction) was observed in a number of treatments at 50°C (Ponce and others 1999). Another study used consecutive combinations of nisin (antibacterial peptide produced by *Lactococcus lactis* subsp. *lactis)* with HHP and ultrasound with high HHP to increase microbial (*Listeria seeligeri* and *E. coli*) inactivation in liquid whole egg product. The HHP processing conditions were either 250 MPa for 886 s or 300 MPa for 200 s at 5 °C, which have been recognized as the optimum HHP conditions based on microbial inactivation kinetics and coagulation of egg proteins (Lee and

others 2003). The combined applications of supplemented liquid whole egg with nisin (1.25 and 5 mg/L) and HHP treatment at 450 MPa also inactivated *E. coli* and *Listeria innocua* (Ponce and others 1998).

Generally, HHP changes the structure of proteins and polysaccharides and modifies the texture, physical appearance, and functionality of foods (Knorr 1993). However, maintaining the fresh natural properties of foods is also an important goal in food safety and preservation (Manvell 1997). The concept of minimal processing describes different preservation techniques that assure the safety of food products while preserving the original natural, fresh quality (Manvell 1997). The minimal processing technique is based on a hurdle concept (Leistner 2000), which involves the development of combined effects of different conventional preservation techniques. Based on the hurdle concept, combined conventional preservation techniques at lower individual severities produce combined antimicrobial results with fewer adverse effects on the nutritive characteristics of the food (Leistner 1992). Different studies have been conducted to define HHP boundary conditions and intensities when used alone or combined with different preservation methods to evaluate the lethal effectiveness of processing techniques. The pressure-assisted thermal processing (PATP) technique has been used to inactivate bacterial spores of *Bacillus stearothermophilus* in egg patties (Rajan and others 2006). The effect of PATP at 700 MPa and 105 °C was comparable to thermal treatment at 121 °C for destruction of *B. stearothermophilus* spores in egg products. About a 4 log reduction in 5 min was observed for PATP compared to a 1.5 log reduction in 15 min for the thermal treatment. The highest reduction of *E. coli* in liquid whole egg was obtained from the combined application of pressure and heat at 50 °C, and the organisms were more resistant to the combined effect of pressure and heat at 20 and –15 °C than at 50 and 2 °C (Ponce and others 1998). At lower pressures (such as 350 MPa) a cycling treatment was more efficient than a continuous treatment (Huang and others 2006). For the inactivation of *E. coli* the HHP treatment was applied at 400 MPa for 0 to 60 min and the *D* values were 14.1 min at 2 °C and 9.5 min at 20 °C (Huang and others 2006).

Kinetic studies on isothermal HHP (250 to 400 MPa) inactivation of *E. coli* in liquid whole egg were studied at 5 and 25 °C, and they produced the characteristic tailing inactivation curves of a first-order biphasic model (Lee and others 2001). HHP treatments (250 and 300 MPa) at 5 °C inactivated more *E. coli* than at 25 °C, and this was considered as effective as a traditional pasteurization technique (Lee and others 2001). Another study applied HHP using 2-2-4 min cyclic treatments at 138 MPa and 20 °C, which was effective in inactivating *S. enteritidis*. The effect of cyclic treatment was much greater than the single treatment (Huang and others 2006). The researchers used a combination of ultrasound and HHP treatments and this resulted in a significant mean log reduction of 3.23 log cycles of *S. enteritidis* (Huang and others 2006).

It has been demonstrated that microbial growth can also be inhibited by adding plant-based antimicrobial compounds (Pina-Pérez and others 2009). The combined effects of antimicrobials and HHP treatments were investigated to assess the possibility of reducing the pressure or the amount of antimicrobial compounds required to inactivate microorganisms (Ross and others 2003). Several natural antimicrobial ingredients have been considered safe, including cinnamon, anise, vanilla, and cocoa powder. These compounds have been examined as antimicrobial additives combined with HHP processing to inactivate *B. cereus* cells in a liquid whole egg (20% v/v) and skim milk (80% v/v) mixed beverage (Pina-Pérez and others 2009). The maximum inactivation level of *B. cereus* occurred at 300 MPa for 12 min, with and without antimicrobial additives. The microbial inactivation obtained in supplemented beverages was related to the synergistic effect of the hurdle technology (Pina-Pérez and others 2009). Hydrogen peroxide (H_2O_2) is another antimicrobial agent approved by the FDA and it has been used in egg products combined with HHP processing (Işiker and others 2003). The HHP treatment of 250 MPa for 5 min combined with 0.5% H_2O_2 in liquid whole egg reduced *S. enteritidis* by 7 to 8 log, similar to the liquid whole egg treated for 5 min at 450 MPa at 20 °C. The authors indicated that egg proteins were protected from coagulation by H_2O_2 which was added in the medium of egg samples before HHP treatments (Işiker and others 2003).

