HIGH PRESSURE THERMAL STERILIZATION OF EGG PRODUCTS

By

PABLO JULIANO

A dissertation submitted in partial fulfillment of
the requirements for the degree of

DOCTOR OF PHILOSOPHY
(Engineering Science)

WASHINGTON STATE UNIVERSITY
Department of Biological Systems Engineering

MAY 2006

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of PABLO JULIANO find it satisfactory and recommend that it be accepted.

_____
Chair

_____

_____

_____

_____

ACKNOWLEDGMENTS

I would like to acknowledge the emotional, academic, and financial support provided by my advisor Dr. Gustavo V. Barbosa-Cánovas, as well as his guidance and encouragement since I started at Washington State University. My gratitude also goes to my Graduate Committee members Dr. Barry G. Swanson, Dr. Juming Tang, and Dr. Carter Clary, for their support and direction. Special thanks to Dr. Stephanie Clark for her many contributions and continuous support during this research work.

I also would like to thank those with whom I shared research time in this multidisciplinary project, mainly to: (a) Frank Younce, Dr. Biansheng Li, Dr. Mònica Toldrà, Mahamoudou Ouattara, Michael Costello, Dr. Kunchalee Luechapattanaporn, Subba Rao Gurram, Galina Mikhaylenko, Esteban Mejía, and all those who participated as sensory panelists at Washington State University, (b) Dr. Tatiana Koutchma and Ilona Setikaite at the National Center for Food Safety and Technology, (c) Dr. V. M. Balasubramaniam at The Ohio State University, (d) Dr. C. Patrick Dunne at the Natick Soldier Systems Center, (e) Jason Mathews and Sherry Lewis at Michael Foods Egg Product Company, (f) Dr. Ed Ting, Curtis Anderson, Dave Monserud, and Stephanie Armstrong at Avure Technologies, (g) Erin Larson and Eric Cudnohoske at ALCAN packaging, and (h) Dr. Glenn Froning at the American Egg Board. I am also very grateful to Sharon Himsl for revising and editing every chapter. My gratitude also goes to the Department of Biological Systems Engineering staff and faculty.

I gratefully appreciate the financial support provided by the Organization of American States (OAS) for my first two years of study in the US, as well as the Uruguayan government for

granting the scholarship and extending my period of stay to complete my doctoral studies. This project work was supported by the CORANET (Combat Rations Network, US Defense Logistics Agency) and Washington State University IMPACT (International Marketing Program for Agricultural Commodities and Trade) programs.

HIGH PRESSURE THERMAL STERILIZATION OF EGG PRODUCTS

Abstract

by Pablo Juliano, Ph.D.
Washington State University
May 2006

Chair: Gustavo V. Barbosa-Cánovas

High pressure low temperature processing technology is an industrial reality as witnessed by the production of a number of pasteurized and extended shelf life foods offered today in the world market. However, the development of shelf stable low-acid foods requires combining high hydrostatic pressure with high temperature to achieve commercial sterility. This dissertation includes a general overview of high pressure high temperature technology (HPHT), with special focus on the development of HPHT treated shelf stable egg based products.

Chapter 1 reviews the *state of the art* of the technology for the production of low-acid shelf stable products. It includes the latest on design requirements for HPHT processing equipment, microbial spore inactivation, and quality retention. In particular, this chapter provides are review on comparisons between HPHT treated and canned products in terms of processing time and quality degradation. HPHT process validation tools to demonstrate microbial efficacy and facilitate regulatory approval of novel HPHT treated products are also described.

A comprehensive feasibility study was conducted to evaluate HPHT treatment as an alternative to conventional thermal processing to stabilize egg-based products. This study was initiated

based on a US Army need to substitute retorted scrambled eggs, unappealing to military consumers, due to green color formation and off-flavor development after thermal processing. Chapters 2, 3, and 4 cover some of the outputs from this study on selected precooked scrambled egg patties focusing on HPHT process design and end product quality.

Chapter 2 presents results on quality evaluation of precooked egg patties after high pressure treatment at low and high temperatures. The importance of egg formulation modification with xanthan gum and flavors to maintain initial quality after high temperature pressurization is demonstrated. Chapter 3 analyzes selected methods for improving texture profile and water retention of scrambled egg patties after HPHT treatment. Among tested methods, addition of cheese particles into the egg mix, lower product porosity than the standard product, and low vacuum packaging contributed in different extents to improve texture and water retention in HPHT treated patties.

Chapter 4 evaluates consumer acceptability of commercial egg patties after HPHT treatment and in-pouch retort processing. Egg patties with added process cheese was the most acceptable formulation after treatment at 700 MPa/105ºC, maintaining most quality parameters after pressurization. Overall results demonstrated that thermal pressurization processing can be a promising alternative to conventional thermal processing for the development of novel shelf stable egg-based products.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

Dedication

To my wonderful family and friends.

GENERAL INTRODUCTION

High pressure sterilization of egg based products

Pablo Juliano

High Pressure Processing (HPP) is a postpackaging technology applied for safety assurance, shelf life extension, and nutrient preservation of prepackaged foods. A number of commercial products such as sliced meats, vegetable and salsa dips, fruit juices, seafood products, and ready-to-eat chilled products are available today in the worldwide market. HPP is known for its potential to manufacture novel value-added foods with retained heat labile nutrients, flavors, and aromas, from individual to institutional size packages (e.g., 6 kg sliced turkey breast package). The use of industrial scale HPP equipment has been suggested for stabilizing precooked egg based products, such as individual and family size "Spanish tortillas" (NC Hyperbaric, 2005), among other foods such as stabilized scrambled egg patties, egg-based sticks, and egg in pasta wraps or pockets (Ting, 2005). In fact, the production of refrigerated (extended shelf-life) and shelf-stable precooked egg patties is finding a new niche in the ready-to-eat meal market, especially as military/humanitarian rations and outdoor food items.

Manufacturing of acceptable commercially sterilized egg products using conventional thermal processing is not yet feasible, as retort processing yields undesirable flavors, greenish-black discoloration, and detrimental changes in texture and syneresis (Luechapattanaporn et al., 2005). In fact, the U.S. Army recently stopped the production of retorted scrambled eggs (net weight 2.7 kg) in plastic institutional trays due to the dissatisfaction found by military consumers with respect to the quality of this benchmark product (Dunne, 2005).

High Pressure High Temperature (HPHT) sterilization, or pressure assisted thermal processing (PATP), is emerging as an alternative for the development and production of low-acid shelf-stable food products. It is based on the combination of high pressures greater than 600 MPa and initial temperatures greater than 70ºC to eliminate vegetative and spore-forming microorganisms, and eventually inactivate spoilage enzymes, in a shorter time than conventional thermal processing (Matser et al., 2004). Microbial spore inactivation studies on scrambled egg patties demonstrated that instantaneous and volumetric temperature increase due to pressurization yields accelerated spore inactivation (Rajan et al., 2006a,b; Koutchma et al., 2005).

Efforts to demonstrate HPHT process feasibility in the development of shelf-stable egg-based products have been condensed into a project granted by the Combat Rations Network (CORANET, US Defense Logistics Agency) started in July 2003. This short-term project surged from a need identified among the US Army and Marine Corps for acceptable group or individual shelf-stable egg-based breakfast rations. Three academic institutions, Washington State University, The Ohio State University, and the National Center for Food Safety and Technology, put together a number of tasks to determine process feasibility in various grounds: (a) egg-based product and HPHT process development, (b) microbial challenge studies, (c) economical feasibility studies, and (d) development of a roadmap for regulatory filing. The project was carried out with the collaboration of partners from industry and government, namely, Michael Foods Egg Product Company, Avure Technologies, the US Army Soldier Systems Center (Natick), shelf-stable rations manufacturers, and the American Egg Board.

Part of the output of this project is contained in this dissertation, mainly focusing on quality and process design aspects. Chapter One portrays an overview of HPHT processing technology,

covering recent findings in terms of spore inactivation, regulatory status, potential products, quality studies, and HPHT system and process design. In particular, features needed for process time optimization and system modification for compression heat retention are also explained. Chapter Two focuses on evaluating the overall quality of egg based products after high pressure processing in combination with low and high temperatures. Special attention is given to appearance, flavor/aroma, and texture of both untreated and treated scrambled egg patty formulations using trained panelists. Textural problems identified in egg patties at high pressure high temperature conditions lead to Chapter Three, where methods for texture and water retention improvement are tested. Chapter Four concludes by evaluating consumer acceptability and accelerated shelf life studies of modified egg patty formulations.

This dissertation demonstrates the application of product and process characterization concepts for the identification and selection of egg-based products suitable for HPHT conditions. Approaches employed in product reformulation and process modification aim to ultimately obtain safe and shelf-stable products acceptable to consumers. These approaches can also be used as a baseline for product development and process design in other low-acid foods to be stabilized by combining high pressure and heat.

References

Dunne CP. 2005. U.S. Army Natick Soldier Center, Department of Defense. Personal communication. August 5.

Koutchma T, Guo B, Patazca E, Parisi B. 2005. High pressure – high temperature inactivation of Clostridium sporogenes spores: from kinetics to process verification. Journal of Food Process Engineering. 28(6): 610-629.

Matser AM, Krebbers B, van den Berg RW, and Bartels PV. 2004. Advantages of high pressure sterilization on quality of food products. Trends in Food Science and Technology, 15 (2): 79-85.

NC Hyperbaric. 2005. High pressure processing. Technology that makes sense. [Commercial CD]. Burgos, Spain.

Luechapattanaporn K, Wang Y, Wang J, Tang J, Hallberg LM, Dunne C.P. 2005. Sterilization of scrambled eggs in military polymeric trays by radio frequency energy. Journal of Food Science. 70(4):288-294.

Rajan S, Ahn J, Balasubramaniam VM, Yousef AE. 2006a. Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores in mashed egg patty mince. Journal of Food Protection. In press.

Rajan S, Pandrangi S, Balasubramaniam VM, Yousef AE. 2006b. Inactivation of Bacillus stearothermophilus spores in egg patties by pressure assisted thermal processing. LWT-Food Science and Technology. In press.

Ting E. 2005. Avure Technologies. Personal communication. December 5.

CHAPTER ONE

Food sterilization by combining high pressure and thermal energy

Pablo Juliano and Gustavo V. Barbosa-Cánovas

1. HIGH PRESSURE PROCESSING: AN INDUSTRIAL REALITY

High pressure processing (HPP) is an industrially tested technology that offers a more natural, environmentally friendly alternative for pasteurization or shelf life extension of a wide range of food products (Welti-Chanes et al., 2005). Commercial high pressure, low temperature methods achieve inactivation of vegetative microorganisms by subjecting vacuum-sealed food in flexible packaging to treatment at hydrostatic pressures of 600 MPa (or less) and initial temperatures lower than 40ºC for one to fifteen min depending upon the product and use. The use of lower temperatures has allowed better retention of sensory attributes characteristic of "fresh" or "just prepared," as well as food nutritional components (Cano and de Ancos, 2005). As a result, HPP has become a post packaging technology convenient for foods whose quality would otherwise be altered by heat pasteurization.

Among many advantages, HPP can add significant value to low cost or heat sensitive raw materials and other prepared foods. Furthermore, similar quality levels can be reached when processing large volumes or larger samples. Different from heat penetration, hydrostatic pressurization allows "instant" pressure transmission in fluids and semisolids within the pressure vessel, thereby achieving reduced product damage from lower temperatures. Moreover, HPP can

add significant shelf life to an existing refrigerated product (Hjelmqwist, 2005). In fact, it has the potential to deliver chemical- or additive-free products, with minimum impact on shelf life.

Like any other food preservation processes, HPP is product specific, making shelf life extension dependent on food composition, the presence of enzymes, and on the actual bacterial species/strains present in a given food factory. Another use of HPP is in texture modification of foods with high protein content. Modified dairy or egg-based ingredients of varied functionality, and tenderized meats, are some examples of the benefits observed in texture modification (Montero and Gómez-Guillén, 2005; Guamis et al., 2005).

The US Food and Drug Administration (FDA) and Department of Agriculture (USDA) have approved HPP as a post package pasteurization technology for manufacture of shelf stable high acid foods and pasteurized low-acid food products, and developed guidelines and regulations for those products (21CFR114 and 21CFR113). Furthermore, the European Commission on food regulations has adapted existing legislation on novel foods to products processed by HPP (EC258/97; European Commission, 2002; Barbosa-Cánovas et al., 2005a).

The term “pasteurization,” originally specifying the destruction of non-spore-forming foodborne pathogens by heat, has been extended to HPP and other validated pathogen-lethality technologies. The National Advisory Committee on Microbiological Criteria for Foods (NACMCF, an advisory group chartered by the USDA’s Food Safety and Inspection Service, the FDA, the Centers for Disease Control and Prevention, the National Marine Fisheries Service, and the Department of Defense Veterinary Service Activity) have jointly established this revised definition of pasteurization, providing specific considerations for a number of thermal (cooking,

microwave processing, ohmic/inductive heating, steam and hot water treatments) and nonthermal (HPP, UV radiation, pulsed electric fields, etc.) preservation processes (NACMCF, 2004; 2005).

Moreover, the FDA and USDA have promoted the implementation of Good Manufacturing Practices (GMP) as well as procedures for Hazard Analysis and Critical Control Point (HACCP) in HPP production facilities for refrigerated or pasteurized foods to ensure product safety. A food safety management standard has also been developed by the International Standardization Organization (ISO) that can be implemented when designing an HPP factory (Tapia et al., 2005; Surak, 2006).

High pressure processing is provides new opportunities for the food industry to develop new products for consumers. About 80 full scale HPP units in the world are run by 55 companies worldwide (Leadley, 2005; Hendricks, 2005). Most applications in Europe are in Italy, Spain, and Portugal, whereas others are spread around the United States, Mexico, and Japan. A number of companies are forming consortia to develop new products and new applications using high pressure technologies, such as low-acid food sterilization (de Heij et al., 2005). Among these companies are Kraft, Hormel, Unilever, Basic American Foods, Stork Food and Dairy Systems, Washington Farms, ConAgra, and Avomex.

HPP has demonstrated strong potential for the delivery of a wide range of high quality chilled products with extended shelf life. Among these, rare and cooked meats, fruits, vegetables, fresh herbs, and a variety of products prepared with these ingredients can be mentioned. Some of the products already commercialized in the worldwide market (Table 1) are fresh-like foods (e.g., new varieties of avocado products and juices) and ready-to-eat (or heat and serve) meat/meals.

The food industry has shown interest in using HPP technology for the development of modified dairy-based items (desserts, puddings), sauces and savory foods, whole muscle meats (partially precooked), and for the improvement of shucking efficiency and seafood safety.

HPP technology, as commercially defined today, is unable to produce low-acid shelf stable products, since bacterial spore inactivation requires high pressures of at least 800-1700 MPa at room temperature, far in excess of what is commercially feasible (Farkas and Hoover, 2000; Leadley, 2005). Even foods with pH lower than 4.5 require refrigerated storage and other preservation hurdles to prevent enzymatic degradation reactions and to inhibit spore germination. It was not until the early 1970s when studies on *Clostridium* species demonstrated the need to combine pressure and heat to achieve spore inactivation (Sale et al., 1970; Heinz and Knorr, 2001). Furthermore, inactivation data on *C. botulinum* spores date from the late 1990s (Rovere et al., 1998; Reddy et al., 1999; Heinz and Knorr, 2001), where pressures in the range of 690 to 900 MPa were combined with initial temperatures between 50 and 70ºC. The following section will provide an in depth description of the potential application of high pressure combined with heat in commercial sterilization.

2. HIGH PRESSURE THERMAL STERILIZATION

High pressure high temperature (HPHT) processing, or pressure-assisted thermal processing (PATP), involves the use of moderate initial chamber temperatures between 60 and 90ºC, which through internal compression heating at pressures of 600 MPa or greater, in-process temperatures can reach 90 to 130ºC. The process has been proposed as a high-temperature short-time process, where both pressure and compression heat contribute to the process's lethality (Leadley, 2005).

In this case, compression heat developed through pressurization allows instantaneous and volumetric temperature increase, which, in combination with high pressure, accelerates spore inactivation in low-acid media.

Several patents recently developed show a number of approaches for the attainment of commercial food sterility in selected low-acid foods (Meyer et al., 2000; Wilson and Baker, 2000; van Schepdael et al., 2002; März 2002; 2003; Wilson and Baker 2003; Cooper et al., 2004). Some of these microbial spore inactivation approaches proposed combining (de Heij et al., 2003; Leadley, 2005): (a) two low pressure pulses at 200-400 MPa (the first one for spore germination and the second for germinated cell inactivation); (b) a low pressure pulse at 200 to 400 MPa for spore germination followed by a thermal treatment at 70ºC for 30 min for vegetative cell inactivation; (c) package preheating above 75ºC and pressurization at 620 to 900 MPa for 1 to 20 min; and (d) package preheating above 70ºC, and applying two or more pulses at 400 to 900 MPa for 1 to 20 min.

Three of the above-mentioned approaches have proven inconvenient from either a microbiological or an economical perspective. When applying low pressures between 200 and 400 MPa, combined with moderate temperature [cases (a) and (b)], residual dormant spores have been detected after treatment (van Opstal et al., 2004; Leadley, 2005), making this option unlikely for a commercial process. Moreover, a high pressure multiple pulse approach [case (d)] is not recommended, as additional cycles decrease equipment lifetime and increase maintenance costs (de Heij et al., 2003). Hence, application of a single pulse above 600 MPa for 5 min or less [case (c)], combined with initial temperatures above 60ºC, would be more cost-effective and a safer approach for industrial purposes (de Heij et al., 2005). As will be explained later, success of

this processing approach depends on the efficient use of compression heat in achieving nearly adiabatic conditions.

2.1. Advantages of HPHT Processing

Recent publications claim that the main advantage of HPHT treatment is its shorter processing time compared to conventional thermal processing in eliminating spore-forming microorganisms (Fig. 1; Matser et al., 2004). This shorter process time and ultimate pressurization temperatures lower than 121ºC have resulted in higher quality and nutrient retention in selected products. For example, better retention of flavor components in fresh basil, firmness in green beans, color in carrots, spinach and tomato puree have been found after HPHT processing (Krebbers et al., 2002; Krebbers et al., 2003). Nutrients such as vitamins C and A have also shown higher retention after HPHT processing in comparison to retort methods (Matser et al., 2004). One more benefit of HPHT processing is its use to process non-pumpable foodstuffs like soups containing solid ingredients such as noodles, barley, and/or cut-up vegetables and meat (de Heij et al., 2005).

As mentioned earlier, high pressure low temperature processing provides direct product scale-up and higher efficiency for larger volumes of food, compared to thermal processing, due to "instant" hydrostatic pressure transmission. Similarly, HPHT processing is suitable for larger sizes as compression heating to high temperatures is instantly achieved throughout the entire package volume. Nevertheless, the preheating step, or the period of time necessary to reach initial product temperature before pressurization, needs to be considered when evaluating overall processing time. A large preheating time, especially in a large container, may lower product quality retention at the end of the HPHT process.

Although the HPHT process can be seen as advantageous due to its shorter time, lower processing temperatures cannot yet be assured for *C. botulinum* inactivation until optimal temperature/pressure/time combinations are identified. Section 1.5. will highlight some of the latest findings in terms of HPHT processing conditions required for *C. botulinum* and surrogate inactivation.

2.2. Potential HPHT Processed Foods

Production of shelf stable foods intended for outdoor, military, or humanitarian use have shown a tremendous increase since the late 1970s, when the concept of Meals Ready-to-Eat (MRE) packed in flexible plastic retort pouches was introduced (Mermelstein, 2001; Hirsch et al., 2005). However, the quality and nutrition challenges encountered in the development of certain foods have led to considering alternative manufacturing processes. There are a number of foods that cannot be turned into shelf stable products by means of retort processing due to the non-acceptable or low quality values obtained after long exposure to high heat. Nonetheless, some of these foods show potential for commercial sterilization using HPHT treatment.

Products stabilized using HPHT processing can be categorized as long life, chill stable, and shelf stable. The chill stable category includes meat snacks, vegetables, and ready-to-eat meals, or heat and serve meats among many products (Franceschini et al., 2005). Potential HPHT shelf stable products may include egg-based breakfast items, meat joints, pot roasts/stews, high quality soups, ready-to-drink teas/coffees, dairy desserts/smoothies, cheese/cream sauces, low-acid pasta sauces, high quality fruits/vegetables, and liquid flavors/herbs (Stewart, 2005). The quality, acceptability, and nutritional value of these products will not only depend on the developed

formulation, but also on the design of the process, i.e., the preheating equipment, the high pressure system, and the packaging material chosen.

3. DEVELOPING A HIGH PRESSURE HIGH TEMPERATURE SYSTEM

A high pressure system designed for commercial sterilization purposes must at least be able to withstand high pressures within the range of 600-800 MPa, chamber temperatures up to 98ºC, and retain product temperatures created during compression up to 130ºC. This can mainly be accomplished by building a pressure chamber of appropriate thickness, adapting an insulated polymeric liner with a sample carrier, and a pumping system that rapidly injects preheated compression fluid. Sections below will describe in more detail requirements for existing pressure systems working at HPHT conditions.

3.1. Available Equipment and System Requirements for HPHT Conditions

A typical batch high-pressure machine system is made of a thick wire wound cylindrical steel vessel with two end closures, a low pressure pump, an intensifier which uses liquid from the low pressure pump to generate high pressure process fluid for system compression, and necessary system controls and instrumentation (Farkas and Hoover, 2000). For HPHT processing (or pressures over 400 MPa), pressure vessels can be built with two or more concentric cylinders of high tensile strength steel. The outer cylinders compress the inner cylinders such that the wall of the pressure chamber is always under some residual compression at the design operating pressure. In some designs, cylinders and frame are prestressed by winding layer upon layer of wire under tension. The tension in the wire compresses the vessel cylinder so that the diameter is reduced (Hjelmqwist, 2005). This special arrangement allows an equipment lifetime of over

100,000 cycles at pressures of at least 680 MPa. Preferred practice is to design high pressure chambers with stainless steel food-contacting parts so that filtered (potable) water can be used as the isostatic compression fluid (Farkas and Hoover, 2000).

During pressurization at high temperature conditions, a temperature increase is produced in both the compression fluid and food (Ting et al., 2002). However, since compression heating in the system steel vessel is almost zero (Ting et al., 2002; de Heij et al. 2003), there is heat loss towards the chamber wall. In theory, heat generated by compression is dissipated by a combination of conduction and convection within the pressurizing fluid in the chamber and transfer of heat across the chamber wall into the surroundings (Carroll et al., 2003). Heat dissipation may cause cooling down of the sample during both come up and holding time, which may thereby decrease spore inactivation effectiveness (de Heij et al., 2002; Ardia et al., 2004). Thus, it is important to avoid heat loss through the chamber system.

A number of high pressure systems specified for high pressure (600-1000 MPa) and high temperature (130ºC) have been developed. There are several designs for vessel volumes ranging from micro/laboratory scale (0.02-2 L) to pilot scale (10-35 L). However, not all systems fulfill equal requirements in terms of pumping speed, compression heat retention, and type of compression fluid used (Balasubramaniam et al., 2004). In most cases, vessels are heated to initial temperature by means of an internal heater (jacket or coils), which also controls the temperature. However, this is not enough to retain compression heat generated during pressurization.

Modern systems are required to use several features for heat loss prevention by mainly: (a) adapting a dense polymeric insulating liner with a free moving piston at the bottom or valve to allow adequate pressure transmission, (b) preheating the inflowing pressurization fluid and pipes, and (c) preheating the vessel at a temperature higher than the initial fluid/sample temperature. Successful installation of these features can make the system close to adiabatic and, in this way, maximize preservation efficacy at chosen HPHT conditions.

High pressure vessels insulated from the interior by means of a cylindrical liner can prevent heat losses through the steel structure. In this case, a material with low thermal conductivity (less than 1 W/m/K) is required as part of the vessel design (de Heij et al., 2003). This product container (5 mm or more in wall thickness) can be made of polymeric materials such as dense polypropylene, polyoxymethylene, polyetheretherketone, or ultra-high-molecular-weight polyethylene to provide intended heat retention (de Heij et al., 2003). Compression heating rates of these materials have not been determined yet, however, empirical testing has proven their benefit for heat loss prevention. Fig. 2 shows the temperature profile of water in a white polypropylene liner (25 mm wall thickness) during pressurization in a 1.7 L vessel.

Some laboratory scale systems require using compression fluid mixtures such as food-approved oil or water containing FDA- and USDA-approved lubricants, and anti-corrosion agents. Water solutions of castor oil, silicone oil, and sodium benzoate are sometimes used as pressure-transmitting fluids (Ting et al., 2002). For HPHT processing of foods typical fluids used in pressure vessels include water with glycerol, edible oils, and water/edible oil emulsions (Meyer et al., 2000). High compression heating of oil-added compression fluids can be of aid for

additional compression heating retention. However, for commercial purposes the use of potable water is the most recommended compression medium.

Pressure vessel size also plays an important role in the compression heating retention (Ting et al., 2002), since larger size non-insulated vessels have been shown to retain more heat during holding time (Hartmann and Delgado, 2003). There are at least four 35 L pilot sterilization units in the world built, each designed by Avure Technologies (USA, The Netherlands, Italy, and Australia), and no machine has been designed yet for industrial use. The latest 35 L vessel design receives compression water from the intensifier after being passed through an ultra-high pressure heater to reach initial target temperature. Another heat retention aid is the addition of preheated water from a fill tank once the product carrier, which is inserted into a cylindrical polymeric liner, is placed inside the vessel to avoid residual air after chamber closure. A series of automatic controls recirculate water prior to starting pressurization to assure initial target temperature is reached in both the compression fluid and samples.

Ideally, a proper HPHT system requires thermocouples that provide reliable in-package temperature readings during the pressurization process. For this purpose, unpublished research has been performed by high pressure equipment manufacturers, who have screened thermocouples for types K (chromel/alumel), J (iron/constantan), and T (copper/constantan) in terms of accuracy, precision, and signal response, when exposed to HPHT conditions. Furthermore, systems that hermetically fix thermocouples inside the pouches are being tested. There are also pH measurement devices being developed to work under HPHT conditions.

The stage after pressure release is also an important part of the process. Assuming spore inactivation depends on preheating and pressurization steps, it is important to optimize the cooling phase in order to prevent overheating and to maximize quality retention. In this case, samples can immediately be removed and transported into a turbulent low temperature water bath at the end of the pressure cycle.

3.2. HPHT Processing and Critical Steps

A single pulse HPHT process involves six main process time intervals: (i) sample vacuum packaging and product loading, (ii) preheating to target temperature, (iii) product equilibration to initial temperature, (iv) product temperature increase to pressurization temperature by means of compression heating, (v) product temperature decrease during decompression, and (vi) product cooling to ambient temperature. Each of these steps (illustrated in Fig. 3) marks the temperature evolution of the process. However, reaching preheating target temperature inside the food, maintaining it up to the pressure pump starts, achieving constant target pressure, and retaining heat inside the product during pressure come up and holding time are all critical to achieving consistent product sterility.

When looking at the overall process flow chart (Fig. 3), two main control points can be distinguished as critical to safety: preheating and pressurization. As observed before, target pressurization temperature in all pouches inside the chamber or liner, as well as in all parts of the food, depends on the target preheating temperature before pressure is initiated. As a result, process variables must be controlled in different sections of the preheating/pressure system to assure that conditions required for spore inactivation are met.

3.2.1. Critical Factors

During an HPHT process, the amount of heat received is determined by three main conditions: target preheating/equilibration temperature, selected pressure, and pressure/temperature holding time. Furthermore, there are other inherent factors such as the presence of lower temperature sites in the vessel, pressure come up rate, pressure holding, decompression rate, and food/package properties. All need to be accounted for when evaluating sterilization performance.

Temperature Distribution

In terms of temperature distribution within a non-insulated system, "cold spots" are located close to the vessel wall. This may cause the product fraction located near the wall to cool down and not match the final temperature obtained at the center of the vessel (de Heij et al., 2002; Ting et al., 2002; Otero and Sanz, 2003; Ardia et al., 2004).

Another factor influencing vessel temperature distribution is the (preheated) liquid entering the vessel (2-30 mL, depending on vessel dimensions), which can cool down the transmission fluid located inside the vessel. Three-dimensional numerical simulations have illustrated temperature gradients created by incoming fluid at the entrance of the vessel (Hartmann et al., 2002).

In order to decrease cooling caused by the inflowing pressurizing fluid, the system can be modified according to the following (de Heij et al., 2003; Balasubramaniam et al., 2004): (a) an internal pressure intensifier can be incorporated to decrease the amount of liquid entering the machine; (b) the pressurizing fluid, the high-pressure pipes, and the external intensifier system in the high-pressure pump can be preheated to a higher initial temperature, and (c) insulation with a special liner can be added to prevent contact between packages and entering fluid. These

solutions can be executed according to (i) the pressure vessel dimensions, (ii) the initial temperature specified for the preheating system, (iii) the compression fluid used by the specific machine, and (iv) the intensifier system, including the incoming fluid. If all processing aids are simultaneously applied, temperature loss during come up and holding times can reach a minimum. However, up until now there has been no existing equipment that can guarantee a heat retention efficiency of 100%.

The difference between the temperature of the fluid/package system before and after high-pressure processing can indicate the extent of heat loss during processing (Ting et al., 2002). Thus, based on what was discussed above, the following parameters can be used to account for the overall process performance concerning product safety:

- Target preheating/equilibration temperature
- Target temperature at maximum (constant) pressure
- Temperature at the end of holding/pressurization time
- Temperature at the end of pressure release

Rates of preheating, compression (pressure come up time), and cooling can be associated with the process performance in terms of processing time, or time of exposure of the food package to high heat.

Pressure come up time

The pressure rise period has proven important concerning spore inactivation, since bacterial spores can be inactivated at zero holding time when combined with temperature (Koutchma et al., 2005). In this case, come up time, or compression rate, is determined by setting the power of

the low pressure pump (driving the intensifier) and the target process pressure (Farkas and Hoover, 2000; Balasubramaniam et al., 2004). For a specific process, variability in the compression rate must be determined and tolerance should be specified for the inactivation process. Moreover, effects on compression rate variation in spore inactivation are still unknown, and its determination could help establish a compression rate tolerance.

Constant Pressure

Maintaining a constant pressure during pressurization is also essential for compression heat retention. In modern high pressure systems, pressure intensifiers and automatic pressure control maintains pressure constant during the holding time. High pressure equipment allows controlling pressure within ± 0.5% (e.g., ± 3.4 MPa at 680 MPa) and recording it to the same level of accuracy (Farkas and Hoover, 2000).

Decompression Rate

It is unclear whether the decompression rate is critical for spore inactivation. However, from a process efficiency perspective, it would be desirable to achieve ambient pressure recovery in the shortest possible time. Control of decompression rate should be recommended based on the results of the effect of pressure release rate on spore inactivation, which is still unknown. Single stroke intensifiers may be used to control the decompression rate of a system (Farkas and Hoover, 2000).

Food Package-related Factors

Factors that are food-related include the package dimensions and weight, food molecular properties (pH, composition, water activity), and thermophysical/structural properties (volume,

shape, density/porosity, compressibility, specific heat, thermal expansion coefficient). Variation of these properties may affect temperature uniformity in the treated food. Uniform initial target temperature of the food sample is desirable to achieve a uniform temperature increase in a homogenous system during compression (Farkas and Hoover, 2000; Meyer et al., 2000). Non-homogeneities in terms of composition, presence of food pieces, or uneven preheating (e.g., from microwaves) may affect the temperature distribution during pressurization. Hence, it is important to analyze and quantify the effect of each factor on the overall process performance and to evaluate the most critical ones.

3.3. Compression Heating in HPHT Processing

In general, compression/decompression temperature and pressure curves are nearly linear and, therefore, compression rate can be assumed constant for a given time interval. Once target temperature is fixed, a constant compression rate should provide a constant compression heating. However, as indicated by the compression heating equation (Eq. 1), this will depend on how the volumetric expansion coefficient α_p (1/K), the density ρ (kg/m^3), and the isobaric heat capacity C_p (J/kg.K) of both the food and liquid will change during pressurization time (Carrol et al., 2003).

$$\frac{dT}{dP} = \frac{T\alpha_p}{\rho C_p} \tag{1}$$

where T is the temperature (K) of the food or compression fluid.

Both the temperature of the product and compression fluid may rise 20–40ºC during high-pressure treatment, whereas, as stated before, the steel pressure vessel is not subjected to significant compression heating (De Heij et al., 2002; Ting et al., 2002). As shown in Fig. 4,

several food components provide variable compression heating rates. Therefore, temperature increase may vary in foods with relatively complex composition. In fact, the compression heating rates of fats and oils can be up to three times higher than in water (Ting et al., 2002; Rasanayagam et al., 2003).

Not much data has been reported on the compression heating rate for food products at HPHT conditions. Balasubramaniam et al. (2004) reported compression heating of water at 4.0, 4.6, and 5.3°C/100 MPa and initial temperatures of 60, 75, and 90°C, respectively. However, Rasanayagam et al. (2003) observed little or no increase in compression heating in oils and fats due to higher initial temperature.

Eq. 1 has been applied using small pressure intervals of ΔP (around 10 MPa) to predict compression heating of water in the range of 0.1 up to 350 MPa and initial temperatures of 22 and 62°C (Otero et al., 2000). The authors reported the decompression cooling rate (rather than compression heating) after holding time, and good agreement was found with experimental data when using values for ρ, α, and C_p taken from literature (Ter Minassian et al., 1981).

Moreover, Ardia et al. (2004) predicted temperature rise in water, sugar solutions, and orange juice by comparing data from the National Institute of Standards and Technology (NIST; Harvey et al., 1996) and experimental measurements done in a high pressure machine. They found no significant deviations between NIST and experimental results for water obtained from the Multivessel Model U111 (Unipress, Warsaw, PL) for distilled water, even at sterilization conditions (initial liquid temperature of 80°C at 600 MPa).

For predictions in sugar solutions and orange juice, Eq. 1 was rewritten using α for water and implementing NIST formulations for the regressive calculation of each property (Eq. 2).

$$\Delta T = \int_{P_0}^{P_1} \frac{\alpha}{\rho_{mixture} \cdot C_{p\ mixture}} \cdot T \cdot dP \tag{2}$$

For sugar solutions, mixing rules (Eqs. 3 and 4) were used to express density and the specific heat of a pure solid/water mixture, $\rho_{mixture}$ and $C_{p\ mixture}$, as a function of temperature:

$$\rho_{mixture} = \left[\frac{[W]}{\rho_{water}} + \frac{[S]}{\rho_{solid}} \right]^{-1} = \left[\frac{[W]}{\rho_{water}} + \frac{[S]}{\left[\frac{1587.9}{1 + 0.000107(T - 15)} \right]} \right]^{-1} \tag{3}$$

$$C_{p\ mixture} = [W] \cdot C_{p\ water} + [S] \cdot C_{p\ solid} = [W] \cdot C_{p\ water} + [S] \cdot \xi \cdot (1622 + 7.125 \cdot T) \tag{4}$$

where [W] and [S] are the relative amounts of water and solid (in percentage). Since mixing rules equations are applicable at ambient pressure, an empirical correction factor was used to correctly fit the experimental results for $C_{p\ mixture}$:

$$\xi = \frac{C_{pwater}(T,\ 0.1\ MPa)}{C_{pwater}(T,\ p)^{0.75}} \tag{5}$$

where the numerator is represented by C_p of water calculated at atmospheric pressure and the denominator denotes C_p of water at higher pressure conditions.

Ardia et al. (2004) found no significant deviations for sugar solutions in the range of 0.1 to 600 MPa, while good continuity was found when extrapolating between 600 and 1400 MPa. When simulating the heat of compression in orange juice with a 9% solid content, no evident deviations from measured temperature were detected. Thus, the model produced satisfactory and reproducible compression temperature rise even at sterilization conditions. This means that the α, ρ, and C_p values of pure water determined from the NIST database were also accurate for predicting the increase in temperature, even at sterilization conditions (Eq. 2).

3.4. Packaging for HPHT Processing

Packaging materials selected for shelf stable products must meet a number of requirements in terms of seal strength, overall integrity, and barrier properties to oxygen and water vapor. However, little is known about the effect of HPHT conditions on polymeric laminates. Recent studies have found that aluminum foil laminated retort pouches designed to withstand a temperature of 121°C may delaminate and blister after a thermal pressure process; particularly at chamber temperatures >90°C and >200 MPa (Schauwecker et al., 2002; Caner et al., 2004).

Delamination during pressurization has been mainly observed in areas in contact with trapped air (undergoing higher compression heating) and in folded parts (suffering higher localized stress at hydrostatic compression). Some researchers claim that HPHT conditions decompose portions of the adhesive layer causing other layers (e.g., polyethylene and foil laminate in retort pouches) to separate. Research on several laminates to identify packaging materials suitable for HPHT

conditions is ongoing. Special attention is being given to the effect of HPHT treatment on packaging components and the contribution of air retained in packages (or headspace) to delamination.

Another aspect to consider about a packaging material is its behavior as a barrier to heat and pressure transfer. During preheating and cooling steps, the container material constitutes an intermediate barrier in the transfer of heat from the heating medium into the product and from the product to the cooling medium. The thickness of the material, as well as its thermal diffusivity, can affect the rate of heat conduction to (or from) the product during preheating (or cooling). During the pressurization stage, packaging material such as polypropylene has been shown to act as an insulating barrier, preventing heat loss from the product (Hartmann and Delgado, 2003). Other than the laminate composition and thickness, the headspace left inside the package as well as internal stresses from the vacuum created are additional factors to consider in the transfer of pressure and heat.

4. PREHEATING STEP: DESIGN AND QUALITY OPTIMIZATION

Correct performance of the preheating step is critical to ensure the temperature of the food product matches the target initial temperature. As already mentioned, a uniform initial target temperature throughout the food sample is desirable to achieve a uniform temperature increase in a homogenous system during compression (Farkas and Hoover, 2000; Meyer et al., 2000). If "cold spots" are present within the food, some areas in the product will not reach the target process temperature during pressurization, thereby posing a safety risk in those areas with lower

temperature (Meyer et al., 2000). However, an extended preheating time can also affect the quality characteristics of the food product, so in order to maximize quality retention, it is desirable to minimize the duration of preheating needed to reach the target initial temperature (Barbosa-Cánovas et al., 2005b; Juliano et al., 2006).

4.1. Preheating Design for Optimal Quality Retention

Several alternatives have been proposed to save preheating time, for example, the use of still water baths set at temperatures higher than the target temperature, and other heat transfer aids such as steam, steam injection in water, water circulation pump systems, or dielectric heating (Hoogland et al., 2001; Juliano et al., 2006a). However, care must be taken when adopting faster preheating methods, as they can affect initial temperature distribution within the food package. Since faster preheating methods provide less uniformity, they require a longer time for equilibration to achieve temperature homogeneity.

In practice, carrier operation time can be reduced by using a two-stage preheating approach, i.e., by having samples preheated at a lower temperature in a separate vessel. In this case, the sample should undergo two equilibration steps: (1) equilibrating in a still water bath at a lower temperature (e.g., 60ºC), (2) placing samples in a carrier, preheating up to target temperature, and equilibrating at target temperature. An alternative approach in step 2 is to preheat the food to a temperature greater than the target temperature. This will shorten equilibration time and assure all parts of the food reach the initial temperature (Barbosa-Cánovas et al., 2005b). In both cases, two-stage preheating could result in improved productivity, especially in larger packages. Choosing this approach will depend on product composition and its sensitivity to long time exposure to moderate temperatures. As will be discussed further, the dimensions and thermal

properties of the test sample and vessel determine equilibrium time (step 2) inside the chamber (Balasubramaniam et al., 2004).

