CONSUMER ACCEPTANCE OF OZONE-TREATED WHOLE SHELL EGGS

THESIS

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By

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ABSTRACT

In-shell thermal pasteurization of eggs has adverse effects on the appearance and functionality of eggs. Ozone-based processing is an alternative technology with potentially fewer adverse effects as it is less thermally intrusive. There are no consumer acceptance studies published on either the appearance or the taste of ozone-treated eggs. This study examines whether consumers can detect differences when comparing ozone-treated eggs to commercially available pasteurized eggs and to fresh unpasteurized eggs. A visual difference test evaluating the appearance of freshly cracked uncooked eggs and a consumer affective test evaluating microwave scrambled eggs made from freshly cracked whole shell eggs was conducted. Visual panelists evaluated eggs on cloudiness of the albumen and yolk, spread of the albumen and yolk, height and color of yolk and overall visual appeal using 10-point linear scales. The yolks and albumens of the thermal treated and ozone-treated eggs were perceived as significantly cloudier than the unpasteurized control while the ozone-treated were perceived as more similar to the control ($p < 0.05$). The yolks of the ozone-treated eggs had a significantly lower heights and greater spreads than the other treatments ($p < 0.05$). Despite these differences, overall visual appeal of ozone-treated eggs was not significantly different from control. In the consumer affective test, panelists used 9-point hedonic scales to evaluate overall liking, appearance, aroma, flavor, and texture. Just-About-Right scales were used to rate

the appeal of color, moistness and texture. There was no treatment effects on degree of liking on any of the attributes. The major difference in egg appeal was in moistness, where thermal treated and ozone-treated eggs were perceived as drier relative to the control but not compared to each other. This did not affect overall liking scores. This shows no adverse effects on consumer acceptance of eggs treated by the ozone process, with acceptance not different from unprocessed control on overall appearance or taste. These findings are useful when considering ozone-pasteurization to enhance the safety of fresh whole shell eggs to meet the goals of the U.S. Egg Safety Action Plan.

DEDICATION

Dedicated to my family.

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CHAPTER 1

INTRODUCTION

In the first decade of the 21st century, there has been an increased awareness of egg safety as a significant public health concern. In the United States it is estimated that 2.3 million shell eggs or 1 out of every 20,000 eggs may be contaminated with the pathogen, *Salmonella enterica* serovar Enteritidis (Ebel and Schlosser, 2000). If the contaminated eggs are subsequently temperature abused, there is a potential for significant bacterial growth that would pose a serious health risk for people who consume these eggs undercooked or raw. Based on Centers for Disease Control and Prevention (CDC) surveillance data, an estimated 174,356 illnesses, 1,440 hospitalizations, and 75 deaths a year are attributed to *Salmonella* Enteritidis-contaminated eggs (USDA-FSIS, 2005).

In 1999, in recognition of the risks and the need for actions to be taken by the egg industry, the Egg Safety Task Force (under the auspices of the President's Council on Food Safety) developed the Egg Safety Action Plan. The plan was based on findings from the 1998 *Salmonella* Enteritidis Risk Assessment (SERA) for shell eggs and egg products prepared by the Food and Drug Administration (FDA) and the USDA's Food Safety and Inspection Service (FSIS). Using a comprehensive farm-to-table risk assessment approach, this report recommended the implementation of key egg safety measures by the egg industry to substantially reduce the risk of illness. The report set forth the goal for the elimination of foodborne illnesses associated with the consumption of *Salmonella* Enteritidis eggs by the year 2010 (President's Council on Food Safety, 1999). Similarly, in the Healthy People 2010 report, 2010 is also their deadline for achieving a 50% reduction (from 1997 baseline data) in *Salmonella* Enteritidis outbreaks (U.S. Department of Health and Human Services, 2000).

Since the report, an updated FSIS risk assessment (USDA-FSIS, 2005) and a proposed rule in the 2004 Federal Register (FDA, 2004) have called for the implementation of mandatory science-based performance standards for the entire egg industry. As a solution, the implementation of a Hazard Analysis Critical Control Point (HACCP) plan with a pasteurization step to kill *Salmonella* Enteritidis was proposed. Egg products (out of the shell) are already, by law, required to be pasteurized under the Egg Products Inspection Act of 1970. Mandatory pasteurization has been successful in eliminating reported cases to the CDC of illnesses associated with egg products. Similarly, in-shell pasteurization is predicted to drastically reduce the incidence of illnesses (FSIS, 2005).

Therefore, with the imminent target of 2010, there is a need within the egg industry to take actions to be in compliance with the plan and any future federal regulations. Irradiation and thermal pasteurization are currently the only two FDA-approved in-shell pasteurization processes. Processors must provide the FDA with evidence of a 5-log reduction in internal *Salmonella* Enteritidis in order to label their eggs as pasteurized (USDA-AMS, 1997). Of the two processes, thermal pasteurization is the only method used at this time for pasteurizing shell eggs available in the U.S. retail market. However, the time and temperatures required to thermally pasteurize shell eggs were reported to adversely affect the quality and functionality of the eggs. The albumen of the eggs appears cloudier, more viscose, and requires longer whipping times (Li-Chan *et al.* 1995; Hou *et al.*, 1996; Schuman *et al.*, 1997).

These changes, if noticeably significant, may take precedence over any safety benefits in consumers' purchase criteria and can deter acceptance. This may be one reason why there is not a great demand for in-shell pasteurized eggs. If processors are not able to pass along the extra production costs associated with pasteurization as a premium to consumers for safer products, they will be reluctant to invest in pasteurization technologies as a control measure.

The economic consequences of association with a foodborne illness can impact the entire egg industry. Consumers may lose confidence in the safety of the egg supply and negatively impact egg sales. According to economic analysis, adverse information about the risk of illness from shell eggs was associated with a 1% decrease in consumption (Morales and McDowell, 1999). The egg industry would thus greatly benefit from innovative solutions that not only produce safe eggs but also overcome the limitations seen with current thermal pasteurization technology.

Ozone-based technology, developed by Yousef and Rodriguez-Romo (2004) at Ohio State University, is proposed as a promising alternative technology that will produce a superior quality egg acceptable by both consumers and the egg industry. The sequential application of heat and pressurized gaseous ozone is intended to lessen the thermal effects on the heat-sensitive albumen proteins. However, the ozone-based process involves exposing eggs to vacuum, pressure, and a powerful oxidant so there is the possibility of changes in sensory quality. At this time, there has been no studies published on consumer acceptance of shell eggs processed using this ozone-based technology. The objectives of this research are to conduct discrimination and consumer acceptance testing of visual and flavor attributes of ozone-treated eggs. If acceptance is favorable, ozone-based technology has the potential for adoption by the egg industry to enhance the safety of shell eggs in order to meet the goals set by the U.S. Egg Safety Action Plan.

CHAPTER 2

LITERATURE REVIEW

Federal Egg Safety Regulatory Agencies and Authorities

The responsibility of overseeing U.S. egg safety regulations is shared by the FDA's Department of Health and Human Services (HHS), USDA's Food Safety Inspection Service (FSIS) and state agencies. The FDA regulates shell eggs while FSIS regulates the processing of egg products. Egg products refer to any dried, frozen, or liquid eggs with or without added ingredients (USDA-AMS, 2008). While the definition of eggs for food consumption include eggs from other birds (turkey, duck, goose or guinea), shell eggs and egg products discussed in this paper refer only to the eggs from domesticated chickens (*Gallus domesticus)*. The FDA's jurisdiction covers whole shell eggs from the producer and processor level to the distribution (wholesale and retail) of eggs. The FDA is responsible for developing standards for egg producers and then state agencies are to conduct inspections and enforce those standards (President's Council on Food Safety, 1999). Egg producers are the persons engaged in the operation of egg production and egg processors are the persons engaged in the operation of assembling, receiving, grading, or packing shell eggs for commercial sale or distribution (ORC, 2008).

There are several agencies under the USDA that oversees the poultry and egg industry. The Animal and Plant Health Inspection Service (APHIS) monitors for the prevalence of *Salmonella* Enteritidis in layer flocks using the National Animal Health Monitoring System. The Agricultural Research Service (ARS) and official state agencies work jointly toward the reduction of disease incidence by certifying poultry breeding stock and hatcheries as *Salmonella* Enteritidis-free through a voluntary National Poultry Improvement Plan (NPIP) (FDA, 2004). The FSIS sets the performance standards for the egg processing industry that include regulating the pasteurization times and temperatures required for egg products. FSIS also oversees proper labeling of shell eggs and consumer education efforts (FSIS, 2005). The Agricultural Marketing Service (AMS) provides a voluntary fee-based grading service for shell eggs in egg-packing plants. The eggs are certified as being processed and packaged under federal supervision based on official U.S. standards, grades and weight classes for shell eggs (USDA-AMS, 2000b).

The FDA, FSIS, CDC, state and local health departments all coordinate to conduct surveillance and monitoring of foodborne outbreaks. Nontyphoidal *Salmonella* spp. infections are national notifiable diseases and there are three programs to specifically track *Salmonella* cases, isolates and outbreaks. They are: Foodborne Diseases Active Surveillance Network (FoodNet), National *Salmonella* Surveillance System through the Public Health Laboratory Information System, and the Foodborne Disease Outbreak Reporting System (President's Council on Egg Safety, 1999).

In addition to federal agencies, egg producers may also choose to participate in state or industry-sponsored egg quality assurance programs (EQAPs). Pennsylvania, Maryland, New York, Ohio, South Carolina, Alabama, Oregon, California and the New England region operate such programs (FDA, 2004). The trade association, United Egg Producers (UEP) provides an UEP Certified program for producers with guidelines based on responsible, science-based production methods (UEP, 2008). The requirements for each program may vary but all are based on HACCP measures and current good manufacturing practices to reduce risk of *Salmonella* Enteritidis-contaminated eggs (Mumma *et al*. 2004). The Layers '99 study conducted by APHIS reported over half of all farm sites surveyed participated in a program (USDA-APHIS, 2000). Mumma *et al*. (2004) determined (through regression analysis) that a 1% increase in number of eggs produced under an EQAP was associated with a 0.14% decrease in *Salmonella* Enteritidis incidence in humans.

Egg Safety Regulations

In 1997, at the request of President Bill Clinton, the FDA, USDA, Environmental Protection Agency and CDC prepared the National Food Safety Initiative. This initiative outlined the steps needed to improve the safety of the nation's food supply and to reduce the incidence of foodborne illness to the greatest extent feasible. The plan's budget allocated for the expansion of food safety research, risk assessment, training and education (FDA, 1997). President Clinton also established the President's Council on Food Safety to "develop a comprehensive strategic food safety plan for Federal food safety activities." This group of experts was to identify and prioritize the areas of food safety that posed the greatest risk to public health. Egg safety was one area of concern that they identified as requiring immediate action. As a result, an Egg Safety Task Force composed of representatives from federal food safety agencies (FDA, CDC, FSIS, APHIS, AMS, and ARS) were commissioned to develop the Egg Safety Action Plan in 1999 (President's Council on Food Safety, 1999).

Using the findings from the comprehensive 1998 farm-to-table *Salmonella* Enteritidis Risk Assessment, the action plan recommended the implementation of key egg safety measures by the egg industry to substantially reduce the risk of illness. The plan proposed two equivalent risk reduction strategies intended for egg producers, egg packers, and egg processors to reduce the incidence of *Salmonella* Enteritidis-contaminated eggs. One strategy called for *Salmonella* Enteritidis testing on farms and the implementation of a system to divert eggs from flocks testing positive to breaker plants for further pasteurization and processing as liquid egg products (President's Council on Food Safety, 1999). The economic impact to processors is the price differential between shell eggs and breaker eggs. This can be anywhere from $0.06 to $0.14 cents per dozen. Eggs from *Salmonella* Enteritidis-positive flock are discounted an additional $0.05-0.08 cents per dozen (Morales and McDowell, 1999).

The alternative strategy requires egg packers or processors to include a FDA-approved pasteurization step as part of their HACCP process (President's Council on Food Safety, 1999). The FDA requires pasteurization processes to achieve a 5-log reduction in *Salmonella* found inside of the egg (USDA-AMS, 1997). For smaller egg processors, the initial cost of implementing a system may be cost prohibitive.

Pasteurization of shell eggs was estimated to cost an additional $0.20 cents per dozen but this may be regained in profits from the higher selling price pasteurized eggs command (Mermelstein, 2001).

The Egg Safety Action Plan also called for the implementation of consistent science-based standards from production to consumption in the egg industry. In response, FDA published the proposed rule, "Prevention of *Salmonella* Enteritidis in Shell Eggs During Production" in the 2004 Federal Register. If approved, this proposal will require all shell egg producers to implement mandatory *Salmonella* Enteritidis preventive measures. These measures include: (1) Procurement provisions of chicks and pullets; (2) biosecurity program; (3) pest and rodent control program; (4) cleaning and disinfection plans of poultry houses testing positive (environmental or egg); (5) egg testing if environmental testing results in a positive test and (6) refrigerated storage of eggs held at the farm. An exemption from the measures (except the refrigerated storage) is permitted if producers (with 3,000 or more laying hens) choose to add a pasteurization step (FDA, 2004).

The Egg Product Inspection Act of 1970 requires the mandatory pasteurization of all liquid egg products. According to FSIS's 2005 risk assessment, mandatory pasteurization was successful in eliminating the number of reported cases of illnesses from *Salmonella* in egg products to the CDC. In contrast, only 0.5% of all shell eggs in the United States are currently being processed as pasteurized shell eggs (USDA-FSIS, 2005). It is believed that if similar measures were adopted by the shell egg industry, this would also drastically reduce the risk of illnesses. FSIS used risk characterization models based on dose-response relationships for *Salmonella* to predict the likelihood of Salmonella *Enteritidis*-contaminated eggs, frequency of illness from eggs and to examine the outcomes of different shell egg pasteurization scenarios. Their model predicted mandatory shell egg pasteurization to achieve a 5-log reduction in *Salmonella* Enteritidis would reduce the annual incidence of illness associated with shell eggs from an estimated 130,000 cases to 19,000. Based on their findings, FSIS recommended pasteurization as the "principal risk management measure" to eliminating internally *Salmonella* Enteritidis-infected eggs (USDA-FSIS, 2005).

U.S. Egg Industry

Eggs are a popular commodity in the United States with over 95% of American households purchasing eggs and eating eggs an average of 1.8 times a week (American Egg Board, 2003). Since 2000, the estimated annual per capita consumption of eggs has held steady at around 250 eggs (USDA-NASS, 2008).

This popularity amongst consumers has made eggs a $4.4 billion industry with yearly egg production totaling 90.2 billion eggs. Table (or shell) eggs account for 76.8 billion of the total eggs (USDA-NASS, 2009). Approximately 60% of the total shell egg production is sold in the retail market. The remainder of the eggs either go for foodservice use (9%), export (<1%) or are further processed into various egg products used in foodservice, manufacturing, retail and export (30%) (American Egg Board, 2008). Based on the average number of layers, Ohio ranks second in all states (Iowa ranks first) for egg production with approximately 26.2 million layers producing 7.2 million eggs (table and hatching) (USDA-NASS, 2009). The industry trend is now towards larger scale operations. In the United States, there are approximately 235 egg producers with flocks of 75,000 layer hens or more, 63 egg producers with over 1 million layers and 15 producers with over 5 million layers (American Egg Board, 2008).

An "in-line" operation is when producers handle both the collecting and the packing of eggs at one centralized facility. After the eggs are laid by the hen, they are carried by conveyor to another building for processing. An "off-line" operation is when producers collect eggs and then ship them to another facility for processing and packaging (Froning *et al.*, 2001). Most modern operations are now in-line (USDA-AMS, 2000a). Once the eggs are collected, they are conveyed through automated washing equipment that washes, sanitizes and dries the eggs. In USDA-approved plants, the minimum temperature of the wash water must be at least 32.2°C and with a temperature differential of 6.7°C between the internal egg temperature and the water temperature (USDA-AMS, 2008). The wash water must be warmer than the egg temperature or else negative pressure could form inside the shell and pull any contaminants from the wash water or present on the surface of the egg into the shell via the pores. The wash water must be changed approximately every 4 hours and at the end of each shift. An USDA-approved sanitizer such as chlorine-based compounds or quaternary ammonium compounds is used at concentrations between 50 to 200 ppm (Zeidler, 2002a; USDA, 2008). The eggs are then dried, candled, sorted, graded, packed, palletized, stored in coolers, and then shipped to their final destinations under refrigerated storage conditions ($\leq$ 7.2°C). Shell eggs typically take three days from processing and distribution until they reach retail outlets or institutions. Eggs are then typically consumed within two to three weeks after being laid (Zeidler, 2002a).

Labeling

Approximately one-third of the nation's shell egg processors participate in the USDA voluntary fee-based grading program. These processors provide three-fourths of the nation's eggs (USDA-AMS, 2006). The packaging cartons of graded eggs display an USDA shield with a grademark designating official U.S. consumer grade standard (AA, A or B). The quality criteria for grading shell eggs (shown in Table 2.1) is based on the quality of the shell, white, yolk, and the size of the air cell. Grading is performed by USDA-licensed graders who are supervised by state, regional, and national supervisors (USDA-AMS, 2000a). There are no official U.S. grade standards for pasteurized shell eggs so these products may carry a "produced from" grademark if they are processed using a FDA-approved process and prepared from U.S. Grade AA or A shell eggs (USDA-AMS, 2000b). The egg carton must have a letter "P" followed by the plant number and a Julian date which is the date the eggs are packed. The Julian date is a three-digit code and represents the consecutive days of the year (001 = January 1 and 365 = December 31) (American Egg Board, 2007). Eggs must not be more than 21 days old at the date of packaging and cannot have been previously shipped for retail sale. This means repackaged eggs are not eligible for U.S. grades (USDA-AMS, 2006). Expiration dates are optional and must not be more than 30 days from the date of pack. "Use before," "Use by," and "Best before" dates are also optional and these indicate the maximum time frame for expected quality. This date must not be more than 45 days from the date of pack (USDA-AMS, 2000a).

Table 2.1: Summary of U.S. standards for quality of shell eggs (modified from USDA-AMS, 2000b and American Egg Board, 2000).

	Grade AA	**Grade A**	**Grade B**
Break Out Appearance	Covers a moderate area. Haugh unit of 72 or higher.	Covers a moderate area. Haugh unit of 60 to < 72.	Covers a wide area. Haugh unit < 60.
Air cell	1/8 inch or less in depth. Unlimited movement and free or bubbly.	3/16 inch or less in depth. Unlimited movement and free or bubbly.	Over 3/16 inch in depth. Unlimited movement and free or bubbly.
Albumen appearance	Clear, firm, stands fairly high; chalazae prominent.	Clear, reasonably firm, stands fairly high; chalazae prominent.	Small amount of thick white; chalazae small or absent. Appears weak and watery.
Yolk Appearance	Firm, round and high.	Firm and stands fairly high.	Somewhat flattened and enlarged.

In 2000, the FDA published the final rule in the Federal Register entitled, "Food Labeling, Safe Handling Statements, Labeling of Shell Eggs; Refrigeration of Shell Eggs Held for Retail Distribution." All raw, shell eggs (with the exemption of pasteurized shell eggs) are required to have the following safe handling instructions on the carton: "SAFE HANDLING INSTRUCTIONS: To prevent illness from bacteria: keep eggs refrigerated, cook eggs until yolks are firm, and cook foods containing eggs thoroughly" (FDA, 2000a).

Nutritive Value and Functionality of Eggs

Eggs are nutrient-dense. One large egg provides 13 essentials nutrients, 12.6% (6.3 grams) of the Recommended Daily Value for protein in adults and children ≥ 4 years of age and 5 grams of fat (mainly monounsaturated and polyunsaturated fats) in only 72 calories (USDA-ARS, 2007). The nutrient composition of a large egg is shown in Table 2.2. Eggs are considered an "excellent" source of choline and selenium and a "good" source of vitamin B_{12}, phosphorus and riboflavin. The protein found in eggs is highly digestible with a biological value of 94%. Eggs are considered one of the highest quality proteins available and are used as the standard to compare protein quality in other foods. The yolk contains vitamins A, D, E, and K as well as folic acid, pantothenic acid and zinc (ENC, 2004). Eggs are also popular as ingredients in dishes. They are multifunctional and are used for coagulation, foaming, emulsifying, and for contributing color and flavor to dishes (Yang and Baldwin, 1995)

Table 2.2: Nutrient composition of a large whole egg, white and yolk[1] (modified from USDA-ARS, 2007).

Nutrient	Whole Egg	White	Yolk
Energy (kcal)	72.00	16.00	54.00
Water (g)	37.90	28.90	8.90
Protein (g)	6.30	3.60	2.70
Carbohydrate (g)	0.40	0.24	0.61
Total lipid (g)	5.00	0.06	4.51
Saturated fat (g)	1.60	0.00	1.60
Monounsaturated fat (g)	1.90	0.00	1.90
Polyunsaturated fat (g)	0.70	0.00	0.70
Trans fat (g)	0.05	0.00	0.05
Cholesterol (mg)	212.00	0.00	210.00
Minerals			
Calcium (mg)	26.00	2.00	22.00
Iron (mg)	0.92	0.03	0.46
Magnesium (mg)	6.00	4.00	1.00
Phosphorus (mg)	96.00	5.00	66.00
Potassium (mg)	67.00	54.00	19.00
Sodium (mg)	70.00	55.00	8.00
Zinc (mg)	0.56	0.01	0.39
Copper (mg)	0.051	0.008	0.013
Manganese (mg)	0.019	0.004	0.009
Selenium (mcg)	15.80	6.60	9.50

Continued

Table 2.2 continued

	Whole Egg	White	Yolk
Vitamins			
Thiamin (mg)	0.035	0.001	0.030
Riboflavin (mg)	0.239	0.145	0.090
Niacin (mg)	0.035	0.035	0.004
Pantothenic acid (mg)	0.719	0.063	0.508
Vitamin B6 (mg)	0.071	0.002	0.059
Folate (mcg)	24.00	1.00	25.00
Choline (mg)	125.50	0.40	116.00
Vitamin B12 (mcg)	0.65	0.03	0.33
Vitamin A (IU)	244.00	0.00	245.00
Lutein and zeaxanthin (mcg)	166.00	0.00	186.00
Vitamin E (mg)	0.48	0.00	0.44
Vitamin D (IU)	18.00	0.00	18.00
Vitamin K (mcg)	0.10	0.00	0.10

[1]Discrepancies in nutrient levels between the white and yolk versus whole egg are due to sampling error.

The albumen or white accounts for 66% of an egg's total liquid weight and contains the majority of the egg's proteins. The albumen is opalescent until it is beaten or cooked and then appears white (American Egg Board, 2007). There are four alternating layers to the albumen; chalaziferous or inner thick white, inner thin white, outer thick white and outer thin layer (Figure 2.1). It is the quantity and viscosity of the thick layers that gives an egg its functionality and quality grading (Stadelman, 1995). When the albumen is beaten vigorously, a foam is created that increases the volume up to eight times the original volume (American Egg Board, 2007). The protein, ovomucin makes up the majority of the thick layers and when heated, forms insoluble films to stabilize foams (Froning *et al.*, 2001; Zeidler, 2002b). Other proteins found in the albumen include (listed in descending order): ovalbumin, conalbumin (ovotransferin), ovomucoid, lysozyme, ovomucin, and avidin (Yang and Baldwin, 1995). Dishes such as meringues, sponge cakes, angel food cakes, soufflés, fluffy omelets, and confectioneries rely on the structure the foams provide to attain the desired volume and stability needed (Ziedler, 2002c). In confectionaries, egg whites are also used to prevent sugar crystallization (Froning *et al*., 2001).