Structural and Functional Modifications of HHP-Treated Egg and Egg Products

Eggs are composed of different proteins with high biological, functional, and nutritional properties, and they are used in numerous food products (Lai and others 2010). Egg white (albumin) is composed of 9% proteins, 0.03% lipids, 0.9% carbohydrates, 0.05% ash, and 90% water (Awade 1996). Thermal treatments change the structural properties of ovalbumin or other egg white proteins, which directly affect the functional properties. Thermal treatment of egg white at 50 to 85 °C unfolds the proteins and exposes hydrophobic and sulfhydryl (SH) groups buried in the protein core (van der Plancken and others 2005). Heat treatment decreases protein solubility in egg white and this subsequently increases turbidity in egg white samples. This phenomenon is due to interactions between hydrophobic molecules caused by high pressure, which promotes disulfide (SS) bond formation through SH-SS exchange reactions and SH oxidation (van der Plancken and others 2005). Egg yolk contains 16% proteins, 32% lipids, 1% carbohydrates, 1% ash, and 50% water (Awade 1996). In egg yolk, different solids, such as the spheres, granules, LDLs, and myelin structures, are mixed with liquid plasma (Anton 2007). More than 68% of the yolk's dry matter is composed of LDL, plus 16% high-density lipoproteins (HDLs), 10% livetins, and 4% phosvitins (Anton 2007). LDL in egg yolk provides the characteristic emulsifying properties by creating a thin layer at the interface of oil and water phases (Anton 2006). The composition of this thin layer between oil and water droplets (structure, interactions, charge, and viscoelasticity) controls the physical stability of egg yolk emulsions (Speroni and others 2005). LDL forms globular molecules with phospholipids and apoproteins at the surface and a neutral lipid in the core. The reduction of triglycerides and cholesterol in LDL molecules does not change the emulsifying properties of yolk (Bringe and others 1996). Proteins (apoproteins) in LDL molecules play an important role in determining the yolk's emulsifying properties (Speroni and others 2005). The LDL in egg yolk is heat-sensitive. Heat treatment over 69 °C decreases protein solubility, thereby increasing the apparent viscosity (Anton and Gandemer 1997). Because of these significant heat-induced changes, demand is high for a suitable microbial stabilization technique for yolk solutions with less denaturing effect than thermal pasteurization.

The structural alterations of biomolecular complexes caused by high pressure treatments could change the functional properties of the complexes (Messens and others 1997). It is important to define specific parameters for pressure treatment based on specific

characteristics of egg components in food formulations to achieve the desired color, foaming, flow, and textural properties.

Alterations in proteins caused by treatments can modify the rheological characteristics of food systems (gels and emulsions). Therefore, it is important to estimate the flow properties of HHP-treated egg. After pressurization of egg yolk emulsions at 2 different pHs (3 and 7) and pressures (200 and 500 MPa), the viscosity of emulsions at pH 7 increased, while no changes were observed in emulsions at pH 3 (Anton and others 2001a).

Specific characteristics of globular proteins (such as compact hydrophobic core, surface area with low hydrophobicity, and ion pairs among subunits) (Perutz and Raidt 1975; Jaenicke 1991a 1991b, 1975b) make them vulnerable to changes in tertiary and quaternary structures under HHP treatment (Gross and Jaenicke 1994). The application of HHP at 500 MPa for 5 min induced protein denaturation with partial coagulation of both liquid components of the eggs (Andrassy and others 2006). Pressurization at 400 and 600 MPa applied to egg yolk and egg white resulted in the formation of stiff gels; however, the gels were more elastic and rigid than the heat-induced egg gels (Hayashi and others 1989; Okamoto and others 1990).