4.2. Factors Affecting Preheating

Factors related to package geometry, product characteristics, and heating system can affect preheat time (NFPA, 1985) and are organized in Table 2 for each element in the preheating system.

In thermal processing, pouches usually have a slab geometry design to achieve a more rapid heat penetration due its thin profile and high surface area to volume ratio (NFPA, 1985). Therefore, container thickness can be considered the main factor affecting heating rate. If containers are placed in hot water, the water vapor released and residual gas retained inside may expand the container, altering its thickness. In retort processing, thickness is controlled with overpressure, which also ensures seal integrity. In a HPHT process, internal product temperatures can be as high as 90ºC in preheating systems, in which case, package thickness can be controlled by other physical means. For example, polymeric racks, grid trays, and cassettes can be specially designed to hold the container in place, avoiding sample mobility and maintaining pouch thickness.

In laboratory scale HPHT processes, samples are generally preheated in selected baths in a carrier and immediately transferred inside the liner for product equilibration. This liner can be later located inside the high pressure machine for subsequent pressurization. Another option, currently used in pilot scale vessels, is to directly perform preheating inside the liner, while having samples stacked in the carrier. Liners should include steam/air injection and circulation

pumps for stirring of the heating media. If higher preheating fluid temperature is used, the liner system needs a means of decreasing fluid temperature at the end of preheating near to the target product temperature (e.g., from 100 to 80ºC). Temperature decrease of the preheating fluid inside the liner will avoid temperature overshooting during product equilibration and pressurization steps while the liner is located inside the pressure chamber. One alternative would be to partially remove the heating fluid and mix it with water preheated at initial pressurization temperature. In this case, highly controlled automatic devices that regulate the inflow/outflow of fluid until the system reaches initial target pressurization temperature need to be designed.

In order to ensure proper temperature exposure to all packages in the system, the racking system should be designed in a way that water circulation is parallel to the container length or width. Furthermore, the separation between each container layer should be calculated to permit water/steam/air mixture circulation and assure temperature uniformity. Given the importance of this critical step, a reference temperature device, similar to the mercury-in-glass thermometer used in retorts, should be added in the system.

Other factors related to the container geometry, e.g., fill weight, container size, and product characteristics (Table 2), need to be identified when adapting a preheating system to a particular product and processing. Container geometry is defined by the package shape and distribution of the food and its particulates, which can affect preheating rate. As previously mentioned, heating rate is also a function of the food's physical and chemical properties, and variation of these properties mainly depends on product formulation. If the product is a semisolid, minor variations in formulation might not significantly change the rate of heat penetration. Moreover, heat transfer rate can be associated with the thermal diffusivities of the package and food components

of the semisolid (Palazoglu, 2006). On the other hand, foods containing sufficient free liquid to promote convection may change heat transfer drastically according to their composition (e.g., due to added starch or other thickening agents), thereby changing heat penetration rate.

4.3. Heat Penetration Evaluation

Evaluation of heat penetration during preheating is important for comparative purposes, particularly when characterizing a system modified for faster temperature rise, during scale-up studies using multiple pouch stacks, or when testing larger size products. Primarily, an adequate temperature login system must be installed to provide reliable temperature profiles used to determine preheating efficiency. Plastic stuffing boxes can help fixing the thermocouples at the center of the pouch (or at the slowest heating position) for accurate determination of heat penetration profiles. Other thermocouple positioning devices shown in guidelines from the US National Food Processors Association can also be adapted (NFPA, 1985).

Heat penetration can be evaluated by using the heating rate index f_h and heating lag factor j_h (Holdsworth, 1997). The heating rate index f_h and heating lag factor j_h can be determined by using Eqs. 6 and 7 (Holdsworth, 1997):

$$\log u = \log\left(\frac{T_R - T}{T_R - T_0}\right) = -\frac{t}{f_h} + \log j \tag{6}$$

where u is the reduced temperature, T is the temperature at the geometric center of the package, T_0 is the initial product temperature, T_R is the reference temperature of the heating medium, and j is the extrapolated lag factor. The corrected heating lag factor j_h can be determined from the 58%

come up time (t_{58}), which corresponds to an additional 42% of come up time needed to reach the target temperature.

$$j_h = 10^{-\frac{t_{58}}{f_h} + \log j} \tag{7}$$

The lag factor j_h is related to the lag time needed to reach uniform heating rate values. The accuracy of heat penetration determination depends, among other factors, on the headspace inside the pouch, as well as the internal vapor pressure created due to increased temperature, which can influence the j_h value due to convective currents in contact with the product surface. Accuracy of temperature readings may also be affected by the type of thermocouples used, thermocouple entry system into the package, and the use of connectors and extension wires (NFPA, 1985).

When comparing different preheating methods, the heat transfer coefficient can provide additional information on preheating efficiency. It can be calculated either by empirical correlations between dimensionless numbers (e.g., Nussell and Reynolds number) or by fitting simplified Fourier balances to experimental temperature histories. The instant heat transfer coefficient h can be determined by solving the following overall heat balance equation (Varga and Oliveira, 2000):

$$\rho C_p V \frac{dT^t_{ave}}{dt} = h_t A (T^t_{\infty} - T^t_{surf}) \tag{8}$$

where T_∞, T_{surf}, and T_{ave} are the heating medium and product surface and volume average temperatures, respectively. Since this expression defines an instant value, the superscript and subscript *t* indicate that these variables are a function of time. Varga and Oliveira (2000) showed that more reliable results can be obtained from the derivatives of average heat transfer coefficient $\overline{h}_t$, instead of calculating h_t values for short time steps in the preheating process. The average external heat transfer coefficient $\overline{h}_t$ between zero and time *t* was defined as:

$$\overline{h}_t = \frac{\int_{t_0}^{t} h_t dt}{t - t_0} \tag{9}$$

where t_0 is the initial time for the preheating stage (depending on whether a one or two-stage preheating is considered). Varga and Oliveira (2000) offered two solutions to determine the average external heat transfer coefficient from t_0 to several values of *t* considered: (a) integrating the heat balance (Eq. 8) or (b) using the residual sum of the square method to minimize the difference between experimental and model temperatures. A detailed description of this methodology escapes the scope of this manuscript and can be found in the mentioned reference.

5. MICROBIAL ENGINEERING AND REGULATORY IMPLICATIONS

Bacterial spore inactivation depends on pressure applied and initial temperature of the food and vessel. Most thermal baro-resistant spores are inactivated at pressures 600 MPa or greater, in combination with initial temperature above 60ºC. However, relatively low pressures (below 200 MPa) can trigger spore germination (Patterson, 2005; Leadley, 2005). Furthermore, studies on

target microorganisms for inactivation and related safety assurance of canned food products, such as for *Clostridium botulinum,* have shown large variation in the pressure resistance of different spore strains (Margosch et al., 2004; Margosch, 2005; Gola and Rovere, 2005).

5.1. Inactivation of *C. botulinum* Strains by HPHT Processing

Until now, the resistance to pressure and temperature has been studied with at least five *C. botulinum* strains in varied conditions and media (Reddy et al., 1999; Reddy et al., 2003; Margosch et al., 2004; Gola and Rovere, 2005). *C. botulinum* spores type E in pH 7 buffer, for example, have been reduced 4.5 logs at 50°C/758 MPa/5 min and 5 logs at 40°C/827 MPa/10 min (Reddy et al., 1999). Furthermore, *C. botulinum* type A was reduced by more than 3 log units in pH 7 buffer and crab meat following treatment at 75ºC/827 MPa/20 min (Reddy et al., 2003). Other studies on several *C. botulinum* strains (types A, B, F proteolytic, B non-proteolytic) in mashed carrots were carried out by Margosch et al. (2004) and Margosch (2005). They found that treatment at 80°C/600 MPa/1 s reduced strains by more than 5.5 log cycles to none at all. In particular, non-proteolytic *C. botulinum* type B was the least resistant strain and was reduced by more than 5.5 log cycles after 80ºC/600 MPa/1 s. In comparison, proteolytic *C. botulinum* type A had more than 5 log reductions after 80ºC/600 MPa/12 min treatment, and proteolytic *C. botulinum* type B spores were inactivated by less than three orders of magnitude at 80ºC/600 MPa/60 min.

Since spore resistance also proved to be product dependent, more research is needed to find the optimum inactivation pressure/temperature/time conditions for *C. botulinum* strains in particular products, as the type of medium may influence spore germination rates (Margosch, 2005).

Moreover, understanding how HPHT conditions affect bacterial neurotoxins produced is worth consideration (Margosch et al., 2005).

5.2. Inactivation of Microbial Spore Surrogates by HPHT Processing

Regarding other spore-forming microorganisms, microbial studies have proven that an initial chamber/product temperature of 75-85ºC and pressure 600-827 MPa can effectively inactivate target heat resistant spore-forming bacteria commonly used as indicators of food safety and shelf stability (Heinz and Knorr, 2001). In particular, *Bacillus stearothermophilus* spores were reduced 5 log cycles in phosphate buffer and beef broth at 70ºC/700 MPa/3 min (Gola et al., 1996; Rovere et al., 1998), and at least 4.5 log cycles in meat balls in tomato puree at 90ºC/700 MPa/30 s (Krebbers et al., 2003). Furthermore, *B. stearothermophilus* spores were inactivated in egg patties at 700 MPa and 105°C (Rajan et al., 2006a; Rajan et al., 2006b; Koutchma et al., 2005). Koutchma et al. (2005) also showed that 700 MPa/105°C/4 min was sufficient to destroy 6 logs of *B. stearothermophilus* in spore strips in egg patties, whereas 6 logs of *C. sporogenes* PA 3679 at 700 MPa/110°C/5 min were destroyed in the same media.

Other spore genii such as *Bacillus licheniformis* spores suspended in pH 7.0 buffer were also inactivated at 60ºC/600 MPa/20 min (Taki et al., 1991), and reduced 6 logs in pH 7 buffer and beef broth at 70ºC/700 MPa/5 min (Gola et al., 1996; Rovere et al., 1998). *Bacillus cereus* spores were also reduced 8 logs in pH 7 buffer at 60ºC/690 MPa/1 min with previous sporulation at 37ºC (Raso et al., 1998), and 5 logs in beef broth at 70ºC/700 MPa/5 min (Rovere et al., 1998). Similarly, *Bacillus subtillis* spores were inactivated at 827 MPa and a process temperature ranging from 102 to 107ºC, yielding up to a 6 log reduction (Balasubramaniam and Balasubramaniam, 2003).

Moreover, *B. amyloliquefaciens* was higher in HPHT resistance than *C. botulinum* strains (Margosch, 2005), becoming a potential surrogate for future research in HPHT sterilization (Margosch et al., 2004). Ahn et al. (2005) obtained up to 7-8 log reductions with *B. amyloliquefaciens* after treatment at 700 MPa and 121°C for less than 1 min.

It is worth mentioning that data reported by several authors on spore inactivation were not obtained at the same conditions due to heat loss experienced in the chamber using with machines of different sizes, the compression fluids, and heat retention configurations (de Heij et al., 2002; Balasubramaniam and Balasubramaniam, 2003; Ardia et al., 2004; Balasubramaniam et al., 2004). Thus, in many cases, data may not be comparable. Previous research has proven by means of numerical simulation the difference in inactivation reduction of *Alicyclobacillus acidoterrestris* spores at 50ºC/800MPa (Ardia et al., 2004) and *B. stearothermophilus* (de Heij et al., 2002) at 700 MPa/121ºC/2-90s pulses between the center and inner side-wall of pressure vessel. Particularly, Ardia et al. (2004) predicted that a difference of 3–4ºC between these two crucial points resulted in a difference of approximately 6 log reductions between the center and inner side-wall of a polyethylene container located inside the pressure vessel. De Heij et al. (2002) predicted a 15ºC difference between the center and vessel side-wall, which roughly gave a difference of 5 log cycles.

5.3. Regulatory Perspective

During the last five years, high pressure sterilization initiatives have been started by two consortia, one in the US and one in Europe, offering a new technology that will rival or complement conventional canning. Although 21CFR113 was not intended for pressure-processed

low-acid vegetables and seafood, it is likely to be applicable since heat is included during compression (Sizer et al., 2002). Other parts in the US Code of Federal Regulations (9CFR318.300 and 9CFR381.300) applicable to the sterilization of food products containing 3 percent or more raw (2 percent cooked) meat or poultry could also be used for this technology.

A standard scenario for commercial sterilization using high pressure processing can be defined as the combination of 700 MPa and a final process temperature of 121ºC (initial temperature 90ºC) with a holding time of 3 min. In this case, an F_0 value of approximately 3 min (corresponding to a 12D reduction of *C. botulinum* spores) is obtained by only accounting for the thermal component and neglecting the pressure effect on microbial inactivation (Sizer et al., 2002). This standard scenario would allow filing HPHT treatment as a thermal process according to 21CFR113, as a first approach, avoiding the use of large volumes of microbial inactivation data and special kinetic models to account for the combined effect of temperature and pressure. On the other hand, various researchers are gathering safety data on microbial inactivation, as well as process data, to model the kinetics of *C. botulinum* inactivation that will allow identifying process performance criteria for process validation and process filing with regulatory agencies such as the FDA.

5.3.1. FSO Concept in High Pressure Sterilization

The concept of Food Safety Objective (FSO) has been introduced by the Codex Committee on Food Hygiene (Codex, 2004) as the "maximum frequency or concentration of a hazard in a food at the time of consumption that provides or contributes to the appropriate level of protection (ALOP)" (NACMFS, 2005). This concept is also emerging as a regulatory parameter for

evaluating the efficacy of novel technologies to inactivate target pathogenic microorganisms. An inactivation performance criterion can be expressed as follows (NACMFS, 2005):

$$H_0 - \Sigma R + \Sigma I \leq FSO \text{ (or PO)} \quad (10)$$

where FSO is the food safety objective, PO is the performance objective, H_0 is the initial level of the hazard, ΣR is the total (cumulative) reduction of hazard on a $\log_{10}$ scale, and ΣI is the total (cumulative) increase of hazard on a $\log_{10}$ scale.

Alternatively, the Performance Objective (PO), or "the maximum frequency and/or concentration of a hazard in a food at a specified step in the food chain prior to consumption that provides or contributes to an FSO or ALOP, as applicable" (Codex, 2004), can be used in Eq. 10 to establish process performance. In the context of risk analysis, an FSO would be based on a public health goal that provides an ALOP or "reasonable certainty of no harm." Since the most resistant microorganism of public health significance in HPHT sterilization of low-acid foods is *C. botulinum*, an FSO could be defined as the achievement of a <100 cfu *C. botulinum*/g in a food at the point of consumption.

5.4. Process Performance Criteria Accounting for Temperature History and Inactivation Kinetics

As mentioned before, the 21CFR113 primarily stipulates minimum temperature requirements for commercially sterilizing low acid foods. However, inactivation of *C. botulinum* has not been validated for thermally pressurized foods, not only due to the lack of inactivation data for several strains, but also because the microbial performance or process outcome criterion has not yet been

established for comparison purposes (Koutchma et al., 2005). This process performance criterion should include inactivation kinetic parameters of *C. botulinum*, and other parameters that account for the pressure and temperature profile during the established HPHT sterilization process. The following paragraphs will describe approaches to determining parameters that simultaneously account for uniformity and the sterility of a HPHT process and that can therefore be applied in process validation.

5.4.1. Conventional Thermal Processing Approach

Koutchma et al. (2005) showed that the HPHT process may be validated by applying concepts such as decimal reduction time (D_T, D_P) and temperature sensitivity (Z_T, Z_P) traditionally used in conventional thermal processing of low-acid foods. The authors based their research on evidence reported on the linearity of microbial inactivation curves (semilog scale) of classical surrogates, namely *B. stearothermophilus* and *C. sporogenes* PA 3679, at a pressure range of 600-800 MPa and process temperature range of 91-108°C. They found that thermal sensitivity of PA 3679 spores (Z_P values) did not vary with pressure, nor did pressure sensitivity (Z_T values) vary with temperature at these ranges. They were able to calculate F_0 values for the HPHT sterilization process by adapting a concept established by Pflug (1987) to calculate the process lethality, which included the initial microbial load. Nevertheless, research is ongoing since Z values of *C. botulinum* spores at a reference sterilization temperature 121°C are necessary for quantifying the additional contribution of pressure in a HPHT process in terms of lethality and overall thermal death time. In order to separate the pressure effect, care must be taken when comparing an HPHT process with a conventional thermal process, since processing factors (i.e., package geometry, food media, temperature measurement, etc.) should be reproducible at only-thermal and thermal pressurization conditions. Furthermore, F_0 values accounting for the thermal components should

coincide in both processes. Due to the asymmetric reduced temperature semilog scale profile obtained in a HPHT process, the General Method (Holdsworth, 1997) could most accurately determine F_0 values from temperature profiles in commercial software packages.

5.4.2. Weibullian Approach

Peleg et al. (2005) identified a theory to redefine the concept of "thermal death time" for non-isothermal heat treatments like HPHT processing that was an alternative to the F_0 value concept, by describing *C. botulinum* inactivation kinetics using a power law or "Weibullian" model with temperature dependent parameters:

$$\log\left[\frac{N(t)}{N_0}\right] = -b(T)\,t^{n(T)} \tag{11}$$

where N_0 is the initial number of microorganisms and $N(t)$ is the number of surviving microorganisms at time t. The parameters $b(T)$ and $n(T)$ are a function of the applied temperature. A similar model can be used to describe the HPHT process using model parameters, b and n, as functions of both pressure and temperature (Campanella and Peleg, 2001). Thus, the combined effect of pressure and temperature of the process can be accounted for in this expression:

$$\log\left[\frac{N(t)}{N_0}\right] = -b(P,T)\,t^{n(P,T)} \tag{12}$$

The relationship between parameters *b* and *n* with pressure and temperature can be determined from experimental survival curves obtained at combined pressures and temperatures. In order to know the effects of pressure and temperature on parameters *b* and *n*, the temperature [i.e., T(t)] and pressure [i.e., P(t)] histories must be known. Thus, an analytical heat transfer model to express T(t) and P(t) needs to be incorporated into Eq. 12. Once parameters *b(t)* and *n(t)* are known, inactivation of microorganisms at selected pressure and temperature can be predicted. Peleg et al. (2003) and Peleg et al. (2005) proposed a methodology to estimate survival parameters *b* and *n* for conditions of variable pressure and temperature. However, as said before, no sufficient *C. botulinum* data exists that validates the model. Once data is collected for a tolerable number of *C. botulinum* strains, these parameters could eventually be used to validate an established HPHT sterilization process.

The advantage of this approach is that parameters will depend on the actual thermal pressurization history; therefore, more accurate conditions to reach sterilization can be defined to establish a given HPHT process. This approach allows process optimization to minimize over processing, and increases the chances of yielding higher quality foods with increased nutritional content (Peleg et al., 2005).

5.4.3. Alternative Performance Criteria

Design of thermal process operations requires the use of heat transfer models to determine process parameters that account for temperature in terms of its distribution within the food or equipment during treatment (Nicolaï et al., 2001). This concept can be extended to build models that not only account for temperature distribution, but also for microbial survivor distribution. Numerical heat transfer models that incorporate inactivation constants as a function of pressure

and temperature have been proposed to account for the extent of combined temperature and pressure inside the pressure vessel (Denys et al., 2000 Hartmann and Delgado, 2003).

For instance, the temporal and spatial distribution of activity of *Bacillus subtillis* α-amylase (Hartmann and Delgado, 2003) and cfu-concentration of *E. coli* (Hartmann et al., 2003) was described with the first order inactivation kinetics equation in terms of continuum mechanics and scalar transport (Ludikhuyze et al., 1997):

$$\frac{\partial A}{\partial t} + u\frac{\partial A}{\partial x} + v\frac{\partial A}{\partial y} + w\frac{\partial A}{\partial z} = -K(P,T)A \tag{13}$$

where A is the relative activity (actual activity related to the initial activity, varying between 100% with values close to zero), $K(P,T)$ is the inactivation rate constant, and u, w, and v are the components of the fluid velocity vector in the x-, y-, and, z-directions, respectively. Velocity vectors were taken from thermal and fluid dynamic conservation equations of mass, momentum, and energy (Hartmann and Delgado, 2003). Eq. 13 represents the coupling between the activity A and the flow field (i.e., the velocity of the vessel fluid and fluid inside packages) and the coupling of A and the temperature distribution. In this way, the pressure-temperature-time profiles, calculated through the model, were integrated through a numerical scheme and the activity retention could be evaluated at any point in time and vessel space.

This model allowed obtaining inactivation distribution per package located at specified volumetric regions in the vessel. For this purpose, a volume-weighted averaging can be carried out for each package and arithmetic averaging of all packages can provide an idea of global

inactivation effectiveness (Hartmann and Delgado, 2003). Furthermore, a process uniformity parameter, Λ, has been defined in terms of the average relative activity retention, i.e.,

$$\Lambda = \frac{A_{ave\_\min}}{A_{ave\_\max}} \tag{14}$$

where A_{ave_min} is the minimum average activity retention and A_{ave_max} is the maximum average activity retention (Hartmann and Delgado, 2003).

A similar inactivation distribution analysis can be performed by means of thermal and fluid dynamic models that include inactivation kinetic equations for pressure/temperature resistant spore-forming bacteria such as *C. botulinum*. In this case, microorganisms should be assumed as a transport quantity varying over space and time (Hartmann et al., 2003). This would allow calculations of temperature uniformity during HPHT sterilization processes, of special use in scale-up studies.

A few works have already integrated thermodynamic, heat transfer, and spore inactivation kinetic models to predict the effect of temperature evolution at selected points in the pressure vessel on spore inactivation (de Heij et al., 2002; Ardia et al., 2004). A mathematical model integrating thermodynamics and inactivation kinetics of *Bacillus stearothermophilus* was built to predict temperature distribution in the high-pressure vessel (de Heij et al., 2002). In this case, an axi-symmetric one-dimensional finite element model based on heat conduction was developed, which predicted temperature at the vessel wall and at the center. A first order kinetic constant *k*

was found from spore inactivation data and fit into a modified Arrehnius-Eyring equation (Eq. 15), expressed as follows as a function of pressure and temperature:

$$k = k_{ref} e^{\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)} - V_a\left(P - P_{ref}\right) \tag{15}$$

where volume V_a is the activation volume, E_a is the activation energy, and R is the universal gas constant (8.314 J/mol/K). Although this contribution does not clearly specify results in terms of microbial reduction, as already mentioned, the model could predict temperature profiles at selected vessel points and illustrate the lower log cycle reduction achieved near the vessel walls after a two-cycle 700 MPa/121ºC/90s process.

Similarly, Ardia et al. (2004) modeled compression heating using a finite element method, also based on heat conduction in radial coordinates. The model, based on Eq. 1, included dT/dP as a time dependent heat source during the compression or de-compression phase. The numerical routine implemented NIST formulations (Harvey et al., 1996) for regressive calculation of the thermal expansion coefficient α, density ρ, and specific heat C_p. To account for temperature increase due to compression, a microbial inactivation model was included into the finite difference scheme, yielding the degree of inactivation (log cycle reductions) for any radial position. In this case, the time dependent inactivation was mathematically expressed assuming n^{th} order inactivation kinetics:

$$\frac{dN}{dt} = -k \cdot N^n \tag{16}$$

The inactivation rate constant was then expressed as a function of pressure and temperature using the Arrhenius-Eyring equation:

$$\ln(k) = \frac{1}{RT}\left(E_a - \Delta V^* P\right) \tag{17}$$

where the activation volume ΔV^* is the characteristic parameter for the pressure dependence of the rate constant. This modeling approach predicted the inactivation of *Alicyclobacillus acidoterrestris* spores at 50ºC/ 800 MPa in geometrically defined locations, finding (as mentioned before) a difference of 6 log cycles inactivation at the center of the pressure chamber and closer to the chamber walls (due to a 3-4ºC difference at the end of pressurization).

5.5. Synergistic Approach

A number of foods composed of heat-labile components, relevant in terms of product quality and nutrition, can be affected by HPHT conditions necessary for sterilization (Matser et al., 2004; van Loey et al., 1998). In this case, additional synergies can be tried by using food additives and preservatives, such as bacteriocins (inactivation by membrane pore formation), surfactants like sucrose esthers (affecting both proteins and biomembrane structure and function), acidulants (providing specificity in antimicrobial inactivation), humectants, chelating agents, and others.

Kalchayanand et al. (2003) showed that, at moderate pressure, bacterial spores can be induced to germinate and outgrow, at which stage they can be killed by bacteriocin-based biopreservatives. In this study, inactivation of *Clostridium laramie* spores or a mixture of 4 clostridial spores (*C. sporogenes*, *C. perfringens*, *C. tertium*, and *C. laramie*) in roast beef with added preservative mixtures (pediocin, nisin, lysozyme, Na-EDTA, and BPy) were subjected to treatment at

60°C/345 MPa/5 min. It was found that a combination of HP with bacteriocins extended the shelf life of inoculated roast beef up to 7 days at 25°C and up to 84 days at 4°C. Stewart et al. (2000) showed that *B. subtilis* and *C. sporogenes* ATCC 7958 were especially sensitive to nisin addition. A marked synergistic decrease in spore count was detected with pressurization at 250-300 MPa combined with 45°C and pH less than or equal to 6.0 in the presence of sucrose laureate fatty esther.

Moreover, Shearer et al. (2000) investigated the addition of sucrose laurate and combined treatment at 45°C/392 MPa/10-15 min, finding 3-5.5 log reductions for *B. subtilis* in milk, *B. cereus* in beef, *B. coagulans* in tomato juice (pH 4.5), *Alicyclobacillus* sp. in tomato juice (pH 4.5), and *Alicyclobacillus* sp. in apple juice. In this study, sucrose laurate appeared to be inhibitory rather than lethal to spores. However, its application to food as an extra hurdle may allow the use of lower temperatures in combination with pressure.

6. QUALITY OF SELECTED HPHT PROCESSED FOODS

Even though extensive research has been done on bacterial spore inactivation, quality validation studies of low-acid foods after HPHT treatment have been barely performed. Furthermore, no consumer data on HPHT processed products have yet been reported, nor have there been comparisons with retort processing on consumer acceptability. Information on appearance, texture, and flavor/aroma mostly exists in terms of analytical evaluations of HPHT treated products such as broccoli juice, green beans, tomato puree, and meat sauce (van Loey et al., 1998; Rovere et al., 2000; Krebbers et al., 2003; Matser et al., 2004). Indeed, a number of high pressure, high temperature (HPHT) treated low-acid foods such as meat, milk, and vegetable

products showed more desirable texture, color, and flavor/aroma retention in comparison to retorted products and, in some cases, to frozen products (Hoogland et al., 2001; Krebbers et al., 2002; Krebbers et al., 2003; Matser et al., 2004).

Color, it has been shown, can be retained in selected vegetables (Matser et al., 2004). For instance, spinach has been found to retain higher color intensity than conventionally sterilized spinach after two pulses at 90ºC/700 MPa/30s. Furthermore, tomato puree treated at 90ºC/700 MPa/30s also retained color, whereas color degradation was greater after conventional retort treatment. This data was supported by a sensory panel that found higher color acceptability in tomato puree treated at HPHT conditions, in comparison to retorted samples. Lycopene content was maintained in this case, while a lower content was found after retort treatment (Krebbers et al., 2003). Moreover, Juliano et al. (2006b) indicated that HPHT treatment at 70ºC/700 MPa/5 min can maintain color and appearance of selected scrambled egg patty formulations (as evaluated by trained panel).

Regarding texture, firmness of HPTS treated green beans after 75ºC/1000 MPa/80s was much higher than those conventionally retorted, dried, or frozen (Krebbers et al., 2002). HP treated tomato puree (90ºC/700 MPa/30s/121ºC) displayed lower serum separation and higher viscosity than retorted product. Previous studies have also identified that utilization of lower pressurization temperatures can significantly improve the texture and water retention of scrambled egg patties (Juliano et al., 2006b). In fact, previous studies on high pressure formation of gels from whole liquid eggs, egg white, egg yolk, and egg yolk/white (Ma et al., 2001; Ahmed et al., 2003; Lee et al., 1999) have shown that pressures greater than 600 MPa not only increase apparent viscosity, but also provide instantaneous gelation of egg yolk and egg white.

Flavor components in fresh basil were best retained after HPHT processing (two pulses of 85ºC/700 MPa) in comparison to freezing, conventional retorting, and drying. Egg flavor retention has also been reported in high pressure high temperature treated egg patties at 700 MPa/105ºC/5 min (Juliano et al., 2006b).

6.1. Shelf Stable Egg-Based Products Processed by HPHT: A Case Study

Manufacturing of acceptable shelf stable egg products using conventional thermal processing remains a challenge, as retort processing yields undesirable flavors, greenish-grey discoloration, and detrimental changes in texture and syneresis (Baliga, et al., 1969; Wesley et al., 1982; Luechapattanaporn et al., 2005). In fact, the US Army recently stopped the production of retorted scrambled eggs in plastic institutional trays due to the dissatisfaction found by military consumers with respect to the quality of this benchmark product (Dunne, 2005).

At present, Washington State University (WSU), in conjunction with The Ohio State University (OSU) and the National Center for Food Safety and Technology (NCFST) has been directing a short-term Combat Rations Network (CORANET, US Defense Logistics Agency) project for the development of shelf stable egg products using HPHT processing. The project, run under the guidance of the US Natick Soldier Systems Center, has been part of a series of efforts carried out to identify processing alternatives for the manufacture of acceptable shelf-stable egg-based products. Alternatives include the use of high temperature short time retort processing, radiofrequency heating, freeze-drying, and refractance windows drying. The HPHT processing project had the active collaboration of experts from industry in various fields, including the high pressure equipment design team from Avure Technologies, the egg product development team

from Michael Foods Egg Products Company, the high pressure processing packaging development team from ALCAN, and military ration developers from Wornick, Ameriqual, and Sopakco.

Among existing precooked egg products, scrambled egg patties were identified as an adequate product for high pressure processing, especially during HPHT processing, due to their semisolid homogeneous structure (Juliano et al., 2006b). During the first phase of this project, several efforts have been directed in terms of formulation development, microbial challenge studies, identification of adequate processing conditions, as well as packaging studies.

The team focused on identifying the effects of HPHT conditions on commercial and modified egg patty formulations. Two processing strategies were initially proposed for egg-based product commercial sterilization and *C. botulinum* inactivation: (a) standard thermal sterilization treatment at process temperature 121ºC accelerated by the application of 700 MPa with holding time at 3 min, and (b) thermal HPHT treatment using lower temperature than in (a) with increased holding time. Option (a) was considered with the purpose of facilitating FDA approval as a thermal process. In both cases, microbial challenge studies have helped in the selection of processing conditions. Furthermore, inactivation kinetics studies, incubation tests, packaging identification, and compression heating studies gave a deeper understanding of the process. Results of these endeavors, after iterative steps, are highlighted in the following sections.

6.1.1. Egg Patty Formulation Identification for HPHT Conditions

Michael Foods has played a key role in the development and production of 6 egg patty formulations that were supplied to WSU, NCFST, and OSU for testing. Trained and consumer

sensory panels at WSU, and additional physical tests (texture profile analysis, quantification of syneresis, and instrumental color measurements) have helped identify a formulation suitable in flavor, aroma, and appearance.

A six-member descriptive panel trained at WSU analyzed a basic formulation (whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dried milk, and citric acid) and other formulations with added water, cheddar cheese particles, xanthan gum, EDTA, and flavors. Formulations with added xanthan gum, EDTA, and flavors, treated at 700 MPa/105ºC/5 min, showed higher tones of butter flavor as well as lower tones of rancid, unclean and retort flavor than the basic formula after pressure (Juliano et al., 2006b).

Texture was initially identified as one of the most challenging problems in the quality of the egg products after pressurization at process temperatures greater than 100ºC (Juliano et al., 2006b). It was found that the addition of xanthan gum and water significantly decreased the hardness of high pressure treated patties by 30-55% after 700 MPa/105ºC/5 min (Juliano et al., 2006a). Furthermore, addition of xanthan gum reduced the water loss (or syneresis) after pressure.

Results of a 40-member consumer panel verified previous studies on the effects of process temperature on product quality after HPHT processing. It was found that decreasing the high pressure process temperature from 121 to 105ºC at 700 MPa improved the overall acceptability of HPHT treated formulations with added xanthan gum. A formulation containing 20% Cheddar cheese and treated at 105-110ºC and 700 MPa obtained ratings similar to the untreated egg patty controls (Juliano et al., 2005).

Testing in the DUST 35 L machine (QUINTUS Food Autoclave Type 35L-600, Avure Technologies, Kent, WA) showed that texture can be improved by using lower vacuum packaging conditions, whereas texture of patty was not affected when processing in smaller 1.5 L chambers (Quintus Food Processor-6, Flow Autoclave systems, Columbus, OH) and 1.7 L chambers (Engineered Pressure Systems, Inc., model #914-100, Haverhill, MA). Furthermore, the three centers (WSU, NCFST, OSU) studied the effect of HPHT processing on the pH of the egg basic formulation and formulation with added xanthan gum, detecting no significant changes after processing.

6.1.2. Preheating Studies

By working with different preheating systems, trials were done to reduce preheating times and to minimize decreased quality and excessive use of heat due to long preheating periods (Juliano et al., 2006a). Comparisons were made between preheating with an electrical heater, a water kettle with steam jacket, a water kettle with steam injection, or microwaves, showing that microwaves and steam injection elevated patty temperatures up to 75ºC the fastest. Considering microwave heating showed significant temperature gradients within the patties (proven by means of infrared imaging), direct steam injection showed to be advantageous in reducing preheating time. One other effect found was that the type of packaging material influenced preheating times. Aluminum foil-based laminates provided better penetration rates than non-foil ones.

6.1.3. Compression Heating of Egg-Based Products

Determination of compression heating properties of egg mixtures gave no significant differences with water. The compression heating factor of egg patties ranged from 3.3ºC/100 MPa to 4.8ºC/100 MPa at the initial process temperatures of 25ºC and 80ºC, respectively. Therefore, no

temperature gradients between the compression fluid and the egg patties are expected due to different compression heating.

6.1.4. Microbial Challenge Studies

Microbial inactivation studies were carried out by NCFST and OSU. Inactivation of *B. stearothermophilus* was studied after different stages in the process: (a) baking of egg mix to form patty, (b) after preheating, (c) after HPHT processing. *B. stearothermophilus* spore inoculated in the egg mix showed a one log cycle reduction after baking. After treatment at 700 MPa/105ºC inactivation of *B. stearothermophilus* (ATCC 7953) spores in egg patties was accelerated (Rajan et al., 2006b). The resistance of *B. stearothermophilus*, given by its D-values, also proved to be much lower when using pressure. Inactivation of *B. stearothermophilus* in spore strips located between two egg patties can be reduced by at least 6 log cycles at 688 MPa/ 105ºC/5 minutes (Koutchma et al., 2005). However, *Clostridium sporogenes* (PA 3679) bioindicator spores were more resistant than *Bacillus stearothermophilus* and needed a process temperature of at least 110ºC.

6.1.5. Identification of Packaging Materials for HPHT Processing

Throughout the first phase, project partners worked with different packaging companies to identify suitable individual flexible pouches (clear and foil laminates). Selected plastic and foil-laminated pouches from Kapak, Pyramid, ALCAN, and Smurfit-Stone manufacturers were screened for their ability to withstand HPHT and retort treatments. Overall packaging integrity, oxygen permeability (determined from a Mocon-Oxtran unit), and seal strength (determined from tensile tests using an Instron texture analyzer) were evaluated before and after treatments at

NCFST facilities. In addition, pouches were tested for delamination, flex damage, or other treatment- related anomalies.

It was proven that foil laminates from Pyramid and Smurfit-stone (48 ga. polyethylene/ adhesive/ 0.0005" Aluminum foil/adhesive/ 4 mL polyolefin) retained their barriers during steam injection preheating and HPHT treatment. However, a statistically significant loss of barrier was found in clear plastic pouches from Pyramid without Aluminum foil. Seal strength of all packaging materials was not significantly affected by HPHT and HP low-temperature treatments, while retort at 121ºC decreased seal strength. Furthermore, WSU, in partnership with ALCAN Packaging, identified a pouch made of coextruded laminates that provided almost no blistering and low oxygen permeability after pressure under a worst-case scenario condition (700 MPa/121ºC/3 min). ALCAN packaging material composition was a 60 ga. biaxial nylon/adhesive/5.0 mL ethylene vinyl alcohol (EVOH) coextruded sealant.

6.1.6. Incubation Tests – Shelf Stability Studies

Egg patties (basic formulation) were treated in WSU's 1.7 L machine at pressures between 200 and 700 MPa, with holding time of 5 minutes and initial temperature of 90ºC for storage testing. Treated and untreated packages were stored at room temperature and 37ºC for one year. Results indicated that at pressures above 400 MPa no pouches showed production of gas or decomposition. HPHT treated patties stored at room temperature for one year maintained its initial color, hardness, and aroma. Moreover, other non-inoculated formulations tested in the NCFST/Avure 35 L pilot machine also remained stable (no gas formation) for six months stored at 37ºC after 700 MPa/105ºC treatment.

Another set of samples were inoculated with *B. stearothermophilus* spores ($7.5x10^6$ spore/g) at OSU laboratories and treated at 700 MPa/105ºC/5 min. The product remained stable after at least two months storage at 37ºC.

7. FINAL REMARKS

The concept of combining high hydrostatic pressure and heat to commercially sterilize low-acid foods emerged in the early 1970s and is scaling up from the laboratory bench to the pilot plant. At least four pilot 35 L high pressure vessels located around the world are being used today as part of various industrial/government consortia projects to identify the benefits of HPHT processing for several products. Patents have been published proposing different approaches, among which the application of a single pressure pulse of 600 MPa or greater, combined with temperature between 90 and 130ºC, seems most appropriate from a food safety and economic point of view. This approach defines a high temperature short time sterilization process, which has proven provides improved flavor, texture, color, and nutrient retention in selected food components, in comparison to retort.

At this stage of development, HPHT technology can be claimed advantageous for its shorter processing time. However, lower processing temperatures than 121ºC cannot yet assure sterilization. For this purpose, additional microbial inactivation data on many *C. botulinum* strains as well as surrogate spore-forming microorganisms of higher resistance (to be identified) are greatly needed. Hence, based on the current knowledge, regulatory approval can only be obtained by filing this technology as a thermal process, following the guidelines established in the 9CFR318.300, 9CFR381.300, and 21CFR113.

Once *C. botulinum* inactivation data on several strains is gathered (together with process data) kinetic models can be developed for HPHT conditions. In fact, microbial kinetic models in combination with heat transfer models could be used to express overall process performance in terms of energy use. Overall parameters obtained for these models would completely account for (or be directly related to) individual performance parameters such as preheating rate, pressure come up time, target preheating/equilibration temperature, target temperature at maximum pressure, temperature at the end of holding/pressurization time, and temperature at the end of pressure release.

Synergistic approaches through the addition of natural antimicrobial preservatives such as bacteriocins can help reduce the HPHT conditions needed to reach sterilization, providing opportunities for the development of products with more heat labile components. The FSO concept can help establish optimal conditions and ingredient addition for sterilization from a regulatory standpoint.

Attainment of optimal sterilization conditions is also related to the efficient use of compression heat developed during pressurization. Equipment modification with heat retention aids such as insulating polymeric liner at chamber walls, preheated pressurization fluids, and an internal pressure intensifier to decrease the amount of inflowing pressurization fluid, can yield more uniform temperature distribution across the chamber volume. If a nearly adiabatic state is achieved inside the liner, pressure holding times may be decreased as temperature will be uniform, even near the steel chamber walls.