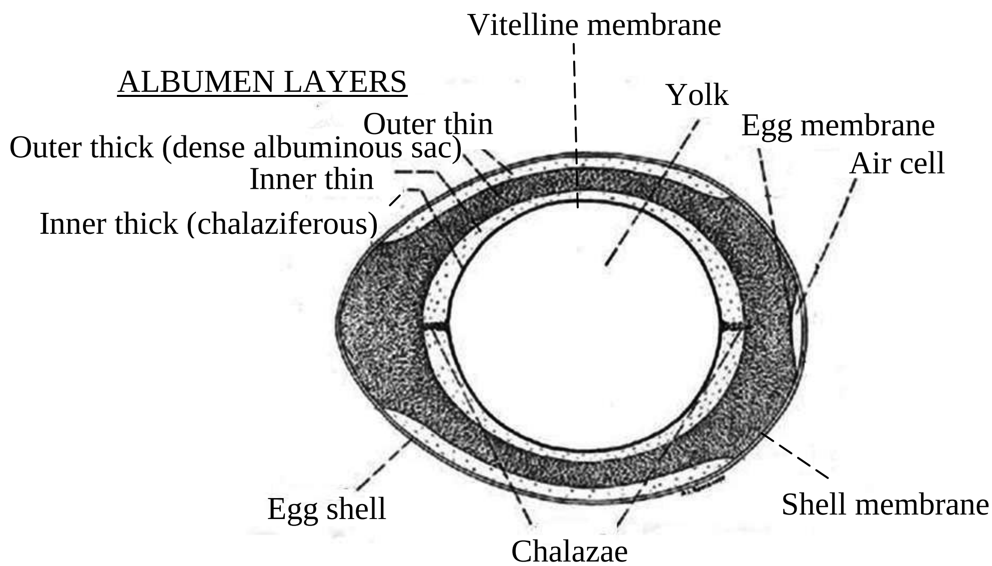


Figure 2.1: Structure of a hen's egg (modified from Romanoff and Romanoff, 1949).

The albumen is judged by its clarity and firmness or thickness (USDA-AMS, 2000b). The Haugh unit is the height of the thick albumen (measured by micrometer) relative to its weight and is a common measurement used in the egg industry to determine albumen quality. A higher Haugh unit corresponds to a higher quality (Stadelman, 1995). The storage conditions of the eggs (length and temperature) inversely affect the egg's Haugh unit. As the storage time and temperature increases, carbon dioxide and moisture diffuses out through the eggshell's pores causing the pH of the egg to rise. The carbon dioxide is responsible for the cloudy appearance of the albumen and so when the egg ages and carbon dioxide loss increases, the albumen becomes clearer. This is why fresher eggs may appear cloudier than older eggs. When the pH rises to around 8.8, the albumen begins to thin and decrease in viscosity (Stadelman, 1995; Froning *et al.*, 2001).

The yolk (30-33% of the total liquid egg weight) is composed of all of the egg's triglycerides, phospholipids, and sterols and is the major source of an egg's calories and nutrients. It is a good source of unsaturated fatty acids (linoleic and oleic) and fat-soluble vitamins (Watkins, 1995). The phospholipids, lecithin and other lipoproteins in the yolk are important emulsifiers for making mayonnaises, salad dressing and other dishes. Specifically, the lipovitellin, livetin, and lipovitellenin act as surface active agents to stabilize films around oil globules in order to form an emulsion (Froning *et al*., 2001). The yolk is also used for its coagulating abilities by providing structure to custards. It also contributes color to foods such as egg noodles and breads (Zeidler, 2002c).

The yolk's yellow-orange color is derived from the fat-soluble carotenoids called xanthophylls, specifically, lutein and zeaxanthin. The color varies according to the nutrient composition of the hen's diet (Li-Chan *et al.*, 1995). A hen fed feed containing yellow corn or alfalfa meal lays eggs with medium yellow yolks. Hens fed wheat or barley produces eggs with lighter-colored yolks (American Egg Board, 2007).

A thin vitelline membrane separates the yolk from the albumen. It is the strength of this membrane that gives the yolk its spherical shape. As an egg ages, the membrane absorbs water from the surrounding albumen and gradually weakens. As a result, when the egg is broken out onto a flat surface, the yolk appears flatter and more spread out than a fresh egg. Therefore, the freshness of an egg is often determined by calculating the yolk index which is the ratio of the yolk's height to diameter. A higher yolk index corresponds to a higher quality fresher egg (Stadelman, 1995).

Specialty Egg Market

In today's marketplace, a wide array of specialty eggs beyond the conventional white shell eggs is available to consumers. Specialty eggs are thought to fulfill consumer needs such as a specific quality attribute, emotional need or health benefit (Patterson *et al.* 2001). These specialty eggs can be categorized into the following groups: nutrient-enhanced, pasteurized, and eggs from specific flock management systems (Zeidler, 2002a). Nutrient-enhanced eggs can be enriched with omega-3 fatty acids, lutein, or vitamin E by adding nutrients to the feed of the hen. Examples of specific flock management eggs are: free-range, cage-free, fertile, vegetarian, and organic eggs (ENC, 2005). There are no set definitions for specialty eggs so there is disagreement within the industry on whether brown shell eggs should be considered specialty eggs since they do not have any value-added benefits. The breed of the hen determines the shell color and brown eggs are not different in nutritional value or wholesomeness from conventional white eggs. However, hen breeds that lay brown eggs are typically larger in size and require more feed so the eggs are more expensive to produce (American Egg Board, 2007).

Specialty eggs are sold at higher prices compared to conventional eggs because of higher production, processing, procurement, and distribution costs (Oberholtzer *et al.*, 2006). At the time of this writing, the retail price of a dozen USDA organic brown eggs were approximately 3.8 times, omega-3 white eggs 2.7 times, cage-free white eggs 3.4 times and vegetarian white eggs 3 times as much as the price of a dozen conventional white grade A eggs (USDA-AMS, 2009). Despite the significantly higher retail prices for specialty eggs, in a national survey of retail eggs where researchers measured interior egg qualities, white eggs were superior to specialty eggs. Specialty eggs were reported to be on average 5 days older (based on carton dating) and had a greater percentage of leakers. Among the specialty eggs, organic eggs had the poorest interior and shell quality yet cost the most (Patterson *et al.*, 2001).

Despite the higher retail prices and lack of evidence of higher quality using traditional methods, there is still a growing demand for specialty eggs. According to the ERS, organic eggs were the fastest growing subtype of specialty eggs and captured almost 1% of the fresh egg market in 2004. Sales were $161 million in 2005 which was an increase from $140 million in 2004. Between 2000 and 2005, there was an average annual growth rate of 19% with future annual growth estimated to be 8-13% and $260 million in annual sales (Oberholtzer *et al.*, 2006).

Eggs and *Salmonella*

Salmonella are gram-negative, facultative aerobes naturally found in the intestinal tract of humans and animals. They can be spread from animal to animal and from animal to humans by poor food-handling practices and consumption of raw or undercooked foods of animal origin (Banwart, 1989). There are more than 2500 serotypes of *Salmonella* but *Salmonella enterica* serotype Enteritidis is of primary concern with poultry and eggs. It is the most prevalent serotype implicated in egg-borne illness in humans (CDC, 2008).

The association between eggs and *Salmonella* Enteritidis was first reported in 1988 and was based on epidemiological studies of outbreaks during the years 1976-1986 (St. Louis *et al*., 1988). The researchers determined 77% of the reported outbreaks with an identified food vehicle were caused by grade A shell eggs or dishes containing eggs. More recently, Braden (2006) studied 997 reported *Salmonella* Enteritidis infection outbreaks in the United States from 1985-2003 and reported that among the outbreaks with a confirmed food vehicle, 75% were still associated primarily with eggs or egg containing dishes. Therefore, the proportion of outbreaks associated with eggs remains steady. In 2006, *Salmonella* Enteritidis was the second most common serotype identified in all *Salmonella* outbreaks and has remained in the top four since 1995 (CDC, 2008). Using data from FoodNet, Schroeder *et al*. (2000) developed a risk model to estimate the number of shell egg-associated *Salmonella* Enteritidis illnesses that occurred in 2000. It was estimated that the consumption of *Salmonella* Enteritidis-contaminated eggs caused 182,060 illnesses, 2,000 hospitalizations, and 70 deaths (Schroeder *et al.*, 2005). This is similar to the FSIS estimate of 174,356 illnesses, 1,440 hospitalizations, and 75 deaths based on surveillance data and the Joint Expert Meetings on Microbiological Risk Assessment (JEMRA) risk model to estimate illness from exposure (USDA-FSIS, 2005).

Salmonella Enteritidis has been recovered from the shells, albumens, and yolks of intact shell eggs from naturally infected and artificially inoculated hens (Humphrey *et al*., 1989b; Gast and Beard, 1990; Humphrey *et al*., 1991; Humphrey, 1994; Keller *et al*., 1995; Gast and Holt, 2001). Researchers artificially inoculated pathogen-free flocks with *Salmonella* Enteritidis and were able to recover *Salmonella* from both the albumen and yolk from the infected hens' eggs in greater percentages than from the shell surface (Gast and Holt, 2001). Previously, it was thought the main bacterial transmission route was through the shell. However, a lack of correlation between shell surface contamination and internal contamination led researchers to propose a transovarian route as the primary route of *Salmonella* Enteritidis internal contamination. By this route, the eggs are contaminated prior to shell formation by the hen's infected reproductive system (Humphrey, 1994). To support this theory, Keller *et al*. (1995) found 73% of *Salmonella* Enteritidis-positive forming eggs from artificially inoculated hens were associated with colonized ovarian tissue or upper oviduct tissue. The incidence of freshly laid eggs being positive for *Salmonella* Enteritidis was only 0-0.6% in their study which indicated some intrinsic factor of the egg prevented survival and growth of the bacteria.

If contamination were to occur in the nutrient-rich yolk, it would be reasonable to expect the presence of pathogens in high numbers as seen by Saeed and Koons (1993) when they inoculated yolks with *Salmonella* (20 CFU per egg) and reported significant growth (1 x 10^9 CFU per ml of egg) within 2-3 days when stored at 23°C. Schoeni *et al.* (1995) also reported a 3-5 log increase in *Salmonella* Enteritidis after 24 hours when artificially-inoculated yolks were stored at 25°C. When stored at 10°C, growth occurred at a slower rate and at 4°C there was only sporadic growth (Schoeni *et al.*, 1995).

Instead, the number of *Salmonella* Enteritidis in internally contaminated eggs has been reported as less than 10-40 cells per contaminated egg (Humphrey *et al.*, 1989a; Humphrey *et al.*, 1991; Hope *et al.*, 2002). This provided more evidence of the albumen or the vitelline membrane being the main site of contamination. However, a small number of cases were reported where eggs were found to contain high numbers (10^4-10^5 CFU) so yolk-contamination should not be ruled out completely (Humphrey *et al.*, 1991).

Regardless of the site of contamination, internal contamination is still a greater concern because *Salmonella* survive the cleaning and disinfection process. *Salmonella* Enteritidis-infected laying hens are asymptomatic and infected hens do not always produce *Salmonella* Enteritidis-positive eggs. Contaminated eggs appear no different from non-contaminated eggs making it difficult to distinguish between the two without extensive testing (Keller *et al.*, 1995; Gast and Holt, 2001).

Salmonella growth inside an egg depends on the initial contamination site, bacterial count and the internal egg temperature (USDA-FSIS, 2005). The egg has several natural defenses to prevent growth of pathogens. A newly laid egg has a pH of 7.6-7.8 which then rises to 9.1-9.6 after 7-10 days of storage due to the diffusion of carbon dioxide through pores of the eggshell. This creates an inhospitable environment for bacterial growth (Froning *et al.*, 2001). In addition, the proteins in the albumen possess antimicrobial properties. Ovotransferrin chelates metal ions (iron, copper, zinc) required for growth, ovomucoid inhibits trypsin, and lysozymes hydrolyze cell walls of gram positive bacteria (Zeidler, 2002b). The albumen's thick viscosity also physically impedes bacterial migration towards the nutrient-rich yolk as well as center the yolk to maximize the distance any surviving bacteria have to travel. The vitelline membrane surrounding the yolk acts as a final barrier to yolk access (Froning *et al*., 2001).

Although growth of *Salmonella* Enteritidis may be restricted in the albumen, it has been shown to survive and grow exponentially as the length of time and storage temperatures increase (Hammack *et al.*, 1993; Humphrey, 1994). *Salmonella* growth is inhibited by temperatures below 15°C and does not grow below 7°C (Banwart, 1989). However, if improper storage temperatures were to occur, *Salmonella* has the potential to overcome the antimicrobial defenses of the albumen and grow. Schoeni *et al.* (1995) inoculated the albumen of eggs with *Salmonella* Enteritidis before storing for up to a week at temperatures ranging from 4-25°C. After 24 hours, storage at the higher temperatures resulted in a 3-5 log increase in bacteria with continued growth upon prolonged storage. Lower temperatures (4 and 10°C) inhibited growth but *Salmonella* still managed to survive. Murase *et al.* (2005) also observed the survival of *Salmonella* Enteritidis in inoculated albumen stored at 25°C for up to 6 days. Researchers have identified the gene, YafD, in *Salmonella* Enteritidis that they believe confers enhanced resistance to antimicrobial effects of the albumen by repairing DNA damage (Lu *et al.*, 2003).

If survival in the albumen is possible, then concerns arise about whether migration of *Salmonella* Enteritidis to the yolk can occur. As the storage time and temperature increases, the egg's vitelline membrane weakens. This allows for a greater permeability of nutrients to pass from the yolk to the albumen. Bacteria then utilize these nutrients in order to grow (Garcia *et al.*, 1983). Braun and Fehlhaber (1995) investigated whether this could occur when they inoculated the albumen of intact eggs with *Salmonella* Enteritidis and stored then under varying temperatures (7-30°C) for up to 4 weeks. There was a positive correlation between contamination dose, temperature, egg age and frequency of migration. Seventy-two percent of 4-week old eggs inoculated at low doses (10 cells/ml) in the albumen and stored at 20°C for up to 28 days had *Salmonella* present in the yolk (Braun and Fehlhaber, 1995). Gast and Holt (2001) inoculated the exterior surface of the yolk membrane and after 24 hours at 25°C were able to recover *Salmonella* Enteritidis in 75% of the yolk contents. Storage at 15°C for 72 hours resulted in 20% of the yolk contents testing positive. Therefore, storage temperature impacted the frequency of penetration of *Salmonella* Enteritidis into yolks.

Salmonella is a concern because of the disease, salmonellosis, that can occur in humans upon the ingestion of the pathogen. Symptoms include fever, vomiting, nausea, diarrhea, cramps and headaches. These symptoms develop within 8-72 hours and last 4-7 days. In severe cases, malabsorption and nutrient loss may occur due to damage to the mucous membrane of the small intestine and colon. Treatment for severe cases usually involves supportive therapy to restore a patient's fluid and electrolyte balance. Antibiotics are not recommended (Poppe, 1999).

The infective dose and severity of symptoms are dependent on the dose and virulence of the strain, person's age and immune status. Approximately 20% of the U.S. population is considered susceptible to the disease and these include: infants, elderly, pregnant women, and those with compromised immune systems (D'Aoust, 1989). It is estimated that 94% of those suffering from illness recover without the need for medical care, 5% require a visit to the physician, 0.5% require hospitalization and less than 1% die. Approximately 3% of those infected develop recurring joint pain and reactive arthritis as a result of the disease (Hope *et al.*, 2000). However, the risks for the susceptible population are higher with infected persons 1.4 times more likely to require medical treatment than the normal population, 2.4 times more likely to require hospitalization and 4.3 times more likely to die from the disease (USDA-FSIS, 1998). For the egg industry, this is an important safety consideration because children and the elderly are key demographics for egg consumption. The American Egg Board's survey estimated that over half of all egg sales are made to families with children. Also, individuals over the age of 50 are among one of the fastest growing consumers of eggs (American Egg Board, 2003).

Salmonella is easily killed at temperatures greater than 55°C but eggs are frequently consumed undercooked or raw. Studies of past outbreaks have suggested the infectious dose for *Salmonella* could be as low as 25 microorganisms per serving (Vought and Tatini, 1998). Unsafe egg handling practices such as temperature abuse, pooling of eggs for large volume cooking that is then held at unsafe temperatures or inadequate cooking may increase the risk of illness by allowing *Salmonella* to grow to dangerously high levels (USDA-FSIS, 2005). Lee *et al.* (2004) evaluated restaurants that prepared breakfast egg entrees and found a high prevalence of high risk egg-preparation practices. The researchers conducted interviews and brief site evaluations in 153 restaurants in 7 U.S. states. They reported 54% of restaurants pooled raw shell eggs not intended for immediate service, nearly 26% stored eggs at room temperature before cooking and employees in 42% of the sites reported sanitizing utensils used to prepare eggs less than once every 4 hours. There was also limited use of pasteurized eggs. Of the restaurants that used shell eggs for egg entrees (84-95% depending on the entrée), unpasteurized shell eggs were used by 78% of restaurants to prepare runny or soft fried eggs, poached eggs by 74% omelets by 42% and scrambled eggs by 58% of restaurants (Lee *et al.*, 2004).

Consumer preparation and handling also play key roles in controlling *Salmonella* and preventing illnesses. Lin and Morales (1997) analyzed data from national consumer surveys of food consumption patterns and assessed the prevalence of unsafe egg preparation and consumption practices. The study reported 27% of all egg dishes were likely undercooked. This is significant because common cooking methods such as sunny-side up and poached eggs may not adequately kill *Salmonella* Enteritidis if present at high levels. Humphrey *et al*. (1989b) and Saeed and Koons (1993) have both reported survival of *Salmonella* Enteritidis when cooked under simulated domestic cooking conditions if initially present in large populations. This was especially true of cooking styles where the yolk remained liquid. Boiling inoculated eggs for up to 10 minutes, frying, preparing over-easy, sunny-side up or scrambling eggs slowly at moderate temperatures were not able to guarantee destruction of all bacteria (Humphrey *et al.*, 1989b). Storage abuse was also a factor in the increased heat resistance of *Salmonella* Enteritidis in contamination eggs (Saeed and Koons, 1993). Chantarapanont *et al*. (2000) also reported that the cooking time to eliminate large numbers of *Salmonella* (10^7 CFU) depended on egg size and initial temperature with some survival of *Salmonella* possible even after being boiled for 8 minutes with a 15 minute hold time before analysis.

Of all the *Salmonella* Enteritidis outbreaks during 1985-1999 where eggs were implicated, 28% were from foods that contained raw eggs (homemade ice cream, Caesar salad dressing tiramisu, and eggnog), 27% from traditional egg dishes (omelets, French toast, pancakes, foods made with egg batter such as crab cakes, chiles rellenos, egg rolls, Monte Cristo sandwiches), 26% from dishes that contained eggs (lasagna, ziti, stuffing) and 15% from "lightly cooked" egg dishes (hollandaise sauce, meringues, cream pies) (Patrick *et al*., 2004). On average, people ate undercooked eggs twenty times a year and 53% ate dishes that contained raw eggs (Lin and Morales, 1997). Similarly, in 2001, 42% of respondents ate raw eggs in a consumer food safety survey conducted by FDA and FSIS. The survey, conducted in 1993, 1998 and 2001, also indicated people were less likely to follow recommended safe handling practices for eggs as compared to other recommended practices with fish, meat, or chicken. This suggested that consumers had a lower relative perceived risk when it came to egg safety (Fein *et al*., 2002). Consumers who are less likely to follow safe handling practice and eat eggs undercooked or raw may be putting themselves at greater risk for illnesses from *Salmonella* Enteritidis-contaminated eggs.

Due to the risks associated with undercooked eggs, shell eggs have been identified since 1990 as a potentially hazardous food in the Food Code issued by the FDA's Center for Food Safety and Applied Nutrition (CFSAN). The Food Code is provided as a guide for state and local authorities to govern retail establishments on the handling and storage of potentially hazardous foods. In the provisions for egg safety, all shell eggs, with the exemption of pasteurized eggs, are required to be kept at temperatures no greater than 7.2°C during storage and display to prevent the rapid growth of any existing pathogenic microorganisms to dangerous levels. Their recommendations also state, "pasteurized eggs and egg products shall be substituted for raw eggs in the preparation of foods such as Caesar salad, hollandaise or Béarnaise sauce, mayonnaise, meringue, eggnog, ice cream and egg-fortified beverages that are not cooked." In addition, "partially cooked animal food such as soft-cooked eggs and meringue may not be served or offered for sale in a ready-to-eat form for food establishments that serves highly susceptible population" (FDA-CFSAN, 2005). This switch to liquid pasteurized egg products would mean certain populations may have to forgo their favorite cooking style of eggs unless pasteurized shell eggs are available.

Consumer Response to Egg Safety

In a survey of 300 Ohio consumers conducted by a professional consumer research company (AZG Research, 2006) under contract to this laboratory, over half of the respondents had heard about people becoming ill from *Salmonella* specifically from consuming eggs. However, 94% of the respondents did not decrease their egg consumption and 91% did not have any concerns about using fresh eggs. Similar findings were reported in a separate study that involved consumer focus group discussions where participants expressed little concern when asked about eggs and foodborne illness. The investigators concluded that the lack of concern was based on lack of sufficient knowledge, specifically that *Salmonella* can be found inside of eggs (Roe *et al.* 2001). On the other hand, the American Egg Board's survey found 27% of consumers attributed the risk of *Salmonella* as a reason why they did not eat more eggs (American Egg Board, 2003).

In the AZG survey, 20% of consumers did not know what the word "pasteurized" meant. Of those who did, they defined it as "cooked." Over 75% were unaware of *Salmonella*-free eggs. When asked to list the advantages of *Salmonella*-free eggs, 44.3% mentioned "safe to eat," 13.3% mentioned "health benefits" and 9.7% mentioned "bacteria free." Over 50% expressed a "somewhat" to "very likely" intent to purchase *Salmonella*-free eggs. However, cost was the biggest barrier and disadvantage to the purchase of *Salmonella*-free eggs for 47% of those surveyed. (AZG, 2006). In a separate study, when consumer focus groups were shown a sample food safety label that identified eggs as being subjected to in-shell pasteurization and asked to give their reaction, concerns that the product taste or texture might be altered were expressed (Roe *et al.*, 2001). In the AZG study (2006), 2.7% mentioned taste as the biggest disadvantage of *Salmonella* free eggs. These findings may indicate that further education is necessary in order for consumers to perceive pasteurization as a positive benefit and to overcome any hesitation over negative quality changes.

Quality Criteria Associated with Egg Purchasing Decisions

In the AZG study (2006), consumers ranked in order of increasing importance; “taste,” “nutritional value,” “healthy,” and “cost” as the most important items to them when they made food purchases. When asked specifically about egg purchases, consumers mentioned “freshness,” “color,” “cost,” and “taste.” This is similar to national results from the American Egg Board survey where freshness (dating), price, safety (quality) were the three most important factors when buying eggs (American Egg Board, 2003). In a survey with European consumers, safety and freshness were also the most important quality factors. In their survey, the definition of egg quality was based on tangible characteristics such as shell strength, e.g. “strong” and “clean,” albumen consistency, e.g. “consistent” and “clear,” and intense yolk color (higher Roche color fan value) (Hernandez, 2005).