HHP may significantly modify the viscoelastic composition of egg yolk dispersions, generally at pH values near the isoelectric point of the proteins (5.5 to 5.8) (Aguilar and others 2007). These modifications begin with denaturation and hydrophobic aggregations that increase with increasing pressure. A pressure of 320 MPa can generate fluid-like behavior in egg yolk dispersions. Increasing the pressure to 420 MPa induces a gel-like transition (Aguilar and others 2007). Changes in the pressure-induced viscoelasticity of egg yolk dispersions were related to denaturation and disruption of LDL proteins (Moussa and others 2002). The hydrophobic interactions cause aggregation, and, based on the pressure level and the size of the aggregates, a thickening effect with linear viscoelastic properties may result in the association of 3-dimensional structures and the development of a complex elastic gel (Aguilar and others 2007).

Egg white protein has excellent foaming abilities as desired by the food industry. It is important to pasteurize an egg white product because of the possibility of contamination with pathogenic bacteria (including *Salmonella* spp.). Thermal pasteurization can induce protein denaturation and changes in the functional properties of proteins. HHP treatment, on the other hand, has the potential to secure microbial safety without inducing denaturation of egg white proteins. The effect of heat treatment (50 to 85 °C; 20 min) and high pressure treatment (400 to 700 MPa; 10 to 60 °C; 20 min) on the foaming properties of egg white proteins (10% v/v or 9.64 mg protein/mL) was determined at 2 pHs (pH 7.6, equivalent to the pH of fresh egg white and pH 8.8, equivalent to that of older egg white). Heating (50 to 85 °C) foams resulted in protein denaturation due to exposure of hydrophobic groups (buried in the protein core) and sulfhydryl (SH) groups. The pH of the medium strongly affected the structure of egg white foams. Heat and HHP treatment of egg white foams at pH 8.8 resulted in voluminous foams, whereas at pH 7.6 dense and very stable foams were formed. Foams prepared from HHP-treated egg white were moist and creamy, like the foams prepared by thermal treatment (65 °C). Extreme HHP treatments (such as 700 MPa) formed very sticky and dense foams (van der Plancken and others 2007).

Since HHP treatment of egg white results in better foaming characteristics compared to heat treatments (Iametti and others 1998; Iametti and others 1999), HHP was applied to the preparation of other egg products. Researchers have demonstrated that the use of HHP treatment for scrambled egg patties (a breakfast item) yields a high quality product (Juliano and others 2006a, 2006b; Knoerzer and others 2007). The application of pressure (675 to 700 MPa) with thermal treatment (30 to 121 °C, which is sterilization temperature) on breakfast items led to the development of scrambled egg patties with improved taste and shape, and a confirmed stable shelf-life of 6 mo (Juliano and others, 2006a, 2006b; Knoerzer and others 2007). The foaming property of egg white is related to ovalbumin, which is the egg protein most studied under HHP treatment. Any changes affecting the 4 SH groups in ovalbumin can alter its structure. HHP-treated albumin had higher viscosity, but it retained its foaming properties as the result of protein unfolding (Iametti and others 1999).

HHP at 400 to 980 MPa (30 min, 25 °C) was used to treat fresh egg white and yolk. The HHP-treated white and yolk formed gel-like structures (Hayashi and others 1989). Egg white and yolk formed a hard gel at or above 600 and 400 MPa. However, both gels were softer and more elastic than gels formed by thermal treatments. Pressure-induced gels did not have a cooked taste, and their vitamins were preserved (Hayashi and others 1989). HHP does not destroy small molecules; therefore, vitamins, flavor compounds, and pigments are preserved after HHP processing. Unlike thermal processing, HHP-treated food can preserve quality and nutritional value. Some compounds can irreversibly change with HHP treatments. Thermally processed hard-boiled egg is visually similar to pressure-treated egg, but pressure-treated egg tastes like raw egg (NFL 2013). HHP treatment did not induce flavor changes (chemical reactions) like the thermal treatment, creating new possibilities from a product development standpoint (NFL 2013).