The preheating step has been identified as a critical control point in the overall process since it determines the achievement of the target pressurization temperature in all food packages. A number of factors intervening in the preheating step have been listed, among which the preheating method used and package geometry seem the most relevant. Furthermore, the equilibration step after preheating is important to assure temperature homogeneity inside the food packages before pressure come up time. From a quality perspective, minimization of preheating times, by selecting faster preheating methods, could help improve food attributes at the end of the HPHT process. A two-stage preheating approach has been proposed to save carrier operating time.

HPHT technology has the potential to manufacture shelf stable egg, vegetable, meat, and dairy products, but more information is needed in terms of the sensory quality of HPHT products and consumer preferences. Further studies on the effect of HPHT conditions on separate food components and developed formulations will allow identifying specific study cases. Once products are identified, characterization in terms of shelf life can be carried out. In this case, shelf stability of developed HPHT processed foods will not only depend on the treatment applied but also on the barrier provided by the selected packaging material. Efforts are ongoing to identify packaging materials that meet the requirements for overall integrity, specific seal strength, and gas permeability after HPHT treatment.

In conclusion, defining the conditions *of C. botulinum* inactivation is fundamental to establishing further product development studies on novel HPHT treated foods to satisfy the shelf stable ready-to-eat markets. In addition, validation of process performance criteria related to *C. botulinum* inactivation, and pressure and temperature history, will not only allow process filing

with regulatory agencies, but will help establish a business case for transforming HPHT processing into an industrial reality.

References

9CFR318.300. 2002. Animals and animal products. Entry into official establishments; reinspection and preparation of products. Canning and Canned Products. In: *Food Safety and Inspection Service, US Department of Agriculture. Code of Federal Regulations (CFR),* Federal Register, US Government Printing Office, Washington, DC, Title 9, Chapter III, Part 318, Subpart G.

9CFR381.300. 2003. Animals and animal products. Poultry products inspection regulations. Canning and Canned Products. In: *Food Safety and Inspection Service, US Department of Agriculture. Code of Federal Regulations (CFR),* Federal Register, US Government Printing Office, Washington, DC. Title 9, Chapter III, Part 381, Subpart X.

21CFR113. 2005. Food and Drugs. Food for human consumption, Thermally processed low-acid foods packaged in hermetically sealed containers. In: *US Food and Drug Administration, Department of Health and Human Services, Code of Federal Regulations (CFR)*, Federal Register, US Government Printing Office, Washington, DC, Title 21, Chapter I, Subchapter B, Part 113.

21CFR114. 2005. Food and Drugs. Food for human consumption. Acidified foods. In: *US Food and Drug Administration, Department of Health and Human Services. Code of Federal Regulations (CFR)*, Federal Register, US Government Printing Office, Washington, DC, Title 21, Chapter I, Subchapter B, Part 114.

Ahmed, J., Ramaswamy, H.S., Alli, I. and Ngadi, M. 2003. Effect of high pressure on rheological characteristics of liquid egg. *Lebensm. Wiss. Technol.* **36**(5), 517-524.

Ahn J., Balasubramaniam, V.M., and Yousef, A.E. 2005. Effect of pressure-assisted thermal processing on the inactivation of selected *Clostridium* and *Bacillus* surrogate spores [abstract]. *Nonthermal Processing Workshop*, September 15-16, Philadelphia, PA, USDA Eastern Regional Research Center, USDA ARS Eastern Regional Research Center, Wyndmoor, PA, Abstract no.# p. 24.

Ardia, A., Knorr, D., and Heinz, V. 2004. Adiabatic heat modeling for pressure build-up during high-pressure treatment in liquid-food processing. *Food Bioprod. Process.* **82**(C1), 89-95.

Balasubramaniam, S., and Balasubramaniam, V.M. 2003. Compression heating influence of pressure transmitting fluids on bacteria inactivation during high pressure processing. *Food Res. Int.* **36**(7), 661-668.

Balasubramaniam, V.M., Ting, E.Y., Stewart, C.M., and Robbins, J.A. 2004. Recommended laboratory practices for conducting high-pressure microbial inactivation experiments. *Innov. Food Sci. Emerg. Technol.* **5**(3), 299-306.

Baliga, B.R., Rao, A.S., Lahiry, N.L. 1969. Prevention of browning in hard boiled eggs during canning. *J. Food Sci. Technol.* **6**(3), 200-204.

Barbosa-Cánovas, G.V., and Rodríguez, J.J. 2005. Thermodynamic aspects of high hydrostatic pressure. In: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, eds. CRC Press, New York, pp.183-206.

Barbosa-Cánovas, G.V., Juliano, P., and Keener, L. 2005a. Legislative issues with respect to processed food, in: *Global Harmonization of Legislation of Food Products and Processes*. Institute of Food Technologists Conference. New Orleans, LA. July 2005, 27-3.

Barbosa-Cánovas, G.V., Juliano, P., Koutchma, T., Balasubramaniam, V.M., Mathews, J.W., and Dunne, C.P. 2005b. High pressure thermal sterilization of precooked egg patties: factors affecting preheating efficiency. In: *High Pressure Processing*, American Institute of Chemical Engineers Annual Meeting. Cincinnati, OH. November, 2005, 572c.

Campanella, O.H., and Peleg, M. 2001. Theoretical comparison of a new and the traditional method to calculate *Clostridium botulinum* survival during thermal inactivation. *J. Sci. Food Agr.* **81**, 1069-1076.

Caner, C., Hernandez, R.J., Pascall, M., Balasubramaniam, V.M., and Harte, B.R. 2004. The effect of high-pressure food processing on the sorption behavior of selected packaging materials. *Packaging Technol. Sci.* **17**(3), 139-153.

Cano, M.P., and De Ancos, B. 2005. Advances in use of high pressure processing and preservation of plant foods. In: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, eds. CRC Press, New York, pp. 283-310.

Carroll, T., Chen, P., and Fletcher, A. 2003. A method to characterize heat transfer during high-pressure processing. *J. Food Eng.* **60**, 131-135.

Codex. 2004. *Procedural Manual of the Codex Alimentarius Commission*, 14th Ed., Codex Alimentarius Commission. Rome, Italy.

Cooper, K.L., Call, M.K., and Meyer, R.S., inventors. 2004. Ultra-high pressure vegetable sterilization method and product, patent: U.S. 20040191382.

De Heij, W.B.C., van den Berg, R.W., van Schepdael, L., Hoogland, H., and Bijmolt, H. 2005. Sterilization - only better. *New Food* **8**(2), 56,58-61.

De Heij, W.B.C., van Schepdael, L.J.M.M., Moezelaar, R., Hoogland, H., Matser, A.M., and van den Berg, R.W. 2003. High-pressure sterilization: maximizing the benefits of adiabatic heating. *Food Technol.* **57**(3), 37-42.

De Heij, W., van Schepdael, L., van den Berg, R., and Bartels, P. 2002. Increasing preservation efficiency and product quality through control of temperature profiles in high pressure applications. *High Pressure Res.* **22**(3–4), 653 –657.

Denys, S., van Loey, A.M., and Hendrickx, M.E. 2000. A modeling approach for evaluating process uniformity during batch high hydrostatic pressure processing: combination of a numerical heat transfer model and enzyme inactivation kinetics, Innov. *Food Sci. Emerg. Technol*. **1**(1), 5-19.

Dunne, C.P. 2005. U.S. Army Natick Soldier Center, Department of Defense, personal communication, August 5.

EC258/97. 1997. Novel foods and novel food ingredients, Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997, Official Journal L 043, 14/02/1997, pp. 0001-0006.

European Commission. 2002. Implementation of Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients, Directorate General Health and Consumer Protection (SANCO D4), European Commission. (March 10, 2006); http://europa.eu.int/comm/food/food/biotechnology/novelfood/initiatives_en.htm.

Farkas, D.F., and Hoover, D.G. 2000. High pressure processing. In: *Kinetics of Microbial Inactivation for Alternative Food Processing Technologies*, Chicago, IL: J. Food Science Supplement, pp. 47-64.

Franceschini, B., Gola, S., Rovere, P.P., and Frustoli, M. 2005. Application of high hydrostatic pressure to increase the safety and the shelf-life of ready-to-eat (RTE) traditional meals. *Industria Conserve*. **80**(4), 391-409.

Gola, S., Foman, C., Carpi, G., Maggi, A., Cassara, A., and Rovere, P. 1996. Inactivation of bacterial spores in phosphate buffer and in vegetable cream treated with high pressure. *High Press Biosci. Biotechnol*. pp. 253-59.

Gola, S., and Rovere, P.P. 2005. Resistance to high hydrostatic pressure of some strains of *Clostridium botulinum* in phosphate buffer. *Industria Conserve*. **80**(2), 149-157.

Guamis, B., Pla, R., Trujillo, A.J., Capellas, M., Gervilla, R., Saldo, J., and Yuste, J. 2005. High pressure processing of milk and dairy and egg products. In: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, eds. CRC Press, New York, pp. 343-360.

Hartmann, C., and Delgado, A. 2002. Numerical simulation of convective and diffusive transport effects on a high-pressure induced inactivation process. *Biotechnol. Bioeng*. **79**, 94–104.

Hartmann, C., and Delgado, A. 2003. The influence of transport phenomena during high-pressure processing of packed food on the uniformity of enzyme inactivation, *Biotechnol. Bioeng.* **82**(6), 725-735.

Hartmann, C., Delgado, A., and Szymczyk, J. 2003. Convective and diffusive transport effects in a high pressure induced inactivation process of packed food. *J. Food Eng.* **59**(1), 33-44.

Harvey, A.H., Peskin, A.P., and Sanford, A.K. 1996. NIST/ASTME – IAPSW Standard Reference Database 10, version 2.2.

Heinz, V., and Knorr, D. 2001. Effect of high pressure on spores. In: *Ultra High Pressure Treatment of Foods*, M.E.C. Hendrickx and D. Knorr, eds. Kluwer Academic/Plenum Publishers, New York, pp. 77-116.

Hendrickx, M. 2005. Pathways to commercialization: from academia to the marketplace academic incubators and innovation (European Model). In: *Commercializing Nonthermal Technologies*, Institute of Food Technologists Continuing Technical Education Committee. New Orleans, LA, July 15-16.

Hirsch, E.S., Kramer, F.M., Meiselman, H.L. 2005. Effects of food attributes and feeding environment on acceptance, consumption and body weight: lessons learned in a twenty-year program of military ration research. US Army Research (Part 2). *Appetite* **44**(1), 33-45.

Hjelmqwist, J. 2005. Commercial high pressure equipment. In: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, eds. CRC Press, New York, pp. 361-374.

Holdsworth, S.D. 1997. *Thermal processing of packaged foods*. Blackie Academic & Professional, New York, pp.112-120.

Hoogland, H., de Heij, W., and van Schepdael, L. 2001. High pressure sterilization: novel technology, new products, new opportunities. *New Food*, **4**(1), 21-6.

Juliano, P., Clark, S., Ouattara, M., Mathews, J., Dunne, C.P., Koutchma, T.N., and Barbosa-Cánovas, G.V. 2005. Consumer and trained panel evaluation of high pressure, Institute of Food Technologists Conference, New Orleans, LA, July 15-20, 99E-21.

Juliano, P., Toldrà, M., Koutchma, T., Balasubramaniam, V.M., Clark, S., Mathews, J.W., Dunne, C.P., Sadler, G., and Barbosa-Cánovas, G.V. 2006. Texture and water retention improvement in high pressure thermally sterilized scrambled egg patties. *J. Food Sci.* **71**(2), E52-61.

Juliano, P., Li, B., Clark, S., Mathews, J.W., Dunne, C.P., and Barbosa-Cánovas, G.V. 2006b. Quality and sensory analysis of precooked egg products after high pressure processing combined with low and high temperatures. *J. Food Qual.* In press.

Kalchayanand, N., Dunne, C.P., Sikes, A., and Ray, B. 2003. Inactivation of bacterial spores by combined action of hydrostatic pressure and bacteriocins in roast beef. *J. Food Saf.* **23**(4), 219-231.

Koutchma, T., Guo, B., Patazca, E., and Parisi, B. 2005. High pressure – high temperature inactivation of *Clostridium sporogenes* spores: from kinetics to process verification. *J. Food Process. Eng*. **28**(6), 610-629.

Krebbers, B., Matser, A.M., Koets, M., and van den Berg, R.W. 2002. Quality and storage-stability of high-pressure preserved green beans. *J Food Eng*. **54**(1), 27-33.

Krebbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M.M., and van den Berg, R.W. 2003. Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innov Food Sci Emerg Technol.* **4**(4), 377-85.

Leadley, C. 2005. High pressure sterilisation: a review. *Campden & Chorleywood Food Research Association* **2005**(47), 1 - 42.

Lee, D.U., Heinz, V., and Knorr, D. 1999. Evaluation of processing criteria for the high pressure treatment of liquid whole egg: rheological study. *Lebensm -Wiss Technol.* **32**(5), 299-304.

Ludikhuyze, L., van den Broeck, I., Weemaes, C.A., and Hendrickx, M.E. 1997. Kinetic parameters for temperature-pressure inactivation of *Bacillus Subtilis* α-amylase under dynamic conditions. *Biotechnol. Prog.* **13**, 617–623.

Luechapattanaporn, K., Wang, Y., Wang, J., Tang, J., Hallberg, L.M., and Dunne C.P. 2005. Sterilization of scrambled eggs in military polymeric trays by radio frequency energy. *J. Food Sci.* **70**(4), 288-294.

Ma, L., Chang, F.J., Barbosa-Cánovas, G.V., and Swanson, B.G. 2001. Comparison study of pulsed electric fields, high hydrostatic pressure, and thermal processing on the electrophoretic patterns of liquid whole egg. In: *Pulsed Electric Fields in Food Processing: Fundamental aspects and applications*, *G*.V. Barbosa-Cánovas and H. Zhang, eds. Technomic Publishing Co., Lancaster, PA, pp. 225-240.

Margosch, D. 2005. *Behavior of Bacterial Endospores and Toxins as Safety Determinants in Low Acid Pressurized Food*. Doctoral Dissertation. Technischen Universität Berlin, Germany.

Margosch, D., Moravek, M., Gaenzle, M.C., Maertlbauer, E., Vogel, R.F., and Ehrmann, M.A. 2005. Effect of high pressure and heat on bacterial toxins. *Food Technol. Biotechnol.* **43**(3), 211-217.

Margosch, D., Ehrmann, M.A., Gaenzle, M.G., and Vogel, R.F. 2004. Comparison of pressure and heat resistance of *Clostridium botulinum* and other endospores in mashed carrots. *J. Food Prot.* **67**(11), 2530-2537.

März, A. 2002. Method for inactivating microorganisms using high pressure processing. Patent number: EP 1 201 252 A1.

März, A. 2003. Method for inactivating microorganisms using high pressure processing. Patent number: US 6635223.

Matser, A.M., Krebbers, B., van den Berg, R.W., and Bartels, P.V. 2004. Advantages of high pressure sterilization on quality of food products. *Trends Food Sci Technol.* **15**(2), 79-85.

Mermelstein, N.H. 2001. Military and humanitarian rations. *Food Technol.* **55**(11), 73-75.

Meyer, R.S., Cooper, K.L., Knorr, D., and Lelieveld, H.L.M. 2000. High-pressure sterilization of foods. *Food Technol.* **54**(11), 67-68, 70-72.

Montero, P., and Gómez-Guillén, M.C. 2005. High pressure applications on myosystems. In: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, eds. CRC Press, New York, pp. 311-342.

NACMCF (National Advisory Committee on Microbiological Criteria for Foods). 2004. Requisite scientific parameters for establishing the equivalence of alternative methods of pasteurization. (Adopted August 27, 2004, Washington, DC; last visited March 10, 2006) http://www.fsis.usda.gov/About_FSIS/NACMCF/index.asp

NACMCF. 2005. Consideration for establishing safety-based consume-by date labels for refrigerated ready-to-eat foods. *J. Food Prot.* **68**(8): 1761-1775.

NFPA. 1985. *Guidelines for Thermal Process Development for Foods Packaged in Flexible Containers*. National Food Processors Association (NFPA), New York.

Nicolaï, M.B., Scheerlinck, N., Verboven, P., and Baerdemaeker, J.D. 2001. Stochastic finite-element analysis of thermal food processes. In: *Food Processing Operations Modeling. Design and Analysis,* Irudayaraj, J., ed. Marcel Dekker, New York, pp. 265-304.

NC Hyperbaric. 2004. *High pressure processing. Technology that makes sense.* [Commercial booklet], Burgos, Spain.

Otero, L., Molina-Garcia, A.D., and Sanz, P.D. 2000. Thermal effect in foods during quasi-adiabatic pressure treatments. *Innov. Food Sci. Emerg. Technol.* **1**,119-126.

Otero, L., and Sanz, P.D. 2003. Modeling heat transfer in high pressure food processing: a review. *Innov. Food Sci. Emerg. Technol.* **4**(2), 121-134.

Patterson, M.F. 2005. A review: microbiology of pressure-treated foods. *J. Applied Microbiol.* **98**, 1400–1409.

Palazoglu, K. 2006. Influence of convective heat transfer coefficient on the heating rate of materials with different thermal diffusivities. *J. Food Eng.* **73**(3), 290-296.

Peleg, M., Normand, M.D., and Campanella, O.H. 2003. Estimating microbial inactivation parameters from survival curves obtained under varying conditions - The linear case. *Bull. Math. Biol.* **65**, 219-234.

Peleg M, Normand MD, Corradini MG. 2005. Generating microbial survival curves during thermal processing in real time. *J. Appl. Microbiol.* **98**, 406–417.

Pflug, I.J. 1987 Using the straight-line semi logarithmic microbial destruction model as an engineering design model for determining the F-value for heat processes. *J. Food Prot.* **50**(4), 342-246.

Rajan, S., Ahn, J., Balasubramaniam, V.M., Yousef, A.E. 2006a. Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores in mashed egg patty mince. *J. Food Prot.* In press.

Rajan, S., Pandrangi, S., Balasubramaniam, V.M., and Yousef, A.E. 2006b. Inactivation of *Bacillus stearothermophilus* spores in egg patties by pressure assisted thermal processing. *LWT-Food Sci. Technol*. In press.

Rasanayagam, V., Balasubramaniam, V.M., Ting, E., Sizer, C.E., Bush, C., and Anderson, C. 2003. Compression heating of selected fatty food materials during high-pressure processing. *J. Food Sci.* **68**(1): 254-259.

Raso, J., Barbosa-Cánovas, G.V., and Swanson, B.G. 1998. Sporulation temperature affects initiation of germination and inactivation by high hydrostatic pressure of *Bacillus cereus*. *J. Appl. Microbiol.* **85**,17-24.

Reddy, N.R., Solomon, H.M., Fingerhut, G.A., Rhodehamel, E.J., Balasubramaniam, V.M., Palaniappan, S. 1999. Inactivation of *Clostridium botulinum* type E spores by high pressure processing. *J. Food. Saf.* **19**, 277-288.

Reddy, N.R., Solomon, H.M., Tetzloff, R.C., and Rhodehamel, E.J. 2003. Inactivation of *Clostridium botulinum* type A spores by high pressure processing at elevated temperatures. *J. Food Prot.* **66**(8),1402-1407.

Rovere, P., Gola, S., Maggi, A., Scaramuzza, N., and Miglioli, L. 1998. Studies on bacterial spores by combined pressure-heat treatments: possibility to sterilize low-acid foods, in: *High Pressure Food Science, Bioscience and Chemistry*, N.S. Isaacs, ed. The Royal Society of Chemistry, Cambridge, UK, pp. 354-63.

Rovere, P., Squarcina, N., Gola, S., Sandei, L., Iametti, S., and Carpi, G. 2000. Effect of thermal treatment under high pressure on the quality of a meat sauce. *High Pressure Res.* **19**, 99–107.

Sale, A.J.H., Gould, G.W., and Hamilton, W.A. 1970. Inactivation of bacterial spores by high hydrostatic pressure. *J. Gen. Microbiol.* **60**, 323-334.

Schauwecker, A., Balasubramaniam, V.M., Sadler, G., Pascall, M.A., and Adhikari, C. 2002. Influence of high-pressure processing on selected polymeric materials and on the migration of a pressure-transmitting fluid. *Packaging Technol. Sci.* **15**, 255–262.

Shearer, A.E.H., Dunne, C.P., Sikes, A., and Hoover, D.G. 2000. Bacterial spore inhibition and inactivation in foods by pressure, chemical preservatives, and mild heat. *J. Food Prot.* **63**(11), 1503-1510.

Sizer, C.E., Balasubramaniam, V.M., and Ting, E. 2002. Validating high-pressure processes for low-acid foods. *Food Technol.* **56**(2), 36-57.

Stewart, C.M., Dunne, C.P., Sikes, A., and Hoover, D.G. 2000. Sensitivity of spores of *Bacillus subtilis* and *Clostridium sporogenes* PA 3679 to combinations of high hydrostatic pressure and other processing parameters. *Innov. Food Sci. Emerg. Technol.* **1**(1): 49-56.

Stewart, C.M. 2005. HPP safety and quality data. In: *Commercializing Nonthermal Technologies*, Institute of Food Technologists Continuing Technical Education Committee. New Orleans, LA, July 15-16.

Surak, J.G. 2006. Global harmonization: foods safety management standards. *Int. Rev. Food Sci. Technol.* Winter 2005/2006, 34-36.

Taki, Y., Awano, T., Toba, S., and Mitsuura, N. 1991. Sterilization of *Bacillus sp.* spores by hydrostatic pressure. In: *High Pressure Science for Food*, R. Hayashi, ed. Sanei Pub. Co., Kyoto, Japan, pp. 217-24.

Tapia, M.S., Arispe, I., and Martínez, A. 2005. Safety and quality in the food industry. in: *Novel Food Processing Technologies*, G.V. Barbosa-Cánovas, M.S. Tapia, and M.P. Cano, eds. CRC Press, New York, pp. 669-680.

Ter Minassian, L., Pruzan, P., and Soulard, A. 1981. Thermodynamic properties of water under pressure up to 5 kbar and between 28 and 120ºC. Estimations in the supercooled region down to -40ºC. *J. Chem. Phys.* **75**, 3064-3072.

Ting, E., Balasubramaniam, V.M., and Raghubeer, E. 2002. Determining thermal effects in high-pressure processing. *Food Technol.* **56**(2): 31-35.

Van Loey, A., Ooms, V., Weemaes, C., van den Broeck, I., Ludikhuyze, L., Indrawati, Denys, S., and Hendrickx, M. 1998. Thermal and pressure-temperature degradation of chlorophyll in Broccoli (*Brassica oleraces L. italica*) juice: a kinetic study. *J. Agr. Food Chem.* **46**, 5289–5294.

Van Opstal, I., Bagamboula, C.F., Vanmuysen, S.C.M., Wuytack, E.Y., and Michiels, C.W. 2004. Inactivation of *Bacillus cereus* spores in milk by high pressure and heat treatments. *Int. J. Food Microbiol.* **92**, 227-234.

Van Schepdael, L.J.M.M., de Heij, W.B.C., and Hoogland, H. 2002. Method for high pressure preservation, patent: PCT WO 02/45528 A1.

Varga, S., and, Oliveira J.C. 2000. Determination of the heat transfer coefficient between bulk medium and packed containers in a batch retort. *J. Food Eng.* **44**(4), 191-198.

Welti-Chanes, J., López-Malo, A., Palou, E., Bermúdez, D., Guerrero-Beltrán, J.A., Barbosa-Cánovas, G.V. 2005. Fundamentals and applications of high pressure processing of foods, in: *Novel Food Processing Technologies*, Barbosa-Cánovas, G.V., Tapia, M.S., Cano, M.P., eds. CRC Press, New York, pp.157-182.

Wesley, R.D., Rousselle, J.R., Schwan, D.R., and Stadelman, W.J. 1982. Improvement in quality of scrambled egg products served from steam table display. *Poultry Sci.* **61**(3), 457-462.

Wilson, M.J., and Baker, R. 2000. High temperature/ultra-high pressure sterilization of foods, patent: US 6,086,936.

Wilson, M.J., and Baker, R. 2003. High temperature/ultra-high pressure sterilization of low acid foods, patent: EU 1 295 537 A2.

Table 1. High pressure commercial chilled products (adapted from NC Hyperbaric, 2004)

Product types	Countries*	Shelf life achieved (4ºC to room temperature)
Juices and beverages	Japan, France, Mexico, USA, Lebanon, UK, Portugal, Italy, Ireland, Czech Republic	21 d. to 12 mo.
Vegetable products	Japan, USA, Italy, Canada	1 to 6 mo.
Meat products	Japan, Spain, USA, Italy	21 d. to 2 mo. (cooked products)
Seafood products	Japan, USA, Australia, Canada, Spain	10 d. to 2 mo.

*Countries are listed in order of product appearance in the market

Table. 2. Factors affecting heat transfer during preheating of packaged foods.

Process variable	System element	Process Factors	Parameters
Fluid temperature	Preheating system	• Type of system • Ratio of fluid mass: product mass (number of packages) • Racking system (separation between container, circulation between layers, package restraint to specified thickness) • Heat transfer aids (steam, steam/vapor, microwaves, radiofrequencies, circulation pumps)	• Heat transfer coefficient • Heating rate
Product initial temperature Target preheating temperature	Container geometry	• Packaging material (composition, thickness) • Package thickness • Fill weight • Sample confining system (racks, trays, cassettes) • Container headspace (amount of air in the package) • Container shape • Distribution of food particulates	• Package thermal diffusivity* • Time to reach target temperature • Temperature equilibration time
	Product characteristics	• Composition of ingredients • Particulate size • Soluble solids • Physical state (fresh/ cooked, liquid, semisolid, frozen) • Food structure (homogeneity) • Occluded gases • Viscosity	• Product thermal diffusivity*

*****Thermal diffusivity γ is known as the ratio of the heat conducted to the heat stored and is calculated using the following expression: $\gamma = \frac{k}{\rho \cdot C_p}$, where k is the thermal conductivity, ρ is the specific density, and C_p is the specific heat.

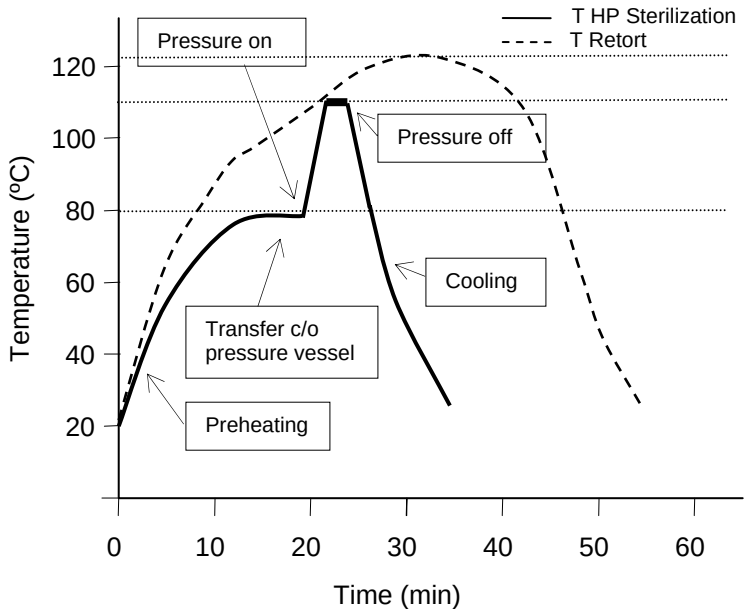

Fig. 1. Typical product temperature profiles in a retort and a HPHT process. Processing steps needed during pressurization.

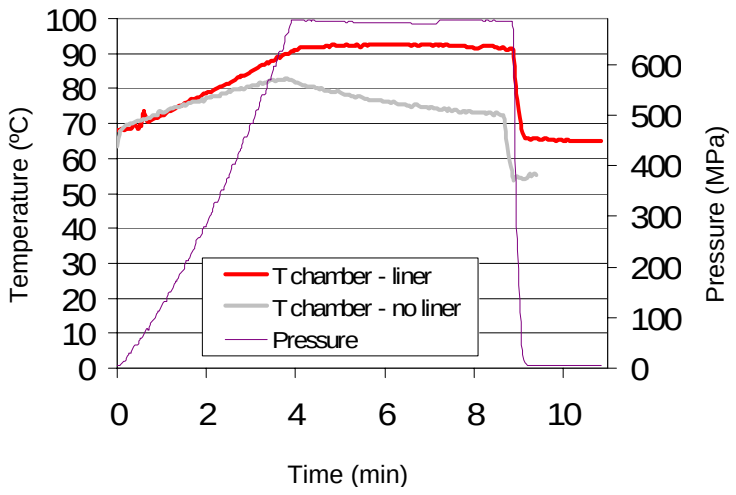

Fig. 2. Temperature profiles of pressurized water at 70ºC initial temperature and 680 MPa when: a) compressed water is in the pressure vessel, b) compressed water is in a preheated polypropylene liner. Data was extracted from a cylindrical liner made (internal diameter 75 mm, external diameter 100 mm, height 21.5 mm) with a movable lid inserted into a 1.7 L high pressure chamber (Engineered Pressure Systems, Inc., model #914-100, Haverhill, MA).

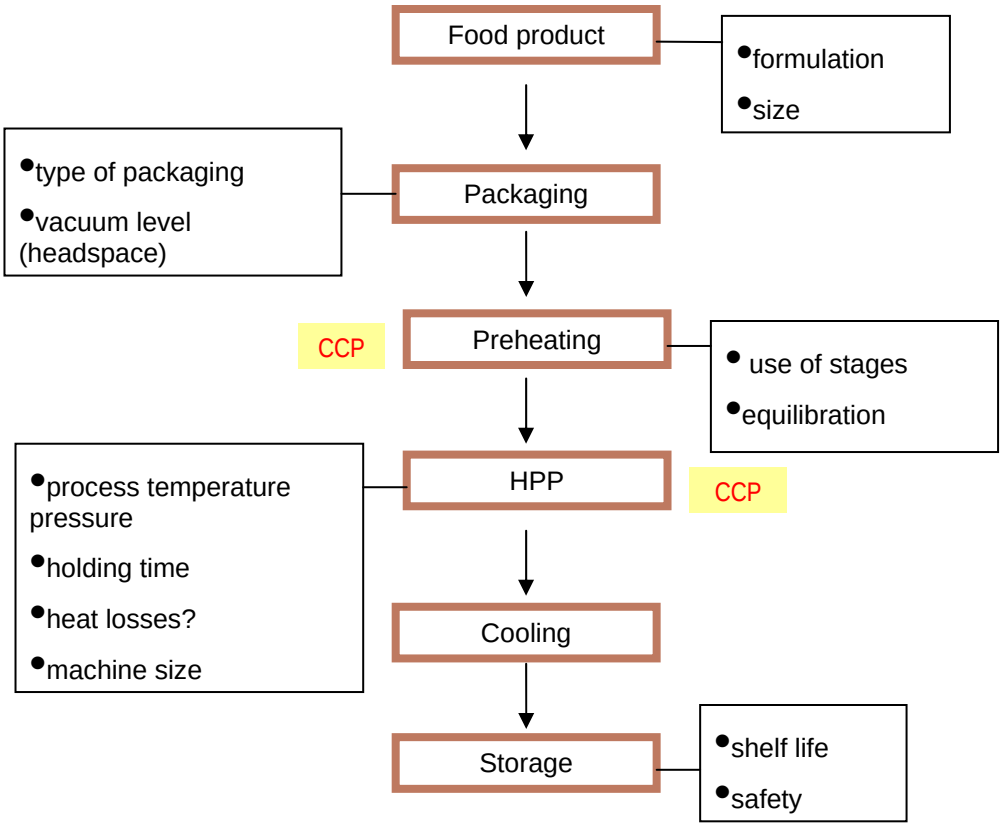

Fig.3. Flow chart of a HPHT process showing critical control points (CCP) for food safety as well as other process variables.

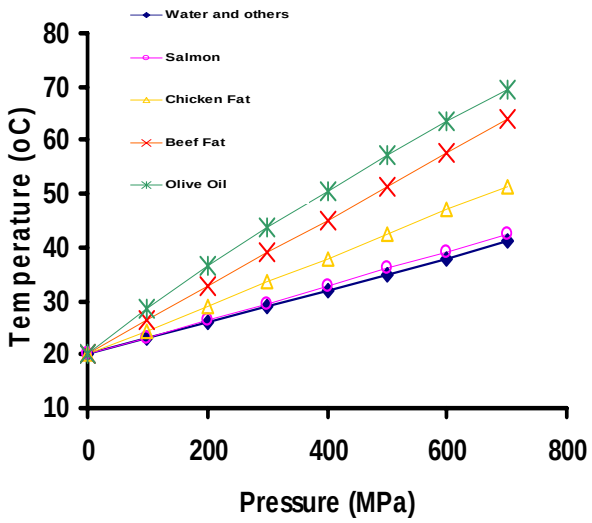

Fig. 4. Temperature elevation from room temperature due to pressurization up to 700 MPa (modified from Barbosa-Cánovas and Rodríguez, 2005).

CHAPTER TWO

Descriptive analysis of precooked egg products after high pressure processing combined with low and high temperatures

Pablo Juliano, Biansheng Li, Stephanie Clark, Jason Mathews,

C. Patrick Dunne, and Gustavo V. Barbosa-Cánovas

1. Introduction

Precooked specialty egg products such as egg patties, omelets, or cook-in-bag scrambled eggs are mainly commercialized in frozen form to fast food outlet chains in the food service industry (Baker and Bruce, 1995). However, precooked egg products, to be stored at room temperature, are not yet available in the market. In fact, only a few companies offer ready-to-eat scrambled eggs and whole hard-cooked/peeled eggs with longevity of 6 to 12 weeks at refrigerated conditions (AEB, 2003). The main challenge is to assure product safety during cooling, packaging, and post packaging stages. This requires either additional thermal treatment, or an alternative treatment that has minimal effects on the product's final quality. Furthermore, egg patties and omelets are often vacuum packaged in multiple amounts. If a thermal post-packaging treatment is applied, these products will need to handle long periods of heat exposure, to reach the target process temperature throughout entire package volume.

Long time exposure to temperatures higher than 70ºC can cause deleterious effects on the quality of products, such as color degradation, texture changes, and syneresis or liquid separation (Wesley et al., 1981). In fact, the development of shelf stable egg-based breakfast items through commercial sterilization techniques has been a significant challenge due to the deleterious effect on egg product appearance (Baliga et al., 1969; Luechapattanaporn et al., 2005). During retort

process, undesirable phenomena such as green-gray discoloration of egg products from formation of ferrous sulfide (Song and Cunningham, 1985), the development of off-flavors, and syneresis after heat treatment (Cotterill, 1995) can occur.

High pressure processing (HPP) is commercially used today as a nonthermal preservation method for prepackaged processed products such as ham. Use of low or mild heat prevents flavor and nutrient content reduction compared to conventional heat treatments (San Martín et al., 2002). HPP has the advantage of volumetric pressurization throughout entire package volume, due to instant pressure "penetration", allowing the same pressurization times for larger packages. Pressure vessels at initial room temperature and pressures 200-800 MPa have been used to inactivate vegetative pathogenic and spoilage bacteria (Margosch et al., 2004).

When elevated initial temperatures (e.g., between 60-90ºC) are combined with pressures greater than 600 MPa in a pressurization vessel, product sterilization can be accelerated using much shorter processing times compared to retort processes (Matser et al., 2004). The main reason for bacterial spore inactivation is due to internal compression heating, which can reach in-process temperatures ranging from 90ºC to greater than 121ºC, depending upon pressure applied and initial temperature of the food and vessel.

Microbial studies have proved that initial chamber/product temperature 75-80ºC and pressure 600-827 MPa can effectively inactivate target heat resistant spore-forming bacteria commonly used as indicators of food safety and shelf-stability (Heinz and Knorr, 2001). For example, *Bacillus stearothermophilus* spores were reduced 5 log in phosphate buffer and beef broth at 70ºC/700 MPa/3 min (Gola et al., 1996; Rovere et al., 1998), and at least 4.5 log for meat balls in

tomato puree at 90ºC/700 MPa/30 s (Krebbers et al., 2003). *Bacillus licheniformis* spores suspended in pH 7.0 buffer were also inactivated at 60ºC/600 MPa/20 min (Taki et al., 1991), and reduced 6 log in pH 7 buffer and beef broth at 70ºC/700 MPa/5 min (Gola et al., 1996; Rovere et al., 1998). *Bacillus cereus* spores were also reduced 8 log in pH 7 buffer at 60ºC/690 MPa/1 min with previous sporulation at 37ºC (Raso et al., 1998), and 5 log in beef broth at 70ºC/700 MPa/5 min (Rovere et al., 1998). Similarly, *Bacillus subtillis* spores were inactivated at 827 MPa and process temperature ranging from 102 to 107ºC, yielding up to a 6 log unit reduction (Balasubramaniam and Balasubramaniam, 2003).

Studies on target microorganism for inactivation and safety assurance of canned food products, *Clostridium botulinum,* have shown large variation in the pressure resistance of different spore strains (Margosch et al., 2004; Margosch, 2005). *C. botulinum* spores type E in pH 7 buffer, for example, have been reduced 4.5 log at 50°C/758 MPa/5 min and 5 log at 40°C/827 MPa/10 min (Reddy et al., 1999). Furthermore, *C. botulinum* type A was reduced by more than 3 log units in pH 7 buffer and crab meat following treatment at 75ºC/827 MPa/20 min (Reddy et al., 2003). Other studies on several *C. botulinum* strains (types A, B, F proteolytic, B non-proteolytic) in mashed carrots were carried out by Margosch et al. (2004) and Margosch (2005), who found that different *C. botulinum* strains treated at 80°C/600 MPa/1 s were reduced by more than 5.5 log cycles to none at all. In particular, non-proteolytic *C. botulinum* type B was reduced by more than 5.5 log cycles after 80ºC/600 MPa/1 s. In comparison, proteolytic *C. botulinum* type A had more than a 5 log reduction after 80ºC/600 MPa/12 min treatment, and proteolytic *C. botulinum* type B spores were inactivated by less than three orders of magnitude at 80ºC/600 MPa/60 min. Since spore resistance also proved to be product dependent, more research is needed to find the

optimum inactivation pressure/temperature/time conditions for *C. botulinum* strains in precooked egg products, which seem to be a suitable medium for spore germination (Margosch, 2005).

The use of high pressure in combination with heat has been identified as a promising approach for providing commercially sterile precooked specialty egg products with improved appearance and greater appeal than retort products. Indeed, a number of high pressure high temperature (HPHT) treated low-acid foods such as meat, milk and vegetable products showed more desirable texture, color, and flavor and aroma retention in comparison to retorted products and, in some cases, to frozen products (Hoogland et al., 2001; Krebbers et al., 2002; Krebbers et al., 2003; Matser et al., 2004).

Previous studies on high pressure formation of gels of whole liquid eggs, egg white, egg yolk , and egg yolk/white (Ma et al., 2001; Ahmed et al., 2003; Lee et al., 1999; Lee et al., 2003; Messers et al., 1997; Ponce et al., 1998) have shown that pressures greater than 600 MPa not only increase apparent viscosity, but also provide instantaneous gelation of egg yolk and egg white. Pressure formed egg-based gels have shown to retain vitamins, amino acid residues, flavor, and color compared to heat-induced gels (Guamis et al., 2005; Hayashi et al., 1989). However, no previous research has been done on the quality and sensory profile of precooked formulated egg products after high pressure processing. Thus, the need to identify stabilized precooked egg products and acquire data on the quality of these products after high pressure treatment at low and high temperatures has led to this study.