There are a few published European studies conducted on consumer’s willingness to pay for egg safety. Eighty percent of German consumers (n= 449) surveyed were willing to pay a premium for food safety with 30% willing to pay extra for safer eggs (Rohr *et al.*, 2005). Baltzer (2004) surveyed Danish consumers and studied actual consumer egg purchases from supermarkets. Willingness to pay (WTP) was low for pasteurized eggs which also had the highest prices among the specialty eggs studied (free range, organic). The researcher proposed food safety was considered a lower priority and consumers did not perceive their risk as high so pasteurized eggs did not provide any additional safety benefits for them.

Pasteurization Technology

Pasteurization is one method to produce safer eggs. This involves rapidly heating eggs and holding at a specified temperature for a period of time which has been shown to kill *Salmonella* (USDA, 1997). The requirements for pasteurization of liquid eggs are shown in Table 2.3. Despite the increased safety, the pasteurization of shell eggs has not been more widely adopted by the egg industry. This could be due to current technologies relying mainly on thermal heating which may cause adverse changes to egg quality and functionality due to slow heat transfer issues (Li-Chan *et al.* 1995; Hou *et al.,* 1996; Schuman *et al.,* 1997). Heat transfers via convection into the egg shell and then via conduction towards the yolk (Hou *et al*., 1996). The times and temperatures required for pasteurization are for the centrally located yolk where *Salmonella* experience greater heat resistance due to the differences in pH, fat and water content (Chantarapanont *et al*., 2000). This is a problem because pasteurization temperatures are similar to the temperatures that cause denaturation of heat-sensitive proteins in the albumen. At prolonged exposures to temperatures as low as 57.3°C, conalbumin, the least heat stable of the egg proteins, may begin to irreversibly coagulate. If held at higher temperatures of 60°C, the albumen will continue to coagulate and the viscosity increase until irregular clumps form (Yang and Baldwin, 1995). By the time the yolk reaches pasteurization temperatures, the albumen may be over-processed. The result is a safe egg but the egg's albumen has an undesirable turbid appearance.

Table 2.3: Pasteurization requirements for egg products (modified from Froning, *et al.*, 2001).

Liquid egg product	Minimum temperature requirements (°C)	Minimum holding time requirements (minutes)
Albumen (without use of chemicals)	56.7	3.5
	55.6	6.2
Whole egg	60.0	3.5
Whole egg blends (less than 2% added non-egg ingredients	61.1	3.5
	60.0	6.2
Fortified whole egg and blends (24-38% egg solids, 2-12% added non-egg ingredients	62.2	3.5
	61.1	6.2
Salted whole egg (with 2% or more salt added)	63.3	3.5
	62.2	6.2
Sugar whole eggs (2-12% sugar added)	61.1	3.5
	60.0	6.2
Plain yolk	61.1	3.5
	60.0	6.2
Sugar yolk (2% or more sugar added)	63.3	3.5
	62.2	6.2
Salt yolk (2-12% salt added)	63.3	3.5
	62.2	6.2

Currently, the only in-shell pasteurized eggs available commercially in the United States are thermal treated. In 1996, Michael Foods Egg Products Co. (Minneapolis, MN) was the first to introduce pasteurized shell eggs to the retail and foodservice markets (Mermelstein, 2001). Then in 1998, Pasteurized Eggs Corp. (Lansing, IL) started to sell their eggs using a FDA-approved pasteurization process patented by John Davidson under the brand name, Davidson's Pasteurized Eggs™. In 2003, the rights to the egg pasteurization technology patents were sold to National Pasteurized Eggs, Inc. who launched their brand, Davidson's Safest Choice® (National Pasteurized Eggs, Inc., 2008). Other pasteurization technologies that have been approved or are being developed for approval are: irradiation, microwave-heating, combination of steam, microwave-heating and ultrasound, and ozone-based processing.

Thermal Pasteurization

Thermal pasteurization method involves heating eggs using water or another heat source such as steam and holding for varying lengths of time necessary to kill *Salmonella* Enteritidis but not to denature heat-sensitive proteins (Davidson, 2004). Water is the preferential heating method with a heating time to reach 55-56°C of approximately 15-20 minutes versus >60 minutes with hot air heating (Stadelman *et al.*, 1996). Lith *et al.* (1995) used immersion heating at 57°C for up to 30 minutes in an attempt to pasteurize shell eggs. Effective pasteurization was not achieved after 30 minutes and eggs held any longer resulted in adverse internal quality changes. Similarly, Hou *et al.* (1996) achieved only a 3-log reduction of *Salmonella* Enteritidis when eggs were immersed for 25 minutes in a 57°C waterbath. However, a combination of heat with water (25 minutes at 57°C) followed by hot air (1 hour in a 55°C convection oven) was effective in achieving a 7-log reduction of *Salmonella* Enteritidis. Schuman *et al.* (1997) achieved an 8.5 D process or a D-value of 6 minutes when eggs were placed into a 57°C waterbath for 65-75 minutes. Brackett *et al.* (2001) used a humidity controlled hot air convection oven set at 57.2°C for 70 minutes and achieved a 7-log reduction. Despite the improved lethality, the longer total process times reported in these studies may not be feasible in a commercial setting.

Commercially, the Davidson process uses hot water immersion tanks that bring and hold eggs at the proper specified temperatures. A diagram of a variation of the process as outlined in the patent is shown in Figure 2.2 (Davidson, 2004). The eggs travel along a continuous waterbath that is sectioned into different temperature zones (54.4- 63.3°C) with hold times for each section varying in order to achieve at least a 4.6 log reduction of *Salmonella* Enteritidis. Upon exiting the waterbath, the eggs move into a holding section where residual heat continues to raise the temperature of the eggs until a 5-log reduction is reached. The eggs stay in this holding section until pasteurization is achieved. Afterwards, the eggs are spray cooled with an antibacterial fluid. The eggs are also sprayed with a sealant and a red letter P is stamped on the shell before being packaged. According to the patent, the total process time is 39-41 minutes and is effective in killing *Salmonella* and viruses like Avian Influenza (Davidson, 2004).

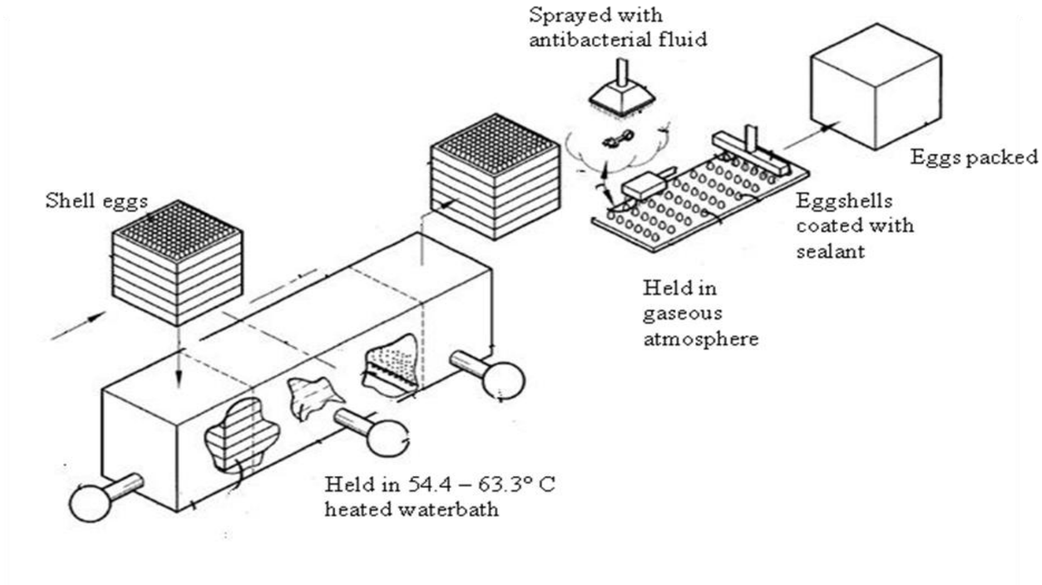


Figure 2.2: Diagram of published thermal pasteurization process (modified from Davidson, 2004).

The main concerns with thermal pasteurization are the quality changes in the egg that may arise after processing. Freshness was cited most often by consumers when asked to list what factors influenced their egg purchasing decisions (American Egg Board, 2003). Egg appearance plays an important role in the consumer's perception of freshness. Therefore, pasteurized eggs must be indistinguishable from the fresh market eggs that they are sold alongside. However the literature shows mixed results on testing the quality of albumens from thermal treated eggs. Hou *et al*. (1996) and Hank *et al.* (2001) reported no changes to Haugh unit while Schuman *et al.* (1997) had observed an increase in Haugh units. There is agreement however that pasteurization increased turbidity (Hou *et al.*, 1996; Schuman *et al.*, 1997; Rodriguez-Romo, 2004), with a "blue" hue reported in thermal treated albumens (Hou *et al.*, 1996). No changes were seen in yolk color or index (Hou *et al.*, 1996; Cox *et al.*, 1999).

Mixed results were also reported when evaluating the extent of albumen denaturation using different instrumental measures. Hank *et al.* (2001) reported eggs subjected to a 55°C hot air oven for 180 minutes did not have any effect on the protein quality as indicated when percent soluble proteins were measured. Rodriguez-Romo (2004) analyzed egg albumens by differential scanning colorimeter and reported a difference in protein denaturation patterns as compared to control.

The results from functionality testing of thermal treated eggs are conflicting. When egg whites were heated, the foaming power decreased, whip times increased, and whip volume decreased (Cunningham, 1995; Schuman *et al.*, 1997; ICMSF, 1980). The ovomucin is responsible for foam stability and may have been denatured by the heat treatment. However, Hou *et al*. (1996) observed a beneficial increase in foaming ability and stability and suggested the enhancement could be due to the unfolding of the protein and increase in surface hydrophobicity. Li-Chan *et al.* (1995) and Froning *et al.* (2001) suggested that eggs at a lower pH (seen when eggs are the freshest) may help in preventing heat-related changes. When liquid pasteurized whole eggs were used to make sponge cakes, there was a noted decrease in volume if heated above 60°C and the cakes had a poorer texture but better volumes. There was no change to the emulsifying power or coagulating ability of the pasteurized yolks (Cunningham, 1995; Cox *et al.*, 1999). There are no formal sensory data of the Davidson pasteurized eggs reported in the literature but their website claims their eggs still retain the freshness and taste of unprocessed eggs (National Pasteurized Eggs, Inc., 2008).

Irradiation

Non-thermal or “cold pasteurization” is proposed as an alternative to heat pasteurization. Low (up to 1 kGy) to medium doses (up to 10 kGy) of ionizing radiation generate reactive hydroxyl ions that then interact with bacterial cell membrane structure and react with the DNA to cause changes to the microorganism’s reproduction ability (Moy, 2005). The source of the radiation can be gamma rays (Cobalt-60 or Cesium-137), converted X-rays, or electron beams. D-values for *Salmonella* spp. in shell eggs range from 0.32- 0.80 kGy depending on the radiation source, species, egg temperature and internal location of *Salmonella* (Fellows, 2000). Gamma irradiation of shell eggs at a dose of 2.5 kGy was found to effectively eliminate *Salmonella* Enteritidis-inoculated yolks (Lith, 1995). Serrano *et al.* (1997) achieved a 4-log reduction of *Salmonella* Enteritidis when using 1.5 kGy of x-ray irradiation.

In 2000, up to 3 kGy of ionizing radiation was officially approved to reduce *Salmonella* in shell eggs (FDA, 2000b). However, irradiated shell eggs are not available in the marketplace due to a strong negative public perception toward irradiation technology used for foods (Moy, 2005).

Pasteurizing shell eggs by irradiation also produced adverse changes in egg appearance and functionality. The ovomucin, the protein that contributes to creating the gel-like structure of the thick white, can denature during irradiation and adversely affect viscosity (Meszaros *et al.*, 2006). Shell eggs irradiated at doses starting at 1.0 kGy, were observed to have decreased albumen viscosity (Pinto *et al.*, 2004; Ma *et al.*, 1990; Meszaros *et al.*, 2006; Al-Bachir *et al.*, 2006; Min *et al.*, 2005; Hale, 1997; Wong *et al.* 2003) and lower Haugh units (Ma *et al.*, 1990; Tellez *et al.*, 1995; Min *et al.*, 2005). Electron beam irradiation (up to 4 kGy) of shell eggs resulted in a loss of 50 Haugh units with further loss seen with extended storage (Wong *et al*., 2003). The thick part of the albumen was described as "runnier" and similar to the thinning seen with older eggs when gamma irradiation up to 3 kGy was used (Meszaros *et al.*, 2006). Hale (1997) compared the decrease seen in viscosity of albumen from irradiated eggs as similar to Grade C quality eggs and concluded that the irradiated eggs would not be suitable for fried or poached eggs. Observations were also made about increased yolk viscosity possibly due to the denaturation and aggregation of lipoproteins (Ma *et al.*, 1990; Pinto *et al.* 2004).

Another adverse effect seen with irradiation was a decreased clarity of the albumen starting at 0.5 kGy. However, tests of the albumen protein of eggs irradiated up to 3 kGy have shown no evidence of denaturation (Ma *et al.*, 1990; Serrano *et al.*, 1997; Pinto *et al.*, 2004). The change could have been due to the yolk membrane becoming weaker after exposure to irradiation which allowed for the diffusion of the yolk into the white (Meszaros *et al.*, 2006). Wong *et al.* (2003) observed this weakening of the membrane after eggs were exposed to electron beam irradiation doses up to 4 kGy. No changes occurred to the color of the yolks at lower doses of irradiation (Serrano *et al.*, 1997; Dvorak *et al.*, 2005; Min *et al.*, 2005). However, at higher doses (beginning at 1.0 kGy), the yolk was described as turbid (Pinto *et al.*, 2004), faded or decreased in yellow hue intensity (Wong *et al.*, 2003, Dvorak *et al.*, 2005; Meszaros *et al.*, 2006) and showing a decrease in Roche yolk color fan values (Ma *et al.*, 1990; Tellez *et al.*, 1995).

There are conflicting results among studies regarding functionality changes due to irradiation. Irradiation affected foaming abilities of the egg white by: increasing stability and increasing angel cake volume (Ma *et al*., 1990), decreasing stability (Meszaros *et al.*, 2006), decreasing stability but not ability (Wong *et al.*, 2003) or decreasing both stability and ability (Min *et al.,* 2005). There was also a decrease in the stability of an emulsion when using the yolks of irradiated eggs (Wong *et al.*, 2003).

In addition to visual and functionality changes of the albumen, the flavor of irradiated eggs may be different from fresh eggs because irradiation has the potential to oxidize the fats in the yolk (Moy, 2005). An off-odor was present in the raw albumen of irradiated eggs (starting at doses of 1 kGy) and in the yolk (Lith *et al.*, 1995, Ma *et al.*, 1990). Min *et al.* (2005) noted an off-odor but this had quickly dissipated when stored under aerobic conditions. Hale (1997) cooked irradiated eggs (up to 1.0 kGy) and described a "cooked" off-odor which was more pronounced when the eggs were cooked (fried) and then reheated by microwave. A visual panel was able to detect differences between irradiated eggs (up to 3 kGy) and untreated controls. However, a separate sensory panel that evaluated irradiated egg whites (less than 2 kGy) found there were no differences (Hale, 1997). When yolks of irradiated eggs (up to 1.5 kGy) were used for mayonnaise, there were no changes observed with taste, flavor, texture or color of the mayonnaise (Al-Bachir *et al.*, 2006).

Microwave Pasteurization

Microwave pasteurization technology generates heat from within the egg itself thus minimizing the come up time needed to achieve pasteurization temperatures. The electromagnetic radiation is converted to heat by the action of the water molecules trying to align themselves to the electric field as it rapidly oscillates at very high frequencies. The effects of microwave processing on microbial inactivation are primarily from the temperature increase associated with the absorption of this energy (Fellows, 2000). Selective heating occurs when solids absorb the electromagnetic energy more effectively as compared to the surrounding fluid. This helps with the uniformity of heating. Microwaves also can cause electroporation or pore formation in the microorganisms' cell membrane as a result of the electrical potential across a membrane. These pores allow cellular contents to leak and lead to microorganism death. In addition, a dielectric cell membrane rupture can occur when the voltage drops across the membrane. The magnetic field energy from the microwaves can also couple with molecules such as proteins and DNA inside cells and cause disruptions that may lead to cell lysis (Kozempel *et al.*, 1998).

Microwaves used for pasteurization are 2450 MHz in frequency. The power penetration depth is the distance from the material surface where 50% of the microwave power is absorbed. When 2450 MHz is applied to an in-shell egg, there is a power penetration depth of 15 mm for albumen and 32 mm for the yolk (Eramus and Rossouw, 2007).

The yolk's high lipid and low moisture content gives it a lower dielectric property than the white so would be expected to heat the slowest. However, Dev *et al.* (2008) observed that the yolk heated up faster than the albumen when whole eggs were heated with microwaves (2450 MHz at different power densities). Because of this, they both reached pasteurization temperature at similar times. Datta *et al.* (2005) proposed that the egg shell's curvature focused the energy and distributed the power evenly among the albumen and yolk. This makes microwave pasteurization an attractive option as it shortens processing times. Eggs heated with microwave energy achieved yolk temperatures of 60°C in only 2 minutes (Stadelman *et al.*, 1996). Lakins *et al.* (2008) studied the use of directional microwave technology that exposed food to horizontal and vertical microwaves sources for more homogenous power distribution within the treatment chamber. The eggs were treated to a 20 second exposure of 2.45 GHz microwaves at 80% magnetron power and a blast of carbon dioxide rapidly cooled the eggs. However, only a maximum 2-log reduction of *Salmonella* Enteritidis was obtained and variations in effectiveness were seen based on the egg's location within the chamber (Lakins *et al.*, 2008).

In 2001, a system to pasteurize shell eggs using microwave technology was introduced by Safe Eggs Ltd. The eggs pasteurized by the Safe Eggs process are available only in South African retail markets. Their claim is that their technology is effective against *Salmonella* Enteritidis as well as viruses such as Avian Influenza and Newcastle disease (Safe Eggs Ltd., 2008). The units use a combination of microwaves (30 kW at 2.45 GHz) and hot air (57-70°C) to achieve pasteurization temperatures. A process diagram adapted from their patent is shown in Figure 2.3 (Erasmus and Rossouw, 2007). The internal temperatures of eggs reach between 50-58°C by simultaneous applying microwave radiation and hot air in the temperature raising and pasteurization stages. In the holding section a blanket of hot air equilibrates the internal and shell temperature and prevents moisture evaporation and heat loss from the shell surface. According to the patent, the eggs achieve an internal target temperature of 58-59°C with a resultant 3-5 log reduction of *Salmonella* Enteritidis in 40-50 minutes total processing time (Eramus and Rossouw, 2007). Safe Eggs Ltd. has a facility that is open to table egg producers for contract processing with a production capacity of 80,000 eggs per day. In 2006, the company began marketing industrial scale units (Safe Eggs Ltd., 2008).

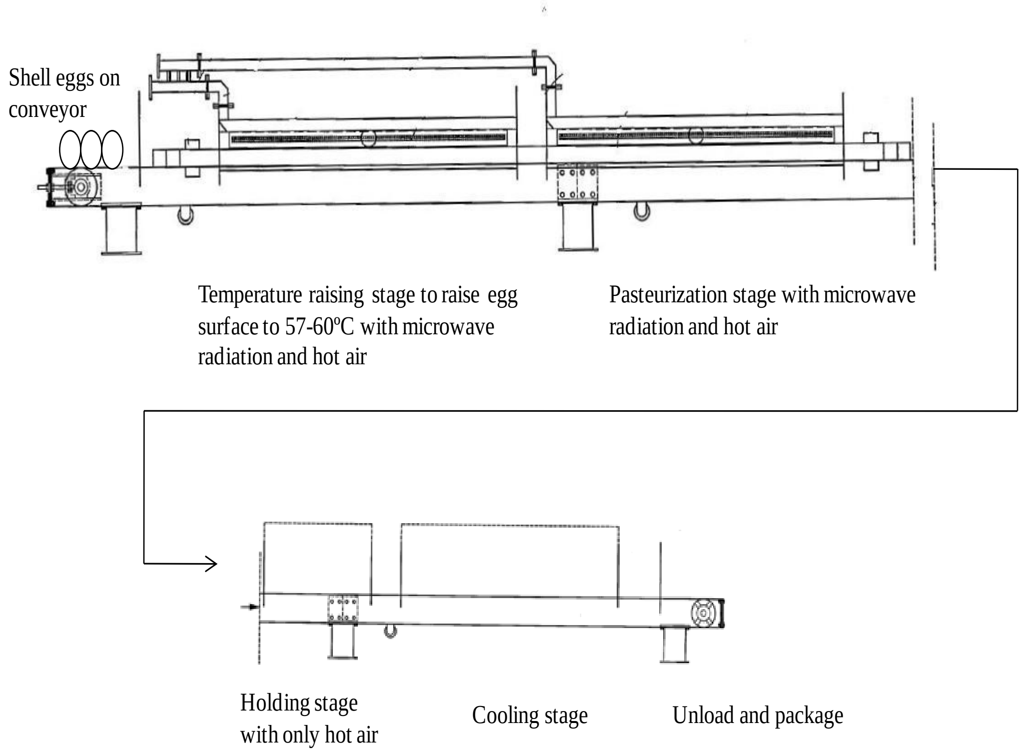


Figure 2.3: Diagram of published microwave pasteurization process (modified from Erasmus and Rossouw, 2007).

According to consumer sensory studies reported by Safe Eggs, there was no significant difference in visual perception of their eggs compared to a fresh egg. The shelf life of their eggs was also extended up to six weeks when stored at ambient (24°C) temperatures. The microwave pasteurized eggs, after 28 days of storage, had more favorable Haugh units as compared to untreated eggs stored under the same conditions with no change to pH after extended storage (up to 6 weeks) (Sluis, 2006). However, they did acknowledge “haziness” to the albumen and a 40% reduction in whip capacity but no change in foam stability of the albumen (Safe Eggs Ltd., 2008).

Dev *et al.* (2008) used a laboratory microwave oven setup and treated whole shell eggs with their own process parameters. The researchers measured changes to the physical properties of the albumens of microwave heated eggs with various instrumental methods. Microwave heated egg whites were more turbid than untreated eggs when absorbance was measured at 650 nm with a spectrophotometer. However, microwave heated egg whites experienced less partial denaturation of the secondary structure of egg white proteins, greater viscosity, better foam stability and density, and was clearer than thermal treated eggs. With the exception of the turbidity, the microwave heated eggs were not different statistically from the untreated eggs (Dev *et al.*, 2007).