Usually, HHP-treated egg white protein transforms into a gel due to changes in protein disulfide bonds that result in changes to egg white structure. Some studies have addressed the textural changes in egg yolk and white due to the effects of high pressure. Okamoto and others (1990) observed that egg yolk formed a very soft gel after being pressurized at 392.3 kg/cm^2, and fresh egg white became opaque and partially coagulated at 490 kg/cm^2 and created hard gels at pressures greater than 588.3 kg/cm^2. The researchers concluded that the gelation mechanism was different for pressure- and heat-induced gels. Pressure denaturation of protein occurs in response to a reduction of volume of the protein solution, whereas heat-induced denaturation of protein results from the vigorous movement of molecules that disrupts noncovalent bonds.

Andrassy and others (2006) used an electronic nose instrument to measure the volatile compounds produced during HHP treatment (400 MPa) in egg white. The quality points of pressure-treated (400 MPa) egg white samples were similar to the quality points of initial control samples and the off-odor of raw albumen was not observed.

Measuring the rheological, thermal, and functional values of HHP-treated whole liquid egg and egg yolk revealed that HHP produces a high viscosity in egg products, which is the result of denaturation and aggregation of egg proteins (Singh and others 2015). Pressure-induced changes in the proteins are reflected in the foaming and functional characteristics of pressurized egg (Singh and Ramaswamy 2015).

Investigations of changes in the structure of egg yolk lipids are limited. Egg yolk color is related to its carotenoid content, which is susceptible to oxidation via the same mechanism as lipid oxidation (Andrassy and others 2006). These authors studied the visible

absorption spectra of chloroform-methanol extracts of HHP-treated (500 MPa, 5 min) egg yolks. They showed that the carotenoid content of yolk did not change; however, lipid oxidation increased, even during storage (11 d). The concentration of the nonsaponifiable part of the lipid extract revealed only minor formation of 7a-hydroxycholesterol and 7b-hydroxycholesterol in the 500 MPa (5 min) pressurized egg yolk samples. These investigations demonstrated that HHP treatment denatured protein and partially coagulated both liquid components of the eggs, relative to the severity of the pressure treatment. The 500 MPa HHP-treated yolk (5 min) formed a stiff gel that was more elastic than heat-treated yolk.

HHP can induce modifications in the quaternary and tertiary structures of a protein, which leads to denaturation, aggregation, and gelation, and this subsequently enhances the texture and mouth-feel of the food product (Balny and Masson 1993; Cheftel and Culioli 1997; Singh and Ramaswamy 2015). A recent study examined the effect of HHP treatment on changes in color and texture of whole liquid egg, egg white, and egg yolk, through a factorial design using several pressure levels (600, 700, 800, and 900 MPa) and treatment times (0, 5, 10, and 15 min) (Singh and Ramaswamy 2013). The HHP treatment was controlled using a pilot-scale unit. The high pressure treatment changed the viscosity and color of the egg products from light yellow to yellow-orange by increasing the pressure level and treatment period. HHP greater than 600 MPa resulted in the development of solid gels for whole liquid egg, egg white, and egg yolk. Pressure-induced egg gels were soft and highly elastic, but by increasing the treatment intensity the resistance and consistencies of all egg constituents increased without changing the flavor of the egg samples. The strength of gels produced from egg white can be increased with increasing pressure between 400 and 800 MPa. The gels treated with 500 MPa for 20 min had a porous, aggregate network structure as demonstrated by Ngarize and others (2005).

Conclusion

The applicability of HHP for treating hen egg products over the last decade was investigated. The effects of high pressure treatment on the constituents and composition of egg fractions has been documented and the mechanisms behind some of these changes have been characterized. However, gaps still exist in assessing the potential use of high pressure techniques in commercial and industrial processing of egg products. It has been established that high pressure treatment can diminish the microbial load of egg products and induce denaturation of egg white proteins, but little information has been published on the use of HHP-treated egg yolk and the properties of egg yolk proteins. Thus, further basic research is needed.

Unlike high-temperature treatment, high hydrostatic processing does not lead to the Maillard reaction or modify covalent bonds, therefore, it does not destroy natural flavors or colorants (Gross and Jaenicke 1994). High pressure processes will certainly create new approaches for producing good-quality egg products. However, more research is needed to provide evidence for the benefits of high pressure techniques for preserving and processing eggs and egg products.

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