The objective of this research was to analyze the adequacy of pre-formulated egg patties treated at high pressure/low and high temperature conditions using quality and sensory descriptors.

2. Materials and Methods

2.1. Preparation of egg-based products

Michael Foods Egg Products Company (Gaylord, MN) provided a round commercial scrambled egg patty (#1, code 46025-30020-00) and a modified formulation (#2, code 03-1426-10). Patties were each 42.5 ± 7.1 g and 88.9 ± 6.4 mm in diameter. Patty #1 is a standard Michael Foods patty and has the following basic ingredients: whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dried milk, and citric acid. Patties #2 had added natural and artificial flavors, xanthan gum, and EDTA and patty formulation #2 had a lower egg: water ratio.

Preparation of precooked scrambled egg products has been reported in different patents developed by Michael Foods (Knipper et al., 2002; Merkle et al., 2003 a, b). In particular, Knipper et al. (2002) explained the production process of precooked egg patties. Whole eggs were mixed with dry and liquid ingredients, after which mixture was pumped into a mold within a flat cooking belt. Egg mixture portions were cooked (or formed) in a convection oven at 180-250ºC for a predetermined time, then frozen and packaged.

Handling and shipping procedure for scrambled egg patties was performed according to an industrial setting, where patties would be stored in a frozen state before HPP treatment. Frozen samples from a single lot were received from Michael Foods (Gaylord, MN) and stored frozen at -30ºC. Each patty was cut in half and repackaged in retort pouches (Smurfit-Stone, Schaumburg, IL) 6.0 x 10.3 cm. The vacuum packaging machine was a tabletop vacuum chamber (KOCH 15-EasyPack™, Kansas City, MO) used at 400 mbar absolute pressure. Retort packaging material

composition was 48 ga. PET/Adhesive/Aluminum foil/Adhesive/4.0 mL Polyolefin. Samples were kept frozen until high pressure treatments.

2.2 Preheating and high pressure treatments

Egg patties were preheated using a water bath at boiling temperature (98ºC) (corresponding to 715 m above sea level) in a tilting steam kettle (DLT-40-1EG, Groen, DI Food Service Companies, Jackson, MS). Temperature of patties was measured using a thermocouple (T-type, Omega Engineering Inc., Stamford, CT) affixed by a stuffing box (Ecklund Harrison Technologies, Fort Meyer, FL) at the center of egg patties.

Table 1 shows the factorial design of the two experiments performed. The first experiment combined different products/initial chamber temperatures for different formulations treated at 675 MPa/5 min. The main purpose was to identify the effect of temperature on the quality of different egg formulations at high pressure conditions which, combined with selected temperatures, can pasteurize (low temperature) or sterilize (high temperature) egg products. Initial high pressure chamber and product temperatures were the same. Comparisons were made with non-pressure-treated controls preheated up to the same initial product temperatures. Samples were preheated in boiling water bath for 2.5 ± 0.2 min to reach 30ºC, 3.4 ± 0.2 min to reach 50ºC, 6.5 ± 0.5 min to reach 70ºC, and 15 ± 1.0 min to reach 90ºC inside the half patty. Patties were placed in water baths at each respective preheat temperature for 5 min to equilibrate, and then loaded into a cylindrical liner made of white polypropylene (internal diameter 75 mm, external diameter 100 mm, height 21.5 mm; McMaster-Carr, Atlanta, GA). The liner was also previously temperature equilibrated and filled with water at the corresponding temperature to maintain temperature of product/water/liner system at chamber temperature.

After equilibration, the liner containing egg patties was inserted into a 1.7 L high pressure chamber (Engineered Pressure Systems, Inc., model #914-100, Haverhill, MA) with 5% Houghton Hydrolubic 123B soluble oil/water solution (Houghton & Co, Valley Forge, PA, USA) as pressure medium. The system was equipped with two thermocouples (T-type, Omega Engineering Inc., Stamford, CT), which controlled water temperature inside liner. Times for pressure come up averaged 2.5 ± 0.3 min for 300 MPa, 3.5 ± 0.5 min for 500 MPa, and 4.2 ± 0.5 min for 675 MPa. Following the pressure treatment, pouches containing egg patties were cooled in an ice bath. The total process time, including preheating step in boiling water bath, ranged from 18 to 21 min. Table 2 shows the liner temperatures before, during pressurization, and after pressure release.

Second experiment (Exp. 2) was performed in the same way by only using formulation #1 at initial product/liner/chamber temperature 90ºC for different pressures (Table 1). As shown in results section, an initial product temperature of 90ºC combined with 675 MPa, i.e., conditions with potential for sterilization, significantly affected hardness and syneresis in egg patty formulation #1. This experiment aimed to show the effect of pressure levels, lower than 675 MPa, in the final quality of egg patties when combined with a high initial temperature of 90ºC.

2.3 Product analyses

Quality of egg products before and after treatment was evaluated by a trained sensory panel, Texture Profile Analysis (TPA), serum content, color, and pH analyses described as follows.

Trained descriptive sensory panel

A six-member descriptive sensory panel was trained to use twenty nine terms for appearance, texture, and flavor attributes (Table 3). All sensory parameters were based on the standard 0-14 unstructured line scale arranged in a sensory ballot. Training was based on trial sessions, and ability of panelists to discriminate and reproduce results was tested in replicate tests on freshly scrambled eggs and preheated egg patties. Freshly scrambled eggs were given to panelists at the beginning of each session to normalize the score of each attribute in the sensory ballot. Control patties, defrosted in boiling water for 20 min, were compared with the same high pressure treated formulations. All samples were coded at random 3-digit numbers, and served to panelists in randomized complete block design.

Texture Profile Analysis

TPA tests were performed with a TA-XT2 Texture Analyzer (Stable MicroSystems Ltd., White Salmon, WA), fitted with a 5 kg load cell. Measurements were carried out using a cylindrical probe of 50.8 mm diameter on cylindrical pieces (25 mm diameter and 8.2 ±1.5 mm thickness) of egg patty at 20ºC. The samples were compressed to 50% of initial height as per Montejano et al. (1985) for protein gels, Paraskevopoulou and Kiosseoglou (1997) for egg yolk gels, and Gujral et al. (2003) for sponge cakes. The cross-head speed (and post-test speed) was 1 $mm \cdot s^{-1}$ according to Woodward and Cotterill (1986) in egg white gels. The TPA parameters hardness, adhesiveness, springiness, cohesiveness, and resilience were obtained through numerical routines established in the software package of the texture analyzer, which calculated each parameter as defined by Bourne (2002) from the two compression cycles applied to the samples. Hardness (N) was calculated by measuring the peak force obtained during first compression cycle. Adhesiveness was determined (N*mm) from the work necessary to pull probe away from

sample, which corresponds to negative area after first cycle. Springiness (dimensionless) was obtained from the ratio of two distances: distance to reach second force peak during second compression cycle and distance to reach first force peak during first cycle. Cohesiveness (dimensionless) was determined by dividing the positive force area during second compression cycle between the area corresponding to during first compression. Resilience (dimensionless) describes how the product regains position after first compression cycle, and was calculated as the ratio of the area corresponding to force withdrawal to the area of compression up to peak force reached.

Syneresis

Water loss (i.e., syneresis) in % weight loss was evaluated by weighing egg patty before packaging and after treatment. The following formula was used as adaptation of serum formula developed by Woodward and Cotterill (1986) for evaluating percentage of serum in heat-formed egg white gels, and by Feiser and Cotterill (1982) for evaluating cooked-frozen-thawed-reheated scrambled eggs:

$$\%\ \ weight\ \ loss = \frac{ipw - fpw}{ipw} \times 100 \qquad (1)$$

where *ipw* and *fpw* are the initial and final weights of patty, respectively. A similar formula was used to determine expressible moisture as indicator of water holding capacity in omelets (O'Brien et al., 1982) as well as percentage of weight loss in chicken meat batters with egg white before and after high pressure (Fernández at al., 1998).

Color measurement

Testing of color degradation was through CIE System evaluation using a colorimeter (Minolta CM-2002 Spectrophotometer, Camera Co., Osaka, Japan). Color was evaluated through lightness L* and color intensity or Chrome calculated using Eq. (2).

$$Chrome = \sqrt{a^2 + b^2} \tag{2}$$

where +a* represents the red direction, -a* the green direction, +b* the yellow direction, and –b* the blue direction in the L*a*b* color space.

pH measurement

pH was measured before and after pressure or heat treatment using a pH meter (Model 420A, ORION Research Inc., Boston, MA) with a glass electrode. Egg patties were blended and diluted with distilled water in a 1:10 ratio using a 10.0 ± 0.1 g sample.

2.4 Statistics

A factorial design for egg panel descriptors, TPA parameter values, weight loss, and color measurement and pH (Table 1) was studied using General Linear Models procedure in SAS statistical package (SAS/STAT Language, SAS Institute Inc., Cary NC., 2004) to perform analyses of variance (ANOVA), Least Square Means ($\alpha = 0.05$), and regression analyses. Sensory results were also evaluated by ANOVA (Minitab, State College, PA).

3. Results and Discussion

3.1. Effect of initial chamber temperature and 675 MPa treatment on quality of formulation #1

Round patty #1 is a commercial Michael Foods egg product and its quality after high pressure processing at different initial temperature conditions was unknown. This section describes the appearance, texture/mouthfeel, and flavor/aroma profiles of selected egg patties after high pressure treatment at low and high temperature conditions.

3.1.1. Color and appearance of patty formulation #1

The sensory panel did not detect significant differences in gloss and green between #1 egg patties treated at 30ºC/675 MPa/5 min and the preheated control. This coincided with lightness L* values and Chrome found with the colorimeter (Table 4). Gels from egg white and egg yolk induced by pressures above 500 MPa have been found to give a more lustrous surface than heat induced gels (Hayashi et al., 1989). Since egg patties were previously heat formed, it is not surprising there were no significant differences detected after pressure.

When pressurized at 675 MPa at chamber temperatures equal to 70ºC, the sensory panel did not find differences in gloss with respect to the control, further supported by no significant changes in L* value. However, the sensory panel found slight, though significant ($P<0.05$), differences in green color when patties were treated at high pressure high temperature conditions. Even though the patty was formulated with an acidifying agent, citric acid, as well as iron chelator to prevent discoloration (Cotterill, 1995), the combination of high pressure and temperatures greater than 70ºC yielded green compounds. Cotterill (1995) explained that lower pH is needed to avoid greening. Furthermore, Feiser and Cotterill (1982) reported that cooking, freezing, thawing, and

reheating increases pH. There is also evidence that high pressure induces pH changes in water, buffers, or food compounds due to changes in dissociation constants of acids and bases (Stippl et al., 2004). However, no significant changes in pH (average 6.9 ± 0.1) were detected after thermal pressurization ($P>0.05$). Cotterill (1995) reported that formation of green FeS compounds is likely in cooked egg products at pH higher than 8.2. However, high pressure thermal treatment favored formation of green compounds at lower pH.

Variations in color were also determined using the Chrome values, a function of color parameters +a* and +b* (Eq. 2). High pressure/high temperature conditions mostly influenced the +b* value (data not shown), which indicated yellow intensity. The #1 egg patties treated at 30ºC/675 MPa/5 min gave Chrome values not significantly different from the control, Chrome values, however, were slightly but significantly decreased at pressure chamber temperature 70ºC or higher (Table 4). Changes in chrome of egg patty formulation #1 after treatment at high pressure/high temperature conditions supported changes seen in color intensity by sensory panel.

Guamis et al. (2005) observed that liquid egg yolk treated at 500 MPa with no heat maintains a yellow color. Moreover, Hayashi et al. (1989) found that pressure induced gels of egg yolk to be vividly yellow even if treated above 800 MPa. The yellow color in egg products results from xanthophylls, in particular carotenoids lutein, zeaxanthin, and crypotaxanthin (Yang and Baldwin, 1995). Pressures up to 500 MPa, treatment time up to 5 min, and temperature up to 40ºC are not detrimental to carotenoids (Cano and de Ancos, 2005). Carotenoids are heat sensitive and can be degraded at conventional sterilization temperatures through oxidative reactions, which will provoke bleaching and subsequent loss in color (Elbe, 1986; Luechapattanaporn, 2005). However, the degradation effect of these yolk compounds due to

combined pressure and temperature has not been previously reported. It is worth mentioning that Indrawati (2004) stated that high pressure treatment slightly affects the carotene content in food products, reporting 5% losses after treatment at 75ºC/600 MPa/40 min in carrot homogenates. Krebbers et al. (2003) reported insignificant losses in lycopene from tomato paste after two pulses at 90ºC/700 MPa/30 s. No other significant appearance differences, including surface homogeneity and crumbly appearance (Table 4), were noted.

3.1.2. Texture and syneresis of patty formulation #1

Texture profile of patty #1 after treatment at 30ºC/675 MPa/5 min was similar to preheated control, whereas #1 patties pressure treated at 70 and 90ºC scored significantly higher ($P<0.05$) in firmness, density, particle size, and mouthfeel roughness (Fig. 1). TPA analysis (Table 5) gave a similar profile comparing the high pressure low temperature treated patty and the control. Changes in hardness values were noticeable at initial chamber temperature 50ºC and were significantly higher above 70ºC and pressure 675 MPa.

Pressure level of 675 MPa is enough to provide complete and instantaneous gelation of egg yolks and egg whites, occurring above 600 MPa and 25ºC (Palou et al., 1999). Okamoto et al. (1990) tested pressure induced egg white gels at 25ºC/600 MPa/30 min and obtained hardness and cohesiveness values within the same range as #1 egg patty control and #1 patty treated at 30ºC/675 MPa/5 min. Even though egg proteins were already coagulated during cooking process, high pressure conditions at initial temperatures above 50ºC induced proteins to further aggregate, providing a firmer structure. Temperatures above 56ºC are enough for fractional precipitation and above 73ºC to allow egg protein coagulation (Cunningham, 1995). Thus,

combining pressure and temperature accelerated gelation process towards a denser, more cohesive, and harder texture.

On the other hand, descriptors astringency, greasy, pasty and mouth coating did not change significantly ($P>0.05$) even after pressurization at 90ºC/675MPa/5 min, with respect to #1control patty (data not shown).

Panelists found syneresis in #1 patties after 70ºC/675 MPa/5 min and 90ºC/675 MPa/5 min (Table 6) treatments to be slightly higher than the control and patty treated at 30ºC/675 MPa/5 min. This was supported by results in weight loss showing an increase in water loss of six to eight times greater than the control when initial chamber temperatures were higher than 50ºC and 675 MPa. Thus, increase in syneresis is caused by a combination of temperature and pressure. As explained by Yan and Baldwin (1995), an increase in firmness of the egg coagulum can squeeze liquid out of the protein matrix, thereby increasing syneresis. The sensory panel also found #1 patties to be significantly dryer (low moisture release at chewing) when treated above protein gelation temperature 70ºC (Fig. 1).

3.1.3. Flavor of patty formulation #1

High pressure processing of egg patty formulation #1, at low temperature and high temperature conditions did not significantly affect the flavor descriptors: oily, oxidized, scorch, rancid, and foreign (data not shown). Soybean oil present in formulation was equally perceived before and after high pressure high temperature treatment. Furthermore, no oxidized or other foreign unexpected tones were noted in patty #1 by the panel even after treatment at 90ºC/ 675 MPa/5

min. Scorched flavor tones, developed when overcooking or burning the surface of the egg patties during manufacturing steps, were not altered after pressure treatment. All other flavor descriptors (Table 7) did not significantly change ($P>0.05$) after egg patty formulation #1 was treated at 30ºC/675 MPa/5 min. Thus, flavor profile of #1 egg patties remained unchanged after high pressure/low temperature processing. However, samples treated at 90ºC/675 MPa/5 min scored higher than control and treated patty ($P<0.05$) at 30ºC/675 MPa/5min in overall flavor, sulfur aroma, retort, unclean flavors, and aftertaste (Table 7).

High pressure, in combination with high heat (initial temperature 90ºC), generates new flavor compounds that are perceived similarly to those developed by retort processing and have an unclean lingering flavor. When egg white is heated at temperatures above 60ºC, there is an increase of –SH groups exposed from protein unfolding, which, through subsequent oxidation to S-S, release hydrogen sulfide thereby increasing sulfur aroma (Yang and Baldwin, 1995). Thus, increased sulfur aroma observed after 90ºC/675 MPa/5 min could be attributed to an increased production of H_2S. Changes seen after pressure treatment at temperatures above 70ºC indicated a need to modify the formulation to improve flavor at high pressure sterilization conditions.

3.2 Effect of pressure level on analytical quality of formulation #1 preheated at 90ºC

When #1 egg patties were treated at pressure levels 300 MPa, 500 MPa, and 675 MPa and initial pressure chamber temperature 90ºC, no significant variations were seen ($P>0.05$) in lightness L* value (Table 8) with respect to control. However, changes in Chrome value were seen, though not significant, when combining high temperature and pressure of at least 300 MPa, mostly due to the effect on yellow pigments, as explained before.

TPA descriptors, adhesiveness and springiness, were not affected by pressure processing and remained not significantly different from control (data not shown). On the other hand, TPA hardness, cohesiveness, and resilience (Table 8) were still higher ($P<0.05$) than control at lower pressurization conditions of 90ºC/300 MPa/5 min, yielding similar values to the ones obtained treating #1 egg patties at 90ºC/675 MPa/5 min (Table 8). Pressurization at 90ºC/300MPa/5min, or higher pressures, gave significantly higher serum content than egg patty formulation #1 control in all cases. Hence, even at 300 MPa, the initial high chamber/liner/product temperature of 90ºC significantly affected texture and syneresis of patty #1.

Temperatures higher than 70ºC have been reported to affect conformation of the egg proteins livetins, conalbumins, globulins, and ovomacroglobulin (Ma et al., 2001; Feiser and Cotterill, 1982) in both liquid whole eggs and cooked-frozen-thawed-reheated egg products. On the other hand, only pressures higher than 500 MPa and room temperature were shown to mainly affect the conformation of ovomacroglobulin and γ-livetins in liquid eggs (Ma et al., 2001). During the egg patty cooking process, all heat sensitive proteins in the liquid egg mix were denatured, leading to formation of a semisolid coagulum with a hardness value similar to control. Preliminary testing showed that even though initially frozen egg patties were thawed and reheated up to 90ºC, the TPA hardness values did not change. However, when cooked-frozen-thawed-reheated egg patties were treated at pressures at least 300 MPa, combined with chamber temperature 90ºC, protein gelation within the egg pre-coagulated network was accelerated, leading to a harder structure than the cooked-frozen-thawed-reheated control.

3.3. Formulation modification for quality improvement of scrambled egg patties at treatment conditions with potential for sterilization

In previous sections, it was stated that commercial egg patty formulation #1 provides adequate quality characteristics after high pressure treatment at low temperature conditions (30ºC/675 MPa/5 min) as a prospective post-packaging pasteurization process. This was also found valid for modified egg patty formulation #2. Experimental data on patty #1 showed that the combination of 675 MPa and initial chamber temperatures above 70ºC, i.e., conditions with potential for egg product sterilization, induce a rougher texture and differences in flavor compared to control. Formulation #1 was modified by adding xanthan gum as a plasticizing agent with the aim of improving water retention, thereby yielding formulation #2. The egg:water ratio was set higher in patty formulation #2 to find out if higher water content could reduce hardness and cohesiveness. Furthermore, EDTA was added to improve color retention, as well as natural and artificial flavors to evaluate whether flavor profiles are maintained after thermal pressurization sterilization conditions.

3.3.1. Appearance and color

Pressure treated patty formulation #2 scored similarly in gloss values as controls even after sterilization conditions 90ºC/675 MPa/5 min, where a process temperature of 121ºC was achieved (Table 2). This was opposed to pressure treated patty formulation #1 that increased gloss score after treatment at these sterilization conditions (Table 9). The lustrous appearance of high pressure induced egg gels (Hayashi et al., 1989; Palou, 1999) was less pronounced in the new formulation, probably due to microstructural differences caused by presence of xanthan gum. In this case, lightness (L*) did not differ significantly between formulations or after pressure treatment (Table 9). Surface appearance was not affected by pressure since no

significant differences were found in the descriptors crumbly and surface homogeneity (data not shown).

Chrome value, representing yellowness, decreased for patty formulation #1 after 70ºC/675 MPa/5 min treatment but not for patty formulation #2 at the same conditions, when comparing to the controls. Patty formulation #2 control was initially lower in yolk content, or initial concentration of yellow pigments, due to a lower egg:water ratio, thereby giving a lower initial chrome value with respect to formulation #1 (Table 9). Even though chrome value of patty formulation #2 was maintained after 70ºC/675 MPa/5 min, it decreased after 90ºC/675 MPa/5 min. Thus, xanthophylls contained in patty #2 were significantly reduced only at standard sterilization conditions 90ºC/675 MPa/5 min.

No significant differences in greening were found between patty #2 and controls after treatment at pressure chamber temperature higher than 70ºC and 675 MPa (Table 9). In this case, chelator EDTA was probably effective in formulation #2 in binding iron, preventing formation of iron green compounds after high pressure thermal treatment. Extensive syneresis has been shown to increase area of discoloration in scrambled eggs after thermal treatments (Luechapattanaporn et al., 2005); therefore, as further shown, a lesser extent of water released in formulation #2 due to HPHT treatment could help to prevent discoloration. Gossett and Baker (1981) reported that 0.03% of EDTA was optimum for preventing discoloration in whole liquid eggs with initial pH of 8.5 after cooking at 100°C for 20 min and holding over steam bath for 60 min. Similar EDTA concentrations were found by Song and Cunningham (1985) to prevent greening in retorted whole egg (121ºC, 60 min). Chiang and Yang (1999) reported that xanthan gum also helped inhibit greening in an egg white and egg yolk mixture after heat coagulation.

3.3.2. Improved texture, water retention, and mouthfeel

TPA analysis showed lower hardness values in modified formulation #2 (41% and 25% lower after 675 MPa and initial chamber temperature 70ºC and 90ºC, respectively) than patty #1 (Table 10). However, firmness and density differences (Fig. 2) determined by the panel were non-significant for formulations #1 and #2 at pressure 675 MPa and initial temperatures above 70ºC, being all values higher than controls ($P<0.05$). No previous literature reported the effects of thermal-high pressure treatments on interactions between xanthan gum and egg proteins.

No difference in firmness was found between the controls of formulation #1 and #2, regardless of presence of xanthan gum. However, TPA hardness and cohesiveness were lower in formulation #2 control (Table 10). O'Brien et al. (1982) showed that scrambled egg omelets with added xanthan gum (0.5-1.5%) had higher tenderness levels. Higher water content in formulation # 2 control, where xanthan gum bound water, also helped to provide lower hardness and cohesiveness than in control patty formulation #1. Beveridge et al. (1980) found that firmness of egg coagulum decreases with higher dilution, i.e., a lower egg:water ratio.

Previous work on xanthan gum solutions (0.25%-1.5%) treated at pressures around 400 MPa showed that viscosity of solutions was not affected by pressure treatment (Ahmed and Ramaswamy, 2004). However, xanthan gum's helical structure is temperature dependent (Pelletier et al., 2001) and can stretch at higher temperatures, therefore it can attain higher viscosity values when exposed to combined high pressure and high temperature conditions. A gum with higher viscosity dispersed within the egg matrix, prevented further protein gelation, thereby providing a less hard structure. Hence, even though the descriptive panel did not find

HPHT treated patties with added xanthan gum less firm, the TPA test showed significantly lower hardness in these patties.

The trained panel gave patty #2 a lower score ($P<0.05$) in particle size and mouthfeel roughness than patty #1 when both were treated at 70ºC/675 MPa/5 min (Fig. 1 and 2). Furthermore, formulation #2 treated at 70ºC/675 MPa/5 min did not differ from the controls, probably due to presence of xanthan gum and increased water content. TPA resilience (or elasticity during the first byte) of pressure-treated patty #2, was higher than its control #2. However, TPA adhesiveness and springiness values did not significantly change after pressure treatment (data not shown).

Patty formulation #2, treated at pressure chamber temperature higher than 70ºC and 675 MPa had no significantly different scores in greasy and astringent descriptors with respect to controls, as opposed to HPHT treated formulation #1, which was higher in both descriptors. Xanthan gum and artificial flavors modifying formulation #2 might have masked tangy and tingling sensations present in patty #1 after high pressure thermal treatment. Slimy, silky, and oily sensations associated with a greasy mouthfeel are also related to addition of xanthan gum and flavors. Mouthfeel descriptors pasty and mouth coating did not change significantly ($P>0.05$) after thermal pressurization with respect to controls (data not shown).

The sensory panel found syneresis in #2 patty to be low, although significant differences were detected between pressure treated samples at 90ºC/675 MPa/5 min and controls (Table 11). When quantified as %weight loss, water released due to HPHT conditions was significantly decreased in egg patty formulation #2 with respect to patty #1 by 50-55%. High variability

observed in % weight loss at 90ºC/675 MPa/5 min can be attributed to the higher temperature which, under high pressure conditions, affected water binding components differently, therefore, the extent of water retention within the egg matrix. Previous studies on precooked-frozen-reheated omelets (O'Brien et al., 1982) proved xanthan gum to effectively reduce expressible moisture, supporting results shown in Table 11. This significant increase in water retention found in patty formulation #2 after HPHT treatment coincided with lower dryness compared to formulation #1, mainly observed after 70ºC/675 MPa/5 min (Fig. 1 and 2). Xanthan gum's effect on moisture retention after high pressure thermal treatment can also be associated with decreased values in hardness.

3.3.3. Improved flavor

As previously mentioned in section 3.1.3., patty #1 scores for oily, oxidized, scorched and foreign flavor tones were not significantly affected by HPHT treatment at temperatures above 70ºC (data not shown). The same was observed in formulation #2 after high pressure thermal treatment. Moreover, natural and artificial flavors added in formulation #2 changed the panel's perception of high pressure thermally treated egg patties to flavor/aroma profiles similar to preheated control (Table 12). It is possible that added flavors masked the retort effects, lingering flavors, and aromas developed at high pressure thermal conditions, as noted in the case of formulation #1.

During the preparation of egg patties, xanthan gum was initially dispersed throughout the protein matrix. Xanthan gum allocated within matrix could prevent the free –SH groups formed during protein unfolding (Yang and Baldwin, 1995) from following subsequent oxidation to S-S. In this

way, the release of hydrogen sulfide was decreased, thereby decreasing sulfur tones to scores within the range of standard (commercial) control.

Modification of formulation with added flavors facilitated obtaining high pressure thermally treated products with profiles similar to preheated control. These results show that adequate product formulation can ensure egg products maintain their quality after sterilization treatment using high pressure thermal processing.

4. Conclusions

Scrambled egg patties from standard and modified formulations maintained overall quality (appearance, texture, and flavor) after high pressure low temperature treatment (30ºC/675 MPa/5 min). Hence, postpackaging pasteurization of commercial egg patties has definite potential in applications using high pressure processing at chamber temperatures within the range of 30ºC at 675 MPa.

The standard egg patty formulation tested was not adequate for high pressure/high temperature treatment conditions (pressure chamber temperature ≥70ºC). Even though appearance was maintained, sensory descriptors for color, texture, and flavor were significantly altered. In particular, the firmer, denser, and rougher structure obtained after 70ºC/675 MPa/5 min treatment, also associated with higher syneresis, was indicative of a need to modify patty formulation. High temperature ≥70ºC in combination with applied pressure of 675 MPa enhanced further gelation of pre-coagulated egg protein network. Even at a much lower pressure of 300 MPa, combined with pressure chamber temperature of 90ºC, analytical texture profile analysis descriptors were equally altered giving similar values as for higher pressures.

Modification of standard egg patty formulation by adding xanthan gum, EDTA, and flavors provided better color, texture, and flavor retention after pressure treatment at sterilization conditions (>70ºC and 675 MPa). While xanthan gum addition helped reduce hardness (25-41%) and syneresis (50-55%), addition of EDTA prevented greening. Use of natural and artificial flavors helped to match the flavor tones obtained in egg patties preheated only. Even at initial chamber temperature of 90ºC, which corresponds to a standard sterilization temperature of 121ºC at 675 MPa, quality improvements could be attained for scrambled egg patties through reformulation, giving sensory profiles more similar to the preheated only patties.

Thus, high pressure processing not only offers the possibility of producing pasteurized egg patties intended for storage under refrigerated conditions, but also offers a promising approach for development of shelf stable egg products produced by combining pressures around 700 MPa and chamber pressure temperatures greater than 70ºC ; not yet been accomplished using conventional thermal processing methods. Future studies, should focus not only on verifying the final safety of precooked egg products after high pressure thermal treatment, but also on testing consumer acceptability and shelf life in selected formulations.

References

AHMED, J., RAMASWAMY, H.S., ALLI, I. and NGADI, M. 2003. Effect of high pressure on rheological characteristics of liquid egg. Lebensm -Wiss Technol. *36*(5), 517-524.

AHMED, J. and RAMASWAMY, H.S. 2004. Effect of high-hydrostatic pressure and concentration on rheological characteristics of xanthan gum. Food Hydrocoll. *18*(3), 367-373.

AMERICAN EGG BOARD (AEB). 2003. Egg Products Buyer's Guide, pp. 25-34, American Egg Board, Park Ridge, IL.

BAKER, R.C. and BRUCE, C. 1995. Development of value-added products. In: *Egg science and technology*, (W.J. Stadelman and Cotterill O.J., eds.), pp. 499-524, Food Products Press, New York.

BALIGA, B.R., RAO, A.S. and LAHIRY, N.L. 1969. Prevention of browning in hard boiled eggs during canning. J. Food Sci. and Technol. *6*(3), 200-204.

BEVERIDGE, T., ARNTFIELD, S., KO, S. and CHUNG, J.K.L. 1980. Firmness of heat induced albumen coagulum. Poultry Sci. *59*(6), 1229-1236.

BOURNE, M. 2002. *Food Texture and Viscosity. Concept and Measurement,* pp. 182-186, Academic Press, New York.

CANO, M.P. and DE ANCOS, B. 2005. Advances in the use of high pressure to process and preserve plant foods. In: *Novel Food Processing Technologies,* (G.V. Barbosa-Cánovas, M.S. Tapia and M.P. Cano, eds.), pp. 283-310, CRC Press New York.

ELBE, J.H.V. 1986. Chemical changes in plant and animal pigments during food processing. In: *Role of Chemistry in the Quality of Processed Food*, (O.R. Fennema, W.H. Chang and C.Y. Lii, eds.), pp. 41–64, Food and Nutrition Press, Westport, Connecticut.

GUAMIS, B., PLA, R., TRUJILLO, A.J., CAPELLAS, M., GERVILLA, R., SALDO, J. and YUSTE, J. 2005. High Pressure Processing of Milk and Dairy and Egg Products. In: *Novel Food Processing Technologies,* (G.V. Barbosa-Cánovas, M.S. Tapia and M.P. Cano, eds.), pp. 343-360, CRC Press, New York.

GOLA, S., FOMAN, C., CARPI, G., MAGGI, A., CASSARA, A. and ROVERE, P. 1996. Inactivation of bacterial spores in phosphate buffer and in vegetable cream treated with high pressure. High Press. Biosci. Biotechnol., Kyoto, Japan. 253-259.

GOSSETT, P.W. and BAKER, R.C. 1981. Prevention of the green-gray discoloration in cooked liquid whole eggs. J. Food Sci. *46*(2), 328-331.

GUJRAL, H.S., ROSELL, C.M., SHARMA, S., SINGH, S. 2003. Effect of sodium lauryl sulphate on the texture of sponge cake. Food Sci. Technol. Int. *9*(2), 89-93.

FEISER, G.E. and COTTERILL, O.J. 1982. Composition of serum from cooked-frozen-thawed-reheated scrambled eggs at various pH levels. J. Food Sci. *47*(4), 1333-1337.

HAYASHI, R., KAWAMURA, Y., NAKASA, T. and OKINAKA, O. 1989. Application of high pressure to food processing: Pressurization of egg white and yolk, and properties of gels formed. Agric. Biol. Chem. 53, 2935-2939.

INDRAWATI, VAN LOEY, A. and HENDRICKX, M. 2004. High Pressure Processing. In: *Nutrition Handbook for Food Processors*, (C.J.K. Henry and C. Chapman, eds.), pp. 440-448, Woodhead Publishing, New York.

KREBBERS, B., MATSER, A.M., KOETS, M. and VAN DEN BERG, R.W. 2002. Quality and storage stability of high-pressure preserved green beans. J. Food Eng. *54*(1), 27-33.

KREBBERS, B., MATSER, A.M., HOOGERWERF, S.W., MOEZELAAR, R., TOMASSEN, M.M.M. and VAN DEN BERG, R.W. 2003. Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. Innov. Food Sci. Emerg. Technol. *4*(4), 377-385.

LEE, D.U., HEINZ, V. and KNORR, D. 1999. Evaluation of Processing Criteria for the High Pressure Treatment of Liquid Whole Egg: Rheological Study. Lebensm –Wiss. Technol. *32*(5), 299-304.

LI-CHAN, E.C.Y., POWRIE, W.D. and NAKAI, S. 1995. The chemistry of eggs and egg products. In: *Egg Science and Technology*, (Stadelman W.J., Cotterill O.J., eds.), pp. 105–175, Food Product Press, New York.

LUECHAPATTANAPORN, K., WANG, Y., WANG, J., TANG, J., HALLBERG L. M. and DUNNE, C.P. 2005. Sterilization of scrambled eggs in military polymeric trays by radio frequency energy. J. Food Sci. *70*(4), 288-294.

MA, L., CHANG, F.J., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 2001. Comparison Study of Pulsed Electric Fields, High Hydrostatic Pressure, and Thermal Processing on the Electrophoretic Patterns of Liquid Whole Egg. In: *Pulsed electric Fields in Food Processing: Fundamental Aspects and Applications*, (G.V. Barbosa-Cánovas and H. Zhang, eds.), pp. 225-240, Technomic Publishing Co., Inc., Lancaster, Pennsylvania.

MARGOSCH, D. 2005. Behavior of bacterial endospores and toxins as safety determinants in low acid pressurized food. Dissertation, TU Berlin, Germany.

MARGOSCH, D., EHRMANN, M.A., GAENZLE, M.G., VOGEL, R.F. 2004. Comparison of pressure and heat resistance of *Clostridium botulinum* and other endospores in mashed carrots. J. Food Prot. *67*(11), 2530-2537.

MATSER, A.M., KREBBERS, B., VAN DEN BERG, R.W. and BARTELS, P.V. 2004. Advantages of high pressure sterilization on quality of food products. Trends Food Sci. Technol. *15*(2), 79-85.

MESSENS, W., VAN CAMP, J. and HUYGHEBAERT, A. 1997. The use of high pressure to modify the functionality of food proteins. Trends Food Sci. Technol. *8*, 107–112.

MONTEJANO, J.G., HAMANN, D.D. and LANIER T.C. 1985. Comparison of two instrumental methods with sensory texture of protein gels. J. Texture Stud. *16*(4), 403-424.

OKAMOTO M., KAWAMURA Y. and HAYASHI, R. 1990. Application of high pressure to food processing: textural comparison of pressure- and heat-induced gels of food proteins. Agric. Biol. Chem. *54*(1), 183-189.

PARASKEVOPOULOU, A. and KIOSSEOGLOU, V. 1997. Texture profile analysis of heat-formed gels and cakes prepared with low cholesterol egg yolk concentrates. J. Food Sci. *62*(1), 208-211.

PELLETIER, E., VIEBKE, C., MEADOWS, J. and WILLIAMS, P.A. 2001. A rheological study of the order-disorder conformational transition of xanthan gum. Biopolymers. *59*(5), 339-346.

PONCE, E., PLA, R., MOR-MUR, M., GERVILLA, R. and GUAMIS, B. 1998. Inactivation of *Listeria innocua* inoculated in liquid whole egg by high hydrostatic pressure. J. Food Prot. *61*, 119–122.

RASO, J., BARBOSA-CÁNOVAS, G. V. and SWANSON, B.G. 1998. Sporulation temperature affects initiation of germination and inactivation by high hydrostatic pressure of Bacillus cereus. J. Appl. Microbiol. 85, 17-24.

REDDY, N. R., SOLOMON, H. M., FINGERHUT, G. A., RHODEHAMEL, E. J., BALASUBRAMANIAM, V. M. and PALANIAPPAN, S. 1999. Inactivation of *Clostridium Botulinum* type E spores by high pressure processing. J. Food Saf. *19*, 277-288.

REDDY, N.R., SOLOMON, H.M., TETZLOFF, R.C., RHODEHAMEL, E.J., BALASUBRAMANIAM, V.M. and PALANIAPPAN, S. 2003. Inactivation of *Clostridium botulinum* type A spores by high-pressure processing at elevated temperatures. J. Food Prot. 66, 1402–1407.

ROVERE, P., GOLA, S., MAGGI, A., SCARAMUZZA, N. and MIGLIOLI, L. 1998. Studies on bacterial spores by combined pressure-heat treatments: Possibility to sterilize low acid foods. In: *High Pressure Food Science, Bioscience and* Chemistry, (N.S. Isaacs, ed.), pp. 354-363, The Royal Society of Chemistry, Cambridge.

SAN MARTIN, M.F., BARBOSA-CÁNOVAS, G.V. and SWANSON, B.G. 2002. Food processing by high hydrostatic pressure. Crit. Rev. Food Sci. Nutr. *42*(6), 627-645.

STIPPL, V.M., DELGADO, A. and BECKER, T.M. 2004. Development of a method for the optical in-situ determination of pH value during high-pressure treatment of fluid food. Innov. Food Sci. Emerg. Technol. *5*(3), 285-292.

TAKI, Y., AWANO, T., TOBA, S. and MITSUURA, N. 1991. Sterilization of *Bacillus sp.* spores by hydrostatic pressure. In: *High Pressure Science for Food*, (R. Hayashi, ed.), pp. 217-224, Sanei Pub., Co., Kyoto, Japan.

WESLEY, R.D., ROUSSELLE, J.R., SCHWAN, D.R. and STADELMAN, W.J. 1982. Improvement in quality of scrambled egg products served from steam table display. Poultry Sci. *61*(3), 457-462.

WOODWARD, S.A. and COTTERILL, O.J. 1986. Texture and microstructure of heat-formed egg white gels. J. Food Sci. *51*(2): 333-339.

Table 1. Experimental design for selected formulations and processing conditions.

Exp.	Treatment combinations	Levels	Design
1.	Processing	Control, Pressure (675 MPa, 5 min)	2 x 4 x 2
	x Initial chamber/product temp.	30ºC, 50ºC, 70ºC, 90ºC	factorial
	x Formulation	#1, #2	3 replicates
2.	Processing	Control, Pressure (chamber 90ºC, 5 min)	2 x 4 x 1
	x Pressure	0.1 MPa, 300 MPa, 500 MPa, 675 MPa	factorial
	x Formulation	#1	2 replicates

Table 2. Temperature profile at different temperature-pressure combinations inside liner.

Pressure	Initial (ºC)	In-Process Initial[a] (ºC)	Final (ºC)[b]
300 MPa	90.6 ± 3.0	106.4 ± 3.3	88.2 ± 2.9
500 MPa	90.9 ± 1.3	114.7 ± 1.6	85.8 ± 1.5
675 MPa	30.1 ± 1.5	50.2 ± 1.9	27.6 ± 1.9
675 MPa	51.5 ± 0.5	74.9 ± 1.5	47.4 ± 1.2
675 MPa	70.4 ± 3.2	98.4 ± 3.6	66.8 ± 2.1
675 MPa	91.0 ± 2.4	121.0 ± 3.5	83.3 ± 0.1

[a]Initial temperature at 675 MPa

[b]Temperature after pressure was released

Table 3. Terms used by trained panel for evaluation of appearance, texture/mouthfeel, and flavor/aroma of egg products.