Leda (Bettcher) Technology

In Europe, another new in-shell pasteurization process is being introduced as the first point-of-use pasteurization system for commercial foodservice establishments. This patented technology by Leda Technologies, GmbH (Dierikon, Switzerland) uses a combination of heat, ultrasound and microwave heating. The combination of heat and ultrasonic waves is termed, "thermoultrasonication" (Cabeza *et al.*, 2004). In 2007, the company was acquired by Bettcher Industries, Inc. (Birmingham, OH) and in 2009, was renamed Bettcher Foodservice, GmbH. The company manufactures pasteurization units for sale to commercial kitchens, hotels for breakfast buffet lines and other foodservice establishments (Bettcher Foodservice, GmBh., 2009). The process begins by placing eggs in an oven space and generating ultrasonic waves (1-3 MHz) and steam. The ultrasound waves evaporate the water at lower temperatures which then raise the relative humidity inside the chamber. The vibrations from the ultrasonic waves also position the yolk in the geometric center within the shell. The water then condenses onto the surface of the egg and this allows for better heat transfer. The eggs are heated by microwaves or a combination of microwaves and steam and maintained at 62.5°C for approximately 16 minutes to allow the yolk to reach 60ºC for 3.5 minutes. They are then rapidly cooled by spraying with water. The total process time is approximately 44 minutes (Braeken, 2006). Their model for commercial foodservice establishments has a maximum capacity of 360 eggs and also has a selection of 14 different process settings for preparing various levels of soft-, medium- and hard-boiled eggs (Bettcher Foodservice, GmbH, 2009).

Claims on the Bettcher Foodservice website state that their eggs are pasteurized with a 7-log bacterial reduction process and their eggs cannot be differentiated from fresh raw eggs in nutritional quality, cooking properties and appearance (Bettcher Foodservice, GmbH, 2009). However, there are no published studies regarding this process of pasteurizing eggs.

Ozone

Ozone-based technology is emerging as another viable pasteurization technology. Ozone is naturally formed in the earth's stratosphere by the sun's solar radiation and at ground level from the reaction of oxygen with volatile organic compounds and nitrogen oxides emitted from car engines or industrial operations. Oxygen (O_2) molecules exposed to high electrical input become excited and split into free single oxygen radicals. These can then recombine with any available oxygen atoms to form the triatomic ozone (O_3). Commercially, high concentrations of ozone can be formed by electrical discharge, electrochemical, photochemical, electrolytic, and radiochemical means (Weavers and

Wickramanayake,, 2001). Most commonly, UV radiation at 188 nm or the corona discharge method is used (Guzel-Seydim *et al.*, 2004). The corona discharge method involves passing air or concentrated purified oxygen (dried to a dew point of at least -60°C) between two electrodes separated by a dielectric material (glass or ceramic) (Figure 2.4). A high voltage is applied to the electrodes and an electric field (corona discharge) is created in the gas-containing gap to form ozone in high concentrations (Weavers and Wickramanayake, 2001).

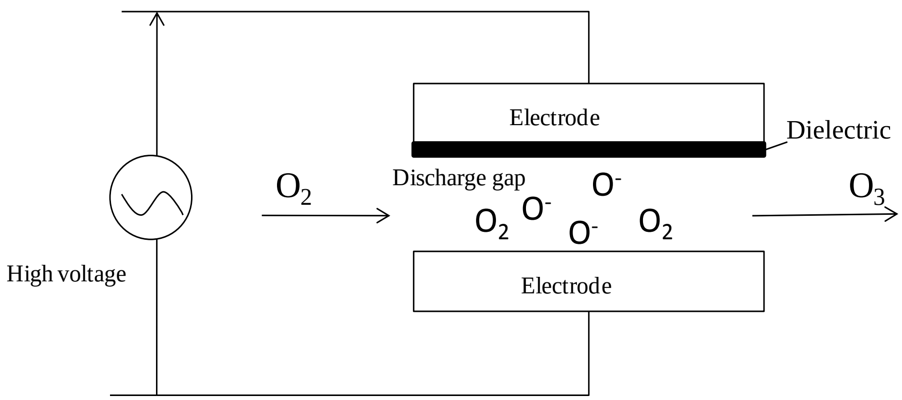


Figure 2.4: Corona discharge method for generation of ozone (modified from Lenntech, 2008).

Ozone is highly unstable and reactive. The third oxygen readily reacts with organic compounds and decomposes rapidly back to the more stable diatomic oxygen form. In the aqueous phase, ozone forms hydroxyls which can then react with other compounds. This gives ozone a standard reduction potential of +2.07 V, making it one of the most powerful oxidizing agents available. Because of its instability, ozone has a short half-life (12 hours in gaseous state at room temperature and 20-30 minutes in pure, clean water with pH of 7-8) (Graham, 1997). Ozone has the advantage of not leaving behind hazardous residues on products after processing. It must be constantly regenerated on-site using oxygen or water (Guzel-Seydim *et al.*, 2004).

Safety precautions must be followed when working with ozone. An environmental ozone monitor is required to protect the safety to personnel from exposure to high levels of ozone. Ozone has a pungent odor and is detectable by humans starting at 0.01-0.05 ppm by volume. The characteristic smell is described as the fresh, clean air smell after a thunderstorm or the "faint garlicky" aroma near televisions, laser printers, and copy machines (Mahapatra *et al.*, 2005). Low concentrations of ozone can be an irritant to the nose, throat and eyes. A tolerance to ozone develops with repeated exposure. Toxicity starts to occur at prolonged exposures (3-6 hours) to high concentrations (0.1- 0.5 ppm) where a person may experience vision loss. The respiratory tract is the primary target and becomes inflamed and causes edema of the lungs. Very high levels (> 1700 ppm) can be lethal in only a few minutes. For this reason, a maximum continuous exposure limit of 0.1 ppm over the course of an 8 hour work day, 40 hour work week is set as the limit by the Occupational Safety and Health Administration. For short term exposure of 10 minutes there is a maximum limit of 0.2 ppm by volume (Mahapatra *et al.*, 2005).

Reviews of ozone in the food industry have shown ozone treatment as effective against vegetative cells of both Gram positive and Gram negative bacteria, spores, viruses, protozoa and fungi (Kim *et al.*, 1999; Kim *et al.*, 2001; Guzel-Seydim *et al.*, 2004; Mahapatra *et al.*, 2005). Ozone can oxidize cellular components such as proteins, unsaturated lipids, respiratory enzymes, peptidoglycans in cell envelopes, and nucleic acids. The polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids of the bacterial cell envelope are targeted. This oxidation of the double bonds of unsaturated lipids and sulfhydryl groups of enzymes causes a disruption to the membrane and leakage of cellular components and ensuing cell death (Khadre *et al*., 2001). There is also potential damage and destruction to viral nucleic acids, spore outer coats and alterations to the polypeptide chains in viral protein coats (Mahapatra *et al.*, 2005).

Ozone received Generally Recognized As Safe status as an antimicrobial agent for the disinfection of bottle water and is approved for use in disinfecting poultry chill water (FDA, 2008). In 2001, ozone in the gaseous or aqueous phase was approved as a secondary direct food additive and was permitted to be used in contact with food in accordance with current good manufacturing practices (FDA, 2001). The gaseous form of ozone is often used for food storage applications. Aqueous ozone is used for surface decontamination, especially of food preparation equipment and packaging materials as an alternative to chlorine (Kim *et al.*, 2003). In the food industry, ozone has been used in a variety of foods such as meat, poultry, eggs, grains, fruits, spices, vegetables, seafood, and mold-inhibited cheeses (Kim *et al*., 1999; Kim *et al.*, 2001; Guzel-Seydim *et al.*, 2004; Mahapatra *et al.*, 2005).

Researchers at The Ohio State University have developed and patented a method of processing shell eggs by sequential application of heat, vacuum, and pressurized gaseous ozone (Yousef and Rodriguez-Romo, 2009). The process begins by immersing eggs into a 57°C waterbath for 25 minutes. They are then transferred to a stainless steel vessel where a vacuum of 10-12 mm Hg is applied and held before ozonation. The relative humidity in the chamber should be 90-95% for optimal microbial inactivation (Kim *et al.*, 2001). Ozone is formed from pure oxygen by passing through a commercial ozone generator using a high voltage electrical discharge. This continuous flow of ozone (10-12% wt/wt ozone in oxygen) is directed into the chamber until a pressure of 10-12 psig is achieved, after which it is held for 40 minutes at a steady state. The pressure facilitates penetration of ozone into the egg shell. The interaction between the sequential application of heating and pressurized ozone was proven effective in microbial inactivation (Kim *et al.*, 2001).

After the treatment, the pressure is released and the residual ozone is directed into a heated catalyst to be destroyed safely. There was 68.1% penetration of ozone through the egg shell and this along with the heat and vacuum was shown to produce a $\geq$ 6.3 log reduction in *S.* Enteritidis (Rodriguez-Romo *et al.*, 2007). The total process time was 65 minutes. Rodriguez-Romo (2004) studied the interior egg quality of treated eggs and reported a slight increase in Haugh units, increased turbidity seen in the albumen, no change to yolk index, and no differences in protein denaturation patterns when analyzed with differential scanning colorimeter. The biggest concern was the significant increase in the production of lipid oxidation byproducts (specifically malonaldehyde concentration) as measured using a 2-TBA test (Rodriguez-Romo, 2004).

More recently, Perry *et al.* (2008) investigated the effect of a similar ozone-based treatment using a larger pilot-scale chamber. A similar procedure was followed where the eggs were immersed in a waterbath at 57°C for 21 minutes and then transferred to the treatment chamber. A vacuum of 67.5 kPa was applied and gaseous ozone directed into the chamber until a final pressure of 184-198 kPa was achieved and held at steady state for 40 minutes. This treatment resulted in a 4.2 log reduction with a total process time of 100 minutes.

Application of Ozone and Effect on Quality of Foods

The occurrence of sensory changes depends on the food's chemical composition, ozone dose and treatment conditions (Kim *et al.*, 2003). In postharvest produce, low concentrations of gaseous ozone have been utilized in cold storage rooms to extend shelf life. Ozone is thought to oxidize ethylene in order to reduce respiration and control spoilage microorganisms (Aguayo *et al.*, 2006). Apples and pears stored under refrigerated conditions with low levels of gaseous ozone showed no signs of ozone injury after 107 days and ozone had effectively reduced ethylene levels inside the storage rooms (Skog and Chu, 2001). Whole and fresh-cut cantaloupe melons maintained good visual quality, aroma and firmness after exposure to gaseous ozone (Selma *et al.*, 2008). Fresh whole scad stored on ice for 10 days under gaseous ozone atmosphere conditions had extended shelf life by 2 days. No signs of rancidity or evidence of fatty acid oxidation had occurred (Silva *et al*., 1998).

However, the application of gaseous ozone was found to adversely affect the quality of some products. The concentration of ozone required is largely dependent on the product's characteristics with high fat foods requiring greater concentrations. For these foods, at higher concentrations, ozone may accelerate surface lipid oxidation. A review by Rice *et al*. (1982) found the oxidative potential of ozone promoted surface lipid oxidation, nutrient degradation and acceleration of spoilage of perishable foods. Ozone-treated freshly cut meats discolored and produced undesirable odors after three days (Fournaud and Lauret, 1972). Exposure to gaseous ozone also appeared to accelerate the oxidation rate of fats found in bacon, butter, dried eggs, and sausages (Van Loeseck, 1949), fish cakes (Chen *et al.*, 1987), milk powder (Ipsen, 1989), and bee pollen (Yook *et al.*, 1998). In ground pistachios, there were no differences to the fatty acid composition except at higher ozone concentrations where a difference in peroxide values was observed (Akbas and Ozdemir, 2006).

Treatment with gaseous ozone was reported to degrade ascorbic acid content of orange juice (Tiwari *et al.*, 2008). However, it slightly increased the ascorbic acid content of whole tomatoes after 15 days of storage (from 0.13 g/L to 0.11 g/L) (Aguayo *et al.*, 2006). Ascorbic acid content in ozone-treated strawberries increased three-fold (0.22 mg/g) when compared to control (0.07 mg/g) after three days of cold storage. The levels were still higher after 5 days but after 15 days, ascorbic acid levels of ozonated strawberries were lower than control (0.13 mg/g versus 0.18 mg/g) (Perez *et al.*, 1999). Perez *et al.* (1999) proposed the increase in ascorbic acid seen in plants could be due to the plant's oxidative stress response to ozone exposure. The thiamine content of wheat flour used in a packaged raw noodle decreased but there were no appreciable changes to sensory quality of the product (Naitoh *et al.*, 1989).

It has been suggested in the literature that ozone may oxidize carotenoids and chlorophyll. Studies were reported where ozone produced color changes in bee pollen (Yook *et al.*, 1998), orange juice (Tiwari *et al.*, 2008), blackberry juice (Tiwari *et al.*, 2009a), broccoli, cucumbers, mushrooms (Skog and Chu, 2001), and carrots (Liew and Prange, 1994). Ozone concentration (up to 7.8% w/w) and treatment time (up to 10 minutes) were critical factors that influenced significant anthocyanin and color degradation for blackberry juice and strawberry juice (Tiwari *et al.*, 2009a, Tiwari e*t al.*, 2009b). However, the use of up to 5% gaseous ozone for 128 minutes did not have any effect on color of whole strawberries and raspberries (Bialka and Demirci, 2007). Whole carrots exposed to low levels of gaseous ozone over an extended period had pitted surfaces with the appearance of dry white blotches and visible injury to the carrot leaves (Liew and Prange, 1994). Ozone was investigated in the treatment of dried spices and fruits for spore reduction. However, an increase in oxidation of volatile compounds was observed in ground black peppers (Zhao and Cranston, 1995). Ozone also reduced the emission of volatile compounds. Ozonated strawberries experienced a 40% reduction of volatile esters (Perez *et al.*, 1999). The aroma of tomatoes treated with ozone was evaluated as below the marketability limit after 15 days storage at 5°C by a trained sensory panel (Aguayo *et al.*, 2006). High concentrations of ozone had a slightly negative effect on sensory scores for flavor, appearance, overall palatability for flaked red peppers (Akbas and Ozdemir, 2008b). No differences in sensory characteristics were seen with dried figs (Akbas and Ozdemir, 2008a).

It is uncertain if using gaseous ozone will affect the sensory characteristics of shell eggs. At this time, there have not been any studies published on either consumer acceptance or discrimination using ozone-based technology. It remains to be determined if consumers can differentiate ozone-treated eggs from untreated eggs based on sensory attributes.

CHAPTER 3

CONSUMER DISCRIMINATION TESTING

3.1. Introduction

In the United States, although the pasteurization of liquid egg products is mandatory and widespread, the egg industry has been slow to adopt technology to pasteurize whole shell eggs. Currently, only 5% of all eggs produced for the shell egg market are pasteurized (USDA-FSIS, 2005). With the proposal of federal regulations calling for increased safety measures in the egg industry, this may change. To be called pasteurized, shell eggs must undergo processing to achieve a 5-log reduction of internal *Salmonella* Enteritidis (USDA, 1997). This treatment is predicted to significantly reduce the public health risk associated with *Salmonella* Enteritidis-contaminated eggs from an estimated 130,000 annual illnesses down to 19,000 (USDA-FSIS, 2005). Presently, only one company, National Pasteurized Eggs, Inc., produces pasteurized shell eggs for the national retail market under the brand, Davidson's Safest Choice® Pasteurized Shell Eggs (National Pasteurized Eggs, Inc., 2008). While pasteurized shell eggs provide increased safety benefits there may be adverse changes in the eggs' functional and quality properties after pasteurization (Li-Chan *et al.* 1995; Hou *et al.,* 1996; Schuman *et al.*, 1997). The egg industry is therefore seeking alternative solutions to overcome barriers to existing technology.

The current shell egg pasteurization process, referred to as the Davidson process, involves heating eggs using water or steam. *Salmonella* Enteritidis has been isolated from both the yolks and albumen of shell eggs so pasteurization times and temperatures must target the yolk (Humphrey *et al*., 1989a; Gast and Beard, 1990; Humphrey *et al*., 1991; Humphrey, 1994; Keller *et al*., 1995; Gast and Holt, 2001). Heat transfer then becomes an issue because the yolk is located in the geometric center of the egg and thus will be the last to reach the required temperature. While there are no changes to the egg shells outward appearance, exposures to high temperatures can denature the albumen's heat-sensitive proteins. As a result, the albumen appears more opaque and cloudier than those from unpasteurized eggs (Hou *et al*., 1996; Shuman *et al.*, 1997; Rodriguez-Romo, 2004; Dev *et al.*, 2007). If heat exposure is prolonged, the proteins begin to coagulate and viscosity increases. This can be advantageous to an extent because the Haugh unit, an indicator of freshness, then increases (Stadelman, 1995). However, the egg's functional properties such as foaming, contribution to volume and stability are impaired as proteins become increasingly denatured (Li-Chan *et al.* 1995, Dev *et al.*, 2006). The vitelline membrane surrounding the yolk can also weaken so that the yolk appears less structured or flatter when broken out onto a flat surface (Stadelman, 1995). These changes can have an adverse effect on consumer acceptance and perhaps on the functional properties of pasteurized shell eggs.

One potential alternative technology, developed by Yousef and Rodriguez-Romo (2004) at The Ohio State University, involves using a combination of heat, vacuum, and pressurized ozone at concentrations high enough to kill SE. The use of ozone (gaseous or aqueous phase) is approved by the FDA as an antimicrobial agent for the treatment, storage, and processing of foods including raw commodities (FDA, 2001). Ozone is a powerful disinfectant due to its high oxidization potential. It is capable of oxidizing unsaturated lipids and proteins of bacterial cell walls and was shown effective at destroying gram positive and negative bacteria, spores, viruses, protozoa, and fungi (Kim *et al*., 1999; Kim *et al.*, 2001; Guzel-Seydim *et al.*, 2004; Mahapatra *et al.*, 2005). Rodriguez-Romo *et al*. (2004) studied the use of gaseous ozone on whole shell eggs for pasteurization purposes. The researchers developed a process whereby eggs are immersion heated until egg yolk temperatures reach 57°C then transferred to a treatment vessel where vacuum is applied before introducing pressurized ozone. This was shown effective in achieving a $\geq$ 6.3 log reduction in *Salmonella* Enteritidis with a total treatment time of 65 minutes (Yousef and Rodriguez-Romo, 2009).

The sequential application of heat and pressurized gaseous ozone is intended to lessen the thermal effects seen on the heat-sensitive albumen proteins. However, the ozone-based process does require applying vacuum, pressure changes, and exposure to a potent oxidizer so there is the potential for changes to egg quality. Rodriguez-Romo (2004) processed eggs with ozone and reported a slight increase in albumen turbidity and a significant increase in Haugh units. There were no apparent changes in protein denaturation patterns when he analyzed the endotherm data from differential scanning calorimetry as compared to control eggs so it was assumed the proteins were unaffected by treatment. There were no differences between the yolk indices of the eggs in his study but Cox *et al.* (1995) has reported a decrease in yolk indices with eggs treated with an ozone-based process.

A second concern with the use of high concentrations of ozone is the potential to oxidize and degrade desirable color pigments in egg yolk. It remains to be determined if there is detectable color loss from possible oxidation of the egg yolk carotenoids.

The current study was based on prior sensory work done in this lab. A second visual evaluation was necessary because of changes to the Davidson process since the first visual evaluation was conducted. Specifically, National Pasteurized Eggs, Inc. switched to the use of USDA Grade AA eggs instead of Grade A eggs for their pasteurized shell eggs. According to the AMS grading system, USDA grade AA quality eggs must have a firm, sufficiently thick or viscous white with a Haugh unit value of 72 or higher (measured at temperature between 7.2 and 15.5°C) when broken out. Grade A quality eggs must have a reasonably firm, less thick or viscous white with a Haugh unit value ranging from 60 to $\leq$ 72 when broken out (USDA-AMS, 2000b). Grade A eggs are the ones most commonly sold in retail markets as regular and specialty eggs. The change to a higher starting quality could be to compensate for the thermal effects seen with pasteurization or as a strategy to add more value to pasteurized shell eggs. Therefore, another visual panel is necessary to more accurately reflect the pasteurized shell eggs currently available in the market. This also allows for comparisons between the visual sensory panel and a subsequent taste panel. Also, the addition of a question about overall visual acceptability of the eggs gives insight to whether perceived differences seen in some attributes of the eggs affects overall opinion.

The consumer acceptance of eggs processed by ozone is expected to be better than thermal treated eggs. This is because the quality attributes where ozone-treated eggs have differed from control were still minimal compared to the results seen with thermal treated eggs. Therefore, this study is focused on using consumer visual discrimination testing of egg appearance because it is strongly associated with the perception of freshness and quality. In the next section, the first sensory panel is summarized and then the current research for the second sensory panel is presented in more detail.

3.2. Materials and Methods

Sensory Panel One

A consumer discrimination test to determine perception of visual attributes of raw eggs treated with either ozone-based technology, thermal treated or untreated eggs was conducted on untrained panelists. The ozone-treated eggs were subjected to the process parameters developed at Ohio State University (Yousef and Rodriguez-Romo, 2004). This involved eggs immersed into a 57°C waterbath until internal egg temperatures equilibrated with the water temperature (25 minutes). The eggs were then transferred to a custom designed stainless steel treatment chamber. A -10 to -11 psig vacuum was applied to the eggs with a vacuum pump before gaseous ozone (9% wt/wt) was introduced via an inlet valve directly into the chamber until a final pressure of 10 psig was reached. The gaseous ozone was produced using the biosafety level 2 pilot plant's ozone generator (OZAT® CFS-7, Ozonia, Degremont Technologies, Elmwood Park, NJ). The gas inlet valve was then closed and the eggs were held under this pressure for 40 minutes.

The Sensory Science Group at the Department of Food Science and Technology conducted the sensory testing. The thermal treated eggs were purchased from a local grocery store and untreated eggs from a local farm were used for the third treatment sample as well as for the reference. One raw egg from each of the three treatments (ozone-treated, thermal treated, control) and a reference egg were cracked onto a clear plate labeled with a random three-digit code and presented simultaneously on a black background.

The eggs were seen by an untrained panel of consumers (n = 75, 40 male, 35 female, ages 18 to over 65) recruited from students, faculty, staff and visitors at the Columbus, Ohio campus of The Ohio State University. Testing took place at the Sensory Evaluation Laboratory in ten individual sensory booths each equipped with a computer monitor, keyboard, and mouse that panelists used to view and complete the assessment. The panelists viewed the samples under white light. The data were collected using Compusense®five version 4.6 software (Compusense, Inc., Guelph, Ontario, Canada). The test selected was a deviation-from-reference assessment with sample order randomized and counterbalanced across panelists. A 19-point category scale was used to rate each of the eggs against a reference for cloudiness of the whites, spreading of the whites, cloudiness of the yolk, yolk height, and the yolk's yellow color (Figure 3.1). The panelists were also asked to indicate their frequency of egg consumption and answered several demographic questions (gender, age, and ethnicity). For the frequency of egg consumption, assessments were made on a 7-point category scale (1 = once a day, 2 = once a week, 3 = once every two weeks, 4 = once a month, 5 = once every six months, 6 = rarely (less than once every 6 months), and 7= never).

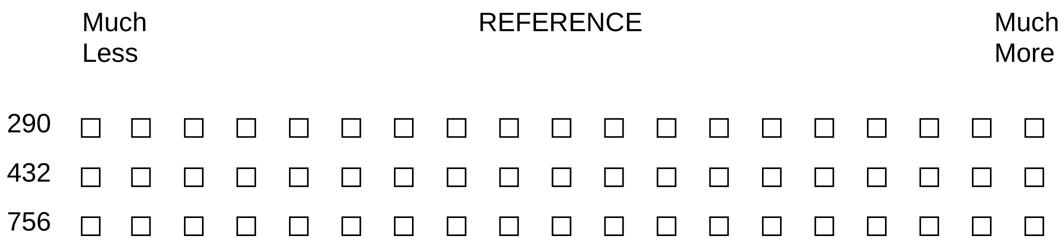


Figure 3.1: 19-point category scale used in sensory panel one to rate visual attributes of egg samples.

Sensory Panel Two

Egg Preparation

Unfertilized, fresh grade AA large shell eggs were obtained from a local farm (Hemmelgarn & Sons, Inc, Coldwater, OH). The eggs were stored at ≤4°C and processed within two weeks of lay. Half of the case (15 dozen) were set aside to be used for the control samples. The day before processing, the egg shells were washed with tap water and allowed to temper at ambient temperature (25°C) for approximately 18 hours. The thermal treated eggs (National Pasteurized Eggs, Inc., Lansing, IL) were ordered

from a local grocery store (Julian date: 233) the week before the sensory panels. The purchased eggs were removed from their plastic cartons and placed on the same fiberboard egg trays as the other treatments and stored in the same cooler.

Ozone Generation

Gaseous ozone was produced from compressed oxygen by electrical discharge using the biosafety level 2 pilot plant's ozone generator (OZAT® CFS-7, Ozonia, Degremont Technologies, Elmwood Park, NJ). Ozone concentration inside of the chamber was monitored continuously with an ultraviolet high concentration ozone monitor (Model Mini-HiCon™, IN USA, Inc., Norwood, MA). During the ozone treatment, environmental ozone concentration was monitored with a low concentration ozone analyzer (IN2000-L2-LC, IN USA, Inc., Norwood, MA). The residual ozone was destroyed by passing the off-gas through a heated catalyst destruct unit (ODT-006, Ozonia, Degremont Technologies, Elmwood Park, NJ).

Ozone Treatment

The ozone treatment followed patented process parameters (Yousef and Rodriguez-Romo, 2004). Three sets of thirteen eggs were placed into three aluminum wire baskets (10 x 6 x 6 in., Fisher Scientific, Pittsburgh, PA) and heated by immersing the baskets into a waterbath (Model 188 and Model 260 circulating, Thermo Scientific Precision, Waltham, MA, USA) filled with distilled water that was set at 57°C. The eggs were held for 25 minutes so that the eggs' internal temperatures equilibrated with the waterbath temperature. Immediately after the heat treatment, the eggs were fitted into custom designed egg holders and transferred into a gasket-sealed stainless steel treatment chamber (300 L, Walker Stainless Equipment Co., Inc., New Lisbon, WI) adapted with a 15-psig pressure gauge (Ashcroft, Dresser Inc., Stratford, CT). Two trials of 36 eggs per trial were run. Boiling water was poured into a reservoir located in the bottom of the chamber in order to raise the relative humidity. Before ozonation, a - 10 to -11 psig vacuum was maintained inside the chamber with a vacuum pump (Model BT7, BESTECH Co., Ltd., Kyunggi-Do, Korea). Gaseous ozone (maximum 9.7% wt/wt ozone in oxygen) was pumped into the chamber through an inlet from the generator until a positive pressure of 10-12 psig was reached. At that point, the ozone generation was stopped, the gas inlet was closed and the eggs were held at a constant pressure for 40 minutes.

At the end of the treatment, the ozone and pressure were slowly released by opening a valve that led directly into the thermal destruct unit. Compressed air was used to purge the chamber of any remaining residual ozone before the chamber was opened and the eggs removed. The eggs were then stored at ≤4°C and used for the sensory panel within one week of processing.

Subjects

Participants were recruited from students, faculty, staff and visitors at the Columbus, Ohio campus of The Ohio State University. Prospective panelists were recruited by email and met the following criteria: 1) at least 18 years of age and 2) willing to participate and available for one of the session times. A $2 gift certificate was offered as an incentive. All participants gave informed consent, in accordance with the policies of The Ohio State University Office of Responsible Research Practices. The panel consisted of 111 volunteers (53 females, 58 males) who ranged in age from 18 to over 65 years of age. The panelists were distributed in the following age groups: four aged 18-20 years, thirty-seven aged 21-25 years, thirty-five aged 26-35 years, twelve aged 36-45 years, eleven aged 46-55 years, nine aged 56-65 years and three over the age of 65 years. Over 80% of the panelists regularly ate eggs with the majority (62%) using eggs at least once per week. Most panelists (76%) had no prior knowledge of egg processing at Ohio State University.

Procedures

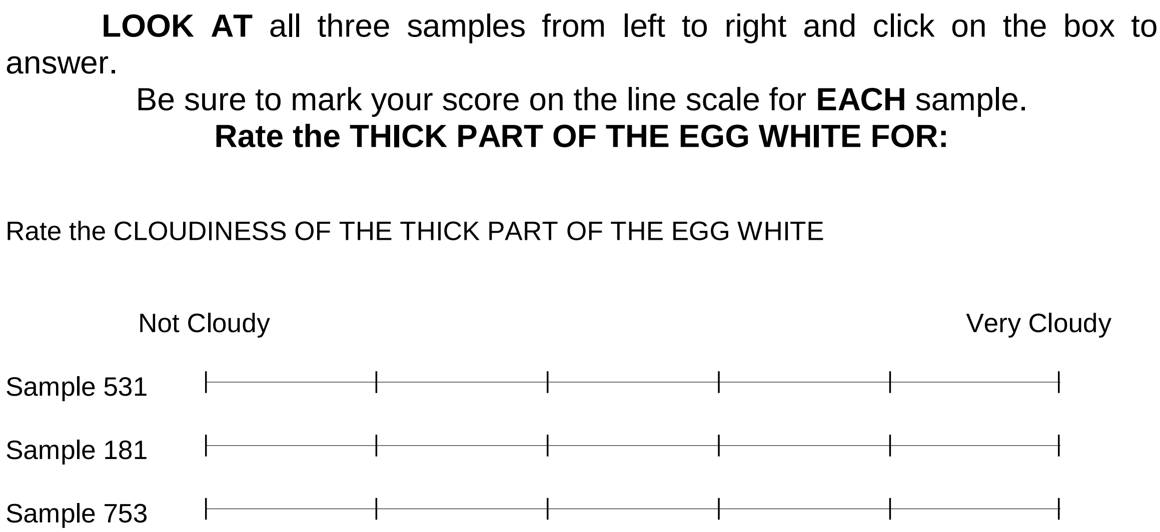
The materials and procedures for this study were reviewed and approved by the OSU Office of Responsible Research Practices (protocol number 2008E0588). Testing took place over two consecutive days in the Sensory Evaluation Laboratory. Ten individual sensory booths were each equipped with a computer monitor, keyboard, and mouse that panelists used to view and complete the assessment. The samples were viewed under fluorescent room plus spot incandescent white lighting. The data were collected using Compusense®five version 4.6 software (Compusense, Inc., Guelph, Ontario, Canada). A consumer discrimination test with a randomized, complete balanced-block design to counterbalance sample order across panelists was conducted. The samples were assigned random three-digit codes and presented simultaneously in one session.

An untrained consumer panel was used to determine whether any perceivable visual differences existed among the eggs using a 10-point attribute intensity linear scale. There were a total of three eggs processed using different treatments seen by the panelists: untreated as control, thermal treated, and ozone-treated. One egg from each treatment was hand cracked onto a clear 6" disposable plate (GFS, Grand Rapids, MI). The three samples were presented side-by-side on trays covered with black placemats

(GFS, Grand Rapids, MI). The setup of the booth as seen by the panelists is shown in Figure 3.2. The order of the eggs on the trays were randomized and changed after each participant. Freshly cracked eggs were prepared every 2 hours.

Figure 3.2: Setup for sensory panel two: Visual discrimination test.

The panelists were instructed to visually examine the samples from left to right without touching, moving or eating the eggs. The panelists were asked to evaluate attribute-by-attribute the samples for intensity and indicate their ratings by marking on a linear scale for the following characteristics: (1) cloudiness of the thick part of the egg white (ranged from 1 = not cloudy to 10 = very cloudy); (2) amount of spreading of thick part of the egg white (ranged from 1 = little spreading to 10 = a lot of spreading); (3) cloudiness of the egg yolk (ranged from 1 = not cloudy to 10 = very cloudy); (4) height of the egg yolk (ranged from 1 = low to 10 = high); (5) color of the egg yolk (ranged from 1 = light to 10 = dark); (6) yellowness of the egg yolk (ranged from 1 = less yellow to 10 = more yellow); (7) spreading of the egg yolk (ranged from 1 = little spreading to 10 = a lot of spreading); and (8) visual appeal of the whole egg (ranged from 1 = not appealing to 10 = very appealing). An example of the linear scale used to rate the cloudiness of the thick part of the egg white is shown in Figure 3.2. At the end, the panelists had the option to type comments on the eggs. The panelists were also asked to indicate how frequently they ate eggs, any previous knowledge of egg processing at Ohio State and answer several demographic questions (gender, age, and ethnicity). For the frequency of egg consumption, assessments were made on a 7-point category scale (1= once a day, 2 = once a week, 3 = once every two weeks, 4 = once a month, 5 = once every six months, 6 = rarely (less than once every 6 months), and 7 = never). For previous knowledge of egg processing, panelists responded by choosing either a yes or a no.



LOOK AT all three samples from left to right and click on the box to answer.

Be sure to mark your score on the line scale for **EACH** sample.

Rate the THICK PART OF THE EGG WHITE FOR:

Rate the CLOUDINESS OF THE THICK PART OF THE EGG WHITE

Figure 3.3: Linear scale used in sensory panel two to rate visual attributes of egg samples.

Statistical Analysis

Compusense software (Compusense, Inc., Guelph, Ontario, Canada) was used to create and collect these data. Analysis was performed separately using the statistical software, SPSS Statistics v. 16.0 (SPSS Inc., Chicago, IL, USA). Treatment effects on the attribute ratings of the eggs were analyzed using repeated measures analysis of variance (ANOVA) with Fisher's least significant difference (LSD) post-hoc test used for pairwise comparisons of the means at a significance level of 5%. Panelists' comments were subjectively grouped by egg treatment and further grouped into positive comments and negative comments within each category.

3.3. Results and Discussion

Seventy five panelists completed the sensory panel one. One hundred and eleven panelists completed sensory panel two. One participant's results were omitted in the second sensory panel because they were unfamiliar with the instructions and could not complete the test. The results and discussion from both panels are discussed together and separated by visual attributes of the albumen and yolk.

Visual attributes of albumen

The mean scores for visual attributes of the albumen are shown in Table 3.1 and Table 3.2 for the first and second sensory panels, respectively. The main outcome of interest was if consumers could detect differences when comparing the albumen of the eggs. The appearance of a high quality egg albumen is described in such terms as clear, viscous, firm, and thick (Stadelman, 1995). Prior studies, using instrumental analysis, reported the thermal treated eggs experienced partial denaturation of heat-sensitive albumen proteins and resulted in a cloudy, more turbid appearance to the resulting albumen (Hou *et al.*, 1996; Shuman *et al.*, 1997; Rodriguez-Romo, 2004; Dev *et al.*, 2007).

Table 3.1: Ratings[1] from sensory panel one on visual attributes of control, ozone-treated, and thermal treated raw, broken-out shell eggs (n=75).

		Control	Ozone	Thermal
Albumen	Cloudiness	9.1 ± 0.4[a]	10.7 ± 0.5[b]	12.7 ± 0.5[c]
	Spread	10.6 ± 0.4[a]	10.9 ± 0.5[a]	9.8 ± 0.6[a]
Yolk	Cloudiness	9.7 ± 0.3[a]	10.0 ± 0.2[a]	11.9 ± 0.4[b]
	Height	10.1 ± 0.3[a]	10.6 ± 0.3[a]	8.9 ± 0.4[a]
	Yellowness	10.2 ± 0.3[a]	10.5 ± 0.3[a]	7.3 ± 0.4[a]

[1]Mean ± standard error of the mean

[2]Attributes evaluated using 19-point deviation-from-reference category scale where 1 = much less and 19 = much more.

Within a row, means followed by the same superscript were not significantly different at $\alpha = 0.05$.

Table 3.2. Ratings[1] from sensory panel two on visual attributes of control, ozone-treated, and thermal treated raw, broken-out shell eggs (n=111).

	Attribute[2]	Control	Ozone	Thermal
Albumen	Cloudiness	3.0 ± 0.2[a]	4.3 ± 0.2[b]	5.5 ± 0.2[c]
	Spread	5.3 ± 0.2[a]	4.8 ± 0.2[a]	3.1 ± 0.2[b]
Yolk	Cloudiness	2.6 ± 0.2[a]	3.2 ± 0.2[b]	3.9 ± 0.2[c]
	Height	5.3 ± 0.2[a]	4.6 ± 0.2[b]	5.3 ± 0.2[a]
	Color	5.2 ± 0.2[a]	5.4 ± 0.2[a]	4.8 ± 0.2[a]
	Yellowness	6.1 ± 0.2[a]	6.2 ± 0.2[a]	5.5 ± 0.2[b]
	Spread	3.7 ± 0.2[a]	5.0 ± 0.2[b]	4.5 ± 0.2[b]
Whole egg	Visual appeal	6.1 ± 0.2[a]	5.9 ± 0.2[a]	5.4 ± 0.2[b]

[1] Mean ± standard error of the mean
[2]Attributes evaluated using a 10-point linear scale. For cloudiness: 1= not cloudy, 10 = very cloudy; for spread: 1 = little, 10 = a lot; for height: 1 = low, 10 = high; for color: 1 = light, 10 = dark; for yellowness: 1 = less yellow, 10 = more yellow, for visual appeal: 1 = not appealing, 10 = very appealing.
Within a row, means followed by the same superscript were not significantly different at $\alpha = 0.05$.

In both sensory panels, panelists' perceived the eggs as being significantly ($p < 0.05$) different from each other in terms of cloudiness. In the first panel, the ozone-treated eggs were rated as more similar to the control and on the second panel, received a mean cloudiness rating of 4.3 that was still towards the "not cloudy" end of the scale. Furthermore, thermal treated eggs were perceived as the cloudiest among treatments. It is important to note that cloudiness of the albumen can also be considered a desirable trait because it indicates freshness. As the storage length of eggs increases, the loss of carbon dioxide via the pores results in egg albumens becoming more translucent. So, fresher eggs appear cloudier than older eggs (American Egg Board, 2007). However, in our study, the thermal treated eggs were older (as determined by the carton pack date) so freshness of the egg would not have been a factor in the differences seen with cloudiness.

The results from the sensory panels correspond to the instrumental analysis results obtained by Rodriguez-Romo (2004) when he used the similar ozone-processing parameters to process eggs and compared the eggs to thermal treated (57-58°C in waterbath for 65-75 minutes) and untreated control eggs. The albumens of the ozone-treated eggs and thermal treated eggs were more turbid as measured using a spectrophotometer. The thermally-pasteurized eggs in his experiment were also the cloudiest. The eggs used to test in his experiment were not the commercially available brand used in this study but similar results should be expected since he used times and temperatures necessary to achieve a 5-log reduction.

In the literature, the spread of the albumen was also reported as different among pasteurized eggs using different pasteurization methods and untreated eggs. The thinning of the thick albumen is an indication of quality loss and flattens a large portion of the albumen to produce a wide arc or halo of liquid surrounding the yolk (Karoui *et al.*, 2006). In this study, the mean scores for albumen spread were not significantly different for any of the treatments in the first panel but in the second panel, the thermal treated eggs were perceived as significantly different from the other two treatments ($p < 0.05$). A possible explanation for the discrepancy between the two panels could be that the consumers were instructed in the second panel to evaluate the thick part of the white. Therefore, the albumen layer on which to focus on was specified in the second panel but not in the first panel. In the second panel, the thermal treated eggs were perceived as having a greater viscosity. This may be because of the coagulation and aggregation of the egg proteins when exposed to heat for prolonged time periods necessary to achieve pasteurization. This is similar to Schuman *et al.* (1997) results where thermal pasteurization increased the Haugh units. Haugh units are determined by measuring the height of the thick albumen relative to its weight (Stadelman, 1995). A higher Haugh unit is a desirable attribute and an indication of egg quality. Overall, the spread of albumens from ozone-treated eggs were still perceived as not different than untreated eggs.

Visual attributes of yolk

The mean scores for visual attributes of the yolk as rated by untrained consumer panels are shown in Table 3.1 and Table 3.2 for the first and second sensory panel, respectively. The egg yolk is more stable and resistant to pasteurization temperatures so changes from heat exposure should be minimal (Zeidler, 2002). Instead, a high quality yolk is described mainly in terms its shape. This is determined by calculating the yolk index which assesses the yolk's spherical condition and membrane strength by taking the ratio of the yolk's height to diameter (Stadelman, 1995). However, irradiation (up to 5 kGy) has induced color changes in egg yolks from a pale yellow to a turbid yellow color so could potentially be a concern (Pinto *et al*., 2004). In terms of yolk cloudiness, in both panels, the thermal treated eggs were perceived as the cloudiest and significantly different from the other two treatments ($p < .05$). In the first panel, there were no significant differences observed between mean scores for control and ozone-treated eggs.

In the second panel, the ozone-treated eggs were rated as significantly different from the other treatments but scores were still closer to the control than thermal treated eggs. All mean ratings among treatments were still below 4 on the intensity scale and indicated cloudiness was not a major concern with these eggs.

The panelists' scores for yolk height and spread among egg treatments were also different between the panels. There are many different variables that changed between panels so it is difficult to say what may have caused the difference in results. In the second panel, only Grade AA eggs were used. According to the American Egg Board, the difference between the grades in regards to the yolk is that AA eggs are rounder and have a high appearance when broken out onto a flat surface whereas A eggs have only a fairly high appearance. Also, because the eggs were store-bought, the age of the eggs could not be matched with the age of the eggs from the other two treatments.

The spreading of the yolk should be related to the height of the yolk with a lower yolk height corresponding to a greater spreading. In the first panel, there were no significant difference in scores between control and ozone-treated for height. Only the thermal treated eggs were significantly different ($p < 0.05$) and were rated as having the lowest height. Yolk spread was not assessed in the first panel. However, in the second study, panelists perceived the yolk height and spread of ozone-treated eggs as significantly lower than control. There were no significant differences between control and thermal treated eggs for yolk height. The mean rating for yolk height of ozone-treated eggs was still 4.6 on the intensity scale so was not drastically different from the other treatments whose mean ratings were both 5.3. There were significant differences amongst all treatments for spread with ozone-treated eggs perceived as having the greatest degree of spreading. However, the mean rating for ozone-treated eggs still remained in the neutral range, with a rating of 5.0 on the intensity scale.

In his experiments, Rodriguez-Romo (2004) did not see any significant differences in yolk index and his procedure was followed for both panels. There is the possibility of the vacuum slightly weakening the vitelline membrane to increase elasticity of the membrane and cause a loss in structural support. Cox *et al.* (1995) reported a change to yolk indices when using a similar process of heating, vacuum and pressurized ozone on shell eggs. They applied a greater vacuum than our procedure and reported that this stretched and weakened the vitelline membrane. Also important to note is that yolk height and spread are related to the thick white layer of the albumen. The thick albumen surrounds the yolk and holds it in position. If the thick albumen is affected and becomes thinner, as discussed previously, then the yolk has a greater spread when broken out (Karoui *et al.*, 2006). In this study, panelists did perceive the spreading of the albumen as greater with ozone-treated eggs than untreated.

The first panel used the term “yellowness” to evaluate whether color differences existed amongst the eggs with the end descriptors being “less yellow” and “more yellow.” For consistency and comparison purposes, we kept the term for the second. However, yellowness may be interpreted differently without a standard reference and thus may not be an appropriate descriptor. Thus we included the more general term “color” with end descriptors of light and dark. When consumers were asked to evaluate egg yolks using color as the descriptive term, there was no significant treatment effects observed. When “yellowness” was used, there was agreement between panels that thermal treated eggs were significantly less yellow ($p < 0.05$) and no significant difference existed between control and ozone-treated. The difference seen with the thermal treated eggs may not be relevant because those eggs came from a different farm than the eggs used for control and ozone-treated. Yolk color varies according to the hen’s diet. More importantly was that there were no differences between ozone-treated and control from which comparisons can be made since they came from the same farm and presumably from hens fed similar diets. This may indicate that consumers did not perceive any noticeable degradation of color pigments as a result of ozonation.

Effect of sensory attributes on overall visual appeal of whole egg

A question about whole egg visual appeal in the second sensory panel was included to determine if significant perceived differences in individual attributes would influence overall appeal. With ozone-treated eggs, there was no significant difference ($p < 0.05$) when compared to the control, indicating favorable acceptance to the standard. However, thermal treated eggs were perceived as significantly different in the negative direction in many different attributes and this seemed to affect their visual appeal score. Thermal treated eggs were rated as less appealing and this difference, although slight, was significant from the other two treatments.

The ozone-treated eggs generated the greatest number of favorable comments and the least number of negative comments as compared to the other treatments (Appendix D). The ozone-treated eggs were described positively with comments such as, “more appealing and easy on the eye,” “looks like a standard fresh egg,” “looks the best and most delicous (*sic*)," "most appealing because it appeared to be fresher," and "has the most promising appearance" (Table D.2). Of the panelists who commented, ozone-treated eggs had the most number of comments describing it as the overall best over the other two treatments. In contrast, thermal treated eggs were described with a greater frequency of negative comments such as, "more cloudy white portion," "doesn't seem natural," "appears to be different from the other two," and "thickest consistency." Several of the comments about the thermal treated eggs described the yolk as being large (Table D.3).

Any differences to the appearance or visual appeal of pasteurized eggs may have still been acceptable to consumers. If comparisons were not available, then they may not have been able to tell the eggs were different. Several panelist comments reflected this idea. Panelists wrote, "I would eat any of these eggs," "I don't usually look at eggs so intently," "I don't think the cloudiness of the egg its (*sic*) crucial for the appeal of the egg," "all 3 egg samples looked normal and I would eat them all," "all 3 eggs are very acceptable from a consumer standpoint," and "If I were to crack open any of these eggs at home, I would probably not notice a difference" (Table D.4). This may indicate that panelists may be willing to substitute pasteurized shell eggs for conventional untreated eggs.

3.4. Conclusion

Results obtained from sensory panels using untrained panelists were suggested as better representations of, and predictors for, consumer preference than expert sensory judgments or physical-chemical product features (van den Heuvel *et al.*, 2007). This may be true for evaluating pasteurized eggs because of the interest in how a typical egg consumer would perceive these eggs during use at home. With appearance consistently ranking high on consumers' purchase criteria for foods, failure to accept pasteurized eggs based on noticeable visual differences can occur and deter future purchases. The results from our two visual discrimination tests indicate that when the albumen and the yolk of eggs are evaluated separately, a panel of untrained consumers can perceive slight differences among treatments. These differences did not adversely affect the overall visual appeal of ozone-treated eggs and these ozone-treated eggs may still be acceptable.

The overall visual appeal of ozone-treated eggs compared favorably to untreated fresh eggs. In the attributes where ozone-treated eggs were perceived as significantly different from untreated eggs, they were still rated better than the thermal treated eggs. These results suggest consumers do not perceive ozone-treated eggs as different from control in visual attributes. Consumers did not perceive ozone-treated eggs as different from the thermal treated eggs (already in the market today). Further work would involve comparing quality attributes of ozone-treated eggs to the newer methods proposed for pasteurization such as microwave heating and the Leda technology. Appearance is a major driver for consumer acceptance and for repeat purchase of foods. Thus the results from this work can help introduce ozone-based technology as a potential pasteurization technology to the egg industry.