Minimum intensity (0)	Maximum intensity (14)
Appearance	
Dull	Glossy
Yellow	Grey-green
Smooth surface	Rough surface
Cohesive	Crumbly
No syneresis	Oily/viscous fluid
Texture/Mouthfeel	
Light (airy)	Dense
Mushy	Firm/Rubbery (Hard)
Smooth mouthfeel	Rough mouthfeel
Tiny particle size	Large particle size
Moist	Dry
No astringency	Astringent/ Mouth drying
Not oily	Oily
Not pasty	Pasty (very tacky/sticky)
No residual mouth coating	Pronounced mouth coating
Flavor/aroma	
Bland flavor	Pronounced overall flavor
Not salty	Salty
No butter	Butter flavor
No oil	Oily
Not sulfurous	Skunky (strong sulfur odor)
Rightly cooked	Over-cooked (scorched)
Not butterscotch	Butterscotch (caramelized)
No acid flavor	Acid
Not rancid	Rancid (hydrolytic)
Not oxidized	Oxidized (fishy)
Not retort	Retort flavor
No black pepper	Black pepper
No unexpected flavor	Foreign
Clean flavor	Unclean (unpleasant) flavor
No lingering flavor	Pronounced aftertaste

Table 4. Color and appearance of patty formulation #1 as indicated by colorimeter (L*, chrome) and sensory panel. Different letters indicate significant differences between mean values ($P<0.05$) within individual columns.

Treatment*	Color (Analytical)		Color (Panel)		Appearance (Panel)	
	L*	Chrome	Gloss	Green	Surface homogeneity	Crumbly appearance
Control	77.3 ± 0.8 a	35.6 ± 0.9 a	4.5 ± 0.6 a	1.0 ± 0.4 a	3.5 ± 1.0 a	0.5 ± 0.1 a
30ºC/ 675 MPa	79.5 ± 0.9 a	34.2 ± 1.1 ab	5.2 ± 1.1 ab	1.4 ± 0.7 a	6.2 ± 2.0 a	0.1 ± 0.3 a
70ºC/ 675 MPa	80.9 ± 0.7 a	32.1± 0.9 b	7.0 ± 1.6 ab	3.4 ± 0.9 b	4.6 ± 2.2 a	0.0 ± 0.3 a
90ºC/ 675 MPa	78.7 ± 0.7 a	32.2 ± 0.9 b	8.8 ± 1.6 b	3.6 ± 0.8 b	6.7 ± 2.2 a	0.0 ± 0.3 a

Table 5. Texture profile analysis of egg patty formulation #1. Comparison between control and high pressure treated patties at 675 MPa and initial temperatures 30ºC, 50ºC, 70ºC, and 90ºC. Different letters indicate significant differences between mean values ($P<0.05$) within individual columns.

Treatment	Hardness (N)	Cohesiveness (dimensionless, 10^2)	Adhesiveness (N.mm, 10^2)	Springiness (dimensionless, 10^2)	Resilience (dimensionless, 10^2)
Control	22.5 ± 2.2 a	69.5 ± 1.2 a	-43.2 ± 9.1 a	87.9 ± 7.8 a	24.0 ± 1.6 a
30ºC/ 675 MPa	23.0 ± 3.2 a	73.3 ± 1.7 b	-29.7 ± 12.8 a	88.0 ± 11.0 a	27.9 ± 2.3 b
50ºC/675 MPa	39.8 ± 2.6 b	72.2 ± 1.4 b	-44.0 ± 10.4 a	90.2 ± 9.2 a	32.1 ± 1.9 b
70ºC/ 675 MPa	51.6 ± 2.0 c	73.9 ± 1.1 b	-37.1 ± 9.1 a	93.1 ± 7.0 a	29.6 ± 1.5 b
90ºC/ 675 MPa	53.6 ± 1.8 c	74.5 ± 1.0 b	-30.6 ± 7.4 a	96.4 ± 6.4 a	30.4 ± 1.3 b

Table 6. Serum measured by panel (syneresis) and calculated using Eq. 1 from weight loss after preheat or treatment at 675 MPa and selected temperatures for 5 min. Different letters indicate significant differences between mean values ($P<0.05$) within individual columns.

Treatment	Serum	
	Syneresis (panel)	Weight loss (%)
Control	1.0 ± 0.6 a	1.7 ± 1.1 a
30ºC/ 675 MPa	0.5 ± 1.1 a	2.3 ± 1.6 a
50ºC/675 MPa	N/A	9.8 ± 1.3 b
70ºC/ 675 MPa	3.5 ± 1.2 b	12.5± 0.8 b
90ºC/ 675 MPa	3.2 ± 1.2 b	13.3 ± 1.0 b

Table 7. Flavor descriptors found significantly different when comparing egg patty formulation #1 control with high pressure treated patties at 675 MPa and 30ºC, 70ºC, and 90ºC. Different letters indicate significant differences between mean values ($P<0.05$) within individual columns.

Treatment	Overall flavor	Sulfur aroma	Retort	Unclean flavors	Aftertaste
Control	7.6 ± 0.5 a	3.9 ± 0.4 a	2.2 ± 0.9 a	1.0 ± 0.6 a	4.6 ± 0.6 a
30ºC/ 675 MPa	8.7 ± 1.0 ab	4.0 ± 0.9 a	0.8 ± 1.8 a	0.5 ± 1.2 a	3.0 ± 1.2 a
70ºC/ 675 MPa	8.8 ± 1.0 ab	6.3 ± 1.0 ab	5.2 ± 2.0 ab	5.4 ± 1.3 b	5.7 ± 1.3 ab
90ºC/ 675 MPa	10.8 ± 1.0 b	7.8 ± 1.0 b	7.5 ± 2.0 b	5.3 ± 1.3 b	8.8 ± 1.3 b

Table 8. Effect of pressure applied on patties treated at initial temperature 90ºC and pressures 300 MPa, 500 MPa, and 675 MPa for 5 min. Different letters indicate significant differences between mean values ($P<0.05$) within individual columns.

Treatment	L* (lightness)	Chrome (dimensionless)	Serum (% weight loss)	Hardness (N)	Cohesiveness (dimensionless, 10^2)	Resilience (dimensionless, 10^2)
Control	77.3 ± 1.2 a	35.6 ± 1.1 a	1.7 ± 1.3 a	22.5 ± 2.6 a	69.5 ± 1.3 a	24.0 ± 1.5 a
90ºC/ 300 MPa	78.0 ± 1.6 a	31.1 ± 1.4 ab	14.3 ± 1.9 b	50.1 ± 3.0 b	74.7 ± 1.4 b	29.1 ± 1.7 b
90ºC/ 500 MPa	75.8 ± 1.6 a	32.6 ± 1.4 ab	13.2 ± 1.9 b	55.2 ± 3.0 b	74.2 ± 1.4 b	33.0 ± 1.7 b
90ºC/ 675 MPa	78.7 ± 1.3 a	31.2 ± 1.0 b	13.3 ± 1.2 b	53.6 ± 2.1 b	74.5 ±1.3b	30.4 ± 1.2 b

Table 9. Comparison of selected scrambled egg patty formulations untreated and treated at 70ºC/675 MPa/5 min and 90ºC/675 MPa/5 min. Different letters show significant differences between mean values ($P<0.05$) within individual columns.

Patty	Treatment	Color (Analytical)		Color (Panel)	
		L* (lightness)	Chrome	Gloss	Green
#1	Control	77.3 ± 0.7 a	35.6 ± 0.9 a	4.5 ± 0.8 a	1.0 ± 0.5 a
#2	Control	77.7 ± 0.8 a	32.3 ± 1.0 b	5.6 ± 1.5 ab	0.7 ± 1.1 a
#1	70ºC / 675 MPa	80.8 ± 0.6 a	32.1 ± 0.7 b	7.0 ± 1.6 ab	3.4 ± 0.9 b
#2	70ºC / 675 MPa	80.4 ± 1.1 a	33.6 ± 1.4 b	6.7 ± 1.4 ab	2.2 ± 1.1 ab
#1	90ºC / 675 MPa	78.7 ± 0.6 a	31.2 ± 0.8 bc	8.8 ± 1.6 b	3.6 ± 0.9 b
#2	90ºC / 675 MPa	79.6 ± 0.9 a	28.8 ± 1.2 c	6.0 ± 1.4 ab	2.6 ± 0.8 ab

Table 10. Texture profile analysis of different scrambled egg patty formulations treated at 70ºC and 675 MPa. Different letters indicate significant differences between mean values ($P<0.05$) within individual columns.

Patty	Treatment	Hardness (N)	Cohesiveness (dimensionless, 10^2)	Resilience (dimensionless, 10^2)
#1	Control	22.5 ± 2.7 b	69.5 ± 1.6 b	24.0 ± 2.0 a
#2	Control	10.8 ± 3.1 a	52.9 ± 1.8 a	19.7 ± 2.4 a
#1	70ºC / 675 MPa	51.6 ± 2.4 e	73.9 ± 1.4 c	29.6 ± 1.8 b
#2	70ºC / 675 MPa	30.0 ± 2.1 c	73.5 ± 1.2 c	34.2 ± 1.6 b
#1	90ºC / 675 MPa	53.6 ± 1.7 e	74.5 ± 1.3 c	33.0 ± 1.6 b
#2	90ºC / 675 MPa	40.0 ± 1.7 d	75.8 ± 1.2 c	30.4 ± 1.6 b

Table 11. Serum measured by panel (visual syneresis) and calculated using Eq. 1 from weight loss after preheat or high pressure thermal treatment in formulations #1 and #2. Different letters indicate significant differences ($P<0.05$) within individual columns.

		Serum	
Patty	Treatment	Syneresis (panel)	Weight loss (%)
#1	Control	1.0 ± 0.6 a	1.7 ± 0.8 a
#2	Control	2.0 ± 1.2 a	1.9 ± 0.9 a
#1	70ºC / 675 MPa	3.5 ± 1.2 ab	12.5 ± 0.8 c
#2	70ºC / 675 MPa	3.2 ± 1.2 ab	6.3 ± 0.8 b
#1	90ºC / 675 MPa	3.2 ± 1.2 ab	13.3 ± 1.0 c
#2	90ºC / 675 MPa	3.8 ± 1.2 b	5.8 ± 1.6 b

Table 12. Flavor and aroma descriptors found significantly different when comparing egg patty formulations #1 and #2 before and after HPHT treatments. Different letters indicate significant differences between mean values ($P<0.05$) within individual columns.

Patty	Treatment	Sulfur aroma	Retort	Unclean flavors	Aftertaste
#1	Control	3.9 ± 0.4 a	2.2 ± 0.9 a	1.0 ± 0.6 a	4.6 ± 0.6 a
#1	90ºC/ 675 MPa	7.8 ± 1.0 b	7.5 ± 2.0 b	5.3 ± 1.3 b	8.8 ± 1.3 b
#2	Control	5.3 ± 1.2 ab	0.3 ± 1.8 a	0.4 ± 1.1 a	4.1 ± 1.3 a
#2	70ºC/ 675 MPa	5.3 ± 1.0 ab	3.1 ± 1.8 ab	1.3 ± 1.1 a	3.9 ± 1.3 a
#2	90ºC/ 675 MPa	6.8 ± 1.2 ab	2.8 ± 1.8 ab	1.1 ± 1.1 a	7.1 ± 1.3 ab

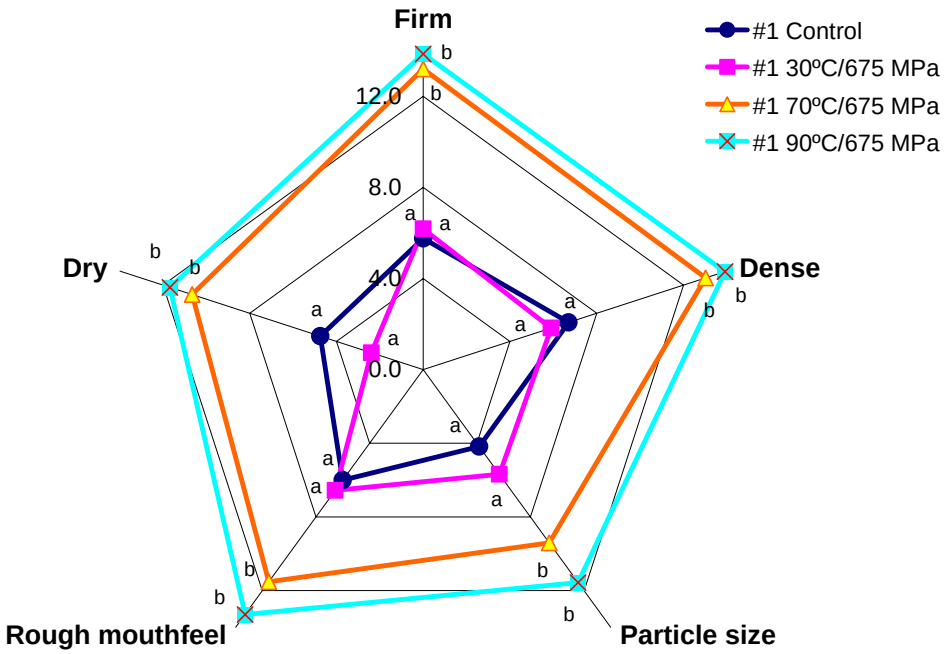

Fig. 1. Texture profile for egg patty formulation #1. Comparison between control and high pressure treated patties at 675 MPa and initial temperatures 30ºC, 70ºC, and 90ºC. Different letters indicate significant differences between mean values ($P<0.05$) in the 0 to 14 scale.

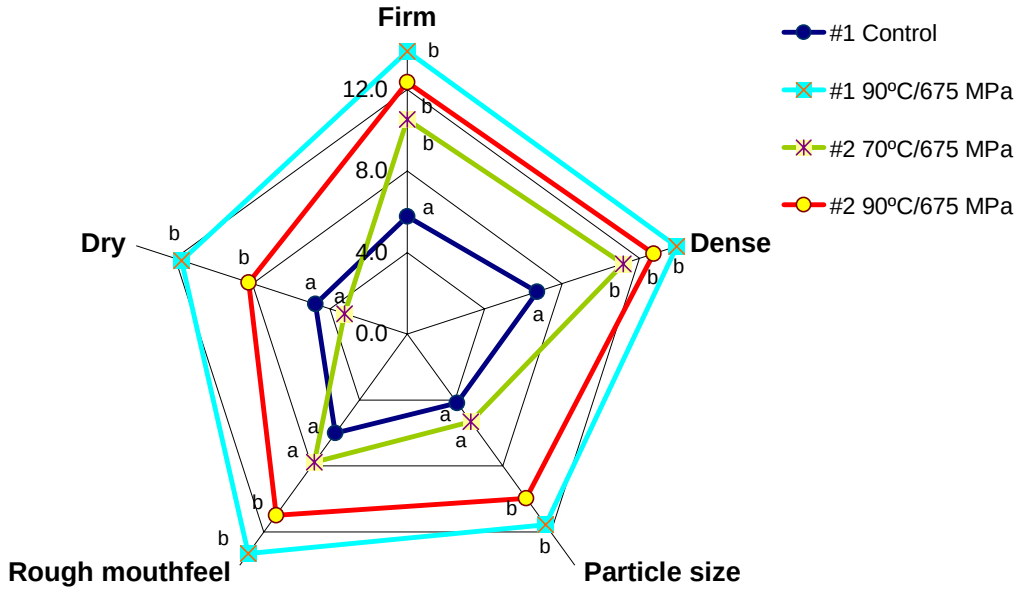

Fig. 2. Texture and mouthfeel profile as detected by the sensory panel. Comparison between control #1, formulation #1 after 90ºC/675 MPa/5min, and #2 treated at 70ºC/675 MPa/5min and 90ºC/675 MPa/5min. Significant differences are indicated using different letters ($P<0.05$) in the 0 to 14 scale.

CHAPTER THREE

Texture and water retention improvement in high pressure thermally treated scrambled egg patties

Pablo. Juliano, Mònica Toldrà, Tatiana Koutchma, V.M. Balasubramaniam, Stephanie Clark, Jason Mathews, C. Patrick Dunne, George Sadler, and Gustavo V. Barbosa-Cánovas

1. Introduction

High Pressure High Temperature (HPHT) treatment or Pressure Assisted Thermal Processing (PATP) is emerging as an alternative processing method for the development and production of low-acid shelf-stable food products. Based on the combination of high pressures of 600-800 MPa, and moderate initial temperatures of 60-90ºC, HPHT treatment is known to eliminate vegetative and spore-forming microorganisms in a shorter time than conventional thermal processing (Matser and others 2004). Meyer and others (2000) showed that combination of elevated pressures and heat (105ºC or greater), in combination with high pressure, yields faster inactivation of bacterial spores. Furthermore, Margosch and others (2004) proved that 600 MPa, at a process temperature of 100ºC for 2 min, can reduce *C. botulinum* spore counts by more than 5.5 log units, and concluded that HPHT processing is promising to produce commercially sterilized foods at reduced temperatures.

B. amyloliquefaciens has proven to be more resistant to the HPHT process than *C. botulinum* strains, and was suggested as a potential surrogate for sterilization studies due to its non-toxigenic nature (Margosch and other, 2004; Margosch, 2005). Rajan and others (2005 a and b) demonstrated that a process temperature of 105 ºC and 700 MPa for 5 min can accelerate the inactivation of *B. amyloliquefaciens* spores and *B. stearothermophilus* spores suspended in egg.

D value of both spores decreased considerably with increased process pressure. These results are in agreement with Koutchma and others (2005), who determined a 6 log inactivation of *B. stearothermophilus* in scrambled egg patties at 105 ºC and 700 MPa for 5 min. They also showed that 110 ºC and 700 MPa for 5 min are sufficient to achieve 6 log reduction of *C. sporogenes* PA3679 in egg patties. Furthermore, Ahn and others (2005) obtained up to 7-8 log reduction of several *Clostridium* and *Bacillus* surrogate spores including *B. amyloliquefaciens* after subjecting them to a combination treatment at 700 MPa and 121°C for less than 1 min.

Other authors have also proven these conditions to be effective to inactivate *Bacillus stearothermophilus* (Gola and others 1996; Rovere and others 1998; Meyer and others 2000, Heinz and Knorr 2001; Krebbers and others 2003), *Bacillus subtillis* (Balasubramaniam and Balasubramaniam 2003), *Bacillus lincheniformis* (Taki and others 1991; Gola and others 1996; Rovere and others 1998), and *Bacillus cereus* (Raso and others 1998; Rovere and others 1998; Meyer and others 2000). A number of low-acid foods such as meat, milk, and vegetable products treated at sterilization conditions were found to provide more desirable texture, color, and flavor and aroma retention in comparison to traditional retorted products, and in some cases to frozen products (Hoogland and others 2001; Krebbers and others 2002, 2003; Matser and others 2004).

Shelf-stable egg breakfast items have found a new niche in the ready-to-eat meal market, especially as military rations. However, commercial sterilization using conventional retort has not been successful due to the green-gray discoloration of the egg products affecting appearance, that is, the formation of iron-sulfur compounds (Song and Cunningham 1985), the development of off-flavors, and the syneresis or exudation of water after heat treatment (Cotterill 1995). HPHT treatment offers a promising alternative for the development of shelf-stable precooked

egg patties since color and appearance of selected scrambled egg patty formulations can be maintained after 700 MPa and a process temperature of 98ºC for 5 min (Juliano and others 2004). However, negative effects on the texture and water retention capacity were detected as the major problem in product acceptability due to the formation of a compact rubbery structure.

It has yet to be proven if addition of selected ingredients can contribute to maintain water holding capacity in scrambled egg-based foods after HPHT treatment. For example, xanthan gum can associate with proteins and improve water holding capacity, thus reducing syneresis (Cotterill 1995; Montero and others, 2001). Cheese is often used as a functional ingredient because of its ability to enhance mouthfeel and improve flavor (Lucey and others 2003). Water addition, either by dilution of the egg mix or addition to the coagulated structure, has been proposed as a way to increase egg coagulation temperature and decrease firmness (Beveridge 1980; Yang and Baldwin 1995).

Vacuum level of the package containing the egg product can also be another factor affecting texture and water release due to the additional vacuum pressure inside the packaging during and after HPHT treatment. Several authors have detected an effect of vacuum packaging at different levels after storing products like Asia bell roots, cheese, and bologna sausage (Park and Lee 2001; Bertola and others 1995; Claus and Hunt 1991).

Another important aspect that might directly influence texture and water retention capacity is the preheating method used prior to HPHT processing, including the subsequent temperature equilibration applied to the food product for cold spot elimination. In order to maximize quality retention, it is desirable to minimize the duration of preheating needed to reach the target

temperature inside the product. Hoogland and others (2001) proposed the use of direct steam injection or microwave heating to shorten preheating time and therefore obtain better quality after HPHT process.

Therefore, the objective of this study was to test selected approaches for improving texture and reducing syneresis of egg products to commercially standard levels after HPHT treatment. The effects of modifying scrambled egg patty formulations, manufacturing conditions, added water, vacuum packaging level, and preheating method, on texture characteristics and degree of syneresis of scrambled egg patties after HPHT processing were evaluated.

2. Materials and methods

2.1 Egg-based products preparation

Michael Foods Egg Products Company (Gaylord, Minn., U.S.A.) provided four types of commercial scrambled egg patties: a basic formulation (#1), a round egg patty added with process cheese (#2), and two egg patties of the same formulation, added with xanthan gum, modified in round and square shapes (#3 and #4, respectively). Table 1 shows the ingredients and dimensions of each 42.5 ± 7.1 g egg patty. Patty #1 is the standard Michael Foods patty and it was used as the basic formulation for developing formulations of patties # 2, 3, and 4.

The preparation of precooked scrambled egg products has been reported in recent patents developed by Michael Foods (Knipper and others 2002; Merkle and others 2003 a, b). In particular, Knipper and others (2002) explained the production process of the precooked egg patties. Whole eggs are mixed with dry and liquid ingredients, and then the mix is pumped in a mold within a flat cooking belt. Egg mix portions are cooked (preformed) in a convection oven

at between 180 and 250 ºC for a predetermined time, and then frozen and packaged. Preparation of patties #3 and #4 (xanthan gum) required two different systems that allowed the liquid egg mix remain with different amounts of air before the cooking process, thereby providing different pore sizes in the egg matrices. This pore size difference was clearly detectable with the naked eye. The square patty #4 was more gel (custard) like and the round patty had a spongy appearance (Table 1).

Handling and shipping procedure for the scrambled egg patties was performed as it would be required in an industrial setting, where patties were stored in frozen state before HPHT treatment. Frozen samples from a single lot were received from Michael Foods (Gaylord, Minn., U.S.A.) and stored frozen at -30 ºC. Each patty was then repackaged in flexible pouches of 127 mm x 127 mm (ALCAN, Chicago, Ill., U.S.A.), and defrosted overnight at 5 ºC. The packaging material composition was Biaxial Nylon / Adhesive / 5.0 ml EVOH/ Coextruded Sealant. Samples were kept refrigerated until HPHT treatments.

2.2 Processing conditions

Besides varying formulations, other processing conditions were tested to gain understanding on texture and water retention improvement of egg patties after HPHT treatment. Four factorial experiments (Table 2) were designed to study the effects of HPHT processing, patty preforming method (egg patty initial porosity), vacuum packaging, water addition, and preheating method on the texture and percentage of weight loss of scrambled egg patties after HPHT processing. Control patties were preheated by placing pouches in boiling water for 20 minutes (as per Michael Foods heating directions) and cooled down in an ice bath.

Vacuum packaging levels, patty shape, and water addition

Patties in experiment 1 (Table 2) were vacuum packaged at a 400 mbar level in pouches, and refrigerated until preheating and HPHT treatment. For experiment 2 (Table 2), frozen patties were packaged at three levels of vacuum packaging (expressed in absolute pressure) using the pouches described above: (a) 10 mbar, (b) 400 mbar, and (c) no vacuum (1013 mbar). The vacuum packaging machine used was a MULTIVAC Vacuum Machine type A 300/41/42 (Kansas City, Mo., U.S.A.). Patties used for this part of the study were #3 patties (round) and # 4 (square) patties with added xanthan gum, both with different porosities. After packaging, patties were stored under refrigerated conditions (5 ºC). After HPHT treatment, vacuum packaged patties were stored no longer than 24 hours under refrigeration conditions, and texture and water loss were evaluated.

For experiment 3 (Table 2), distilled water was applied to the surface of defrosted square xanthan gum added patty #4 (5%, 10%, and 15% of patty weight) in individual dishes. Patties were left standing 5 to 10 minutes in order to allow water absorption throughout the whole structure. After water addition, patties were vacuum packaged at a 400 mbar level in pouches, and refrigerated until preheating and HPHT treatment. Except for preheating experiments (expt # 4), samples were preheated in a water tank using steam injection up to 75 ºC for 8.0 ± 1.5 min.

Preheating methods

Preheating studies (expt #4) were conducted using water baths in a tilting steam kettle (DLT-40-1EG, Groen, DI Food Service Companies, Jackson, Miss., U.S.A.) at 80 ºC and 90 ºC and using a conventional pilot-scale retort (Design RDSW3; Lee Metal Products Co., Philipsburg, Pa., U.S.A.) at 90 ºC and boiling temperature of 98 ºC (corresponding to 715 m above sea level). The

water in the retort was injected with steam at approximately 207 kPa and with air pressure of approximately 138 kPa. Temperature of the patties was measured using a thermocouple (T-type, Omega Engineering Inc., Stamford, Conn., U.S.A.) fixed by a stuffing box (Ecklund Harrison Tech. Fort Meyer, Fla., U.S.A.) at the center of the egg patties. For this experiment, the initial temperature of the patties was 20 ºC and preheating times were measured until temperature of the egg patties reached 80 ºC. Heat penetration was evaluated by finding the heating rate index f_h and heating lag factor j_h as per Holdsworth (1997). Factors were calculated using the temperature of the heating medium as the reference temperature.

Pressure treatment

For all experiments shown in Table 2, preheated patties were subjected to HPHT treatment 700 MPa/105 ºC/5 min. During treatment, due to compression heating, the sample temperature increased from 75ºC to 105 ºC. To investigate the influence of water addition, (expt #3, Table 2) patties were also treated at 700 MPa and 121 ºC for 3 min, where the initial temperature of the pressure vessel was 90 ºC. A Flow Pressure Systems QUINTUS® Food Press Type 35L-600 sterilization machine (Flow International Corporation, Kent, Wash., U.S.A.) was used with filtered municipal water as pressure transmitting medium. Patties were fitted into a metallic carrier structure with seven levels each holding four pouches. Four thermocouples (k-type, Omega Engineering Inc., Stamford, Conn., U.S.A.) were used to measure temperature of the pressure medium as well as temperature inside the patty. Table 3 indicates the time and temperature values corresponding to each step of the process. A slight decrease in temperature was observed at the end of the pressurization time, as no chamber insulation was used (Table 3). The recorded temperature profile at 121 ºC (HPHT2) was used to calculate F_0, as reported in Table 2, by applying the General Method (Holdsworth 1997):

$$F_0 = \int_0^t 10^{\left[\frac{T-121.1}{z}\right]} dt \qquad (1)$$

where T is temperature (°C), t is processing time (min), and z is 10 °C for *Clostridium botulinum*. As a conservative approach, contribution of pressure lethality was not considered in this calculation.

2.3 Product analyses

The quality of the egg products before and after HPHT treatment was evaluated by means of Texture Profile Analysis (TPA) and by measuring the degree of syneresis.

Texture analysis

TPA tests were performed with a Texture Analyzer (Stable MicroSystems Ltd., White Salmon, WA), fitted with a 0.5 kg cell. Measurements were carried out using a 50.8 mm dia cylindrical probe on cylindrical pieces (25 mm dia and 8.2 ±1.5 mm thickness) of egg patty at 20 ºC. The samples were compressed to 50% of the initial height (Montejano and others 1985; Paraskevopoulou and Kiosseoglou 1997; Gujral and others 2003) at a cross-head speed (and post-test speed) of 1 $mm \cdot s^{-1}$ (Woodward and Cotterill 1986). The parameters hardness (N, peak force during first compression cycle), adhesiveness (N*mm, work necessary to pull probe away from sample), springiness (dimensionless, height recovered from product relative to first byte), cohesiveness (dimensionless, ratio of positive force area during second compression to that during first compression), and resilience (dimensionless, area during withdrawal of first compression, divided by area of first compression), were determined as defined by Bourne (2002).

Degree of syneresis

Water loss (or syneresis) in (% weight loss) was evaluated by weighing the egg patty before packaging and after HPHT treatment. The formula used (Eq. 2) was adapted from the serum formula developed by Woodward and Cotterill (1986) for evaluating percentage of serum in heat-formed egg white gels, and also by Feiser and Cotterill (1982, 1983) to evaluate cooked-frozen-thawed-reheated scrambled eggs:

$$\%\ \ weight\ \ loss = \frac{ipw - fpw}{ipw} \times 100 \qquad (2)$$

where *ipw* and *fpw* are the initial and final weights of the patty, respectively. A similar formula was used to determine expressible moisture as an indicator of water holding capacity in omelets (O'Brien and others 1982) as well as percentage of weight loss in chicken meat batters with egg white before and after high pressure (Fernández and others 1998).

In order to evaluate the amount of water retained by the patty after adding water into its structure a *water holding index* was used:

$$Water\ holding\ \ index = 100 - \%\ \ water\ retained \qquad (3)$$

The percentage of water retained can be expressed as:

$$\%\ \ water\ retained = \frac{fpw - ipw}{fpw} \times 100 \qquad (4)$$

In this case, the *ipw* value is the initial patty weight value before water was added. Since the control is preheated and the patty with added water is HPHT treated, the % of water retained will be a negative value. Thus, the water holding index provides an idea of the amount of water added held by the structure after processing.

For experiment 1 (Table 2), preheated patties # 1, 2, and 3 were used as controls and compared with the same HPHT treated formulations at 105ºC and 700 MPa for 5 min.

2.4 Statistical analyses

A factorial design for texture, weight loss, and water retained (Table 2) was studied using the General Linear Models PROC GLM procedure in the SAS statistical package (SAS/STAT Language, Version 9, SAS Institute Inc., Cary, N.C., 2004) to perform analysis of variance (ANOVA), Least Square Means ($\alpha = 0.05$), and regression. All the experiments were replicated (Table 2) on separate dates.

3. Results and Discussion

Results and discussion in the following sections will indicate how texture and water retention of HPHT treated egg patties can be affected by reformulating with cheese or xanthan gum, the use of vacuum packaging level, the addition of water before pressurization, and the preheating method used.

3.1 Formulation selection

Patties in all formulations experienced significant increased hardness, cohesiveness, and resilience after HPHT treatment (Table 4). In this study, springiness values were not influenced

by the type of treatment applied to each formulation and among formulations. Descriptors hardness, cohesiveness, and adhesiveness showed significant interaction between variables formulation and treatment, whereas no interaction was found for resilience and springiness.

Egg patty made using basic formulation (#1), preheated at 75 ºC, showed higher hardness and cohesiveness than egg patty with added xanthan gum (formulation #3). Addition of cheese (formulation # 2) did not influence hardness. O'Brien and others (1982) showed that scrambled egg omelets had higher tenderness levels after adding xanthan gum (0.5-1.5%) into the formulation.

After HPHT treatment, egg patties made using basic formulation (#1) had significantly higher hardness and cohesiveness than egg patty formulations with cheese (#2) or xanthan gum (#3) added. In fact, both modified HPHT treated formulations showed hardness reduction by 33% compared to HPHT treated original formulation (#1), as cohesiveness was reduced by 30% after HPHT treatment. Nevertheless, no previous literature reported the effects of high pressure thermal treatment on the interactions between xanthan gum and egg proteins, and egg proteins with cheese. Laneuville and others (2000) studied the formation of whey protein-xanthan gum complexes, and explained that this anionic polysaccharide shields active protein-protein interaction sites, decreasing the collision rate between molecules, while binding water and providing plasticity. Ahmed and Ramaswamy (2004) did not observe major effects with pressures around 400 MPa and process temperature of 20 ºC on viscosity of xanthan gum solutions (0.25-1.5%), especially at high concentration levels. However, the helical structure of xanthan gum depends on temperature (Pelletier and others 2001) and therefore should change during thermal pressurization at 105ºC.

In addition, heat produces a disordered structure of xanthans with lower viscosity (Speers and Tung 1986). Xanthan gum was dispersed within the proteins during egg patty production when mixing ingredients and provided more flexibility and lower hardness before HPHT treatment. Mixing process maximized protein-polysaccharide interactions within the egg patty's structure (Cotterill 1995; Laneuville 2000), therefore, decreasing protein-protein aggregation during thermal pressurization. The effect of xanthan gum addition on texture will depend on the amount of water retained, mainly by egg proteins, as well as by other water binding ingredients included in the formulation.

HPHT treated egg patties containing process cheese (#2) also had lower hardness and cohesiveness than HPHT treated original patties (#1). Johnston and others (2002) found that hardness of low-fat Cheddar cheese did not change after applying 800 MPa and low temperature. Furthermore, when cheese is heated, there is a dramatic decrease in the total number and strength of casein-casein interactions in the cheese matrix (Lucey and others 2003). In fact, Cheddar cheese fat (approximately 30%) melts after reaching 40 ºC, resulting in loss of elasticity and increase in viscous flow (Lucey and others 2003). During HPHT treatment, cheese, originally distributed as small particles in the egg patty structure, melted and integrated into the egg patty matrix, providing plasticity to the egg protein matrix, and impeded further gelation. Thus, the dispersion of cheese in the scrambled egg structure caused a decrease in hardness and cohesiveness of the product. Koidis and others (2002) found that inclusion of corn oil into low-fat egg yolk gels led to gel network structures of relatively low stiffness.

HPHT treatment only decreased adhesiveness value (that is, increased the absolute value of the negative area) in formulation #2 with process cheese, whereas adhesiveness did not change after HPHT treatment in formulations #1 and #3. Although patties tested were preformed using heat and then pressurized, the lower adhesiveness of HPHT treated patties (formulation #2) can be explained by the increased fat content at the surface of the patty. In fact, adhesiveness of Cheddar cheese depends on the fat content (Bryant and others 1995). Therefore, during thermal pressurization, melted cheese diffused out of the egg matrix and partially leached out at the surface, increasing adhesiveness in the final product.

HPHT treatment yielded lower resilience in #2 patties than in formulations #1 and #3. Resilience in HPHT formulations #1 and #3 was not significantly different ($P>0.05$). This can also be explained by the additional fat content due to cheese, which provides pliability to the structure. Wu and Ockerman (1982) found that the addition of fat reduced resilience in meatballs, affecting the resistance during compression and decompression at the first compression cycle.

Regarding the effects of formulation on percentage of water loss, no significant differences were observed among control patties after preheating up to 75ºC (Fig. 1). However, a significant interaction ($P<0.05$) between HPHT process and formulation as affecting weight loss was found. HPHT treated formulations #1 and #3 had significantly higher water loss than the control. This can be explained as a volume exclusion effect (Fernandes and Raemy 1996; Palou and others 1999), in which protein unfolding occurred due to the combination of pressure and heat, leading to increased aggregation and subsequent water loss.

Formulation #3, containing xanthan gum, was 30% lower in weight loss than formulation #1 after HPHT. O'Brien and others (1982) determined that xanthan gum, at 0.1%, was very effective in reducing expressible moisture in precooked frozen and reheated omelets. Furthermore, Fernandes and Raemy (1996) reported that during the application of high pressure (up to 800 MPa at 30ºC and 50ºC) xanthan gum and whey protein partially unfolded with a subsequent increase in hydration degree. Thus, xanthan gum located within the egg gel matrix grains had a positive effect on moisture retention after HPHT, which can be correlated with decreased values of hardness.

On the other hand, weight loss in HPHT treated formulation #2 was not significantly different from all the preheated controls, being 75% lower than HPHT treated formulation #1. This phenomenon might be explained by an increase in strength of hydrophobic casein-water interactions in the cheese due to increase with temperature (Lucey and others 2003). Increased viscous flow of melted process cheese due to high temperatures allowed more water absorption and retention of water being released. Measurements of expressible serum in Mozzarella cheeses after 200 MPa for 60 min at 20 ºC suggested that internal redistribution of moisture was responsible for pressure-induced changes (Johnston and Darcy 2000). In the case of HPHT treated formulation #2, water being released from the egg protein structure was likely absorbed by the cheese, which also had pressure-temperature induced redistribution throughout the structure.

3.2 Effect of vacuum packaging and patty forming method (initial porosity)

Other than by formulation modification, the improvement of texture and water retention to levels comparable to controls could be reached by modifying selected pretreatment steps before HPHT

treatment. In this experiment, three vacuum packaging levels were used in addition to two forming methods for round and square patty shapes.

The effects of vacuum packaging level and patty forming method on TPA hardness and percentage of water loss are shown in Table 5 and Fig. 2, respectively. HPHT treated square patty #4 showed significantly lower hardness (26-35%) than HPHT treated round patty #3 at each vacuum level condition (Table 5). During production of the egg patties, two different systems deposited the liquid egg mix with different level of injected air into the molds, yielding patty #3 with clearly visible and homogenous pores and patty #4 with a gel-like structure. One of the effects of high pressure processing is the displacement of air bubbles trapped in a solid matrix (Yamakazi and Kinefuchi 2003). After HPHT treatment, patty #3 had higher air loss, which allowed pore collapse and the formation of a denser structure. Since patty #4 had smaller pores than patty #3, there was less air removal and consequent structural modification. This decreased the extent of gelation during pressurization and provided hardness values closer to the controls. These results for #4 patties were supported by the 29% lower water loss values observed at no or intermediate vacuum packaging conditions (Fig. 2).

For patty # 3, hardness was increased by 7% and 19 % with vacuum packaging levels of 400 mbar and 10 mbar, respectively, as compared to no vacuum conditions (Table 5). This can be explained as an additive compression effect during hydrostatic pressurization, where vacuum and hydrostatic pressures add up and enhance the contact between proteins, resulting in greater gelation and greater hardness. Park and Lee (2001) found that vacuum packaging increased the flesh hardness of peeled lance Asia bell roots stored at 4 ºC. In the case of egg patties, preliminary studies showed that vacuum packaging did not affect the hardness after preheating,

showing recovery of the structure after vacuum packaging for a short time. However, high vacuum packaging had a negative effect on texture after HPHT treatment as shown by an increase in hardness.

Hardness in square patty #4 at no vacuum conditions after did not differ from the control HPHT. Furthermore, no significant vacuum level effect was seen after HPHT treatment for patty #4 (Table 5). In this case, hardness of a more compact and less porous egg matrix like #4 might be less affected by the combination of pressure and high temperature than #3.

Vacuum packaging and patty preforming method effects on hardness presented significant interactions ($P<0.05$). However, no effect of vacuum packaging and patty preforming method on adhesiveness was found before and after HPHT. Springiness was only significantly affected by patty preforming method, and not affected by vacuum packaging and HPHT processing. Patty #4 was more elastic than patty #3 before and after HPHT treatment. This difference in springiness can be directly related to the egg patty porosity, where patty #3 had more air (or pores) than patty #4 within the structure. No previous literature on egg products has established the effect of vacuum level on these descriptors.

Syneresis in egg patties #3 and #4 increased significantly after HPHT treatment with respect to controls (Fig. 2). Patty #4 had the highest weight loss percentage at the highest vacuum condition of 10 mbar, 35 % higher than low vacuum and HPHT treated patty #4, whereas no influence of the initial vacuum condition was seen for patty #3. Claus and Hunt (1991) observed that low-fat high added-water bologna sausage resulted in lower water purge when packaging was at a lower vacuum level. The vacuum effect trend on water loss was contrary to that observed for hardness

and cohesiveness values in patties #3 and #4. While vacuum level affected syneresis of a less porous structure like patty #4 at HPHT conditions, the vacuum level effect influenced hardness only in the more porous patty #3. This implies that syneresis and hardness after HPHT treatment are not directly related and they depend on the vacuum level used and the porosity of the patty.

Even though storage time and temperature were not variables in this experiment, previous studies on vacuum packaging level have found that storage time and temperature can also be critical factors on the final texture and water retention capacity (Bertola and others 1995; Montero and others 1996; Park and Lee 2001).

3.3 Effect of water addition

The benefits of water addition on the final texture of the #4 square patties after HPHT treatment were studied. There were two temperature and time conditions used during pressurization: a "minimal scenario" for commercial sterilization HPHT1 (700 MPa, 105 ºC, 5 min) and a standard scenario HPHT2 (700 MPa, 121 ºC, 3 min) to determine texture improvements in the possible operating time-temperature ranges for sterilization at 700 MPa. There was no effect on hardness observed due to water addition within control samples and HPHT1 patties, whereas HPHT2 treated patties showed significantly lower hardness in a 20-25% when water was added (Fig. 3). Water addition had no effect on cohesiveness (data not shown) after HPHT1 and HPHT2 treatments, while control with 0% water added was more cohesive than controls with water added. HPHT1 and HPHT2 treatments did not show significant differences in hardness and cohesiveness.

Compared to the Michael Foods commercial patty #1 control (preheated only), the hardness of #4 patties with added water and treated at HPHT1 and HPHT2 were not significantly different from this control($P<0.05$). Thus, water addition reduced the hardness of HPHT treated patties to standard control values. It has been reported that the previous addition of water provided different hydration modes for the polymers in sol and gel states (Gekko 1994), softening the structure. Furthermore, the temperature required for coagulation of eggs is elevated by water dilution, and the firmness of the coagulum decreases with increased dilution (Beveridge and others 1980).

As pressure and temperature influenced the volume of the patty, yielding a more cohesive and denser structure, availability of free water was changed. In fact, cohesiveness after the HPHT process was increased even with respect to the #1 control patty. Adhesiveness and springiness were not significantly affected after HPHT1, when compared to control #4 and #1. No significant changes were found in adhesiveness, springiness and resilience due to water addition after HPHT treatment. HPHT2 treatment resulted in less adhesive and more resilient patties compared to controls #4 and #1. Data on cohesiveness, adhesiveness, springiness, and resilience are not shown.

Retention of water added in patty #4 was shown by an increase in water holding index (Eq. 3) for all treatments before and after HPHT. However, no significant differences were found between patties with 5, 10, and 15% added water (Fig. 4). In egg patty production the egg proteins are unfolded during cooking and although associated with each other, the added water is absorbed by the proteins and other hydrophilic molecules and components present in the structure (xanthan gum, whey protein, modified food starch, nonfat dry milk).

High pressure (800 MPa and 50 ºC) can induce several conformational changes in egg and whey proteins, which, by unfolding, increase the solvent accessible surface area, increasing hydration (Fernandes and Raemy 1996). In this case, the protein matrix was modified in a way such that it was still able to retain higher amounts of water after HPHT1 treatment (Fig. 4), whereas higher temperatures after HPHT2 treatment gave a lower water holding index than for control patties. Thus, even though the hardness values detected in water added patty #4 after both HPHT1 and HPHT2 were found similar to #1 patty control, water retention decreased at higher pressurization temperatures.

3.4 Preheating method selection

During HPHT treatment, the process of preheating is extremely important to assure that the initial temperature throughout the food samples matches (or is higher than) the initial pressure chamber temperature. In this experiment, two heating systems at two respective temperatures were tested to evaluate the heat penetration rate and its consequence on texture and syneresis. Fig. 5 shows the temperature profiles inside patty #1 obtained using each preheating method.

Table 6 shows that the heating rate index f_h was significantly lower with increasing preheating temperature, indicating a faster rate of heat penetration. However, no significant difference was found between the heating rate index obtained by preheating in the water bath in the steam kettle and the retort with steam/air injection at 90 ºC.

The heating rate index depends on the thermal properties, particularly, on the thermal diffusivity, and dimensions of the food product being heated (Holdsworth 1997). At the same time, thermal

diffusivity is related to the thermal conductivity of the food, the specific heat, and the food density. At 98 ºC, the mixture of injected steam and air accelerated the convective rate of heat transfer to the packaging material and then to the food. The headspace inside the pouch, as well as the internal vapor pressure increased due to higher temperature, also influenced the j_h value due to convective currents in contact with the egg patty surface. For this experiment, single packages were located separated in each carrier during preheating. However, the configuration of multiple pouches stacked together needs also to be considered during preheating since it will decrease the heating rate.

The lag factor j_h, related to the lag time to reach uniform heating rate values, was significantly lower for the 80 ºC water bath and significantly higher for the one 98 ºC in comparison to other temperatures. Part of the lag at 80 ºC was due to slow temperature come up in the water.

The TPA descriptors hardness, adhesiveness, springiness, and resilience showed no significant differences between preheating methods before and after HPHT treatment. However, as shown in Table 7, cohesiveness was increased with higher temperatures and by the injection of air and steam. Contrary to what was found for hardness and resilience, cohesiveness only increased due to HPHT processing after being preheated in an 80 ºC water bath. Thus, a faster preheating might retain the cohesive structure of the egg patty after HPHT treatment.

Even though the preheating conditions tested provided significantly different penetration rates, this was not reflected in textural changes after HPHT process. The same occurred with syneresis values expressed as % weight loss (Table 7). Since different preheating rates provide similar texture parameter values (except for cohesiveness) as well as water retention before and after

HPHT treatment , variations in preheating rates / periods to reach temperatures around 60 ºC to 80 ºC will probably not affect the texture and water retention of patties of this size. This allows the possibility of preheating in two stages without affecting the final texture properties of the product. The use of two stages (for example, initial equilibration of egg patties at 60ºC using water bath and then fast heating up to 80 ºC) will decrease initial temperature gradients within the product, minimizing cold spots.

If the patties were of larger dimensions, the differences found in heating rate index j_h and preheating times for each tested condition would be more pronounced. The same would apply when a high number of pouches placed into the carriers are touching. Reduction of preheating time, by means of a higher preheating rate, in a larger scale egg patty (for example, in an institutional type of pouch) would provide less exposure to heat, especially at the food's surface, and possibly improved texture.

As seen before, higher hardness, resilience and % weight loss were found after HPHT with respect to the preheated control. However, springiness and adhesiveness (data not shown) did not change significantly after HPHT.

The advantage of HPHT with respect to retort processing is that once the initial temperature is reached throughout the entire product, temperature rise during pressurization will be uniform within the whole volume. This benefit can also be used when scaling up the process for larger sized products. In this case, a media with large heat transfer coefficient, such as water injected with steam/air mixtures can help make the process shorter by decreasing come up time, and can eventually yield products with improved texture and water retention. The heat transfer properties

of the packaging material used might also play a role in the heating rate of penetration and the lag factor.

4. Conclusions

Various approaches to improving texture and increasing water retention of scrambled egg patties to commercially desired levels after HPHT treatment were tested. Formulation modification with xanthan gum in a standard formulation reduced hardness, cohesiveness and water loss values after HPHT treatment. Reformulation of egg patties with added process cheese was also beneficial, as it gave similar hardness reduction as xanthan gum and increased water retention by 75% after HPHT treatment, yielding values similar to the preheated control. Low-porosity patty with added xanthan gum, packaged at 400 mbar or no vacuum, had lower hardness and water loss values than the porous egg patty after HPHT treatment, maintaining hardness values similar to the preheated controls.

High vacuum packaging level of 10 mbar gave highest hardness and syneresis in formulation with added xanthan, due to an additive effect of vacuum and HPHT treatments on patty structure. Water addition in egg patties decreased hardness after HPHT treatment at processing temperatures 105 ºC and 121 ºC to similar values to the preheated standard Michael Foods patty, and increased the water holding capacity in the egg matrix. Among the preheating methods, steam injection at 98ºC provided the highest heating rate in comparison to other methods. However, most TPA descriptors and syneresis did not change significantly with preheating rates after HPHT processing.

The advantage of HPHT processing with respect to conventional thermal sterilization methods is the decreased process time due to “instant” compression heating, which retains appearance and flavor of scrambled egg patties, and prevents the formation of green compounds attained with conventional thermal processing. This feasibility study demonstrated how texture and water retention, key quality parameters for the development of egg products, can be maintained or minimized after HPHT processing, showing potential for the production of commercial shelf stable egg items.

References

Ahmed J, Ramaswamy HS. 2004. Effect of high-hydrostatic pressure and concentration on rheological characteristics of xanthan gum. Food hydrocoll 18(3):367-73

Ahn J, Balasubramaniam VM, Yousef AE. 2005. Effect of pressure-assisted thermal processing on the inactivation of selected *Clostridium* and *Bacillus* surrogate spores [abstract]. In: Nonthermal Processing Workshop; September 15-16; Philadelphia, Pa. USDA Eastern Regional Research Center.

Balasubramaniam S, Balasubramaniam VM. 2003. Compression heating influence of pressure transmitting fluids on bacteria inactivation during high pressure processing. Food Res Int 36(7): 661-8.

Bertola NC, Bevilacqua AE, Zaritzky NE. 1995. Rheological behaviour of Reggianito Argentino cheese packaged in plastic film during ripening. Lebensm -Wiss Technol 28 (6):610-5.

Beveridge T, Arntfield S, Ko S, Chung JKL. 1980. Firmness of heat induced albumen coagulum. Poultry Sci 59(6): 1229-36.

Bourne, M. 2002. Food texture and viscosity. Concept and measurement. 2nd ed. New York: Academic Press. 400 p.

Bryant A, Ustunol Z, Steffe J. 1995. Texture of Cheddar cheese as influenced by fat reduction. J Food Sci 60(6):1216-9, 1236.

Claus JR, Hunt MC. 1991. Low-fat, high added-water bologna formulated with texture-modifying ingredients. J Food Sci 56(3):643-7, 652.

Cotterill OJ. 1995. Freezing egg products. In: Stadelman WJ, Cotterill OJ, editors. Egg science and technology. 4th ed. New York: Food Products Press. p 265-88.

Feiser GE, Cotterill OJ. 1982. Composition of serum from cooked-frozen-thawed-reheated scrambled eggs at various pH levels. J Food Sci 47(4):1333-37.

Feiser GE, Cotterill OJ. 1983. Composition of serum and sensory evaluation of cooked-frozen-thawed scrambled eggs at various salt levels. J Food Sci 48(3):794-97.

Fernandes PB, Raemy A. 1996. High pressure treatment of whey protein/polysaccharide systems. In: Hayashi R, Balny C, editors. High pressure bioscience and biotechnology. Tokyo, Japan: Elsevier Science. p 337-42.

Fernández P, Cofrades S, Solas MT, Carballo J, Colmenero FJ. 1998. High pressure-cooking of chicken meat batters with starch, egg white, and iota carrageenan. J Food Sci 63(2):267-71.

Gekko K. 1994. The sol-gel transition of food macromolecules under high pressure. In: Nishinari K, Doi E, editors. Food hydrocolloids: structures, properties and functions. New York: Plenum Press. p 259-64.

Gola S, Foman C, Carpi G, Maggi A, Cassara A, Rovere P. 1996. Inactivation of bacterial spores in phosphate buffer and in vegetable cream treated with high pressure. High Press Biosci Biotechnol. Kyoto, Japan: Sanei Schuppan,. 253-59.

Gujral HS, Rosell CM, Sharma S, Singh S. 2003. Effect of sodium lauryl sulphate on the texture of sponge cake. Food Sci Technol Int 9(2):89-93.

Heinz V, Knorr D. 2001. Effect of high pressure on spores. In: Hendrickx MEC, Knorr D, editors. Ultrahigh pressure treatment of foods. New York: Kluwer Academic/Plenum Publishers. p 77–116.

Holdsworth SD. 1997. Thermal processing of packaged foods. New York: Blackie Academic & Professional. 112 p.

Hoogland H, de Heij W, van Schepdael L. 2001. High pressure sterilization: novel technology, new products, new opportunities. New Food 4(1):21-6.

Johnston DE, Darcy PC. 2000. The effects of high pressure treatment on immature Mozzarella cheese. Milchwissenschaft, 55(11):617-20.

Johnston DE, O'Hagan M, Balmer DW. 2002. Effects of high pressure treatment on the texture and cooking performance of half-fat Cheddar cheese. Milchwissenschaft 57(4):198-201.

Juliano P, Li B, Clark S, Mathews JW, Dunne, CP, Barbosa-Cánovas GV. 2004. Optimal high pressure thermal sterilization conditions for formulated egg products [abstract]. In: IFT Annual Meeting Book of Abstracts; 2004 July 12-16; Las Vegas, Nev.: Institute of Food Technologists. p 136. Abstract nr 49H-20.

Knipper AJ, Beam LS, inventors; Michael Foods Egg Products Company, assignee. 2002 July 2. Enhanced precooked egg product and process for formulation of precooked egg products. U.S. Patent 6,413,572.

Koidis A, Paraskevopoulou A, Kiosseoglou V. 2002. Fracture and textural properties of low fat egg yolk gels containing emulsion droplets. Food Hydrocoll 16(6):673-8.

Koutchma T, Guo B, Patazca E, Parisi B. 2005. High pressure – high temperature inactivation of *Clostridium sporogenes* spores: from kinetics to process verification. Journal of Food Process Engineering. Forthcoming.

Krebbers B, Matser AM, Koets M, Berg RW van den. 2002. Quality and storage-stability of high-pressure preserved green beans. J Food Eng 54(1):27-33.

Krebbers B, Matser AM, Hoogerwerf SW, Moezelaar R, Tomassen MMM, Berg RW van den. 2003. Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. Innov Food Sci Emerg Technol 4(4):377-85.

Laneuville SI, Paquin P, Turgeon SL. 2000. Effect of preparation conditions on the characteristics of whey protein-xanthan gum complexes. Food Hydrocoll 14(4):305-14.

Lucey JA, Johnson ME, Horne DS. 2003. Invited review: perspectives on the basis of the rheology and texture properties of cheese. J Dairy Sci 86(9):2725-43.

Margosch D. 2005. Behavior of bacterial endospores and toxins as safety determinants in low acid pressurized food. TU Berlin, Germany. [Dissertation]

Margosch D, Ehrmann MA, Gaenzle MG, Vogel RF. 2004. Comparison of pressure and heat resistance of Clostridium botulinum and other endospores in mashed carrots. J Food Prot. 67(11): 2530-2537.

Matser AM, Krebbers B, van den Berg RW, Bartels PV. 2004. Advantages of high pressure sterilization on quality of food products. Trends Food Sci Technol 15(2):79-85.

Merkle J, Ball H, Mathews J, inventors; Michael Foods of Delaware, Inc., assignee 2003a June 26. Formulation and process to prepare a premium formulated fried egg. U.S. Patent 30,118,714.

Merkle J, Ball H, Mathews J, inventors; Michael Foods of Delaware, Inc., assignee 2003b July 17. Formulation and process to prepare a premium formulated fried egg. U.S. Patent 30,134,030.

Meyer RS, Cooper KL, Knorr D, Lelieveld HLM. 2000. High-pressure sterilization of foods. Food Technol 54(11):67-68, 70-72 .

Montero P, Solas T, Pérez-Mateos M. 2001. Pressure-induced gel properties of fish mince with ionic and non-ionic gums added. Food Hydrocoll 15(2):185-94.

Montero P, Gomez-Guillen MC, Borderias J. 1996. Influence of subspecies, season and stabilization procedures in gel-forming ability of frozen minced muscle of sardine (Sardina pilchardus). Food Sci Technol Int 2(2):111-22.

Montejano JG, Hamann DD, Lanier TC. 1985. Comparison of two instrumental methods with sensory texture of protein gels. J Texture Stud 16(4): 403-24.

O'Brien SW, Baker RC, Hood LF, Liboff, M. 1982. Water-holding capacity and textural acceptability of precooked, frozen, whole-egg omelets. J Food Sci 47(2):412-7.

Palou E, López-Malo A, Barbosa-Cánovas GV, Swanson BG. 1999. High Pressure Treatment in Food Preservation. In: Rahman MS, editor. Handbook of Food Preservation. New York: Marcel Dekker. p 533-76.

Paraskevopoulou A, Kiosseoglou V. 1997. Texture profile analysis of heat-formed gels and cakes prepared with low cholesterol egg yolk concentrates. J Food Sci 62(1):208-11.

Park YM, Lee JH. 2001. Effects of film materials, vacuum packaging and shelf temperature on the quality of peeled lance Asia bell roots. Food Sci Biotechnol 10(3):331-4.

Pelletier E, Viebke C, Meadows J, Williams PA. 2001. A rheological study of the order-disorder conformational transition of xanthan gum. Biopolymers 59(5):339-46.

Rajan S, Pandrangi S, Balasubramaniam VM, Yousef AE. 2005a. Inactivation of *Bacillus stearothermophilus* spores in egg patties by pressure assisted thermal processing. LWT-Food Science and Technology. Forthcoming.

Rajan, S., J. Ahn, V. M. Balasubramaniam**,** and A. E. Yousef. 2005b. Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores[abstract]. In: Nonthermal Processing Workshop; September 15-16; Philadelphia, Pa. USDA Eastern Regional Research Center. Abstract no. 23.

Raso J, Barbosa-Cánovas GV, Swanson BG. 1998. Sporulation temperature affects initiation of germination and inactivation by high hydrostatic pressure of Bacillus cereus. J Appl Microbiol 85:17-24.

Reddy NR, Solomon HM, Fingerhut GA, Rhodehamel EJ, Balasubramaniam VM, Palaniappan S. 1999. Inactivation of *Clostridium botulinum* type E spores by high pressure processing. J Food Saf. 19:277-88.

Reddy NR, Solomon HM, Tetzloff RC, Rhodehamel EJ. 2003. Inactivation of *Clostridium botulinum* type A spores by high pressure processing at elevated temperatures. J Food Prot 66(8):1402-7.

Rovere P, Gola S, Maggi A, Scaramuzza N, Miglioli L. 1998. Studies on bacterial spores by combined pressure-heat treatments: Possibility to sterilize low-acid foods. In: Isaacs NS, editor. High Pressure Food Science, Bioscience and Chemistry. Cambridge: The Royal Society of Chemistry. p 354-63.

SAS System Version 9. 2004. SAS Institute Inc., SAS Campus Drive, Cary, N.C., U.S.A.

Sizer C, Balasubramaniam VM, Ting E. 2002. Validating high-pressure processes for low-acid foods. Food technol 56(2):36-42.

Speers RA, Tung MA.1986. Concentration and temperature dependence of flow behavior of xanthan gum dispersions. J Food Sci 51(1):96-8, 103.

Song IS, Cunningham, FE. 1985. Prevention of discoloration in retorted whole egg. J Food Sci 50(3):841-2.

Taki Y, Awano T, Toba S, Mitsuura N. 1991. Sterilization of *Bacillus sp.* spores by hydrostatic pressure. In: Hayashi R, editor. High Pressure Science for Food, Kyoto, Japan: Sanei Pub. Co. p. 217-24.

Woodward SA, Cotterill OJ. 1986. Texture and microstructure of heat-formed egg white gels. J Food Sci 51(2):333-9.

Wu FY, Ockerman HW. 1982. Effect of mechanical treatment, fat level and chopping time on the texture of comminuted meatballs. J Food Prot 45(8):729-32.

Yang SH, Baldwin RE. 1995. In: Stadelman, W. J. & Cotterill, O J., editors. Egg science and technology. 4th ed. New York: Food Products Press. p 405-64.

Yamazaki A, Kinefuchi M. 2003. Using high pressure treatment for research in food processing and development of cooked rice. J Appl Glycosci 50(1):89-96.

Table 1. Characteristics of precooked scrambled egg patties.

Formulation	Dimensions [a] (mm)
1. Basic (code 46025-30020-00) Basic ingredients: whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dry milk, and citric acid.	88.9 dia
2. Basic + cheese (code 46025-70019-00) Added ingredients: 20% pasteurized Cheddar process cheese.	82.6 dia
3&4[b]. Basic + xanthan gum (code 03-1426-10) Added ingredients: natural and artificial flavors, xanthan gum[c], EDTA.	(3) round 88.9 dia (4) square 69.9 x 76.2

[a] ± 6.4 mm

[b]Patties #3 and #4 were different in porosity. The cross section of #3 revealed a spongy-like structure whereas #4 was of a less porous (more gel-like) structure, clearly noticeable with the naked eye.

[c]xanthan gum, 0.1 – 1.5% by weight, as per Knipper and others (2002) and O'Brien and others (1982).

Table 2. Experimental design carried out to investigate the effects of modifying formulations and processing conditions on HPHT treated egg patties.

Expt	Pressure treatment	Product/pkg attribute investigated*
1.	Control, HPHT1 (2 x 3 factorial)	Formulation (basic, cheese, flavor)
2.	Control, HPHT1 (2 x 3 x 2 factorial)	Vacuum packaging (10 mbar, 400 mbar, no vacuum) shape (round and square)
3.	Control, HPHT1, HPHT2 (3 x 4 factorial)	Water added (0%, 5%, 10%, 15%)
4.	Control, HPHT1 (2 x 4 factorial)	Preheating (WB1 WB2, SAI1, SAI2)

* With the exception of experiment 1, all experiments had 3 replicates. Experiment 1 had 2 replicates.

HPHT1 – 700 MPa, 105 ºC, 5 min

HPHT2 – 700 MPa, 121 ºC, 3 min

WB1 – Water bath at 80 ºC

WB2 – Water bath at 90 ºC

SAI1 – Steam / air injection at 90 ºC

SAI2 – Steam / air injection at 98ºC (boiling temperature)

Table 3. Temperatures at different steps for HPHT processing conditions at 121ºC and 700 MPa for 3 min (standard scenario for sterilization) and 105 ºC and 700 MPa for 5 min.

Processing step	Processing at Final target T: 105 ºC		Processing at Final target T: 121 ºC Fo=3.3 min*	
	Time	Temperature	Time	Temperature
Defrosting	24 h	5 °C	24 h	20 °C
Preheating	8 min	75 °C	12 min	90 °C
Product loading in pressure chamber	1 min	75 °C	1 min	90 °C
Come up to 700 MPa	3.5 min	105 °C	3.5 min	121 °C
Holding @ 700 MPa	5 min	103 °C	3 min	119 °C
Pressure come down	0.5 min	73 °C	0.5 min	88 °C
Product unloading	1 min	-	1 min	-
Cooling	1 min	20 °C	2 min	20 °C
Total time (from preheating)	20 min		22 min	

*F_0 calculated from the recorded temperature profile

Table 4. TPA descriptors of preheated (control) and HPHT treated egg patties for formulations #1, #2, and #3. Standard errors are shown with $\alpha = 0.05$. Different letters indicate significant differences (n = 2, $P < 0.05$) between treatment (capital letters) and type of patty (lowercase).

Patty #	Treatment	Hardness (N)	Cohesiveness (dimensionless, 10^2)	Adhesiveness (N.mm, 10^1)	Springiness (dimensionless, 10^2)	Resilience (dimensionless, 10^2)
1	Control	22.5 ± 2.8 Aa	69.5 ± 1.7 Aa	-4.32 ± 1.57 Aa	87.9 ± 5.0 Aa	24.0 ± 1.6 Aa
1	HPHT1	52.7 ± 1.7 Ba	74.2 ±1.0 Ba	-3.02 ± 0.95 Aa	94.9 ± 3.0 Aa	30.1 ± 1.0 Ba
2	Control	17.4 ± 1.7 Aab	51.5 ±1.0 Ab	-5.51 ± 0.95 Ab	90.8 ± 3.0 Aa	16.9 ± 1.0 Ac
2	HPHT1	35.2 ± 1.4 Bb	62.6 ± 0.8 Bb	-11.01 ± 0.79 Bb	87.7 ± 2.5 Aa	24.1 ± 0.8 Bb
3	Control	10.8 ± 3.2 Ab	52.9 ±2.0 Ab	-1.99 ± 1.82 Aa	91.3 ± 5.8 Aa	19.7 ± 1.6 Ab
3	HPHT1	35.3 ± 2.3 Bb	73.3 ±1.4 Bb	-4.54 ± 1.29 Aa	89.1 ± 4.1 Aa	29.1 ± 1.0 Ba

Table 5. Hardness of preheated (control) and HPHT treated egg patties with three vacuum packaging levels and formulations #3 and #4. Standard errors are shown by $\alpha = 0.05$. Different letters indicate significant differences ($P < 0.05$) between vacuum levels (capital letters) and patty porosity (lowercase)[a].

Patty #	Vacuum level[b]	Hardness (N)	Cohesiveness[c] (10^2)	Adhesiveness (N.mm, 10^1)	Springiness[c] (10^2)	Resilience[c] (10^2)
3	Control	10.8 ± 2.7 Aa	52.9 ± 0.9 Aa	-1.99 ± 0.82 Aa	91.3 ± 0.3 Aa	19.7 ± 0.2 Aa
3	HPHT1 no vacuum	33.0 ± 1.9 Ba	73.5 ± 0.6 Ba	-3.24 ± 0.58 Aa	90.6 ± 0.2 Aa	33.6 ± 0.2 Ba
3	HPHT1 400 mbar	35.5 ± 1.9 BCa	73.0 ± 0.6 Ba	-4.63 ± 0.58 Aa	88.6 ± 0.2 Aa	28.9 ± 0.2 Ba
3	HPHT1 10 mbar	40.7 ± 1.9 Ca	70.8 ± 0.6 Ca	-5.00 ± 0.58 Aa	87.1 ± 0.2 Aa	28.9 ± 0.2 Ba
4	Control	15.9 ± 2.7 Aa	70.7 ± 0.9 Ab	-4.58 ± 0.82 Aa	94.5 ± 0.3 Ab	30.5 ± 0.2 Ab
4	HPHT1 no vacuum	21.6 ± 1.8 ABb	76.2 ± 0.6 Bb	-3.44 ± 0.54 Aa	96.9 ± 0.2 Ab	32.9 ± 0.1 Aa
4	HPHT1 400 mbar	27.4 ± 2.1 Bb	75.3 ± 0.7 Bb	-3.59 ± 0.64 Aa	96.7 ± 0.2 Ab	31.4 ± 0.2 Aa
4	HPHT1 10 mbar	26.4 ± 1.9 Bb	77.1 ± 0.6 Bb	-3.56 ± 0.58 Aa	93.2 ± 0.2 Ab	35.7 ± 0.2 Ab

[a]Square patty #4 has lower porosity than round patty #3.

[b]Controls were preheated in boiling water for 20 min as per Michael Foods directions.

[c]Dimensionless

Table 6. Heating rate index f_h and heating lag factor j_h of tested preheating conditions. Different letters indicate significant differences (P<0.05) between treatments.

Preheating method	f_h (min)	j_h (10^1, dimensionless)
Water bath at 80 ºC	5.86 ± 0.28 a	0.20 ± 0.30 a
Water bath at 90 ºC	4.00 ± 0.35 b	3.14 ± 0.34 b
Steam/air injection at 90 ºC	3.54 ± 0.30 b	3.44 ± 0.28 b
Steam/air injection at 98 ºC	2.35 ± 0.35 c	5.16 ± 0.31c

Table 7. Comparison of TPA significant descriptors and syneresis after different preheating conditions in control and HPHT treated samples. Standard errors are shown with $\alpha = 0.05$. Different letters indicate significant differences ($P<0.05$) between process (capital letters) and preheating method (lowercase).

Treatment	Preheating method	Hardness (N, ±1.4)	Cohesiveness (dimensionless, 10^2, ±0.6)	Resilience (dimensionless, 10^2, ±1.0)	Weight loss (%, ±0.4)
Control	WB 80 ºC	26.5 Aa	65.4 Aa	23.7 Aa	2.6 Aa
Control	WB 90 ºC	22.7 Aa	68.1 Ab	25.7 Aa	2.0 Aa
Control	SAI 90 ºC	22.7 Aa	70.6 Ac	25.2 Aa	2.0 Aa
Control	SAI 98 ºC	24.2 Aa	70.4 Ac	26.0 Aa	2.7 Aa
HPHT1	WB 80 ºC	49.0 Bb	70.7 Ba	31.3 Bb	12.3 Bb
HPHT1	WB 90 ºC	44.2 Bb	69.7 Aa	30.7 Bb	12.6 Bb
HPHT1	SAI 90ºC	44.2 Bb	71.0 Aa	30.2 Bb	12.6 Bb
HPHT1	SAI 98C	45.4 Bb	69.6 Aa	29.7 Bb	12.2 Bb

WB – Water bath

SAI - Steam / air injection

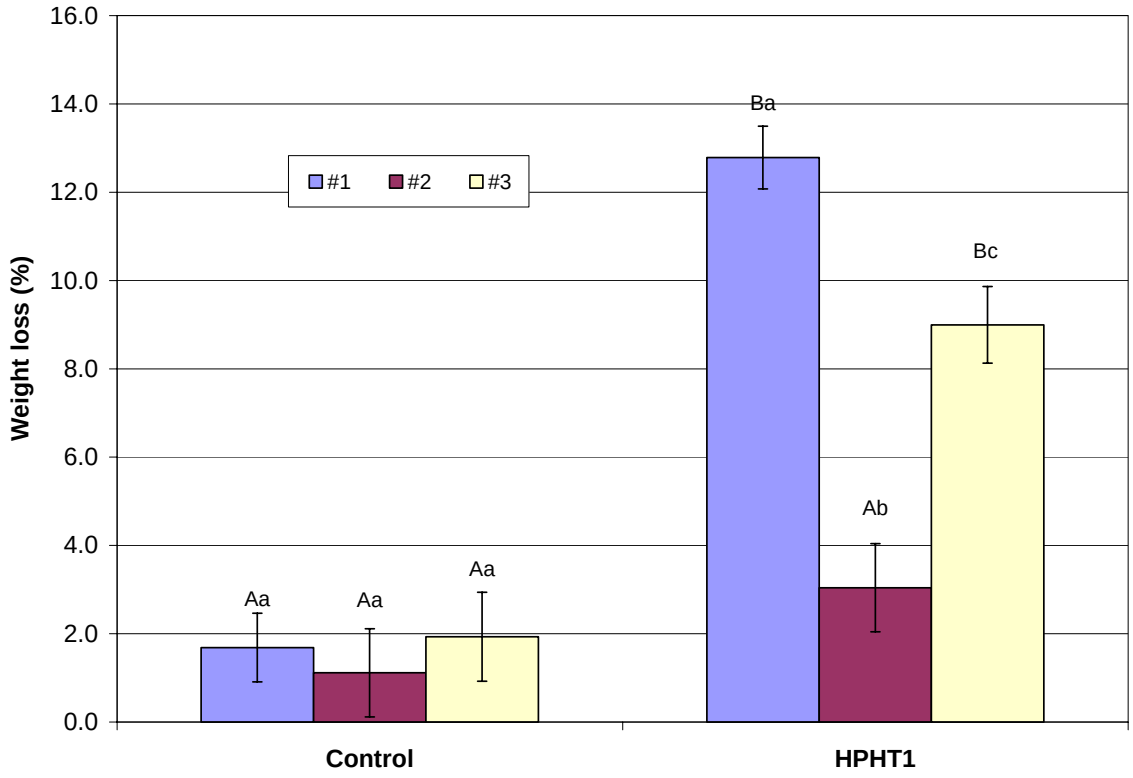

Fig. 1. Weight loss of preheated (control) and HPHT treated egg patties with formulations #1, #2, and #3. Error bars show mean confidence intervals ($\alpha = 0.05$). Different letters indicate significant differences ($P<0.05$) between treatment (capital letters) and formulation (lowercase).

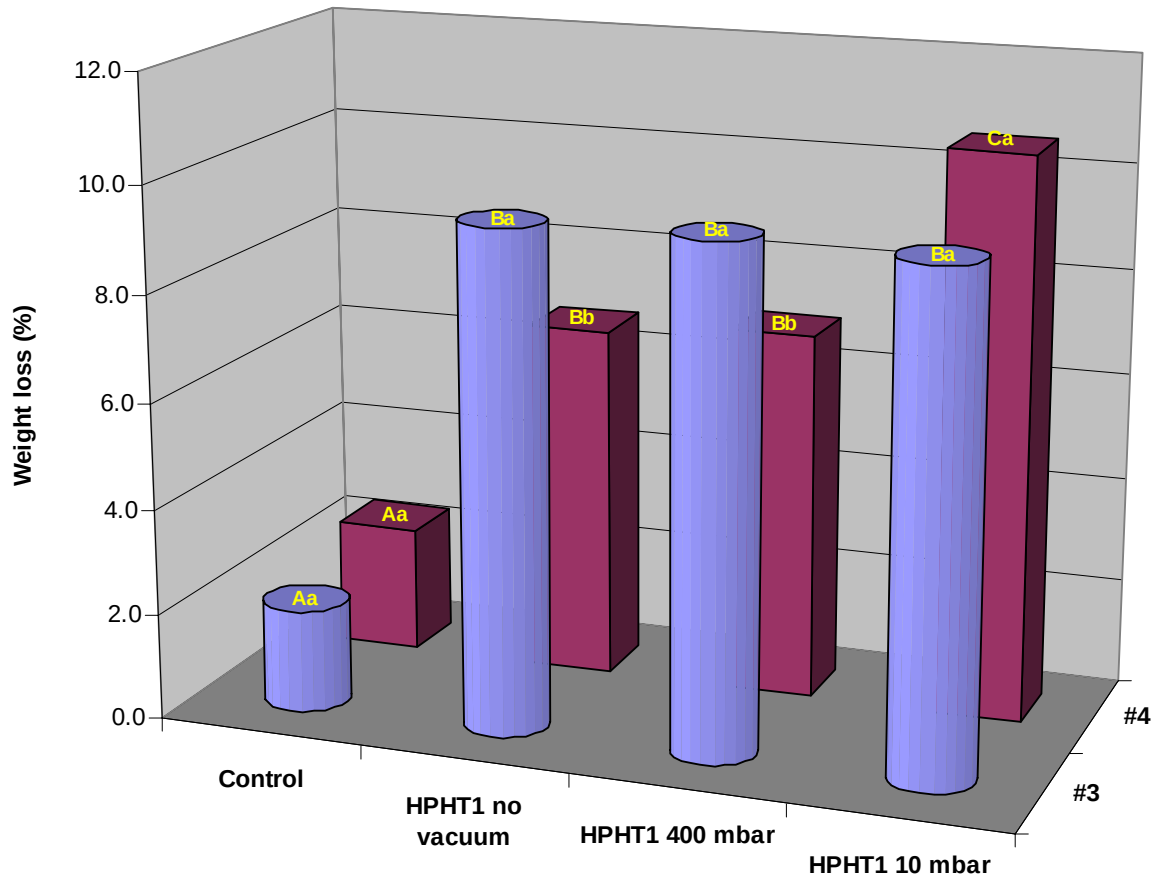

Fig. 2. Percentage of weight loss in preheated (control) and HPHT treated egg patties for formulations #3 and #4, as influenced by vacuum packaging level. Different letters indicate significant differences (P<0.05) between vacuum levels (capital letters) and patty shape (lowercase) for each condition.

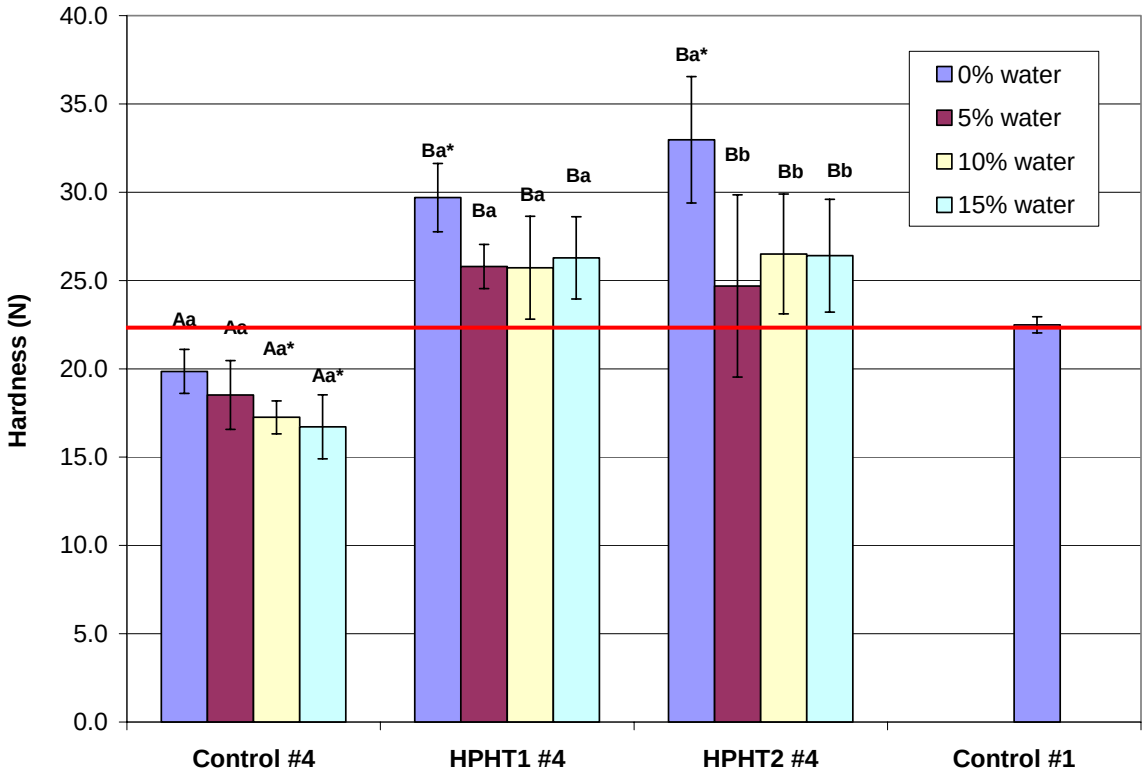

Fig. 3. Hardness of patty #4 before and after HPHT treatment as influenced by percentage of water added. Error bars show confidence intervals ($\alpha = 0.05$). Different letters indicate significant differences ($P<0.05$) between treatments at the same percentage of water added (capital letters) and percentage of water added (lower-case letters) within the same treatment. Stars (*) indicate significant differences with the hardness value for the control patty #1 (red line).