CHAPTER 4

CONSUMER AFFECTIVE TESTING

4.1. Introduction

Consumers have certain expectations and purchasing criteria for foods and flavor is one the primary drivers towards the acceptance of foods. The liking of a product's flavor is a response to many different stimuli such as visual, taste, aroma, and texture (Stone and Sidel, 2004). A strong dislike for any one of these sensory attributes can deter consumer away from purchasing the product. For eggs, the preference is for the cooked egg flavor and odor to be mild or bland (Yang and Baldwin, 1995).

With ozone-treated eggs, consumer acceptance of flavor attributes has not been formally investigated. However, there was sensory research conducted on shell eggs treated with irradiation and the development of undesirable off-odors. Irradiation oxidized the polyunsaturated fatty acids found in egg yolks to acid peroxides which were then further oxidized into secondary reaction byproducts (Al-Bachir and Zeinou, 2006). Since ozone is also a powerful oxidant, there is the potential for oxidation to occur to produce acids, aldehydes, alcohols and ketones. These byproducts contribute to the development of rancid tastes and odors (Rice *et al.* 1982). In sensory terms, the taste of lipid oxidation is described as “papery” or “cardboard-like” (Lawless and Heymann, 1998). Rodriguez-Romo (2004) used a 2-thiobarbituric acid (TBA) test to detect secondary lipid oxidation products (specifically malonaldehyde) in ozone-treated egg yolks as compared to gamma irradiated (6 kGy) yolks. Ozone-treated eggs had a significant increase in malonaldehyde concentration from control eggs but not as high as the irradiated eggs indicating some degree of lipid oxidation had occurred (Rodriguez-Romo, 2004).

There were mixed results seen with the use of gaseous ozone on other foods and flavor quality changes. In a review of the use of ozone for perishable foods by Rice *et al.* (1982), there were studies where exposure to gaseous ozone (50-100 ppm) accelerated the spoilage of fats in bacon, butter, dried eggs, meat and sausages (Van Loesecke, 1949). Lipid oxidation was also reported to have occurred in milk powder (Ipsen *et al.*, 1989), fish cakes (Chen *et al*., 1987), and bee pollen (Yook *et al.*, 1998) and negatively impacted sensory ratings. The oxidation of certain volatile oil compounds after sterilization by ozone and production of new ozone-induced compounds occurred in ground black pepper as detected by gas chromatography analysis (Zhao and Cranston, 1995). Overall sensory quality in tomatoes (Aguayo *et al.*, 2006), cantaloupe (Selma *et al.*, 2008), dried figs (Akbas and Ozdemir, 2008), and pistachios (Akbas and Ozdemir, 2006) were unaffected by gaseous ozone treatment. A trained sensory panel evaluated dried red pepper flakes treated with gaseous ozone (up to 9.0 ppm) for up to 360 minutes and assessed the taste, flavor, and overall palatability. At higher concentrations of ozone (higher than 5.0 ppm) for 360 minutes resulted in slightly negative sensory scores for flavor and overall palatability but not for taste (Akbas and Ozdemir, 2008).

Therefore, in order for successful consumer acceptance of ozone-treated eggs, it is imperative that the flavor (taste and aroma) be indistinguishable from regular eggs. The objective of this study is to determine consumer acceptance of flavor, aroma, and texture attributes of ozone-treated scrambled eggs as compared with unpasteurized eggs and the thermal treated eggs currently available in the retail market. If any of the egg's attributes are disliked by consumers then whether those attributes affect overall acceptance or liking of the eggs was also investigated. The ozone process exposes the eggs to rapid changes in pressure as well as heat exposure so has the potential to result in changes to the shell matrix. Shell strength is one of the physical qualities consumers seek in high quality eggs and one that producers are interested in studying for quality control (De Ketelaere *et al.*, 2002). Therefore, instrumental analysis using compression testing was used to assess whether the ozone-processing affects shell strength.

4.2. Materials & Methods

Egg Preparation

Unfertilized, fresh grade AA large eggs were obtained from a local farm (Hemmelgarn & Sons Inc., Coldwater, OH) and stored at ≤4°C. The eggs for the ozone treatment were used within 2 weeks of lay and one week prior to the taste panel. Half of the case (15 dozen) were set aside to be used as the control eggs. Before processing, the eggs were washed with tap water and allowed to temper at ambient room temperature

(25°C) overnight (at least 8 hours). The thermally processed eggs (National Pasteurized Eggs, Inc. Lansing, IL) were ordered from a local grocery store (Julian date-288) the week of the sensory panels. The purchased eggs were removed from their plastic cartons and placed on the same fiberboard egg trays as the other treatments and stored in the same cooler.

Ozone Generation

A commercial ozone generator (OZAT CFS-03 2G, Ozonia, Elmwood Park, NJ, USA) system designed for non-pathogenic use was custom built for the lab by Tower Tool & Manufacturing, Inc. (Twinsburg, OH, USA). The system design was modeled after the original biosafety ozone generator. Upon delivery, a rotary vacuum pump (RV3, BOC Edwards, Shoreham, UK), 15-psig pressure gauge (Ashcroft, Dresser Inc., Stratford, CT), ultraviolet high concentration ozone monitor (Mini-HiCon, InUSA, Inc., Needham, MA), low concentration room ozone analyzer (IN2000-L2-LC, InUSA, Inc., Needham, MA) and 3-way diversion ball valve were installed to complete the system. High concentrations of ozone oxidize and corrode metals so stainless steel was used for all surfaces and pipes that carry ozone. All tubing, gaskets, and fittings used were made from polytetrafluoroethylene (PTFE). Any remaining ozone after processing was safely destroyed by passing through a heated catalyst destruct unit (ODT-003, Ozonia). As an extra safety precaution, the air coming out of the destruct unit was directed outdoors via an exhaust pipe and fan.

Ozone Treatment

A modified standard operating procedure was developed for the new commercial ozone generator (Appendix A). Four rounds (30 eggs each) of ozone-processing were completed. For each round, two sets of fifteen eggs were placed into two aluminum wire baskets (10 x 6 x 6 in.; Fisher Scientific, Pittsburgh, PA). The baskets were then immersed into a 57°C waterbath with circulating pump (Model 265, Thermo Scientific Precision, Waltham, MA, USA) for 35 minutes. The minimum water level above the shell eggs was 1 inch. The length of the time it took for the internal egg temperatures to equilibrate with the waterbath temperature was determined prior to the trials by inserting thermocouples (Oakton Instruments, Vernon Hills, IL) into the geometric center of the most centrally located egg in each of the baskets and recording the time and temperature. Immediately after heating, the eggs were fitted into specially designed egg holders and transferred into a custom designed gasket-sealed stainless steel treatment chamber (100 L, Alloy Products Corp., Waukesha, WI). A schematic diagram of the setup is shown in Figure 4.2. A flask that contained approximately 200 mL of boiling tap water was placed inside of the chamber to raise the relative humidity. A -10 to -12 psig vacuum was maintained inside of the chamber with a vacuum pump before ozone generation. The ozone generator produced gaseous ozone by electrical discharge from extra dry compressed 99.6% oxygen. In order to attain maximum ozone concentration, the gaseous output was first diverted, using the diversion valve, directly into the ozone destruct unit. After thirty minutes of diversion, the gaseous stream was then directed into the chamber until a pressure of 11-12 psig was reached. At this point, the ozone destruct valve was opened slightly and the gaseous flow was decreased by adjusting the flow valve until a steady pressure state was achieved. The valve that allowed for gas flow to the high concentration ozone monitor was opened so ozone concentration in the chamber could be monitored. This steady state was held for 40 minutes. During the processing, environmental ozone concentration was monitored using a low concentration ozone analyzer. The maximum residual gaseous ozone concentration achieved was 6.25-6.84% wt/wt ozone in oxygen for the four processing trials.

To release the pressure and safely destroy the residual ozone, the ozone destruct valve was opened and the off-gas was directed into the ozone destruct unit. After de-pressurizing, the contents of the chamber were pressurized again with compressed air to purge any remaining ozone until the reading on the ozone monitor read 0.0%. As an extra precaution, the ozone destruct valve was fully opened and compressed air continuously flushed the chamber for ten minutes. The chamber contents were emptied and the eggs were transferred to fiberboard trays and stored in a $\leq$ 4°C cooler.

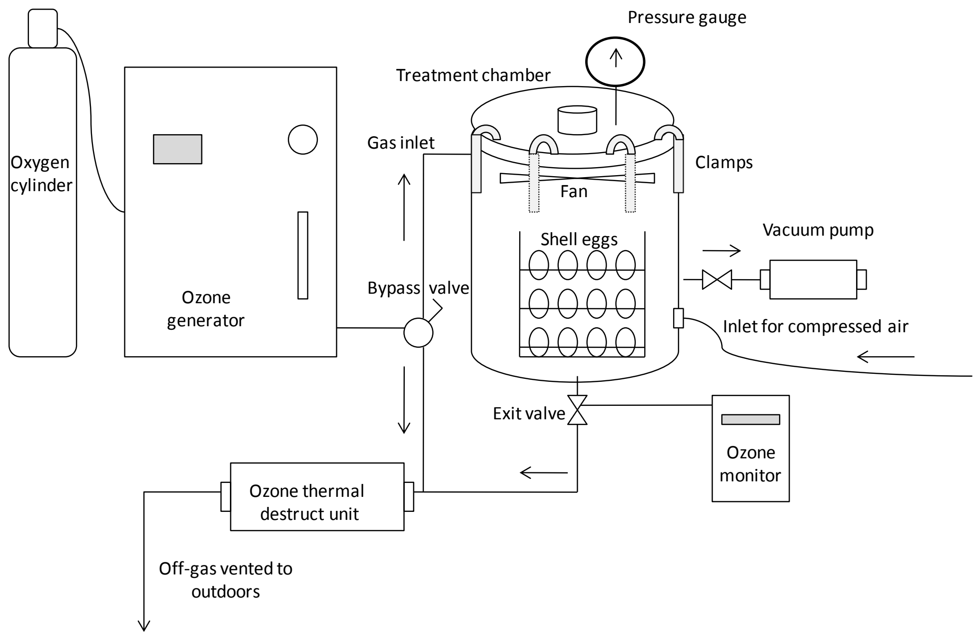


Figure 4.1: Schematic diagram of experimental setup used for ozone-processing of shell eggs.

Subjects

One hundred and thirty two untrained individuals were recruited from students, faculty, staff and visitors to the Columbus, Ohio campus of The Ohio State University. Prospective panelists were recruited by email and met the following criteria: 1) at least 18 years of age, 2) ate eggs, and 3) was willing to participate and was available for one of the session times. All participants gave informed consent, in accordance with the policies of The Ohio State University Office of Responsible Research Practices. A $5 gift certificates was offered as an incentive. The panel consisted of 75 females and 57 males who ranged in age from 18 to over 65 years of age. The panelists were distributed in the following age groups: thirteen aged 18-20 years, thirty-five aged 21-25 years, forty-four aged 26-35 years, eleven aged 36-45 years, nineteen aged 46-55 years, eight aged 56-65 years and two over the age of 65 years. Over 80% of the panelists ate eggs frequently with the majority (69%) eating eggs at least once per week. Over 80% of the panelists had no prior knowledge of egg processing at Ohio State University.

Procedures

The materials and procedures for this study were reviewed and approved by the OSU Office of Responsible Research Practices (protocol number 2008E0594). Testing took place over two consecutive days in the Sensory Evaluation Laboratory. Ten individual sensory booths were each equipped with a computer monitor, keyboard, and mouse that was used by the panelists to view and complete the assessment. The booths were illuminated with standard fluorescent room plus spot incandescent white lighting. The responses were collected using Compusense®five version 4.6 software (Compusense, Inc., Guelph, Ontario, Canada). The test selected was a consumer affective test combining degree of liking questions using 9-point hedonic category scales and questions about egg appeal using 5-point Just-About-Right (JAR) intensity scales. The samples were given computer-generated random three-digit codes. Serving order was randomized and counterbalanced across panelists by selecting complete balanced blocks design. Samples were served to the panelists in sequential monadic order.

In each session, a total of three samples of scrambled eggs prepared from eggs of each treatment (untreated as control, thermally processed and ozone-treated) were presented to each panelist. A protocol was developed beforehand that standardized the mixing, cooking and serving time of the samples. A timer was used during actual sample preparation to strictly adhere to the protocol. One person prepared all the egg samples for all sessions. It was determined that 6 eggs would yield 10 one ounce servings. The eggs were pre-mixed and portioned out for each session the morning before each testing day to minimize preparation time. This involved hand cracking the six eggs into a glass mixing bowl and blending thoroughly on high for 45 seconds with a hand blender with the whisk attachment (Model #59780, Hamilton Beach, Washington, NC). This egg mixture was then poured into appropriately labeled Ziploc food storage bags (SC Johnson, Racine, WI) and stored in a commercial refrigerator (Model 6025-S, Delfield, Mt. Pleasant, MI) at < 4°C until use.

The samples were prepared fresh for each session and cooking began as soon as the panelists were seated. The contents of one bag were poured into a glass mixing bowl and blended on the high setting for 5 seconds using a hand blender with whisk attachment. The eggs were then cooked in the microwave (Model JES1358WL 01, GE, Louisville, KY) for one minute at full power (1100 W), taken out to be blended again on the low setting for an additional 5 seconds before cooking for another minute, then hand mixed with a plastic spatula to break up any large pieces of egg before cooking for the final minute. After removing from the microwave, the bowl was covered with cling film (Wasserstrom, Columbus, OH) and allowed to sit for 30 seconds to complete the cooking process and ensure no liquid remained. Two microwaves of the same model were set up in the testing kitchen so that during this period, the cooking of the next batch of eggs could begin. Before serving, the temperature was taken using a handheld thermometer

(Model 35625-41, Oaktron, Vernon Hills, IL) and recorded. The eggs were portioned out using a one ounce scoop (Vollrath, Sheboygan, WI) onto appropriately labeled white 6 inch foam plates (GFS, Grand Rapids, MI) and served immediately to the panelists. Room temperature distilled water (Ice Mountain, Greenwich, CT) was provided for each panelist as a palate cleanser between samples.

Panelists evaluated the scrambled egg samples for visual liking, aroma, flavor, texture, and overall liking using 9-point hedonic category scales (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). An example of the scale is shown in Figure 4.2. The 5-point Just-About-Right (JAR) scales (Figure 4.3) were used on questions about the appeal of the scrambled egg color (1 = much too dark, 2 = slightly too dark, 3 = just about right, 4 = slightly too light, 5 = much too light), egg moistness (1= much too dry to 5 = much too watery), and egg texture (1= much too tough to 5 = much too tender). At the end, panelists had the option to type comments on the sample. The panelists were also asked to indicate their consumption frequency of eggs, previous knowledge of egg processing at Ohio State and several demographic questions (gender, age, and ethnicity). For the frequency of egg consumption, assessments were made on a 7-point category scale (1 = once a day, 2 = once a week, 3 = once every two weeks, 4 = once a month, 5 = once every six months, 6 = rarely (less than once every 6 months), and 7= never). For previous knowledge of egg processing, panelists responded by choosing either a yes or a no.

Please **LOOK ONLY** at this sample of scrambled eggs and answer the following questions.

Please check only **ONE BOX.**

OVERALL VISUAL LIKING

dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like nor dislike	like slightly	like moderately	like very much	like extremely
1	2	3	4	5	6	7	8	9

Figure 4.2: 9-point hedonic scale used to rate consumer acceptance of scrambled egg samples.

Continue **TASTING** this sample of scrambled eggs and answer the following questions.

Please click on **EACH** box to answer **EACH** question.

Rate the EGG COLOR

Way too Dark	Slightly too Dark	Just About Right	Slightly too Light	Way too Light
1	2	3	4	5

Figure 4.3: 5-point Just-About-Right scale used to rate appeal of scrambled egg samples.

Analytical Testing

Eggs were allowed to temper at ambient room temperature (approximately 25°C) for 24 hours. Twelve eggs from each of the three treatments were randomly selected for testing eggshell strength. A static compression test was performed using the Universal

Materials Testing Instron 5542 (Instron, Norwood, MA, USA) equipped with a 100 N load cell. Bluehill®2 materials testing application software (v.2.17, Instron, Norwood, MA, USA) was used to collect the data. The crosshead speed was set to 150 mm per minute and a continuous force was applied with the two inch compression anvil until the point of eggshell failure as detected by a change in force. To prevent spillage of the egg contents, all the eggs were placed into Ziploc bags prior to testing. The eggs were oriented on its side so that contact with the plate would be along the egg's equator. Maximum force to introduce a crack in the shell was reported as Newton of force.

Statistical Analysis

Compusense software (Compusense, Inc., Guelph, Ontario, Canada) was used to create and collect these data. Analysis was performed separately using the statistical software, SPSS Statistics v. 16.0 (SPSS Inc., Chicago, IL, USA). Treatment effects on the attribute ratings of the eggs were analyzed using repeated measures analysis of variance (ANOVA) with Fisher's least significant difference (LSD) post-hoc test used for pairwise comparisons of the means at a the 5% significance level.

The JAR scale attribute means were calculated and were also subjected to repeated measures ANOVA with Fisher's LSD post-hoc test. The means and standard deviations were calculated for the compression testing results and analyzed for significance using univariate ANOVA and Tukey's HSD to determine significance at the 5% level. Panelists' comments were subjectively grouped into texture, color, flavor, moisture, aroma and other categories and further grouped into positive comments and negative comments within each category.

4.3. Results and Discussion

One participant's data was omitted because they indicated that they never consumed eggs and so did not meet the criteria to participate. Two other participants could not complete the test so their results were also omitted. For the compression testing, one ozone-treated egg was cracked prior to testing so was omitted from the final results.

Visual liking

There were no significant differences ($p > 0.05$) in overall visual liking of scrambled ozone-treated eggs as compared to the other egg treatments. All of the eggs were equally liked with mean ratings of 6.4, that corresponds to the "like slightly" category (Table 4.1). Consumers appeared to have different opinions regarding the appeal of the color according to their mean JAR ratings (Table 4.2). The mean JAR ratings were statistically different ($p < 0.05$) among treatments. The control sample was judged closest to just-about-right with a mean of 3.0 followed by ozone-treated and then thermally-pasteurized with means of 2.9 and 3.2, respectively. The ozone-treated samples were judged slightly more frequently as being slightly too dark whereas consumers considered thermally-pasteurized eggs as slightly too light. However, color comparisons cannot be made between thermal treated samples and the other two treatments because the source of the eggs was different. The color of the yolk will vary according to the hen's diet and this was a variable we could not control for in this experiment because the thermally-pasteurized eggs were purchased from a grocery store. Color comparisons between the yolks of control and ozone-treated eggs are valid as these eggs originated from the same batch at the same farm. When reviewing the comments, the control and ozone elicited similar numbers of positive and negative color comments (Table E.1).

Table 4.1: Consumer acceptance ratings[1] on egg attributes (n = 132).

Attributes[2]	Control	Ozone	Thermal
Overall visual	6.4 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Aroma	6.2 ± 0.1	6.2 ± 0.1	6.3 ± 0.1
Flavor	6.0 ± 0.1	6.2 ± 0.1	5.9 ± 0.1
Texture	6.3 ± 0.1	6.0 ± 0.1	6.1 ± 0.2
Overall liking	6.0 ± 0.1	6.0 ± 0.1	5.8 ± 0.1

[1] Mean ± standard error of the mean
[2]Attributes were rated using a 9-point hedonic scale with 1 = dislike extremely, 9 = like extremely
Within a row, means were not significantly different at $\alpha = 0.05$.

Table 4.2: Consumer ratings[1] on appeal of egg attributes (n = 132).

Attribute[2]	Control	Ozone	Thermal
Color	3.0 ± 0.0^a	2.9 ± 0.0^b	3.2 ± 0.0^c
Moistness	2.7 ± 0.1^a	2.5 ± 0.1^b	2.4 ± 0.1^b
Texture	2.6 ± 0.1^a	2.5 ± 0.1^a	2.5 ± 0.1^a

[1]Mean ± standard error of the mean
[2]Attributes were rated using a 5-point JAR scale. For color: 1 = way too dark, 3 = JAR, 5= way too light; for moistness: 1 = way too dry, 3 = JAR, 5 = way too watery; for texture: 1 = way too tough, 3 = JAR, 5 = way too tender.
Within a row, means not followed by the same superscript were significantly different at $\alpha = 0.05$.

Flavor, aroma, texture

Flavor, aroma and texture all interact to impact an individual's response to taste characteristics so will be discussed together. The main concern was whether exposure to high concentrations of ozone would cause significant lipid oxidation leading to the development of undesirable odors as seen with other high fat containing foods (Van Loesecke, 1949; Fournauad and Lauret, 1972). Egg yolks contain 4.5 grams of fat of which 56-60% is of the unsaturated type and so are susceptible to lipid oxidation (Zeidler, 2002). If the ozone process introduces any oxidation byproducts, there is the potential for consumers to taste an oxidized or rancid flavor and dislike the eggs. If this were to occur, it would be expected to negatively impact hedonic scale ratings. However, in this study, there were no significant differences ($p > 0.05$) in liking of flavor, aroma or texture among the egg treatments. This is especially promising because this experiment used significantly higher concentrations of gaseous ozone than that reported in studies where sensory differences have been observed in other food products. Nickos *et al.* (1997) had previously suggested in the literature that eggs may possess built-in antioxidant characteristics such as phosvitins to prevent lipid oxidation. More research may be needed to identify these protective properties. The occurrence of lipid oxidation in ozone-treated eggs cannot be ruled out. Oxidation could have occurred but at such low levels that were undetectable to consumers. Oxidation may propagate as post-process storage time increases. In this study, eggs were tested one week after processing. It remains to be determined whether similar results would be seen throughout the shelf life of the eggs.

The flavor of all the eggs were rated similarly with the mean ratings ranging from 5.9- 6.2 that correspond to the "neutral" and "slightly liking" range on the hedonic scale. Aroma plays a role in flavor interpretation and consumers rated it similarly among treatments with mean ratings in the "slightly like" category. When reviewing the comments, "bland" and "needs more flavor" were often mentioned (Table E.2). However, this is not necessarily a negative attribute as eggs are characteristically described as mild or bland and a strong egg flavor may not be preferred. Judging from several comments made by panelists, eggs are not often eaten alone but with added ingredients such as salt, pepper or oil which could have influenced their opinions. Ozone-treated eggs generated more negative flavor descriptors used by consumers to describe an unexpected taste. Two panelists described the eggs as tasting of maple, two other panelists mentioned soy or soy sauce taste and three others used "burnt" as a way to describe an off-taste. With aroma comments, six panelists described ozone-treated eggs as having an off-odor and likened it to "plastic" (Table E.3). However, there were no differences seen in hedonic liking of taste or aroma between ozone-treated and control so these comments were not shared by a significantly large number of the panel.

When asked specifically about the appropriateness of the moistness, the mean ratings for moistness of all the eggs were below just-about-right (ranging from 2.4-2.7) indicating consumers considered them as slightly too dry. The mean ratings for thermal treated and ozone-treated were significantly lower than control. In panelists' comments thermal treated and ozone-treated both generated a similar number of negative comments with dryness mentioned most often (Table E.4).