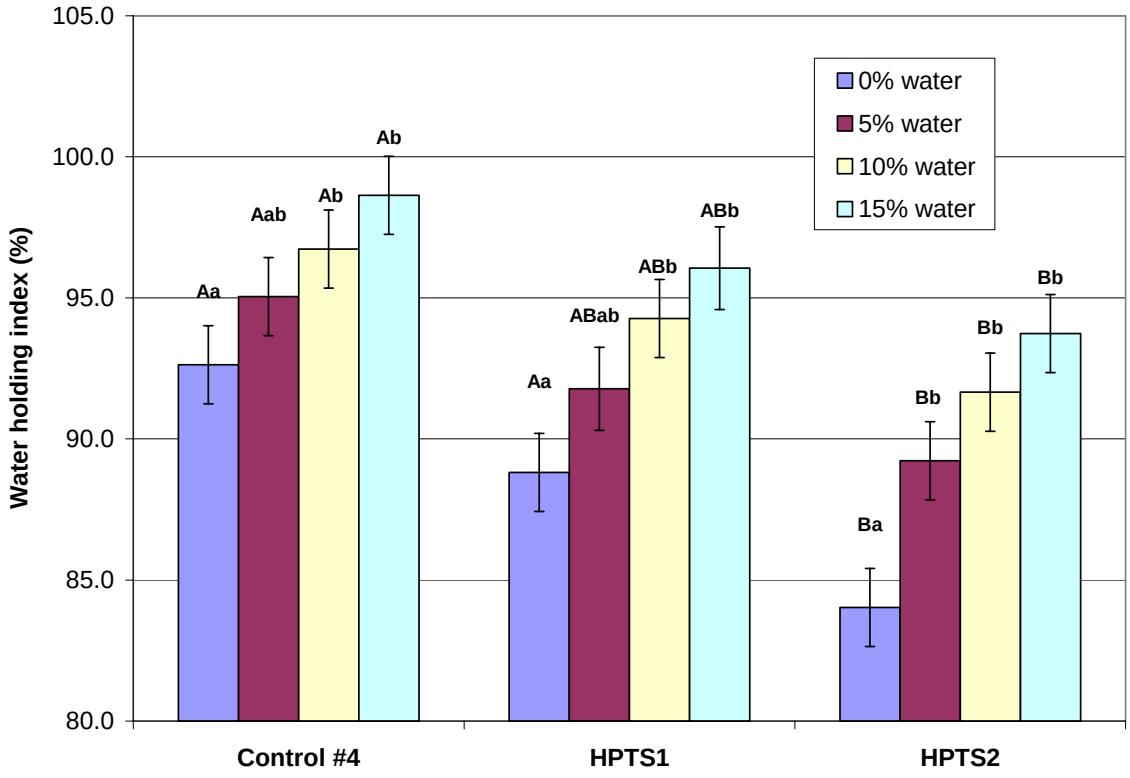

Fig. 4. Water holding index in patty #4 before and after HPHT treatment, as influenced by percentage of water added. Error bars show confidence intervals ($\alpha = 0.05$). Different letters indicate significant differences ($P<0.05$) between treatments (capital letters) and percentage of water added (lowercase).

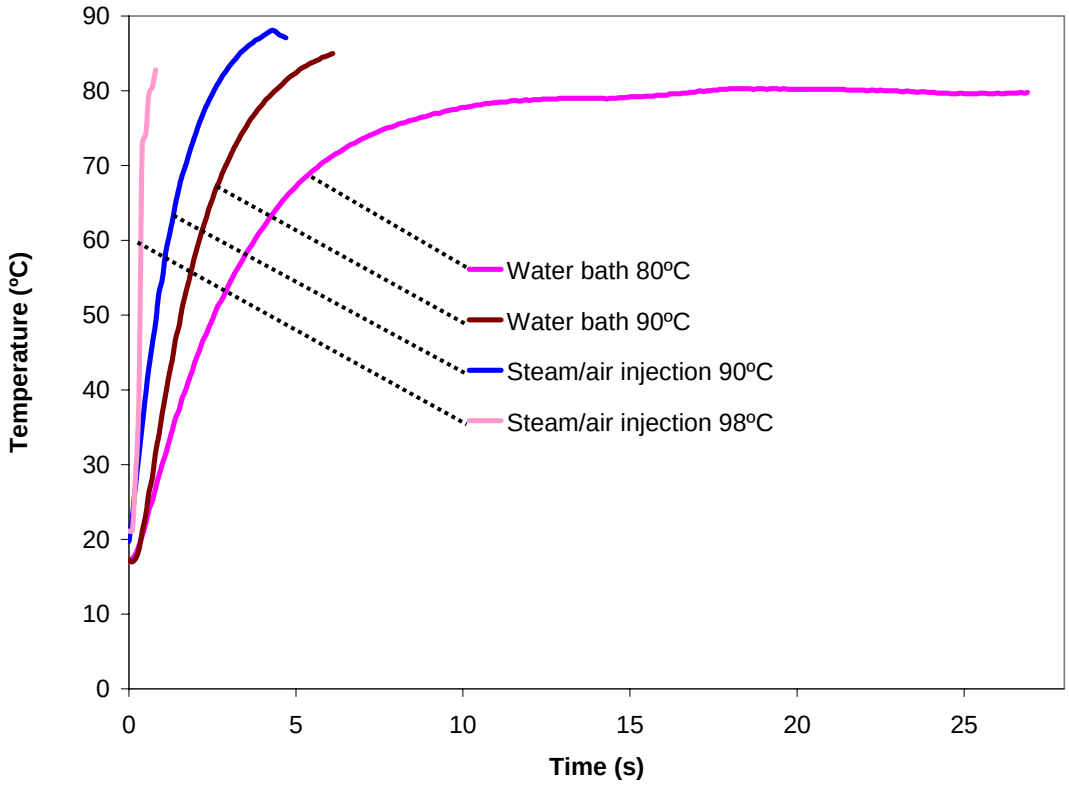

Fig. 5. Typical preheating curves obtained using each tested condition for patty #1.

CHAPTER 4

Consumer and trained panel evaluation of high pressure thermally treated scrambled egg patties

Pablo Juliano, Stephanie Clark, Tatiana Koutchma, Mahamoudou Ouattara, Jason Mathews, C. Patrick Dunne, Gustavo V. Barbosa-Cánovas

1. Introduction

Consumer demand for specialty precooked egg products has increased during the last decade in the United States (American Egg Board, 2004). About one-third of the eggs produced are further processed into egg products for the foodservice and food-manufacturing industries (Turner, 2003). The egg product sector in the US was 15% of the total egg production in 1984, whereas today it has increased to about 30%, or 60.9 million cases of shell eggs broken into egg products (American Egg Board, 2004). In fact, tremendous growth of the use of precooked frozen egg products, such as scrambled egg patties, has occurred in foodservice venues, ranging from gas stations to fast food restaurants, especially as breakfast menu items (Turner, 2003).

In particular, the production of refrigerated (extended shelf-life) and shelf-stable precooked egg patties is finding a new niche in the ready-to-eat meal market, especially as military rations and outdoor food items. Manufacturing of shelf stable egg products using conventional thermal processing remains a challenge, as retort processing yields undesirable flavors, greenish-black discoloration, and detrimental changes in texture and syneresis (Baliga, Rao & Lahiry, 1969; Wesley, Rousselle, Schwan & Stadelman, 1982; Luechapattanaporn, Wang, Wang, Tang, Hallberg & Dunne, 2005). As a matter of fact, the U.S. Army recently stopped the production of

retorted scrambled eggs (net weight 2.7 kg) in plastic institutional trays due to the dissatisfaction found by military consumers with respect to the quality of this benchmark product (Dunne, 2005). On the other hand, alternative food sterilization technologies, specifically, high hydrostatic pressure processing in combination with heat, have potential to make production of shelf stable low-acid egg products feasible and acceptable.

High pressure high temperature (HPHT) processing, or pressure-assisted thermal processing (PATP), involves the use of moderate initial chamber temperatures between 60-90ºC, which through internal compression heating at pressures of 600 MPa or greater, can reach in-process temperatures of 90-130ºC. A number of publications prove the bactericidal effectiveness of 700 MPa and process temperature of at least 105ºC MPa for the accelerated inactivation of selected spores (*C. sporogenes, B. stearothermophilus, B. licheniformis, B. cereus, and B. subtillis*) in selected matrices like phosphate buffer, beef, vegetable cream, and tomato puree (Gola, Foman, Carpi, Maggi, Cassara & Rovere, 1996; Rovere, Gola, Scaramuzza & Miglioli, 1998; Raso, Barbosa-Cánovas & Swanson, 1998; Meyer, Cooper, Knorr & Lelieveld, 2000; Heinz and Knorr, 2001; Krebbers, Matser, Hoogerwerf, Moezelaar, Tomassen & van der Berg, 2003; Balasubramaniam and Balasubramaniam, 2003; Ahn, Balasubramaniam & Yousef, 2005; Koutchma, Guo, Patazca & Parisi, 2005). Moreover, *B. amyloliquefaciens,* proven higher in HPHT resistance than *C. botulinum* strains (Margosch*,* 2005*)* and *B. stearothermophilus* spores were inactivated in egg patties at 700 MPa and 105°C (Rajan, Pragandi, Balasubramaniam & Yousef, 2005a; Rajan, Ahn, Balasubramaniam & Yousef, 2005b; Koutchma, Guo, Patazca & Parisi, 2005). Koutchma et al. (2005) also showed that 700 MPa/105°C/4 min was sufficient to destroy 6 log of *B. stearothermophillus* in spore strips in egg patties. Furthermore, Ahn et al.

(2005) obtained up to 7-8 log reduction in *B. amyloliquefaciens* after 700 MPa and 121°C for less than 1 min.

Even though extensive research has been done on bacterial spore inactivation, few quality validation studies of low acid foods after HPHT treatment have been published. Furthermore, no consumer acceptability data on HPHT processed products have yet been reported; neither have comparisons with retort on consumer acceptability. Ludikhuyze and Hendrickx, (2001) have suggested that the three main characteristics of high-quality foods that determine consumers' acceptance are appearance, texture, and flavor/aroma. Some of these three characteristics have been validated using analytical methods on HPHT treated broccoli juice, green beans, and tomato puree, and meat sauce (Van Loey et al., 1998; Rovere, Squarcina, Gola, Sandei, Iametti & Carpi, 2000; Krebbers, Matser, Koets & van den Berg, 2002; Krebbers et al., 2003; Matser et al., 2004). In particular, sensory and analytical color testing on HPHT treated tomato puree resulted in higher color appreciation values than the retorted samples, which correlated with lower content on lycopene found after retort (Krebbers et al., 2003).

Among the existing precooked egg products, scrambled egg patties have been identified as an adequate product for high pressure processing, especially during HPHT processing, due to their semisolid homogeneous structure (Juliano, Li, Clark, Mathews, Dunne & Barbosa-Cánovas, 2004; Juliano et al., 2005). Previous research on HPHT treated scrambled egg patties showed that reformulation with pasteurized process cheese and xanthan gum significantly improved texture and water retention in egg patties compared to untreated egg patties, thereby providing more adequate egg products for a HPHT process (Juliano et al., 2004; Juliano et al., 2005). Given the color and texture retention observed on selected egg patties after HPHT processing, it

is important to determine whether these HPHT processed egg patties are acceptable to consumers and meet desired shelf stability.

Hence, the objective of this research was to evaluate selected scrambled egg patty formulations, after HPHT treatment, by using trained and consumer panels, analytical methods, and incubation tests.

2. Materials and Methods

The following sections will describe commercial egg-based formulations chosen as well as HPHT processing and retort treatments selected for the experimental design. Tools to characterize the process and descriptions of consumer and trained panels are included.

2.1 Egg-based products

Michael Foods Egg Products Company (Gaylord, MN) provided three commercial scrambled egg patties of 42.5 ± 7.1 g. Patties #1 and #2 were round (88.9 ± 6.4 mm diameter) and patty #3 was square (69.9 ± 6.4 mm x 76.2 ± 6.4 mm). Patty #1, the standard Michael Foods patty (code 46025-30020-00), had the following basic ingredients: whole eggs, water, soybean oil, modified food starch, whey solids, salt, nonfat dried milk, and citric acid. Patty #2 (code 46025-70019-00) had the same ingredients as #1 patty, plus 20 % of pasteurized process Cheddar cheese granules. Patty #3 (code 03-1426-10) also had #1 patty basic ingredients, but natural and artificial flavors, xanthan gum, and EDTA were added.

Precooked scrambled egg products were manufactured as indicated in Michael Foods patents (Knipper & Beam, 2002; Merkle, Ball & Mathews, 2003 a, b) by mixing whole pasteurized eggs

with dry and liquid ingredients. The mix was pumped into a mold within a flat cooking belt and egg mix portions were cooked in a convection oven at 180ºC to 250ºC. After baking, patties were frozen and packaged. Frozen scrambled egg patties from a single lot were received from Michael Foods (Gaylord, MN) and stored at -30ºC. Each patty was repackaged in special flexible pouches of 127 mm x 127 mm (ALCAN, Chicago, IL), and defrosted overnight at 5ºC. ALCAN packaging material was composed of biaxial nylon/adhesive/EVOH (ethylene-vinyl alcohol copolymer film)/co-extruded sealant to provide physical, oxygen, and water vapor barrier. Samples were refrigerated for a maximum of 24 h until HPHT treatments.

2.2 Prepackaged precooked egg-product processing

Formulations and processing conditions were tested to gain understanding of their effect on the quality and acceptability of egg patties after HPHT treatment. Three factorial experiments (Table 1) were designed to: 1) study the effects of HPHT processing on consumer acceptability of formulations #2 and #3, and explain subsequent sensory changes after treatment, as well as quality changes during 6-month storage at 37ºC; 2) compare quality after processing by novel in-pouch retort and HPHT at a standard scenario (121ºC) and at a lower pressurization temperature (105 ºC); and 3) test the effect of water addition on consumer acceptability of scrambled egg patties. Egg patties #2 and #3, with added cheese and xanthan gum, respectively, have proved to provide improved texture (lower hardness and syneresis) after HPHT treatment in comparison to HPHT treated egg patty #1 (Juliano et al., 2005), therefore, it is important to know how consumers perceive these changes.

Part of objective 2 included comparing HPHT treated patties with in-pouch retort processed ones, as in-pouch retort offers shorter processing time than conventional retort processing (due to

higher heat transfer rates through the thin polymeric films and smaller size of the samples). Thus, acceptability of a selected egg patty formulation was compared after in-pouch retort processing and after HPHT treatments at low pressurization temperatures (105ºC), and at standard pressurization temperature for sterilization (121ºC).

Finally yet important, previous studies showed that the harder texture obtained after HPHT treatment could be softened by the addition of water into the structure of the patty before processing (Juliano et al., 2005). The effect of water addition into a selected egg patty formulation upon consumer perception of the product was also tested.

Preheating and processing

For each experiment, the initial temperature of the patties was 5ºC. Patties were preheated in a water bath using steam injection until reaching 75ºC, indicated by thermocouples (K-type, Omega Engineering Inc., Stamford, CT) located at the center. Preheated patties were placed into the high pressure vessel, initially preheated at 75ºC, to reach 105ºC during pressurization at 700 MPa for 5 min (HPHT1). In experiment 2 (Table 1), the pressure chamber was preheated at 90ºC to reach 121ºC at 700 MPa, pressurizing for 3 min (HPHT2). A Flow Pressure Systems QUINTUS Food Autoclave Type 35L-600 Sterilization Machine (Flow International Corporation, Kent, WA) with a 17 L vessel, was used with filtered city water as the pressure medium with no heat insulation. Four thermocouples (K-type, Omega Engineering Inc., Stamford, CT) were used to measure temperature of the pressure medium as well as temperature inside the patty.

High pressure processing temperature and pressure conditions will be expressed in the following forms:

- T(ºC)/P(MPa)/t(min) when expressing initial pressure chamber temperature, pressure level used, and pressure holding time (e.g., 90ºC/700 MPa/3 min)
- P(MPa)/T(ºC)/t(min) when expressing pressure level used, product or transmission fluid temperature during pressurization, and pressure holding time (e.g., 700 MPa/121ºC/3 min)

Egg patties (#3) were packaged in retort pouches (Smurfit-Stone, Schaumburg, IL) following the same procedure as high pressure treated patties and were thermally processed using a steam rotary retort (Steritort, FMC corporation, San Jose, CA). The in-pouch retort treatment was not conventional since it was performed using small egg product packages of about 45 g, allowing for higher heat transfer rate, and thereby a process time of 16 min. Fig. 3 shows the temperature and pressure profile obtained during pressurization (HPHT) together with the temperature profile for the retort treatment. The recorded temperature profiles of both processing methods, HPHT2 and retort, at reference sterilization temperature of 121ºC were integrated into Eq. 3 (Stumbo, 1973) by using the General Method (Holdsworth, 1997) to calculate the sterilization values (F_0):

$$F_0 = \int_0^t 10^{\left[\frac{T-121}{z}\right]} dt \approx \sum_0^t 10^{\left[\frac{T-121}{z}\right]} \cdot \Delta t \qquad (1)$$

where T is the process temperature (°C) inside the egg patty, t is processing time (min), 121 corresponds to the reference temperature (°C), and z, the z-value (or thermal sensitivity) [z for *Clostridium botulinum* is 10°C]. The integral of Eq. 1 was calculated using the General Method (Holdsworth, 1997). An approximation to the integral value was done by using the numerical

quadrature formula shown at the right side of Eq. 1 and short time intervals of 1s and 2s for the HPHT2 and retort processes, respectively.

The relative thermal effect on food quality of the processed scrambled egg patties was also quantified using cook values (C_{100}). The cook values for the retort and HPHT2 were calculated using the following equation (Lund 1986):

$$C_{100} = \int_0^t 10^{\left[\frac{T-100}{z}\right]} dt \approx \sum_0^t 10^{\left[\frac{T-100}{z}\right]} \cdot \Delta t \qquad (2)$$

where the chosen z value for the processed egg products is 33°C as it is generally used to calculate the overall quality loss (Lund 1986; Luechapattanaporn et al., 2005). The reference temperature of 100ºC (instead of 121ºC) used in Eq. 2 has been established for quality degradation studies after thermal processing (Lund, 1986).

2.3 Sensory evaluation

Two types of panels, trained and consumer, characterized egg patties before and after HPHT or in-pouch retort treatment. Consumers gave product acceptability values and trained panelists evaluated egg patties with a number of attributes on appearance, texture, and flavor.

2.3.1 Trained panels

Six committed panelists (Washington State University, students, faculty, and staff) were recruited to participate in a long-term sensory evaluation study. Twenty nine (29) appearance, texture, and flavor attributes, arranged in sensory ballots (Fig. 2), were used to describe products of differing formulations and processing conditions. Several training and refresher training

sessions were set up to develop the different sensory attributes and normalize the panelists according to common perceptions, as described below.

2.3.1.1 Training. Panelists were trained using standard and modified scrambled egg formulations in six one-hour sessions for term generation and calibration for accuracy in interpretation and repeatability.

In Session 1, panelists tasted scrambled eggs with specific highlighted attributes on appearance, flavor, and texture. Panelists were invited to generate terms to describe personal observations. Session 2 consisted of generating additional descriptive terms. In Session 3, redundant descriptive terms were removed and samples exhibiting specific attributes were tasted to fine-tune terms to include on ballots. Session 4 was designed to establish ballot anchors where all attributes and their synonyms were fitted (Fig. 2) on a standard unstructured line scale (14 cm). To assist panelists, terms were used to describe each attribute at low intensity (0 cm) and high intensity (14 cm). In Session 5, the ballots were tested by panelists in private booths with unknown representative samples. Collected data were analyzed by ANOVA (Minitab Release 8, 1991), and panelist deviations were assessed to determine where additional training was needed. During the last session, Session 6, tasting results and panelists' deviations were discussed, and specific terms and anchors were clarified. Additional products were tasted to confirm panelist agreements of terms and anchors. Official product testing began the week following training.

2.3.1.2 Refresher training. In order to assure panel accuracy after extended time between tasting sessions, refresher trainings were conducted one week prior to official tasting sessions.

Sessions were set up to refresh panelists' memories of already familiar term definitions and anchors by using samples representing specific attributes.

2.3.1.3 Evaluation sessions. For official experimental testing, samples were labeled with random three-digit codes, matched with panelist number, in a randomized complete block design. In each session, panelists received a maximum of 5 samples to evaluate. For acclimation, the first sample received only by the trained panelists was fresh grilled scrambled eggs (eggs 77%, skim milk 20%, butter 1%, and salt 0.3% in a total of 436 g per sensory run). Scrambled eggs were prepared by mixing fresh eggs, skim milk, and salt until smooth, and fried at 190ºC on a buttered preheated electric skillet (Model 06829, Presto, Eau Claire, WI) until just firm, with no residual liquid.

The remaining experimental samples were served to panelists in random order. Control patties, initially frozen, were heated in boiling water for 20 min. HPHT and retort treated patties were reheated in a water bath at 50ºC. Freshly scrambled eggs and egg patties were placed in packages labeled with the randomized three-digit codes. All packages were kept in the 50ºC water bath until served. Preheated patties (#s 1, 2 and 3) served as controls, and treatments were the same patties after HPHT or retort processing. Each sample was presented to each panelist in private booths lighted with incandescent lights. Bottled water at room temperature was provided was provided to each panelist between samples.

2.3.2 Consumer panels

Consumer tests were run in three sessions, with 40 untrained consumers, simultaneously with trained panels. Consumers received the same samples described in 2.3.1.3, except not standard

scrambled eggs, as they were used only for trained panel acclimation. Consumer panelists evaluated control and treated egg patties for overall acceptability, appearance, aroma/flavor, and texture using a 9-point hedonic scale (1=dislike extremely, 2=dislike very much, 3= dislike moderately, 4=dislike slightly, 5=neither like nor dislike, 6=like slightly, 7=like moderately, 8=like very much, 9=like extremely).

2.4 Analytical testing

Texture Profile Analysis

Texture Profile Analysis (TPA) tests were performed to monitor firmness stability during storage studies explained in 2.5. A TA-XT2 Texture Analyzer (Stable MicroSystems Ltd., White Salmon, WA), fitted with a 5 kg load cell was used. Measurements were carried out using a cylindrical probe of 50.8 mm diameter on cylindrical pieces (25 mm diameter and 8.2 ±1.5 mm thickness) of egg patty at 20ºC. The samples were compressed until 50% of the initial height, as per Montejano, Hamman and Lanier (1985) using protein gels, and Juliano et al. (2005) using egg patties, at a cross-head speed (and post-test speed) of 1 $mm \cdot s^{-1}$. The parameter hardness (N), determined from the peak force during the first compression cycle (Bourne, 2002), was used to represent changes in firmness of the egg patty structure during storage.

Color measurement

Testing of color degradation after treatment and after storage testing was through CIE System evaluation using a colorimeter (Minolta CM-2002 Spectrophotometer, Camera Co., Osaka, Japan). Color was evaluated through the lightness L* and the color intensity, or *chrome*, calculated using Eq. 3.

$$chrome = \sqrt{a^2 + b^2} \quad (3)$$

where +a* represents the red direction, -a* the green direction, +b* the yellow direction, and –b* the blue direction in the L*a*b* color space. *Chrome* value increases from the center of the chromaticity circle outward, and has been shown to represent yellowness levels on egg patties (Juliano et al., 2004).

2.5 Incubation studies and shelf life tests

Shelf stability of formulations #2 and #3 after HPHT processing was evaluated using the end-point method, which consists of incubating samples at 37ºC and evaluating gas formation or package bulging at selected times (Guan et al., 2003). Ten pouches of each formulation, control and processed at HPHT1, HPHT2, and retort (Table 2), were incubated at 37°C for 3 months. Half of the pouches were removed for testing and the other half continued in incubation for 3 months. The pouches were checked for bulging every 2 to 3 days during incubation. Bulged pouches were indicative of presence of gas forming bacteria. At the end of the incubation period, all pouches were opened, and those without signs of gas formation or putrefaction were tested for TPA hardness, lightness L*, and *chrome* (Eq. 3) to evaluate texture and color degradation during the incubation period.

2.6 Statistical analysis

Minitab statistical package (Minitab Release & State College, PA. 1991) was used to differentiate means of all descriptive sensory terms. The significance level was established at $P \leq 0.05$ in a completely randomized block (panelist) design with a one way treatment structure.

Trained and untrained panelists were the units of replication and panelists were treated as random effects.

Statistical differences between means of analytical texture and color data from accelerated shelf life studies were found using the General Linear Models procedure in the SAS statistical package Version 9 (SAS/STAT Language, SAS Institute Inc., Cary, NC, 2004), which performed analyses of variance (ANOVA), Least Square Means, and determination of standard errors.

3. Results and Discussion

Consumer panel overall acceptability of HPHT treated egg patties and their controls will be presented in the following paragraphs. Sections 3.1, 3.2, and 3.3, will specifically discuss consumer scores for appearance, aroma/flavor, and texture/mouthfeel, respectively (Table 3). Each section includes results from the trained panel and instrumental results to interpret consumers' acceptability for each parameter. Shelf stability results will be described in Section 3.4.

Consumer panelists filled in questionnaires that later allowed characterization of the overall profile of the panel and the panelists' preference for egg products. Results on the survey are summarized in Table 2, showing the panel characteristics in terms of gender, age, and egg consumption.

Mean scores for consumer overall acceptability of control and treated scrambled egg patties are included in Table 3. In general, overall acceptability of controls was greater than HPHT treated egg patties, being acceptability of HPHT treated egg patties #3 (xanthan gum, no cheese) lower

than HPHT treated egg patties #2 (cheese) and the controls. In particular, controls for egg patties #1 (standard, no cheese) and #2 (cheese) received overall acceptability mean scores close to "moderately liked", whereas control #3 (xanthan gum) was closer to being "liked slightly". When egg patty #2 (cheese) was high pressure treated at 700 MPa/105ºC/5 min (HPHT1), it provided similar overall acceptability to control patty #3 (xanthan gum, no cheese). HPHT1 treated patty #2 (cheese) was "slightly liked" by consumers whereas HPHT treated formulation #3 (xanthan gum, no cheese) received significantly lower scores in overall acceptability. When looking at all acceptability parameters (including appearance, aroma/flavor, and texture) for the HPHT1 treated patty #2 (cheese), acceptability values were lower in appearance and texture than the controls (Table 3). Discussions on the effect of HPHT on acceptability are shown in the following sections in terms of acceptability of appearance, flavor/aroma, and texture, by comparing with trained panel attributes and analytical descriptors.

The #3 patties (xanthan gum, no cheese) treated at HPHT1 (700 MPa/105ºC/5 min) showed higher acceptability scores, although not significantly different ($P>0.05$), than when treated at HPHT2 (700 MPa/121ºC/3 min). A higher pressurization temperature affected the overall acceptability of formulation #3 at a standard scenario of 700 MPa/121ºC, by mainly affecting texture, as seen by the lower (though not statistically significant) scores shown. Further sections give special attention to the relation between overall acceptability and the other acceptability parameters.

Rapid in-pouch retort sample #3 was "liked slightly", showing a similar acceptability profile to the HPHT1 treated patty #2. Retort treatment mostly affected appearance acceptability, as it is

further explained. Concerning formulation #3, acceptability was higher after in-pouch retort than after both HPHT1 and HPHT2.

The fact that the in-pouch retort patty underwent a rapid processing at 121ºC, due to the use of small pouches and low thermal load, explains the acceptable results. Conventional retort treatment on scrambled eggs packaged in larger volumes, such as trays, yield a product of brownish color and rubbery and grainy structure (Luechapattanaporn et al., 2005; Song & Cunningham, 1985; Baliga et al., 1969). The use of smaller packages increased heat transfer rate, thereby providing a shorter process time than conventional treatment in cans. In general, the thinner shape of retort pouches offer less resistance to the transfer of heat with respect to cans, thereby decreasing process time and increasing energy efficiency (Barbosa-Cánovas & Juliano, 2004).

An F_0 value of 3.3 min was obtained for HPHT2 using the temperature profile obtained at a standard sterilization scenario of 700 MPa/121ºC/3 min. Processing using rapid retort resulted in a F_0 of 5.6 min. Cook values (C_{100}) for HPHT2 treatment and retort were 16.4 min and 30.4 min, respectively. The lower value for HPHT2 shows that the quality damage due to thermal factor should be lower if treated using HPHT2. However, combined pressure and temperature produced changes in the overall quality of the scrambled egg patties, some of which affected the acceptability of formulation #3 after high pressure high temperature treatment.

Even though 5% to 15% water addition to egg patty #3 improved water holding capacity after HPHT treatment patty (Juliano et al., 2005), consumer panelists determined no difference from the overall acceptability of the HPHT treated patties #3 (plus 5% water). It was expected that

water would soften the egg matrix and improve texture acceptability in formulation #3, thereby improving the overall acceptability score. However, the water-added product remained "neither liked nor disliked" by the consumers after HPHT processing in terms of overall acceptability, appearance, aroma/flavor, and texture. Moreover, all descriptors from the trained panel and the colorimeter did not significantly change due to the addition of water in patty #3.

The mean values for appearance, aroma/flavor, and texture in tested formulations at each treatment condition were, in general, similar to the overall acceptability values (Table 3). However, the formulation #3, treated at HPHT2, received lower scores for texture than for overall, appearance, and aroma/flavor.

3.1 Appearance

Regarding appearance, HPHT treatment showed an effect on acceptability, as controls #1 and #2 ("like moderately") and #3 ("like slightly") were more acceptable than HPHT treated patties, which were "neither liked nor disliked" (Table 3). As already mentioned, lower scores (although not significant; $P>0.05$) in appearance values probably contributed to the slightly lower overall acceptability value found in HPHT treated #2 patty, with respect to controls. The same could be argued for the in-pouch retort treated patty #3, not significantly different from HPHT1 treated #2 patty, which showed lower acceptability scores than controls.

The trained panel only found significant differences among controls and HPHT2 treated egg patty #3 in gloss intensity, whereas differences were also found in yellowness (*chrome*) obtained from the colorimeter (Table 4). It is possible that lower values of yellow intensity in HPHT processed formulation #3 influenced appearance acceptability with respect to the control.

Differences in gloss between controls and HPHT treated patty #3 were not verified by the L* values in egg patties, and did not show significant changes due to high pressure and thermal treatment.

Trained panel scores in patties #3, retort and HPHT treated, did not significantly differ from controls in green (to yellow) color (Table 4). However, scores for in-pouch retort treated patty #3 were four times higher in green color than fresh scrambled eggs and control patty #1, which was indicative of the production of green FeS compounds (Song & Cunningham, 1985). *Chrome* (yellow color) values, were maintained in egg patty #2 after HPHT1, as opposed to lower values found in HPHT and in-pouch retort treated #3 egg patties. Lower *chrome* values in retort are in agreement with Luechapattanaporn et al. (2005), who also reported a decrease on +b* value after retorting freshly scrambled eggs in polymeric trays after a F_0 of 4.6 min (about 80 min processing time).

Xanthophylls lutein, zeaxanthin, and crypotaxanthin, that is, carotenoids that provide yellow pigmentation in egg yolk (Yang & Baldwin, 1995), probably degraded during thermal pressurization in formulation #3. Egg patties #2 maintained their original color due to higher egg yolk content and possibly due to the presence of cheese in the mix. Indrawati, Van Loey and Hendrickx (2004) stated that high pressure treatment slightly affect the carotene content in food products, reporting only 5% losses after a treatment of 75ºC/600 MPa/40 min in carrot homogenates.

3.2 Aroma and Flavor

Aroma and flavor of control egg patties, HPHT1 treated patty #2, and in-pouch retort patty #3 were "slightly liked" by consumers (Table 3). HPHT treated #3 patties were "neither liked nor disliked" and had significantly lower acceptability of aroma/flavor than all other patties. However, the trained panel found no significant differences among controls and HPHT treated patties in most flavor attributes except for butter flavor intensity in formulation #3 control, salty tones in formulation #2, and overcooked flavors in retort treated formulation #3 .

The trained panel detected degradation of flavors in #3 patties after HPHT and retort due to lower butter notes (Table 5), indicative of degradation or volatilization of diacetyl compounds in formulation #3 (Andres, 1983). Little is known about the effect of high pressure on butter flavor compounds, and much less has been studied on the effect of high pressure combined with high temperature. Observations in decreased butter notes coincided with those found by Juliano et al. (2004) after treating formulation #3 at 675 MPa/98ºC/5 min in a 1.7 L machine. Decreased butter flavor likely accounts for the lower aroma/flavor acceptability scores by consumers for HPHT treated patty #3.

The trained panel did not identify significant differences in sulfur notes between HPHT treated egg formulations and the controls (Table 5). Sulfur containing volatiles, hydrogen sulfide being the major component after heating eggs, contribute significantly to the overall flavor of eggs, mainly originating from egg whites (Warren & Ball, 1991; Chen & Hsu, 1981). Compounds such as dimethyl and trimethyl sulfide, identified for providing "sulfurous, bad egg odor" (MacLeod & Cave, 1975), were probably not present in high amounts in HPHT treated patty #3 since the

average consumer panel scores did not reach the lower "slightly disliked" threshold of 4.0 (Table 3).

Retort treated #3 patties were higher in over-cooked flavor/aroma tones than #3 patties HPHT treated and control (Table 5). Nose burn aroma note is related to the production of ammonia and hydrogen sulfide upon heating the proteins of the egg white (Germs, 1973; Warren & Ball, 1991). Overcooked was enhanced after retort processing, but did not occur during HPHT processing (Table 5).

The control and HPHT treated #2 egg patties (cheese), were significantly more salty than egg #3 control, HPHT, and retort treated patties (Table 5). Cheese contained in formulation #2 probably increased the impression of saltiness, and no difference in saltiness was seen in patty #2 (cheese) before or after HPHT processing. Comments from the consumer panel included that HPHT treated patty #2 was "a good match of salty and acidic", which probably led to higher flavor acceptability than formulation #3 after HPHT treatment (Table 3).

3.3 Texture and mouthfeel

Texture of control patties and in-pouch retort patty #3 were "slightly" to "moderately liked" by consumers. Although HPHT1 processed patty #2 was neither liked nor disliked, it was not significantly different from control patties (Table 3). On the other hand, consumers slightly disliked the texture of HPHT treated patty #3. Treatment of egg patties #3 at 700 MPa/105ºC/5min yielded higher texture acceptability scores than when treated at 700 MPa/121ºC/3 min. Thus, higher temperature, in combination with 700 MPa, decreased texture

acceptability of formulation #3. In the fact, trends in both texture and overall acceptability scores of HPHT treated egg patties suggest texture as being the controlling variable.

The trained panel supported the decrease in overall acceptability of HPHT treated patties by finding them significantly higher in firmness than control patty #3 (Table 6). However, no significant differences were found in firmness between HPHT treated samples and controls #1 and #2. Juliano et al. (2004, 2005) reported increases in TPA hardness values after treating selected egg patty formulations at initial chamber temperature of 70-75ºC and pressures greater than 300 MPa, due to accelerated protein gelation in egg patties. Furthermore, a higher firmness in HPHT treated products corresponded to higher product density, as seen when comparing formulations #2 and #3 before and after HPHT processing (Table 6). HPHT treatment also increased particle size perception of #3 patties when masticated compared to the controls (Table 6). Previous research also found higher particle size scores in formulation #3 tested in a 1.7 L machine at 675 MPa/98ºC/5 min (Juliano et al., 2004). Furthermore, TPA cohesiveness also increased for formulation #3 after 700 MPa/105ºC/5 min, as reported by Juliano et al. (2005). Even though firmness increased in egg patty #2 after HPHT treatment, overall and texture acceptability values (Table 3) were not significantly changed. Hence, an increase in firmness was not determinant for the acceptability of formulation #2 after HPHT treatment, possibly due to the enhanced mouthfeel sensation provided by the added cheese.

Lower texture acceptability scores found by consumers in HPHT2 treated patty #3 with respect to in-pouch retorted patty #3 (Table 3), were also reflected in the trained panel. Higher firmness and density values were reported for the HPHT2 treated patty than retort #3 (Table 6). Hence, the

sterilization scenario of 121ºC combined with 700 MPa, induced further gelation and hardening in comparison to a 121ºC treatment only.

In general, no significant differences were found among control and treated egg patties for mouthfeel descriptors rough, dry, astringent, pasty and mouth coating. However, HPHT1 treated #3 patty was significantly more oily than #3 control (Table 6). An oily or slimy mouthfeel might be explained by increased oil leached out to the surface during pressurization (and protein gelation at high chamber temperatures). There is evidence that water leaches out of the egg patty matrix after treatment at egg gelation temperatures of 70ºC combined with pressures greater than 300 MPa (Juliano et al., 2004).

Since oily mouthfeel notes remained low in formulation #2, HPHT conditions might have only had an impact when cheese was not present in the formulation. Alternatively, and more likely, added cheese could have masked the oily mouthfeel. Shelke (2004) mentioned that cheese based ingredients "enhance" viscosity, and thereby creaminess and mouthfeel. In the case of HPHT treated egg patties, a higher acceptability seen in HPHT processed formulation #2 (cheese) with respect to #3, is indicated by a lower oily mouthfeel tone as opposed to the scores obtained for HPHT treated egg patties #3, which are closer to greasy and viscous notes.

HPHT2 treated egg patties #3 received higher mean scores for visual syneresis than control #3 patties. This is supported by findings by Juliano et al. (2004), who observed significantly higher syneresis (measured as % weight loss) in formulation #3 after HPHT treatment. A higher syneresis may have influenced the lower texture acceptability scores in formulation #3 obtained

after HPHT treatment. On the other hand, patty #2 (cheese) maintained visual syneresis scores after HPHT treatment, which was also supported by previous work (Juliano et al., 2005).

3.4 Shelf stability of control and treated egg patties

Incubation tests at 37ºC were performed during three and six months. HPHT processed products did not produce gas or decompose for at least six months (Table 7). Control patties degraded after at least one week of incubation, some of them producing gas and some others undergoing proteolytic reactions probably due to spoilage bacteria (Lake, Graves, Lesniewski & Anderson, 1985). In-pouch retort treated patties #3 had 10% positive samples after three months of incubation. It is possible that some spore forming spoilage bacteria survived the in-pouch retort treatment.

After three months, a darker surface was observed in all patties due to browning, which was reflected by lower L* values, indicating that 37ºC is a harsh condition for storage of egg-based products for long periods of time.

Egg patty #3, retort and HPHT treated, did not change significantly in hardness and *chrome* during incubation at 37ºC for up to six months (Table 8), whereas HPHT1 treated patty #2 showed increasingly higher hardness values and lower yellow color, indicated by L* and *chrome* values. Changes in *chrome* can also be directly related to the browning occurring during storage, however, there are other reasons that could explain changes in texture in egg patty #2: (a) continuing gelation during storage at 37ºC, (b) low serum remaining in the package after thermal pressurization, and (c) permeation of water outside the package.

Gelation did not occur to a great extent in formulation number #3, as shown by hardness values, which remained practically unchanged throughout the storage time (Table 8). A factor that contributed to maintaining or increasing texture could be the water released inside the package after thermal pressurization. Previous work on egg patty formulations #2 and #3, showed that #2 patties gave 2-3% free liquid inside the package immediately after HPHT1, whereas HPHT1 treated formulation #3 gave 8-10% (Juliano et al., 2005). Therefore, a higher serum surrounding the surface of patty #3 could have contributed to maintaining the softness of the patty #3, as opposed to patty #2.

Permeation of water out of the package could be another factor that may have increased the hardness in patty #2 during storage, besides the low residual serum yielded. Little is known about the vapor permeability of packages after HPHT treatment, which might be increased. In fact, after incubation, both formulations were observed to have reduced free liquid remaining in the package (data not shown). The surface of the HPHT1 treated patty #2 looked drier, and no residual fluid was left on it; indicative of slow water diffusion during storage at 37ºC, which thereby created a harder structure. This phenomenon depends not only on the type of packaging film used, but also on the thickness of the sample, which sets its ability to retain water.

The identification of new suitable packaging materials that not only reach complete film and seal integrity, but also provide adequate high vapor and oxygen permeability after high pressure thermal treatment is work in progress. Regardless of this fact, accelerated shelf stability results shown in this document, proved that HPHT process maintained analytical descriptors for texture and color in egg patties, while preventing product decomposition and gas production.

Accelerated shelf life testing has set up the potential of HPHT process to provide extended shelf stability to egg-based products at room temperature conditions. Compression heating not only allows a shorter process, but also gives the appropriate HPHT conditions for inactivation of spore-forming spoilage bacteria. Microbial and quality storage studies at room temperature of HPHT treated egg-based formulations should follow as part of further quality studies needed to validate this process.

4. Conclusions

Commercial egg patty formulations tested as controls, standard formulation (#1), formulation with added cheese (#2), and formulation with added xanthan gum (#3) were acceptable to consumers. After HPHT processing, consumers preferred the formulation with added cheese (#2). Addition of cheese significantly helped maintain color and appearance, flavor/aroma, and texture/mouthfeel after HPHT treatment, regardless of the increased firmness observed by the trained panel. Although addition of xanthan gum in the formulation #3 decreased hardness after HPHT process, the egg patty (#3) with added xanthan gum and flavors was neither liked nor disliked. Degradation of yellow color and flavors, and a firmer texture induced by the thermal pressurization process at 105ºC or 121ºC were the main causes for lower overall acceptability values obtained in formulation #3. Differences detected by trained panelist allowed explaining differences found in overall, appearance, aroma/flavor, and texture acceptability. The main factor controlling overall acceptability was the change in texture acceptability, as evidenced by changes in firmness, density, and TPA hardness. Consumers did not provide higher scores to #3 patties with 5% added water after thermal pressurization, despite the hardness reduction previously detected by analytical methods.

Small packages used for novel in-pouch retort applied to egg patty #3, provided a good scenario for sterilization due to short times needed to reach an F_0 value of 5.6 min, thereby giving products that were slightly liked. This is opposed to previous information on conventional retort treatment on scrambled eggs packaged in trays and cans, where deleterious quality was obtained. It is expected that when sterilizing egg products of larger size at high pressure high temperature conditions, instant compression heating will allow for a shorter process than retort, and therefore better quality. The higher pressurization temperature (121ºC) used as a standard sterilization scenario in formulation #3, did not change acceptability scores with respect to 105ºC, although changes in gloss and texture were detected. Shorter dwell times at high temperature high pressure conditions, in comparison to retort, still gave decreased appearance and texture acceptability in both formulations.

HPHT treated patties showed signs of shelf stability since no decomposition and gas formation were found, as opposed to controls. On the other hand, 10% of in-pouch retort samples decomposed throughout a 3-month period. Identification of suitable packaging that provides oxygen and water barrier after HPHT processing is essential to continuing further studies on shelf stability.

Consumer acceptability of a HPHT treated egg patty formulation with added process cheese proves HPHT processing method as a potential alternative for the production of novel shelf stable egg products for an increasing ready-to-eat meal market. Further consumer acceptability studies should be carried out to evaluate treated egg-based products as ready-to-eat shelf stable, i.e. without presenting untreated controls or benchmark products to consumers. This would

determine their potential as outdoor or military products intended for places with low availability of fresh eggs.

Further data collection on inactivation on *C. botulinum* strains at HPHT conditions will allow validating process sterilization conditions. This will allow guiding processing authorities in the regulatory approval process for this technology. The fact that HPHT processing technology can yield acceptable egg products, a very heat labile material, indicates its potential for the production of prepared shelf-stable low-acid foods containing meats or vegetables.

.

References

Ahn, J., Balasubramaniam, V.M., & Yousef, A.E.. 2005. Effect of pressure-assisted thermal processing on the inactivation of selected *Clostridium* and *Bacillus* surrogate spores. [abstract]. In Nonthermal Processing Workshop. USDA Eastern Regional Research Center, Philadelphia, PA. September 15-16.

American Egg Board. (2004). EGGSolutions™. The complete reference for egg products. Park Ridge, IL.

Andres C. (1983). Concentrated natural butter flavor. *Food Processing*. *44*(12), 76.

Balasubramaniam, S., & Balasubramaniam, V.M. (2003). Compression heating influence of pressure transmitting fluids on bacteria inactivation during high pressure processing. Food Research International. *36*(7), 661-8.

Baliga, B.R., Rao, A.S., & Lahiry, N.L. (1969). Prevention of browning in hard boiled eggs during canning. *Journal of Food Science and Technology*. *6*(3), 200-204.

Barbosa-Cánovas, G.V., & Juliano, P. 2004. Adaptation of classical processes to new technical developments and quality requirements. *Journal of Food Science*. *69*(5), E240-250.

Bourne, M.C. (2002). Food Texture and viscosity: concept and measurement. 2nd Edition. New York: Academic Press Inc.

Chen, T.C., & Hsu S.Y. (1981). Quality attributes of whole egg and albumen mixtures cooked by different methods. *Journal of Food Science*. *46*,984-986.

de Heij, W., van Schepdael, L., van den Berg, R., & Bartels, P.(2002). Increasing preservation efficiency and product quality through control of temperature distributions in high pressure applications. *High Pressure Research.22*: 653-657.

Dunne C.P.(2005). U.S. Army Natick Soldier Center, Department of Defense. Personal Communication. August 5.

Germs, A.C. (1973). Hydrogen sulfide production in eggs and egg products as a result of heating. *Journal of Science and Food Agriculture*. *24*, 7-16.

Guan, D., Gray, P., Kang, D.H., Tang, J., Shafer., B., Ito, K., Younce, F., & Yang, T.C.S. 2003. Microbiological validation of microwave-circulated water combination heating technology by inoculated pack studies. *Journal of Food Science*. *68*(4):1428-1432.

Gola, S., Foman, C., Carpi, G., Maggi, A., Cassara, A., & Rovere, P. (1996). Inactivation of bacterial spores in phosphate buffer and in vegetable cream treated with high pressure. In S. Schuppan, *High Pressure Bioscience and Biotechnology*, (pp. 253-259). Kyoto, Japan.

Gujral, H.S., Rosell, C.M., Sharma, S., & Singh, S. (2003). Effect of sodium lauryl sulphate on the texture of sponge cake. *Food Science and Technology International*. *9*(2), 89-93.

Heinz, V., & Knorr, D. 2001. Effect of high pressure on spores. In M.E.C. Hendrickx and D. Knorr, *Ultrahigh Pressure Treatment of Foods* (pp. 77 – 116). New York: Kluwer Academic/Plenum Publishers.

Holdsworth, S.D. (1997). Thermal processing of packaged foods. New York: Blackie Academic & Professional.

Indrawati, Van Loey, A., & Hendrickx, M. (2004). High Pressure Processing. In C.J.K. Henry & C. Chapman, *Nutrition Handbook for Food Processors* (440-461 p). Woodhead Publishing.

Juliano, P., Li, B., Clark, S., Mathews, J.W., Dunne, C.P., & Barbosa-Cánovas G.V. (2004). Optimal high pressure thermal sterilization conditions for formulated egg products [abstract]. *IFT Annual Meeting Book of Abstracts* (pp. 136, Abstract nr 49H-20, July 12-16). Las Vegas, Nev.: Institute of Food Technologists.

Juliano, P., Toldrà, M., Koutchma, T., Balasubramaniam, V.M., Clark, S., Mathews, J.W., Dunne, C.P., Sadler, G., & Barbosa-Cánovas, G.V. (2005). Texture and water retention

improvement in high pressure thermally sterilized scrambled egg patties. Journal of Food Science. In press.

Knipper, A.J., Beam L.S., inventors; Michael Foods Egg Products Company, assignee. (2002) July 2. Enhanced precooked egg product and process for formulation of precooked egg products. U.S. Patent 6,413,572.

Koutchma, T.; Guo, B.; Patazca, E.; Parisi, B. (2005). High pressure – high temperature inactivation of *Clostridium sporogenes* spores: from kinetics to process verification. Journal of Food Process Engineering. In press.

Krebbers, B., Matser, A.M., Koets, M., van den Berg, R.W. (2002). Quality and storage-stability of high-pressure preserved green beans. *Journal of Food Engineering*, *54* (1), 27-33.

Krebbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M.M., van den Berg, R.W. (2003). Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Science and Emerging Technologies*. *4* (4), 377-385.

Lake, D.E., Graves, R.R., Lesniewski, R.S., Anderson, J.E. (1985). Post-processing spoilage of low-acid canned foods by mesophilic anaerobic sporeformers. *Journal of Food Protection*. *48*(3): 221-226.

Larousse, J., & Brown, B.E. (1997). Food canning technology. New York: Wiley-VCH.

Ludikhuyze, L., & Hendrickx, M.E.G.. 2001. Effects of high pressure on chemical reactions related to quality. In M.E.G. Hendrickx, D. Knorr, *Ultra High Pressure Treatments of Foods* (pp. 167-188). New York: Kluwer Academic/ Plenum Publishers.

Luechapattanaporn, K., Wang, Y., Wang, J., Tang, J., Hallberg, L.M., Dunne, C.P. (2005). Sterilization of scrambled eggs in military polymeric trays by radio frequency energy. *Journal of Food Science*. *70*(4):288-294.

Lund, D.B. (1986). Kinetics of physical changes in foods. In M.R. Okos, *Physical and chemical properties of food.* (pp. 367–381). St. Joseph, Mich.: American Society of Agricultural Engineers.

MacLeod, A.J. & Cave S.J. (1975). Volatile flavor components of eggs. *Journal of Science and Food Agriculture*. *26*, 351-360.

Margosch, D. (2005). Behavior of bacterial endospores and toxins as safety determinants in low acid pressurized food. Dissertation, TU Berlin, Germany.

Margosch, D., Ehrmann, M.A., Gaenzle, M.G., Vogel, R.F. 2004. Comparison of pressure and heat resistance of *Clostridium botulinum* and other endospores in mashed carrots. *Journal of Food Protection. 67*(11), 2530-2537.

Matser, A.M., Krebbers, B., van den Berg, R.W., & Bartels, P.V. (2004). Advantages of high pressure sterilization on quality of food products. *Trends in Food Science & Technology*. *15*(2), 79-85.

Merkle, J., Ball, H., Mathews, J., inventors; Michael Foods of Delaware, Inc., assignee (2003a) June 26. Formulation and process to prepare a premium formulated fried egg. U.S. Patent 30,118,714.

Merkle, J., Ball, H., Mathews, J., inventors; Michael Foods of Delaware, Inc., assignee (2003b) July 17. Formulation and process to prepare a premium formulated fried egg. U.S. Patent 30,134,030.

Meyer, R. S., K. L. Cooper, D. E. Knorr, and H. L. M. Lelievald. High-pressure sterilization of foods. *Food Technology*. *54*,67-73.

Montejano, J.G., Hamann, D.D., & Lanier, T.C. (1985). Comparison of two instrumental methods with sensory texture of protein gels. *Journal of Texture Studies*. *16*(4), 403-24.

Paraskevopoulou, A., & Kiosseoglou, V. (1997). Texture profile analysis of heat-formed gels and cakes prepared with low cholesterol egg yolk concentrates. *Journal of Food Science*. *62*(1), 208-11.

Raso, J., Barbosa-Cánovas, G. V., & Swanson, B.G.. (1998). Sporulation temperature affects initiation of germination and inactivation by high hydrostatic pressure of *Bacillus cereus*. *Journal of Applied Microbiology*. 85, 17-24.

Rajan, S., Pandrangi, S., Balasubramaniam, V.M., & Yousef, A.E. (2005a). Inactivation of *Bacillus stearothermophilus* spores in egg patties by pressure assisted thermal processing. *Lebensmittel-Wissenschaft-und-Technologie*. In press.

Rajan, S., Ahn, J., Balasubramaniam, V.M., & Yousef ,A.E. (2005b). Combined pressure-thermal inactivation kinetics of *Bacillus amyloliquefaciens* spores. [abstract no. 23.] Nonthermal Processing Workshop. USDA Eastern Regional Research Center, Philadelphia, PA. September 15-16.

Rovere, P., Gola, S., Maggi, A., Scaramuzza, N., & Miglioli, L. (1998). Studies on bacterial spores by combined pressure-heat treatments: possibility to sterilize low acid foods. In N.S. Isaacs, *High Pressure Food Science, Bioscience and Chemistry* (pp. 354-363).Cambridge: The Royal Society of Chemistry.

Rovere, P., Squarcina, N., Gola, S., Sandei, L., Iametti, S., & Carpi, G. (2000). Effect of thermal treatment under high pressure on the quality of a meat sauce. *High Pressure Research. 19*, 99–107.

Shelke, K. (2004). Cheese targets all the trends. *Food Processing*. *65*(10), 41-47.

Song, I.S., & Cunningham, F.E. (1985). Prevention of discoloration in retorted whole egg. *Journal of Food Science*. *50*(3),841-842.

Stumbo, C.R. (1973). Thermobacteriology in food processing. 2nd ed. New York:

Academic Press.

Turner, J. 2003. Eggs-traordinary!. Food Product Design. *13*,(8): November supplement.

Van Loey, A., Ooms, V., Weemaes, C., Van den Broeck, I., Ludikhuyze, L., Indrawati, Denys, S., Hendrickx, M. (1998). Thermal and pressure-temperature degradation of chlorophyll in Broccoli (*Brasssica oleraces L. italica*) juice: a kinetic study. *Journal of Agricultural Food Chemistry*. *46*, 5289–5294.

Wesley, R.D., Rousselle, J.R., Schwan, D.R., & Stadelman, W.J. 1982. Improvement in quality of scrambled egg products served from steam table display. *Poultry Science*. *61*(3), 457-462.

Warren, M.W. & Ball, H.R. (1991). Effect of concentration of egg yolk and white on fresh scrambled egg flavor. *Poultry Science*. *70*, 2186-2190.

Woodward, S.A., & Cotterill, O.J. (1986). Texture and microstructure of heat-formed egg white gels. *Journal of Food Science*. *51*(2),333-9.

Yang, S.H., & Baldwin, R.E.. (1995). In W. J. Stadelman & O.J. Cotterill. *Egg science and technology* (pp. 405-464). 4th ed. New York: Food Products Press.

Table 1. Research design.

	Main variable	Design	Levels
1.	Formulation	2 x 3 factorial: 2 replicates	[Control, HPHT1] x [formulations #1, #2, #3]
2.	Processing	4 x 1 factorial: 2 replicates	[Control, HPHT1, HPHT2, Retort] x [formulation #3]
3.	Water added	2 x 2 x 1 factorial: 3 replicates	[Control, HPHT1] x [0%, 5%] x [formulation #3]

HPHT1 – 700 MPa, 105ºC, 5 min

HPHT2 – 700 MPa, 121ºC, 3 min

Table 2. Consumer information collected from the panelists before product evaluation (results summarize means of 3 panels).

Panelist characteristics	
Gender	44 – 53% female
	47 – 56% male
Age range	18 – 56 yr[a]
Have previously eaten scrambled eggs	100%
Eat plain scrambled eggs	52 – 68%;
Add condiments in scrambled eggs	Salt (78-85%); pepper (77-89%); ketchup
	(40-63%); other flavoring (57-67%)
Would purchase shelf stable ready to eat scrambled eggs[b]	53-59%

[a] high proportions were between 27 to 35 years of age (20%) and 41 to 45 years (18%)

[b]before tasting the products

Table 3. Consumer evaluation of preheated and HPHT processed egg patties*. Different letters indicate significant differences between means within a column (P<0.05).

Patty type**	Treatment	Overall	Appearance	Aroma/Flavor	Texture
#1	Control	6.7 ± 1.5 c	6.9 ± 1.6 c	6.5 ± 1.5 c	6.4 ± 1.7 c
#2	Control	6.8 ± 1.6 c	6.8 ± 1.4 bc	6.5 ± 1.7 c	6.8 ± 1.7 c
#3	Control	6.3 ± 1.8 c	6.2 ± 1.7 bc	6.0 ± 2.0 c	6.4 ± 1.8 c
#2	HPHT1	6.0 ± 1.7 bc	5.4 ± 1.8 ab	6.2 ± 1.8 c	5.4 ± 1.9 bc
#3	HPHT1	4.8 ± 1.8 a	5.0 ± 1.8 a	4.6 ± 2.0 a	4.8 ± 1.6 ab
#3 / 5 % water	HPHT1	4.9 ± 1.8 ab	4.7 ± 2.2 a	4.8 ± 2.0 ab	4.8 ± 2.1 ab
#3	HPHT2	4.4 ± 2.0 a	4.8 ± 2.1 a	4.8 ± 1.9 ab	3.8 ± 2.2 a
#3	In-pouch retort♦	6.0 ± 1.5 bc	5.6 ± 2.1 ab	6.0 ± 1.6 bc	5.9 ± 1.8 bc

*acceptability scores of 3 = dislike moderately, 4 = dislike slightly, 5 = neither like or dislike, 6 = like slightly, and 7 = like moderately

**#1 (standard egg patty); #2 (added cheese); #3 (added xanthan gum and flavors)

♦non conventional, due to rapid processing and use of products of thin cross section in small retort pouches

Table 4. Significant appearance descriptors[#] and L* and *chrome* values found for control and HPHT processed egg patties. Different letters indicate significant differences between means within a column ($P<0.05$).

Product	Treatment	Gloss intensity	Green color	L*	*chrome*
#1	Control	5.1 ± 1.1 a	0.1 ± 0.1 a	77.3 ± 4.3 a	35.6 ± 2.0 b
#2	Control	4.2 ± 0.9 a	0.8 ± 0.8 ab	73.3 ± 3.9 a	34.8 ± 2.5 b
#3	Control	5.7 ± 0.7 a	0.6 ± 0.3 a	77.6 ± 2.5 a	35.0 ± 1.2 b
#2	HPHT1	9.4 ± 2.2 ab	1.2 ± 1.2 ab	75.7 ± 2.4 a	33.8 ± 1.2 b
#3	HPHT1	8.8 ± 1.2 ab	1.6 ± 0.5 ab	73.0 ± 2.9 a	26.2 ± 1.4 a
#3	HPHT2	11.5 ± 0.6 b	2.7 ± 1.1 ab	77.3 ± 3.0 a	27.1 ± 1.5 a
#3	In-pouch retort	7.3 ± 1.4 ab	4.3 ± 1.4 b	76.2 ± 3.4 a	29.3 ± 1.6 a

[#] Evaluation by trained sensory panel with 14 cm unstructured line scale

Table 5. Significant flavor descriptors* found for controls and HPHT treated egg patties. Different letters indicate significant differences between means ($P<0.05$) within a column.

Product	Treatment	Butter	Sulfur	Overcooked	Salty
#1	Control	4.2 ± 1.3 ab	3.7 ± 0.8 a	2.3 ± 0.8 ab	0.7 ± 0.3 a
#2	Control	5.8 ± 2.0 ab	3.4 ± 1.1 a	2.9 ± 0.9 ab	4.1 ± 1.9 b
#3	Control	9.0 ± 1.1 b	3.0 ± 0.5 a	0.4 ± 0.2 a	0.8 ± 0.3 a
#2	HPHT1	6.0 ± 2.2 ab	3.4 ± 1.2 a	1.9 ± 1.2 ab	5.2 ± 0.9 b
#3	HPHT1	3.3 ± 1.5 a	7.1 ± 1.1 a	0.1 ± 0.1 a	0.4 ± 0.2 a
#3	HPHT2	1.5 ± 1.4 a	4.7 ± 1.6 a	1.5 ± 1.1 ab	0.6 ± 0.3 a
#3	In-pouch retort	2.0 ± 2.0 a	3.5 ± 1.0 a	5.0 ± 1.9 b	0.4 ± 0.3 a

* Evaluation by trained sensory panel with 14 cm unstructured line scale

Table 6. Significant texture and mouthfeel descriptors* found for controls and HPHT treated egg patties. Different letters indicate significant differences between means ($P<0.05$) within a column.

Product	Treatment	Density	Firmness	Particle size	Oily
#1	Control	8.5 ± 0.8 bc	7.4 ± 0.9 bc	4.8 ± 1.2 ab	4.1 ± 1.4 ab
#2	Control	7.7 ± 1.1 ab	7.6 ± 1.0 bc	4.4 ± 1.7 ab	1.0 ± 1.0 a
#3	Control	4.0 ± 0.8 a	3.2 ± 0.8 a	1.9 ± 0.7 a	1.2 ± 0.7 a
#2	HPHT1	11.9 ± 0.7 c	11.7 ± 0.7 c	7.9 ± 2.1 b	2.5 ± 1.9 a
#3	HPHT1	9.2 ± 1.2 bc	10.5 ± 0.9 c	7.0 ± 1.3 b	8.0 ± 1.4 b
#3	HPHT2	12.5 ± 0.6 c	10.4 ± 1.4 c	8.1 ± 1.7 b	4.1 ± 1.8 ab
#3	In-pouch retort	8.3 ± 2.2 b	6.3 ± 1.6 ab	5.4 ± 1.4 ab	4.7 ± 1.4 ab

* Evaluation by trained sensory panel with 14 cm unstructured line scale

Table 7. Percentage of packages showing gas formation and/or product decomposition, after three- and six-month incubation at 37ºC, of egg patty formulations #2 (cheese) and #3 (xanthan gum) treated at 700 MPa/105°C (HPHT1), and formulation #3 treated at 700 MPa/121°C (HPHT2) and in-pouch retorted.

Patty	Treatment	Storage time at 37 ºC	Gas formation / decomposition
#3	Control	3 mo	Positive (100%)
#3	Control	6 mo	Positive (100%)
#3	HPHT1	3 mo	Negative
#3	HPHT1	6 mo	Negative
#3	HPHT2 (F_0 = 3.3 min)	3 mo	Negative
#3	HPHT2 (F_0 = 3.3 min)	6 mo	Negative
#3	In-pouch retort (F_0 = 5.6 min)	3 mo	Positive (10%)*
#3	In-pouch retort (F_0 = 5.6 min)	6 mo	Positive (10%)*
#2	HPHT1	3 mo	Negative
#2	HPHT1	6 mo	Negative

*Complete product degradation without gas formation

Table 8. Accelerated shelf life test of formulations #2 (cheese) and #3 (xanthan gum) treated at 700 MPa/105°C (HPHT1), and formulation #3 treated at 700 MPa/121°C (HPHT2) and in-pouch retorted. TPA hardness and color values after 0, 3, and 6 mo storage at 37ºC. Different letters within the same treatment indicate significant differences between means ($P<0.05$) within a column.

Patty	Treatment	Storage time at 37 ºC	TPA Hardness (N)	L*	*chrome*
#3	HPHT1	0 mo	35.5 ± 7.0 a	80.3 ± 0.8 c	26.2 ± 0.7 a
		3 mo	35.6 ± 5.4 a	73.3 ± 0.7 b	29.2 ± 0.6 a
		6 mo	46.1 ± 6.1 a	67.6 ± 0.9 a	29.7 ± 0.8 a
#3	HPHT2	0 mo	36.5 ± 5.5 a	78.7 ± 0.8 c	27.1 ± 0.7 a
	(F_0 = 3.3 min)	3 mo	49.9 ± 5.5 a	71.8 ± 0.8 b	27.7 ± 0.7 a
		6 mo	49.7 ± 5.5 a	65.5 ± 0.8 a	28.4 ± 0.7 a
#3	In-pouch retort	0 mo	24.3 ± 5.7 a	76.2 ± 0.9 b	29.3 ± 0.8 a
	(F_0 = 5.6 min)	3 mo	24.7 ± 5.7 a	72.3 ± 0.9 a	29.1 ± 0.8 a
		6 mo	29.5 ± 5.5 a	71.9 ± 0.8 a	28.5 ± 0.7 a
#2	HPHT1	0 mo	35.1 ± 4.5 a	77.1 ± 0.6 c	33.8 ± 0.5 a
		3 mo	73.8 ± 6.1 b	70.9 ± 0.8 b	32.6 ± 0.7 a
		6 mo	120.7 ± 5.5 c	53.2 ± 0.8 a	35.0 ± 0.7 b

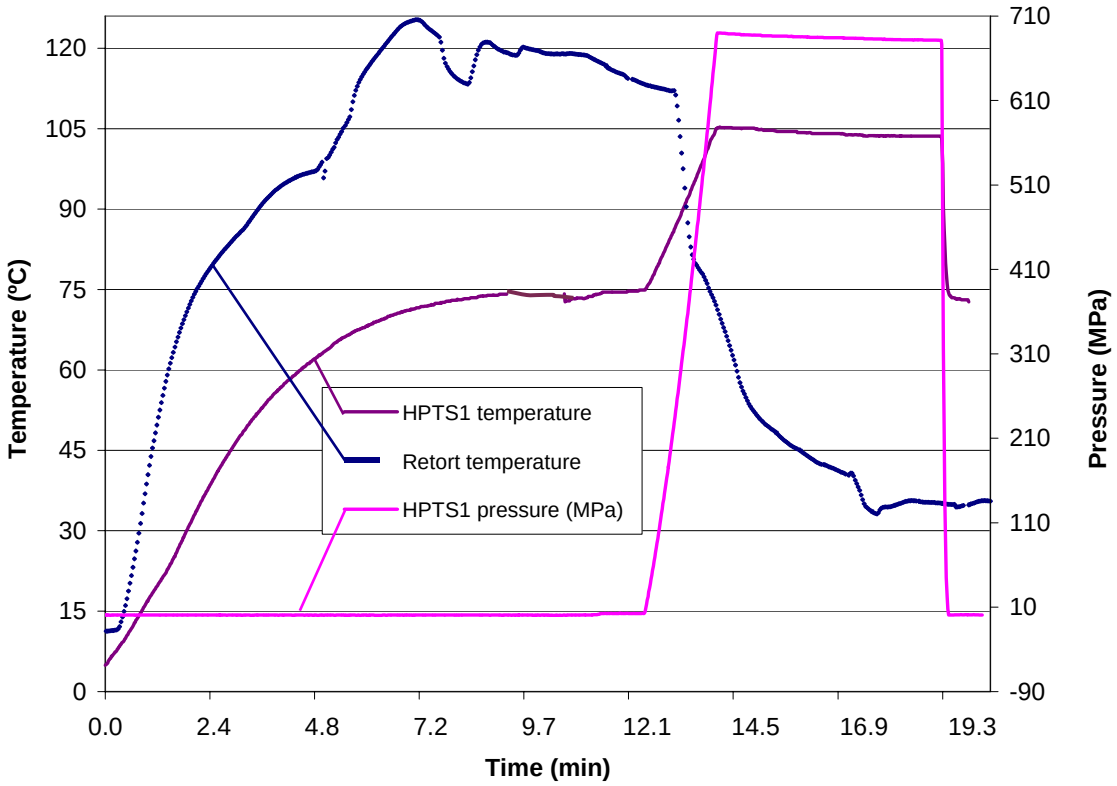

Fig. 1. Typical temperature and pressure profiles during pressurization of the transmission water/egg patty system at 700 MPa/105°C/5 min (HPTS1). Retort temperature profile is also shown as read from the thermocouples inside the tested scrambled egg patties.

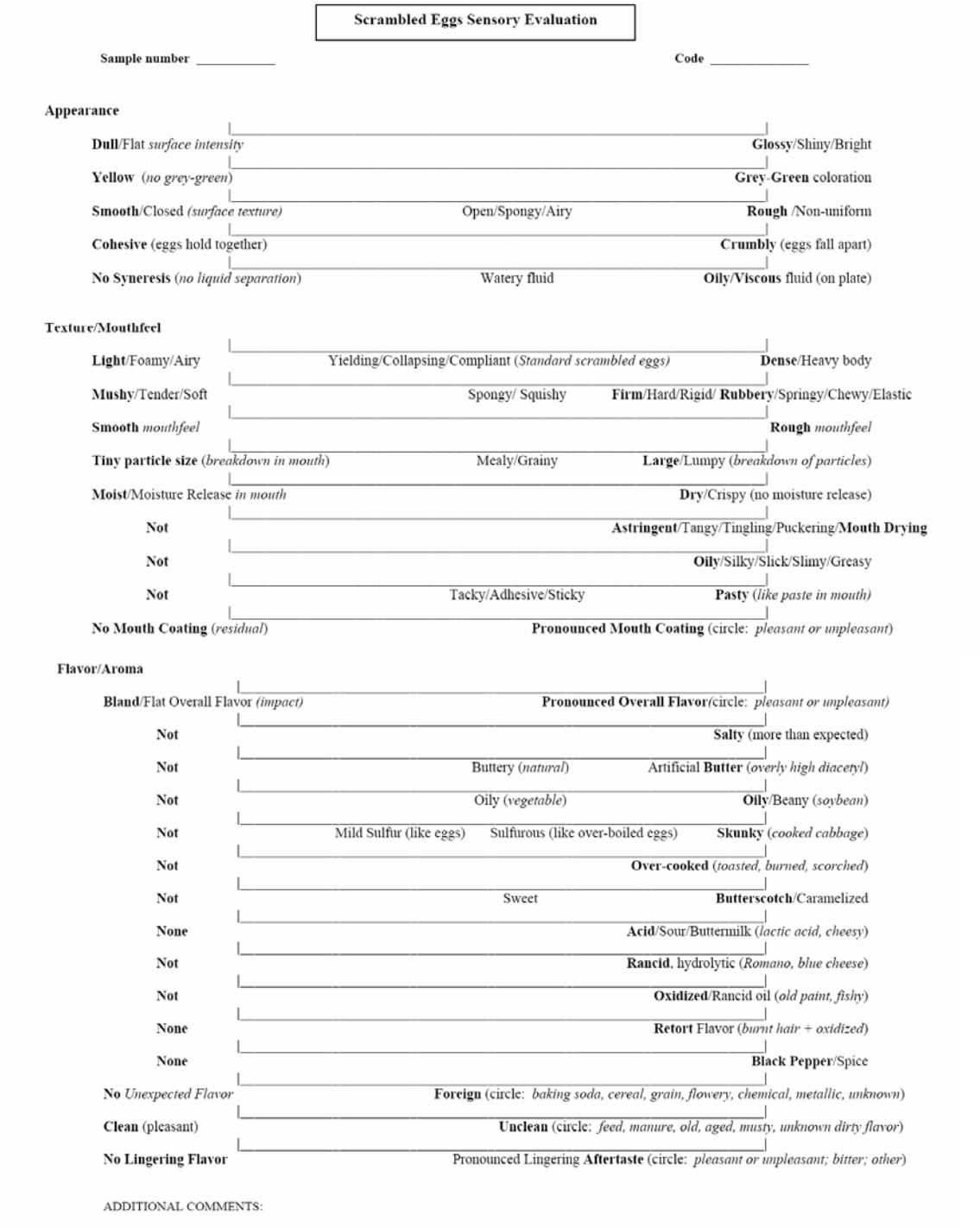

Fig. 2. Descriptive sensory ballots developed during panelist training sessions used for evaluation of egg patties. A 14 cm unstructured line scale was used for each descriptor.

GENERAL CONCLUSIONS

This research aimed to establish the feasibility of HPHT processing for the production of shelf stable egg-based scrambled egg patty formulations. Special attention was given to identifying minimal process requirements for bacterial spore inactivation based on the current knowledge in the literature, egg product selection and characterization, product/process modification for texture improvement, and consumer acceptability of the end product.

The concept of combining high hydrostatic pressure and heat to commercially sterilize low-acid foods is scaling from the laboratory bench to the pilot plant, as four pilot 35 L high pressure sterilization vessels are being used in industrial/government consortia projects. Patents have been published proposing different approaches, among which the application of a single pressure pulse of 600 MPa or greater, combined with temperature between 90-130ºC, seems most appropriate from a food safety and economic point of view.

Based on microbial spore surrogate inactivation data, a process of 700 MPa/105ºC/5 min would suffice commercial sterility. However, at this stage of development, lower processing temperatures than 121ºC cannot assure sterilization due to the lack of microbial inactivation data on many *C. botulinum* strains. Hence, based on the current knowledge, regulatory approval can only be obtained by filing this technology as a thermal process, following the guidelines established in the 9CFR318.300, 9CFR381.300, and 21CFR113.

Attainment of optimal sterilization conditions has been related to the efficient use of compression heat developed during pressurization. Equipment modification with heat retention

aids such as an insulating polymeric liner at chamber walls, preheated pressurization fluids, and an internal pressure intensifier to decrease the amount of inflowing pressurization fluid, can yield more uniform temperature distribution across the chamber volume. If a nearly adiabatic state is achieved inside the liner, pressure holding times may be decreased as temperature will be more uniform, even nearby the steel chamber walls.

From a quality standpoint, selection of preheating methods that provide minimal preheating time may contribute to minimize quality damages after thermal pressure treatment. HPHT process parameters preheating rate, pressure come up time, temperature at the beginning and end of holding/pressurization time, and temperature at the end of pressure release can be used as indicators of process performance in terms of heat retention. These parameters, or heat transfer models accounting for time-temperature (pressure) profiles, will ultimately allow determining microbial inactivation and quality factors for process design and control.

Among tested commercial scrambled egg patty formulations, all survived high pressure low temperature treatments, while maintaining their overall quality. Thus, HPP showed potential to produce stabilized prepackaged egg products intended for refrigerated storage. On the other hand, high temperature ≥70ºC in combination with applied pressure enhanced further gelation of pre-coagulated egg protein network. Egg product reformulation with xanthan gum, EDTA, Cheddar cheese, and flavors provided better color, texture, and flavor retention after pressure treatment at sterilization conditions.

Various approaches tested to improving texture and increasing water retention of scrambled egg patties to commercially desired levels after HPHT treatment were identified. Product

reformulation, egg patty porosity reduction, water addition on the surface, and use of low vacuum pressure helped lowering egg product hardness after HPHT treatment to values similar to the untreated standard formulations. Product reformulation with Cheddar cheese particles showed the largest effect on reduction of syneresis. However, higher preheating rates did not significantly improve product texture in the standard egg patty formulation after HPHT processing.

Consumer evaluation showed that the formulation with added cheese was preferred among the HPHT processed formulations. However, addition of cheese to the basic formulation could not decrease firmness to standard control values after HPHT treatment. In fact, the main factor controlling overall acceptability was the change in texture. As opposed to previous information reported on conventional retort treatment on scrambled eggs packaged in trays and cans, small packages containing egg patties with added xanthan gum were acceptable after in-container thermal processing. No significant differences in acceptability were found in the same formulations treated at standard sterilization scenario (700 MPa/121ºC) and minimal sterilization conditions (700 MPa/105ºC). HPHT treated patties showed signs of shelf stability since no decomposition and gas formation were found, as opposed to controls.

Hence, consumer acceptability of a HPHT treated egg patty formulation with added process cheese proves HPHT processing method as a potential alternative for the production of novel shelf stable egg products. Definition of *C. botulinum* inactivation conditions will allow further egg product development studies. In addition, validation of process performance criteria in terms of *C. botulinum* inactivation kinetics in egg media, and pressure and temperature history, will not

only allow process filing by regulatory agencies, but will also help establish a business case for transforming HPHT processing of egg products into an industrial reality.

RECOMMENDATIONS FOR FUTURE RESEARCH

High pressure thermal sterilization of foods is at the early stages of development. Further research is needed in product and process development from an engineering, microbial, quality, economic, and regulatory perspective. Design of industrial equipment is work in progress, as witnessed by four 35L pilot plant machines strategically located in well known research centers around the world. However, systems with higher volume capacity are needed to compensate for capital and installation costs, eventually operating costs, and depreciation, in order to settle a cost-effective process. Systems that allow variations in preheating modes for preheating optimization, as well as cooling rates, have not yet been engineered. Temperature control systems also require thermocouples that can be hermetically fixed into the pouch and provide reliable measures at HPHT conditions. Maximization of heat retention in chamber during pressurization, proven of utmost importance to assure process uniformity, is work in progress. Moreover, engineering design concepts must be put together to develop more automated, reliable, and flexible micro-, laboratory, pilot, and industrial scale machines.

Extensive additional microbial inactivation data on selected *C. botulinum* strains as well as surrogate spore-forming microorganisms of higher resistance is needed to establish, like in thermal processing, an equivalent definition (or performance parameter) for “thermal death time” to be applied as a standard by processing authorities in process validation. Indeed, microbial kinetic models, in combination with heat transfer models, are a useful possibility to measure process performance in terms of inactivation efficacy. More specifically, heat transfer models accounting for the compression heating effect and conductive and/or convective heat transfer mechanisms can be coupled with spore inactivation kinetic constants, expressed as function of

temperature and pressure, to represent inactivation distribution throughout the pressure chamber. Validation of these models is fundamental for their adoption to assess the reliability of a high pressure low-acid food sterilization process.

Spore inactivation information in the existing literature should be revisited, and should account for the temperature profiles achieved during processing, rather than for values expressed for a particular point in the chamber. In particular, comparison of microbial inactivation results performed in micro-, laboratory, and pilot scale machines should give an idea of process scalability.

Synergistic approaches by addition of natural antimicrobial preservatives such as bacteriocins can help reduce HPHT conditions needed to reach sterilization, thereby giving opportunities for the development of products with more heat labile components. In addition, synergistic inactivation effectiveness could lower processing time, thereby providing economical benefits.

Even though it is unlikely that high pressure sterilization will replace canning or freezing, it will open doors for developing modified or novel products to satisfy specific needs in the shelf-stable ready-to-eat meal markets. Creativity in the development of added value products is key for product identification and commercial success. In particular, HPHT treatment effect on nutritional components and determination of nutritional value on HPHT treated foods is a field that requires further exploration.

More research is needed in regards to consumer preferences to product manufactured using HPHT processing. Comparisons with retort processing should also help identify process

advantages from a quality standpoint, especially when using larger packages or large production runs. In this case, determination of optimal formulations using response surface methodology could help come up with the most adequate novel products.

Based on experimental results presented in this dissertation, the following recommendations could be listed for continuation of the research in high pressure sterilization of egg-based products:

- Formulation and product development

Given that texture of scrambled egg patties after HPHT treatment is the dominant factor for product acceptability, more formulations should be developed and tested. An immediate step would be the combination of xanthan gum and cheese particles to determine if at least there is an additive effect after HPHT treatment. Study of gelation kinetics on heat induced egg mixes at HPHT conditions, supported by microstructural studies, can help identify other approaches for texture improvement, allowing the selection of optimum formulations for selected ingredients. Furthermore, optimum vacuum packaging conditions and preheating conditions can also be determined using kinetic models. Changes and porosity and density after HPHT should also be measured.

Specialized consumer acceptability studies should also be carried out to evaluate other HPHT treated egg-based formulations. It would be interesting to understand the effect of consumer expectations on egg product acceptability. For instance, product perception may vary if consumers do not receive untreated controls or benchmark products in the pool of samples. Instead, comparisons can be done between shelf stable products only. This would determine the

real potential of these products as outdoor, military or humanitarian rations intended for places with low availability of fresh eggs or cooking tools.

- Scale up studies

Process scale up can be evaluated from two perspectives: (a) comparing smaller (e.g., 50 – 100 g packages) with larger sized products (e.g., 200 g - 3 kg packages), (b) comparing laboratory, pilot and industrial size machines. In both cases, it should be determined if significant variations are seen while scaling up. Dimensionless numbers could be applied to characterize and reproduce processing conditions.

- Preheating studies

Information on the effect of preheating rates on larger products after HPHT processing can also contribute to minimize process time and, possibly, quality damage. The heat transfer properties of the packaging material used might also play a role in the heating rate of penetration and the lag factor.

- Packaging studies

Identification of suitable packaging that maintains overall and seal integrity, by providing desired oxygen and water barrier after HPHT processing is essential to continuing further studies on shelf stability.

- Shelf life studies

Shelf stability of HPHT egg products at room temperature needs to be explored. Studies on treated egg product can be done using: (a) non-inoculated products, (b) products inoculated with

selected bacterial spores. Studies can be characterized using both instrumental testing as a first approach and, later on, consumer testing.

In this work HPHT processing technology could yield acceptable egg products, a very heat labile material, therefore, its potential for industrial scale production of prepared shelf-stable low-acid foods containing meats or vegetables seems promising. Once regulatory boundaries are surpassed, HPHT processing technology will gain further industrial support and investment to bring shelf-stable ready-to-eat products to global markets.