The mean JAR ratings for texture were not significantly different for any of the egg treatments and they were all perceived as below just-about-right with a slightly tougher texture. The number of negative and positive comments was similar for the treatments with more negative comments. The descriptors to describe the eggs were "tough," "rubbery," "chewy," and "rough." With the thermal treated and ozone-treated eggs, the word "rubbery" was mentioned slightly more times than control (Table E.5). Thermal exposure during processing has the potential to increase water loss by the denaturation and aggregation of protein. When cooked for the same length of time, these egg proteins may become overcooked and contribute to the perception of a firmer or harder texture when compared to the control. The perceived texture might be indicative of the cooking style rather than a property of the eggs themselves. In the United States, consumers tend to prefer their eggs slightly undercooked or runny which, for safety reasons, could not be served to the panelists (Lin *et al.*, 1997). However, the tendency towards negative judgments regarding the degrees of moistness and texture properties of the eggs were not reflected in the consumers' hedonic score for texture that was still in the "slightly like" category.

Overall liking

The consumers participating in this panel accepted all eggs equally ($p < 0.05$) when asked to rate their overall liking using the hedonic scale. This may signify favorable acceptance of ozone-treated eggs. Frequent consumers of eggs did not negatively perceive any of the flavor attributes of ozone-treated eggs. Consumers also did not differ in their acceptance of ozone-pasteurized eggs as compared to thermal treated eggs. This is promising when introducing ozone-treated eggs into the market. In the previous study, ozone-treated eggs performed better than thermal treated eggs on visual attributes.

Table 4.3: Eggshell compression test results.

Sample	Maximum compressive load (N)		
	Control	Ozone	Thermal
1	30.485	23.345	27.640
2	36.371	*	32.710
3	28.998	37.977	37.240
4	36.008	27.503	33.991
5	36.809	23.538	31.221
6	34.428	27.458	23.328
7	30.085	28.918	34.357
8	29.640	27.511	43.651
9	29.659	30.901	28.349
10	43.109	29.512	37.360
11	40.703	28.701	28.477
12	30.079	32.737	29.044
Mean	33.865[a]	28.918[a]	32.281[a]
SD	4.780	4.088	5.480

*Cracked egg.
Means within a row were not significantly different at $\alpha = 0.05$.

Compression testing

Natural variations in eggs present challenges to physical egg quality assessments. Nonetheless, producers have the need to identify factors that could affect the quality of their products and for quality assurance testing (Jones and Anderson, 2006). The shell breaking force is one of those qualities and is related to the amount and thickness of the egg shell. This is, in turn, influenced by the hen’s diet, specifically by vitamin D, calcium, phosphorus, and manganese. Strength has also associated with hens’ genetic strain, stress, diseases, hen age and egg size. As the hens age, their eggs become larger but the shell weight does not increase proportionally so the shell becomes thinner (Roberts, 2004).

Results from compression testing for eggshell strength in other studies were quite variable. Jones and Musgrove (2005) reported a range of 35 to 38 N of force among replicates (n =113). In our study, effort was made to position the egg so that the force was applied in the same manner and location of the egg but egg shapes are not homogenous so this could have added to the variability in results. Ketelaere *et al*. (2002) has acknowledged in the literature that considerable variation in breaking strength may occur when measured at different locations on the same egg because of variation in thickness, shape, and contact between measurement device and egg.

In this experiment, there was great variability seen between eggs of the same treatment. Comparisons between control and ozone-treated eggs can be made because they came from the same farm whereas comparisons were not made to the locally purchased thermal treated eggs with variations that invalidate any conclusion. There were no statistically significant differences seen between any of the treatments ($p = 0.059$) in terms of force required to introduce a crack in the eggshell.

4.4. Conclusion

The sensory data obtained from this consumer acceptance panel indicated that the preference of flavor, aroma, and texture characteristics of scrambled eggs were unaffected by ozone-processing or thermal pasteurization. Egg yolks may possess intrinsic properties that protect the unsaturated lipids from oxidation. More research may be needed to identify these protective properties and whether consumer acceptance of eggs remains positive throughout the shelf-life of ozone-treated eggs. Preliminary research into the strength of the eggshell suggests the strength of the shell was not affected by additional processing. The results from consumer acceptance testing suggest that based solely on intrinsic factors, ozone-treated eggs may be acceptable to consumers. Assessing the potential for market success may be the next step. Similar to thermally-pasteurized eggs, ozone-treated eggs will be a part of the specialty egg market and will be priced accordingly. However, the results from this study indicating that there were no differences in preference between ozone-treated and thermally-pasteurized eggs already in the marketplace are promising for assessing the potential for entering into and successfully competing for share of the specialty egg market. Further research should determine whether extrinsic factors such as packaging, labeling, and price information influence the willingness of consumers to pay a higher premium for safer eggs.

CHAPTER 5

CONCLUSIONS

Consumers are becoming more aware of the importance of food safety when making purchasing decisions. As a result, the demand for foods providing extra safety benefits is rising. This along with the federal government calling for increased safety in the egg industry makes clear the demand for proven new technologies to ensure egg safety. Ozone-processing is one of the more promising technologies proposed for pasteurization of shell eggs. Before this study, published studies have not been conducted on whether ozone has any effects on the sensory properties of shell eggs as perceived by consumers. This is important to assess because appearance and taste influence consumers' judgments of egg quality and subsequent purchasing decisions.

In this study, two types of sensory testing were conducted with untrained consumers as panelists. In chapter 3, discrimination testing was used to determine whether the appearance of raw, broken out ozone-treated eggs is perceived as different from untreated and thermally-pasteurized eggs. Ozone-treated eggs were perceived as different in terms of albumen cloudiness, yolk height and yolk spread. However, the overall visual appeal scores of the ozone-treated eggs were not significantly different from the control eggs. In chapter 4, consumer acceptance testing was conducted to determine consumer acceptance of the flavor of the eggs when they were cooked as scrambled eggs. There were no differences in acceptance of ozone-treated eggs as compared to untreated eggs with all the samples being similarly liked. There was not any flavor attribute that was disliked strongly enough to influence the consumers' hedonic scoring of the eggs.

The overall visual and flavor acceptability of ozone-treated eggs in these sensory panels were positive and in most cases, the eggs were perceived as better than the competing pasteurized shell eggs currently available in retail markets. This work may facilitate the introduction of ozone-based technology as a potential pasteurization technology, as it retains the fresh quality characteristics important to consumers and to the egg industry. The rapid market growth seen with organic and nutritionally-modified eggs shows there is great potential for other value-added eggs to succeed in a diversifying egg market. Appropriate economic studies can assess potential for market success and future research will determine whether extrinsic factors such as price, labeling, marketing will have an effect on consumer acceptance. Future work may include conducting consumer acceptance studies over the duration of the shelf life of ozone-treated eggs. Additional research may also include comparing ozone-treated eggs with other proposed pasteurization methods such as the microwave-heating process and the combination of microwave, heat and ultrasound of the Leda (Bettcher) Technologies method. If ozone-treated eggs are marketed to capitalize on their fresh appearance and unaltered taste quality, there is promise for wide public acceptance of ozone-treated leading to a successful placement in the specialty egg market.

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APPENDIX A

OZONE GENERATOR STANDARD OPERATING PROCEDURE, OZONE MDS

Working Safely with this Ozone Processor: last revised October 20, 2008

This machine was built by Tower Tools (Larry Lapchinsky) as specified by Ken Lee.133 to implement the invention of Ahmed Yousef.1 via a federal grant by Lee et al. This device produces food grade eggs for human consumption. Another pathogen-only ozone machine is in the biosafety pilot plant in Howlett Hall. Contact Setsuko Kamotani.2 or Ken Lee.133 for more information.

Read and Understand these Safety Items

OZONE SAFETY. Ozone gas is toxic and accelerates combustion. The OSHA permissible exposure level is 0.10 ppm. You can smell ozone at 0.02 ppm and higher, but this detection level may rise from gradual adaptation. Thus pay attention to the room safety ozone detector mounted nearby.

The concentration of ozone inside this closed system is dangerous. This machine is designed to contain pressurized ozone. If there is a rapid release of ozone, **immediately unplug the machine at wall outlet and evacuate the room.** All ozone should exit this system through the heated ozone destruct catalyst that operates at 450 degrees C.

You need not wear gloves, goggles or a respirator. Standard pilot plant hair net and lab coat are recommended. Ozone is highly unstable and decomposes rapidly at room temperature to oxygen gas. See attached ozone MSDS for more information.

VACUUM SAFETY. This machine is designed to contain a high vacuum. Checking for vacuum leaks by hand could pinch or tear, so use plastic wrap. Vacuum pump oil has an ignition point of 300 to 600 degrees C., made significantly lower in the presence of either ozone or pure oxygen. The vacuum pump produces a small oil mist that is trapped by an exit filter. This mist can be explosive in the presence of either ozone or pure oxygen. A plastic mist filter was burnt to a crisp in an August 2008 accident. For these reasons, **the vacuum pump is only used to evacuate air, never allow ozone to enter the vacuum pump.** Conversely, do not operate the vacuum pump with any gas other than air inside the chamber.

PRESSURE SAFETY. This machine is designed to contain pressurized ozone. Tower Tools has tested the chamber to 50 PSIG. The pressure relief valve on the lid opens at 20 PSIG. Do not exceed 15 PSIG of ozone. All pressure comes from the bottled oxygen gas cylinder. This cylinder must be secured on the platform of this machine. Since this machine is on wheels it is unsafe to secure the cylinder independent of the cart**. In case of emergency shut the gas cylinder valve and unplug the machine from the wall.**

ELECTRICAL SAFETY. Single phase 230 volt current at 30 amps enters the machine through a single wire. Both 230v and 115 volt devices with proper ground and independent fuses are on the cart. Each device has an independent on/off switch. Become familiar with the location of each switch before using this machine. The ozone generator contains high voltage capacitors that store current, thus a separate grounding tool is used to discharge them. **Do not open the ozone generator cabinet** unless you know how to use this grounding tool. Water resistant electrical components are used throughout, but do not operate this machine if it is wet. **In case of emergency unplug the machine from the wall.**

MECHANICAL SAFETY. Engage the brake at the chamber end of the cart before operation. Unplug at wall, close main gas cylinder supply, disconnect air, disconnect cooling water and open all drain valves before moving this machine. Never move this machine while pressurized or energized.

E	V	O	**Warm Up.** Two or more trained operators must be present.
Electrical is ON	Vacuum OFF	Ozone OFF / **Ozone ON**	**1.** Connect machine to 230v wall outlet. Switch main power on. Both the hi-con and ambient ozone monitors must display weight % and the ozone destruct unit shows temperature. Connect the water **but do not connect the air.** 2. Turn the cooling water valve on slowly. Ensure fast cool flow into floor drain. The window mounted exhaust fan is on high speed. 3. Switch on the tank top pressure-vacuum gauge, initial reading is zero PSI. 4. All yellow valves are in the perpendicular off position. Set the yellow diversion valve perpendicular, **so all ozone flows into the destruct unit.** ↔ Note arrow. 5. Open main gas supply at oxygen cylinder with regulator at 37 psi. 6. Switch ozone generator on. Front LED panel will show system status. 7. When destruct unit shows >400 degrees or higher, push PSU button on the ozone generator to on. Control and set point switches are "local" and PSU is "100%." 8. Adjust gas flow with hand operated control valve to 10 to 15/100. Turn upper pressure regulating valve on the ozone machine (not the cylinder) to 2.2-2.5 bar.
			Process. Contents of chamber are treated as follows.
	Vac. ON		9. After ~30 minutes, check vacuum oil level, oil color and switch vacuum pump on. There must be zero PSI and no ozone inside the tank. 10. Use two hands to gently open the red vacuum valve. 11. When -10 to -13 PSIG is reached shut vacuum valve. 12. Switch off vacuum pump. Move diversion valve so ozone flows into the tank.
	Vacuum is OFF		13. After pressure is >1 psi positive, open small yellow valve to ozone monitor. There is a T-valve next to the yellow valve. If the flow is wrong turn the T-valve. 14. When ozone pressure is 10-13 psi, turn down hand control valve (set in step 8 above) and slightly open the destruct valve to maintain steady pressure for 40 minutes. Optionally adjust flow through the hi-con monitor to hold pressure.
			Shutdown. Two or more trained operators must sign logbook.

		Ozone is OFF	15. Press PSU off button on ozone control panel and see that pressure is declining. 16. Ensure ozone destruct shows >450 degrees and gradually open yellow destruct valve (exit valve, ~20 degrees from horizontal). 17. When tank pressure reaches zero, press purge on, wait a minute, and turn diversion valve to flush remaining ozone from the diversion line. 18. Turn off main gas supply (CW) at oxygen cylinder. 19. Shut off PURGE and shut off ozone generator at red illuminated toggle switch. 20. Turn cooling water supply valve off and set yellow destruct valve fully open. 21. Connect air supply and re-pressurize to ~10 psi. The air line attaches by the vacuum ball valve. Shut air and allow it to exit through destruct valve. 22. Repeat above step two or more times until the ozone monitor readings are <1.0%. 23. You may also continuously flow fresh air in, with the destruct valve fully open, for several minutes to blow residual ozone from the chamber.
El is OFF			24. Turn off small yellow valve to high concentration ozone monitor. 25. Shut off the main power at red rotary switchbox. Shut off air. 26. Open chamber and empty contents. 27. Disconnect power cable, water supply, exhaust air and compressed air if machine will not run again for several days. 28. The wall mounted ozone detector remains on. It requires a full day warm up for accurate readings. The window mounted exhaust fans stay on. **Clean up area.** 29. Record name, date, conditions and helpful comments in the log book.

OZONE MATERIAL SAFETY DATA SHEET

Effective Date: 06/01/00

Product: Ozone

1. Product Identification

Synonyms: Triatomic oxygen
CAS No.: 10028-15-6
Molecular Weight: 48.0
Chemical Formula: O_3

2. Composition/Information on Ingredients

Ingredient

Ingredient	CAS No	Percent	Hazardous
Ozone gas	10028-15-6	1 - 15%	Yes

3. Hazards Identification

Emergency Overview

Highly reactive, can explode on contact with organic substances, especially strong reducing agents.

Ozone is a powerful oxidizing agent and oxidation with ozone evolves more heat and usually starts at a lower temperature than oxidation with oxygen. It reacts with non-saturated organic compounds to produce ozonides, which are unstable and may decompose with explosive violence. Ozone is an unstable gas which, at normal temperatures, decomposes to diatomic oxygen. At elevated temperatures and in the presence of certain catalysts such as hydrogen, iron, copper and chromium, this decomposition may be explosive.

Potential Health Effects

Inhalation: Causes dryness of the mouth, coughing, and irritates the nose, throat, and chest. May cause difficulty in breathing, headache, and fatigue. The characteristic sharp, irritating odor is readily detectable at low concentrations (0.01 to 0.05 ppm).

Skin: Absorption through intact skin is not expected.

Eye Contact: Ozone is an irritant to the eyes causing pain, lacrimation, and general inflammation.

Ingestion: Not a route of exposure.

Aggravation of Pre-existing Conditions:
Ozone may increase sensitivity to bronchoconstrictors including allergens.

4. First Aid Measures

Inhalation:
Remove to fresh air; if breathing is difficult a trained person should administer oxygen. If respiration stops, give mouth-to-mouth resuscitation. Get medical attention.

Ingestion:
Not an expected route of exposure.

Skin Contact:
Wash skin thoroughly with soap and water.

Eye Contact:
Immediately flush eyes with large amounts of water for at least 15 minutes, while forcibly holding eyelids apart to ensure flushing of the entire eye surface. If irritation, pain, or other symptoms persist seek medical attention.

Acute:
May cause irritation of skin, eyes, and mucous membranes of the respiratory tract. Drowsiness, dizziness, headache, and fatigue have been associated with exposure.

Chronic:
Long term health effects are not expected from exposures to ozone. A partial tolerance appears to develop with repeated exposures.

5. Fire Fighting Measures

Flash Point:
N/D

Auto ignition Temperature:
N/D

Flammable Limits in air, % by volume - Upper: N/D **Lower:** N/D

Extinguishing Media:
Use extinguishing media suitable for surrounding fires.

Unusual Fire and Explosion Hazard: None expected. Since ozone is highly unstable and decomposes under all conditions and is not encountered except at very small levels in the immediate vicinity where formed.

6. Accidental Release Measures

Evacuate danger area. Open doors and windows to allow area to ventilate.
Consult an expert. Ozone should be contained within a chemically compatible piping system.

Ozone is a powerful oxidizing agent and oxidation with ozone evolves more heat and usually starts at a lower temperature than oxidation with oxygen. It reacts with non-saturated organic compounds to produce ozonides, which are unstable and may decompose with explosive violence. Ozone is an unstable gas which, at normal temperatures, decomposes to diatomic oxygen.

8. Exposure Controls/Personal Protection

Exposure Guidelines:
OSHA PEL: 0.10-ppm PEL/TLV

Ventilation Requirements:
General exhaust recommended. Avoid working with ozone generating equipment in enclosed spaces.

Specific Personal Protective Equipment

Respiratory:
Respirators may be used when engineering and work practice controls are not technically feasible, when such controls are in the process of being installed, or when they fail and need to be supplemented. Respirators may also be used for operations which require entry into tanks or closed vessels, and in emergency situations.

Only appropriate respirators shall be provided and used when the use of respirators is the only means of controlling exposure for routine operations, or during an emergency. (Refer to Table 1 of ANSUI/ASTM E591-77 for appropriate respirator selection).

Positive pressure air line with mask or self-contained breathing apparatus should be available for emergency use.

Eye:
Not necessary

Gloves:
Not necessary.

Other Clothing and Equipment:
Not necessary.

9. Physical and Chemical Properties

Specific Gravity (H_2O=1):
2.144 g/L

Molecular Weight:
48.00

Boiling Point:
-111.9°C

Melting Point:
-192.7°C

Vapor Pressure:
N/A

Evaporation Rate (BuAc=1):
N/A

Vapor Density (Air=1):
1.7

Solubility in H_2O % by Weight:
0.49

Appearance and Odor:
Colorless to bluish gas with a characteristic pungent odor similar to the smell after strong lightning storms.

10. Stability and Reactivity

Stability:
Ozone spontaneously decomposes under all ordinary conditions, so that it is not encountered except in the immediate vicinity of where it was formed. The decomposition is speeded by solid surfaces and by many chemical substances.

Hazardous Decomposition Products:
Free radical oxygen. O^-)

Hazardous Polymerization:
Will not occur.

Incompatibilities:
Ozone is a powerful oxidizing agent and reacts with all oxidizable materials , both organic and inorganic. Some reactions are highly explosive. Alkenes, benzene and other aromatic compounds, rubber, dicyanogen, bromine diethyl ether, dinitrogen tetroxide, nitrogent trichloride, hydrogen bromide, and tetrafluorohydrazine.

11. Toxicological Information

Ozone is extremely irritating to the upper and lower respiratory tract. The characteristic odor is readily detectable at low concentrations (0.02 ppm to 0.05 ppm). Ozone produces local irritation of the eyes and mucous membranes and may cause pulmonary edema at high exposure. Systematically, ozone has been reported to mimic the effects of ionizing radiation, and may cause damage to chromosomal structures. A partial tolerance appears to develop with repeated exposures. Although most effects are acute, the possibility of chronic lung impairment should be considered, based upon animal experimentation.

12. Ecological Information

Environmental Fate:
No information found.

Environmental Toxicity:
No information found.

13. Disposal Considerations

Do not dispose of ozone off gas to atmosphere without properly designed off gas destruct unit. State and local disposal regulations may differ from federal disposal regulations.

14. Transport Information

Proper Shipping Name:
N/A
Hazard Class:
N/A
Identification Number:
N/A
Packing Group:
N/A

15. Regulatory Information

SARA TITLE III:
N/A
TSCA:
The ingredients of this product are on the TSCA Inventory List.
OSHA:
Nonhazardous according to definitions of health hazard and physical hazard provided in the Hazard Communication Standard (29 CFR 1910.1200)

16. Other Information

Label Hazard Warning:
HIGHLY REACTIVE. OZONE GAS AFFECTS THE RESPIRATORY SYSTEM.

Label Precautions:
Keep away from heat, sparks and flame. Avoid contact with eyes, skin and clothing. Avoid breathing. Use with adequate ventilation.

Label First Aid:
If inhaled, remove to fresh air. Get medical attention for any breathing difficulty.

Product Use:
Laboratory Reagent.

Revision Information:
Pure. New 16 section MSDS format, all sections have been revised.

More information:

Working safely with ozone:

http://www.ccohs.ca/oshanswers/chemicals/chem_profiles/ozone/working_ozo.html

NIOSH International Chemical Safety Card for Ozone

http://www.cdc.gov/niosh/ipcsneng/neng0068.html

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APPENDIX B

VISUAL DISCRIMINATION TESTING

IRB APPLICATION, RECRUITMENT LETTER AND BALLOT

APPLICATION FOR EXEMPTION
FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

All research activities involving the use of human beings as research subjects must be reviewed and approved by an Ohio State University Institutional Review Board (IRB), unless the Office of Responsible Research Practices (ORRP) determines that the research falls into one or more of the categories of exemption established by federal regulation.

Exempt research is generally **short term** in nature. It must be performed "as written," i.e. the investigators do not make changes in the research design, the selection of subjects, the informed consent process, or the instrumentation during the course of the study. If changes are necessary, re-application is required.

A determination that research is exempt does not absolve the investigators from ensuring that the **welfare of human subjects** participating in research activities is protected, and that methods used and information provided to gain subject consent are appropriate to the activity. **Investigators may not solicit subject participation or begin data collection until they have received approval from the appropriate Institutional Review Board OR written concurrence that research has been determined to be exempt.**

All OSU Investigators who participate in human subjects research must be appropriately trained in human subjects protection. See **http://orrp.osu.edu/irb/training/citi.cfm** for more details.

There is no deadline or timeline for submitting exempt applications for review. Applications are processed as received. Each application **must** include a research proposal. The proposal must include (at a minimum) the following items: the background literature review, the research question, a description of the research methods including sample size and data collection procedures, and a data analysis plan.

Please allow up to three weeks for processing.

If you have questions regarding the application process or the review of exempt protocols, please contact Office of Responsible Research Practices.
Phone: 688-8457 / Fax: 688-0366 / E-mail: exemptinfo@osu.edu

A COMPLETE APPLICATION PACKET INCLUDES THE FOLLOWING MATERIALS:

☐ **Title page** (attached). Identifies the investigators. Lists the protocol title and the source of funding.

☐ **Screening questions** (attached). Identifies the categories of exemption and solicits responses to screening questions.

☐ **Description of the proposed research** (questions #1 through #9, attached). Includes responses to questions about the objective(s) of the research, the methodology that will be used to gain informed consent from the subjects, and the measures taken to protect the confidentiality of information obtained in research.

☐ **Research proposal** (see question #1).
☐ **Grant proposal.** Must be included when externally-sponsored funding is being sought.
☐ **Letter(s) of support** (see question #4).
☐ **Copies of surveys, instruments, questionnaires, interview questions, focus group topics, and/or data collection sheets** (see question #5).
☐ **Recruitment letter** (see question # 8).
☐ **Consent form** (see question #9).

SEND ONE COPY OF YOUR APPLICATION TO:

Office of Responsible Research Practices
300 Research Foundation Building
1960 Kenny Road
Columbus OH 43210-1063
Fax (614) 688-0366

TITLE PAGE - APPLICATION FOR EXEMPTION

FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

For office use only **PROTOCOL NUMBER:**

►**Principal Investigator** University Title: ☒ Professor ☐ Associate Professor ☐ Assistant Professor ☐ Instructor ☐ Other. Please specify. (May require prior approval.)	Name: **Dr. Ken Lee**	Phone:614.292.7797
	Department or College: Food Science & Technology	E-mail:lee.133@osu.edu
	Campus Address (room, building, street address): 215 Parker Food Science Building 2015 Fyffe Road Columbus, OH 43210-1007	
	Signature: Date:8/19/08	Fax:614.292.0218

►**Co-Investigator** University Status: ☐ Faculty ☒ Staff ☐ Graduate Student ☐ Undergraduate Student ☐ Other. Please specify.	Name: **Melody Leidheiser**	Phone:614.688.4793
	Campus Address (room, building, street address) or Mailing Address: 144A Howlett Hall 2015 Fyffe Rd. Columbus, OH 43210	E-mail: leidheiser.10@osu.edu
	Signature: Date: 8/19/08	Fax:614 688-5459

►Co-Investigator	Name: **Setsuko Kamotani**	Phone: 440.539.0684
University Status: ☐ Faculty ☐ Staff ☒ Graduate Student ☐ Undergraduate Student ☐ Other. Please specify.	Campus Address (room, building, street address) or Mailing Address: 110 Parker Food Science Building 2015 Fyffe Road Columbus, OH 43210	E-mail: kamotani.2@osu.edu
	Signature: Date: 8/19/08	Fax:614.292.0218

►Protocol Title	**Commercialization of a system to sterilize shell eggs-Visual**

►Source of Funding	United States Department of Agriculture, CSREES

For Office Use Only		
☐ **Approved.**	►	Research has been determined to be exempt under these categories: ____________________. Research may begin as of the date of determination listed below.
☐ **Disapproved.**	►	The proposed research does not fall within the categories of exemption. Submit an application to the appropriate Institutional Review Board for review.
Date of determination: ________________		**Signature:**______________________________ ***Office of Responsible Research Practices***

The purpose of the Application for Exemption is two-fold: (a) to determine whether the proposed research qualifies for exemption from review and continuing oversight by an Institutional Review Board; and, if so, (b) to ensure that the informed consent process protects the rights and welfare of human subjects in research. Please respond to the following questions and provide the requested documentation.

Have all investigators completed the required web-based course in the protection of human research subjects? ☒Yes ☐No

If No, see http://orrp.osu.edu/irb/training/citi.cfm for more information. EDUCATIONAL REQUIREMENTS MUST BE SATISFIED PRIOR TO SUBMITTING THE APPLICATION FOR IRB REVIEW.

Please check the categories of exemption for which you are applying. The list of categories is located at the end of this application. You may check more than one box.

EXEMPT CATEGORY: 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☒

<u>SCREENING QUESTIONS:</u> If you check <u>YES</u> to any of the questions below, your research is not exempt. Do not complete the exempt application. Submit an application to the appropriate Institutional Review Board for review.

Does any part of the research require that subjects be deceived?	☐ Yes	☒ No
Will research expose human subjects to discomfort or harassment beyond levels encountered in daily life?	☐ Yes	☒ No
Could disclosure of the subjects' responses outside the research reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, or reputation?	☐ Yes	☒ No
Will fetuses, pregnant women, human *in vitro* fertilization, or individuals involuntarily confined or detained in penal institutions be subjects of the study?	☐ Yes	☒ No
For research proposed under category 2, will research involve surveys, interview procedures, or observation of public behavior with individuals under the age of 18?	☐ Yes	☒ No
For research proposed under category 4, will any of the data, documents, records, pathological specimens, or diagnostic specimens be collected or come into existence after the date you apply for exemption?	☐ Yes	☒ No
For research proposed under category 4, will any of the information obtained from data, documents, records, pathological specimens, or diagnostic specimens that come from private sources be recorded by the investigator in such a manner that subjects can be identified directly or through identifiers linked to the subjects?	☐ Yes	☒ No

---

IF YOU CHECKED <u>YES</u> TO ANY OF THE QUESTIONS ABOVE, YOUR RESEARCH IS NOT EXEMPT.

IF YOU HAVE CHECKED <u>NO</u> TO <u>ALL</u> OF THE QUESTIONS ABOVE, YOUR RESEARCH MAY BE EXEMPT. PLEASE CONTINUE WITH THE EXEMPT APPLICATION.

If you have questions about the application or review process, please contact Janet Schulte, Office of Responsible Research Practices. Phone: 688-8457 / Fax: 688-0366 / E-mail: exemptinfo@osu.edu

For purposes of this application, "research" includes the recruitment of human subjects as well as data collection and analysis. None of these research activities may begin until the investigator has received a protocol number AND has received written concurrence that the

proposed research is exempt. The "date of determination" on page one of this application is assigned by the Office of Responsible Research Practices; it indicates the date when research may begin.

Please describe your study clearly and completely, using a style of language that can easily be understood by someone who is not familiar with your research.

GENERAL QUESTIONS REGARDING THE PROPOSED RESEARCH

1. Describe the purpose of the research activity to be undertaken. Describe how it involves human subjects. Respond in the space provided here, or attach a research proposal and/or grant proposal containing the requested information.

Here is a synopsis from the attached and funded USDA proposal.
We seek to commercialize a system capable of applying a sterilizing treatment to whole shell chicken eggs. The process of sterilization was discovered and patented by researchers at OSU and commercial rights are assigned to Egg Tech, Ltd., Versailles, and EISC, Inc. of Toledo. This sterilization process has the capability of producing shell eggs that are free of the Salmonella enteritidis (SE) bacterium, which causes 700,000 cases of foodborne illness in the United States annually. In 1999, the U.S. Egg Safety Action Plan was signed by President Clinton as an executive order. A portion of this plan calls for a "kill step" within egg processing plants to ensure the elimination of SE. Under this plan, it is required that outbreaks of SE attributable to shell eggs be eliminated by the year 2010.

The goal of this project is for the Egg Tech producers to be in compliance with the 2010 Egg Safety Action Plan deadline by producing, processing, and marketing SE-free eggs. Our role is to conduct taste-panel sensory studies in The OSU Food Science and Technology Department's sensory evaluation lab to ensure the eggs' acceptance among consumers.

Sensory studies are employed to determine consumer acceptance of eggs. This study compares the unique sterilization treatment on fresh grocery store eggs to untreated eggs. There are no pathogens or hazards introduced beyond what consumers are exposed to routinely in the free market. In practice, the eggs we test in this study are much safer than the raw eggs commonly sold in the free market. The treatment involves use of gaseous ozone that leaves no residue within the egg. There are no additives and the use of ozone on food for human consumption is already approved by the Food and Drug Administration.

2. Provide a brief description of the subjects you plan to recruit and the criteria used in the selection process. Indicate whether subjects are 18 years of age or older.

Description: Subjects will be recruited from volunteers that are 18 years old and older. They will be recruited based on availability and willingness to participate. Most subjects are employees of OSU that are contacted via college and department email list servers.

3. **Describe how the proposed research meets the criteria for exemption from IRB review and oversight. (Refer to the criteria on the last page of this application that correspond to the category or categories you checked on the screening sheet.)**

Description: This research meets the criteria for exemption under category 6. Subjects will be asked to look at three samples of cracked raw eggs presented on a plate and compare them. The egg samples will not be consumed.

4. **Will your subjects be recruited through schools, employers, and/or community agencies or organizations, and/or are you required to obtain permission to access data that is not publicly available? If the answer is yes, provide a letter of support from the person authorized to give you access to the subjects or to the data in question. More than one letter may be required.**

☒ **Does not apply.**
☐ **Letter(s) attached.**
☒ **Comments:**
Subjects will be recruited from the faculty, staff, and students proximate to the Parker Food Science and Technology building and surrounding areas.

5. **Describe the means you will use to obtain data. Check all boxes that apply.**

☒ **Surveys or questionnaires distributed by mail or in person. I am attaching a copy of the instrument(s).**
☐ **Surveys distributed through the Internet, through listservs, or through E-mail. I am attaching a copy of the instrument(s). Provide the Internet address:**
☐ **Interviews. I am attaching a copy of the interview questions.**
☐ **Focus groups. I am attaching a copy of the questions that will shape the discussion.**
☐ **Observation of public behavior.**
☐ **Observation of activities in school classrooms.**
☐ **Audiotapes. I will obtain consent from the subjects to tape their responses.**
☐ **Videotapes. I will obtain consent from the subjects to tape their activities or responses.**
☐ **Review of existing records, including databases, medical records, school records, etc. I am attaching a copy of the data collection sheet. I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects. All of the information in the records to be reviewed exists as of the date of submission of this application.**
☐ **Tissue specimens. All of the specimens have already been collected**

and are "on the shelf." I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects.

6. **Indicate the date when you plan to begin research, and the date when you anticipate that data analysis will be complete.**

Begin date: 9.1.08 **End date:** 9.1.09

CONFIDENTIALITY

- **Investigators are required to protect the confidentiality of the information obtained during research, unless the subjects (a) explicitly agree to be identified or quoted, and/or (b) explicitly agree to the release of material captured on audiotapes or videotapes for use in presentations or conferences.**

7. **Provide a brief description of the measures you will take to protect confidentiality. Please describe how you will protect the identity of the subjects, their responses, and any data that you obtain from private records or capture on audiotape or videotape. Describe the disposition of the data and/or the tapes once the study has been completed.**

Description: No identifying information will be collected. Individual subject responses are coded and individual responses are identified by random numbers. Demographic information such as gender and age are grouped with no possible traceback to the subject.

INFORMED CONSENT

- **In most cases, investigators are required to obtain informed consent from their subjects before collecting data. Respond to questions #8 and #9 to indicate how you will inform your subjects about the research and how you will obtain and document their consent.**
- **Subjects must be told what they will be asked to do if they agree to participate in research, how long it will take, and how you will protect the confidentiality of the information they provide.**
- **Subjects must be told that their participation is voluntary, they can refuse to answer questions that they do not wish to answer, and they can refuse to participate or they can withdraw at any time without penalty or repercussion.**
- **With few exceptions, written consent of the child's parent(s) or guardian(s) is required if subjects are under the age of 18. In addition, children 14 years of age or older should be asked to give written assent (agreement) to participate. Children younger than 13 years of age should be asked to give verbal assent (agreement) to participate.**

- **Provide a means for subjects to contact the investigator(s) if they have questions or concerns about the research. Make it clear to the subjects that you are affiliated with The Ohio State University.**

8. **What information do you plan to give to your subjects before you ask for their consent? Use a style of language that simply and clearly explains the research to your subjects. Respond in the space provided here, or attach a copy of the information you plan to provide to your subjects and/or their parents or guardians. (Note: if you use more than one method of recruitment, you may check more than one box)**

☐ **Letter(s) attached. I will give each of the subjects a copy of this letter.**
☐ **I will be contacting subjects by phone or in person. I am attaching a script that contains the information I will give them.**
☐ **Does not apply. My data analysis is limited to existing records or tissue specimens.**
☒ **Response:** Information about the study will be presented on the first screen of the Compusense® data collection software (see attached questionnaire). Attached is the recruitment letter that will either be presented on paper or as an email to a list server used routinely to find potential participants.

9. **How do you plan to document informed consent? Read all of the options before checking the appropriate boxes. (A sample consent form is attached to this application.)**

☐ **The subjects are 18 years of age or older. Before collecting data, I will ask them to sign a written consent form. I am attaching a copy of the consent form.**
☐ **The subjects are 18 years of age or older. Before collecting data, I will ask them to give verbal consent to participate in this research study.**
☐ **The subjects are 18 years of age or older. I am distributing a survey or questionnaire to the subjects. They can choose whether or not they want to respond. I am requesting a waiver of written consent.**
☐ **The subjects are <u>under</u> the age of 18. I am attaching a copy of the consent form that I will use to obtain consent from their parents or guardians and assent (agreement) from subjects who are 14 years of age or older.**
☐ **Some of the subjects are 18 years of age or older, and some are younger than 18. I have checked more than one box above to reflect the methods I will use to document informed consent.**
☐ **Does not apply. My data analysis is limited to existing records or tissue specimens.**
☒ **Other. Please explain and provide justification for your request.** Data will be collected via Compusense® software where subjects will indicate their ratings of the samples. Prior to making these ratings, a screen will describe the study and ask panelists to indicate informed consent by clicking "Yes, I consent to participate."
☐ **Comments:**

Recruitment Letter – for distribution by email and flyer

Department of Food Science and Technology
110 Parker Food Science and Technology Building
2015 Fyffe Road
Columbus, OH 43210

Phone 614-292-6281
FAX 614-292-0218

Judges Needed!

The Sensory Analysis Laboratory

Ohio State University ~ Food Science and Technology

Date:	**September 18-19, 2008**
Time:	**By Appointment: 18th- 10:30 a.m.- 2:30 p.m.** **19th- 11:00 a.m.- 12:30 pm.** **Please choose any 30 minute period and email kamotani.2@osu.edu by September 16th with your request.**
Place:	**Parker Building 122** Sensory Laboratory, 2015 Fyffe Road, Columbus OH building 64.
Perk:	**$2 Dairy Store coupon**

Your help is sought to visually evaluate eggs. Anyone allergic to eggs, anyone who does not eat eggs, and anyone under 18 years of age may not participate. In this study, you will be presented with three samples at the same time on clear plates. You will be asked to compare them but will NOT be asked to taste or consume the eggs. You will judge each sample using easily understood prompts from our computer display. You will answer a few demographic questions such as gender and age, but your identity remains confidential.

Your answers go directly into a computer using a mouse and keyboard. This will likely take 15 minutes but there is no time limit. Your participation is voluntary and you may refuse to answer any question, but missing replies will not tabulate and your session will end. Your responses are not linked to your identity. You must answer all questions in order to qualify for a $2.00 gift certificate to the world renowned OSU Dairy Store (just across the hall from our test in Parker).

If you have questions you may contact Melody Leidheiser.10, Ken Lee at lee.133@osu.edu, 614-292-6281 or Setsuko Kamotani at kamotani.2@osu.edu, 614-247-7135. Everyone working on this project is affiliated with The Ohio State University and this work is supported by a federal grant.

Questionnaire:

Welcome to Sensory Testing!
Ken Lee, Principal Investigator

This study is designed to look at consumer perception of raw un-cooked eggs. In this study, you will be presented with a control plus three samples at the same time on clear plates. (*At no time will you be asked to consume and you may* ***NOT*** *taste the raw eggs you evaluate*). You will be asked to compare them visually. At the end, you will be asked a few demographic questions such as gender and age.

Your answers will be entered directly into the computer using a mouse and keyboard. This has been estimated to take less than 20 minutes but you may take as long as you need. Your responses will in no way be linked to your identity. Upon completion of the entire test, you will be compensated with a Dairy Store gift certificate. If you have any questions, please feel free to ask the attendant at any time.

If you wish to participate, please read the following statement and indicate your consent to participate.

INFORMED CONSENT STATEMENT

I understand the purpose, procedures and time requirements of this study. All questions have been answered to my satisfaction. I may withdraw at any time without penalty or compensation. I am 18 years of age or older. I freely and voluntarily give my consent to participate.

I have read the **INFORMED CONSENT STATEMENT** on the previous screen, and in accordance, I voluntarily give my consent to participate by marking **"YES"** below. (Click "Display Instructions" if you wish to read the statement again.

❍ YES
❍ NO

Evaluate (**LOOKING AT ONLY)** the numbered samples from left to right and answer the following questions marking **ONE** box for each sample. **PLEASE DO NOT EAT ANY OF THESE EGGS!**

Question # 1

LOOK AT all three samples from left to right and click on the box to answer.
Be sure to mark your score on the line scale for **EACH** sample.
Rate the THICK PART OF THE EGG WHITE FOR:

Rate the CLOUDINESS OF THE THICK PART

	Not Cloudy				Very Cloudy
Sample 531					
Sample 181					
Sample 753					

Question # 2

Rate the AMOUNT OF SPREADING OF THE THICK PART

	Little Spreading				A Lot of Spreading
Sample 531					
Sample 181					
Sample 753					

Question # 3

LOOK AT all three samples from left to right and click on the box to answer.
Be sure to mark your score on the line scale for **EACH** sample.
Rate the THE EGG YOLK

Rate the CLOUDINESS OF THE EGG YOLK

	Not Cloudy				Very Cloudy
Sample 531					
Sample 181					
Sample 753					

Question # 4

Rate the HEIGHT OF THE EGG YOLK

Low — High

Sample 531

Sample 181

Sample 753

Question # 5

Rate the COLOR OF THE EGG YOLK

Light — Dark

Sample 531

Sample 181

Sample 753

Question # 6

Rate the YELLOWNESS OF THE EGG YOLK

Less Yellow — More Yellow

Sample 531

Sample 181

Sample 753

Question # 7

Rate the SPREADING OF THE EGG YOLK

Little Spreading — A Lot of Spreading

Sample 531

Sample 181

Sample 753

Question # 8

LOOK AT all three samples from left to right and answer the following question on the scale.
Be sure to mark your score at any point on the line for **EACH** sample.
Rate the THE WHOLE EGG

Rate the VISUAL APPEAL THE WHOLE EGG

	Not Appealing	Very Appealing
Sample 531	|----|----|----|----|----|	
Sample 181	|----|----|----|----|----|	
Sample 753	|----|----|----|----|----|	

Question # 9.

Please feel free to comment on the eggs
you have just evaluated
by pulling out your keyboard
and typing in your comments.

Thank you!

Question # 10.

Please indicate **HOW OFTEN YOU EAT EGGS** by clicking on **ONE BOX** below

- ❍ At least once a week
- ❍ Once every two weeks
- ❍ Once a month
- ❍ Once every 6 months
- ❍ Rarely (less than once every 6 months)
- ❍ Never

Question # 11.

Please indicate your **GENDER** by clicking on **ONE BOX** below

- ❍ FEMALE
- ❍ MALE

Question # 12.

Please indicate your **YOUR AGE CATEGORY** by clicking on **ONE BOX** below

- ❍ 18 - 20 years
- ❍ 21 - 25 years
- ❍ 26 - 35 years
- ❍ 36 - 45 years
- ❍ 46 - 55 years
- ❍ 56 - 65 years
- ❍ Over 65 years

Question # 13.

Please indicate your **ETHNICITY** by clicking on **ONE BOX** below

- ❍ American Indian
- ❍ Asian, or Pacific Islander
- ❍ Black, not of Hispanic origin
- ❍ Hispanic
- ❍ White, not of Hispanic origin
- ❍ Other
- ❍ Prefer not to disclose

Question # 14.

Please indicate if **YOU HAVE ANY PREVIOUS KNOWLEDGE OF EGG PROCESSING AT OSU** by clicking on **ONE BOX** below

- ❍ YES
- ❍ NO

APPENDIX C

CONSUMER AFFECTIVE TESTING

IRB APPLICATION, RECRUITMENT LETTER AND BALLOT

APPLICATION FOR EXEMPTION

FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

All research activities involving the use of human beings as research subjects must be reviewed and approved by an Ohio State University Institutional Review Board (IRB), unless the Office of Responsible Research Practices (ORRP) determines that the research falls into one or more of the categories of exemption established by federal regulation.

Exempt research is generally **short term** in nature. It must be performed "as written," i.e. the investigators do not make changes in the research design, the selection of subjects, the informed consent process, or the instrumentation during the course of the study. If changes are necessary, re-application is required.

A determination that research is exempt does not absolve the investigators from ensuring that the **welfare of human subjects** participating in research activities is protected, and that methods used and information provided to gain subject consent are appropriate to the activity. **Investigators may not solicit subject participation or begin data collection until they have received approval from the appropriate Institutional Review Board OR written concurrence that research has been determined to be exempt.**

All OSU Investigators who participate in human subjects research must be appropriately trained in human subjects protection. See **http://orrp.osu.edu/irb/training/citi.cfm** for more details.

There is no deadline or timeline for submitting exempt applications for review. Applications are processed as received. Each application **must** include a research proposal. The proposal must include (at a minimum) the following items: the background literature review, the research question, a description of the research methods including sample size and data collection procedures, and a data analysis plan.

Please allow up to three weeks for processing.

If you have questions regarding the application process or the review of exempt protocols, please contact Office of Responsible Research Practices.
Phone: 688-8457 / Fax: 688-0366 / E-mail: exemptinfo@osu.edu

A COMPLETE APPLICATION PACKET INCLUDES THE FOLLOWING MATERIALS:

☐ **Title page** (attached). Identifies the investigators. Lists the protocol title and the source of funding.

☐ **Screening questions** (attached). Identifies the categories of exemption and solicits responses to screening questions.

☐ **Description of the proposed research** (questions #1 through #9, attached). Includes responses to questions about the objective(s) of the research, the methodology that will be used to gain informed consent from the subjects, and the measures taken to protect the confidentiality of information obtained in research.

☐ **Research proposal** (see question #1).
☐ **Grant proposal.** Must be included when externally-sponsored funding is being sought.
☐ **Letter(s) of support** (see question #4).
☐ **Copies of surveys, instruments, questionnaires, interview questions, focus group topics, and/or data collection sheets** (see question #5).
☐ **Recruitment letter** (see question # 8).
☐ **Consent form** (see question #9).

SEND ONE COPY OF YOUR APPLICATION TO:

Office of Responsible Research Practices
300 Research Foundation Building
1960 Kenny Road
Columbus OH 43210-1063
Fax (614) 688-0366

TITLE PAGE - APPLICATION FOR EXEMPTION
FROM REVIEW BY THE INSTITUTIONAL REVIEW BOARD
The Ohio State University, Columbus OH 43210

For office use only
PROTOCOL NUMBER:

►**Principal Investigator**	Name: **Dr. Ken Lee**	Phone:614.292.7797
University Title: ☒ Professor ☐ Associate Professor ☐ Assistant Professor ☐ Instructor ☐ Other. Please specify. (May require prior approval.)	Department or College: Food Science & Technology Campus Address (room, building, street address): 215 Parker Food Science Building 2015 Fyffe Road Columbus, OH 43210-1007	E-mail:lee.133@osu.edu
	Signature: Date: 8/7/08	Fax:614.292.0218

►**Co-Investigator**	Name: **Melody Leidheiser**	Phone:614.688.4793
University Status: ☐ Faculty ☒ Staff ☐ Graduate Student ☐ Undergraduate Student ☐ Other. Please specify.	Campus Address (room, building, street address) or Mailing Address: 144A Howlett Hall 2015 Fyffe Rd. Columbus, OH 43210	E-mail: leidheiser.10@osu.edu
	Signature: Date: 8/7/08	Fax: 614 688-5459

►**Co-Investigator**	Name: **Setsuko Kamotani**	Phone:440.539.0684
University Status: ☐ Faculty ☐ Staff ☒ Graduate Student ☐ Undergraduate Student ☐ Other. Please specify.	Campus Address (room, building, street address) or Mailing Address: 110 Parker Food Science Building 2015 Fyffe Road Columbus, OH 43210	E-mail: kamotani.2@osu.edu
	Signature: Date: 8/7/08	Fax:614.292.0218

►**Protocol Title**	**Commercialization of a system to sterilize shell eggs- Scrambled eggs**

►**Source of Funding**	United States Department of Agriculture, CSREES

For Office Use Only		
☐ **Approved.**	►	Research has been determined to be exempt under these categories: ____________________. Research may begin as of the date of determination listed below.
☐ **Disapproved.**	►	The proposed research does not fall within the categories of exemption. Submit an application to the appropriate Institutional Review Board for review.
Date of determination: ________________		**Signature:**________________________________ *Office of Responsible Research Practices*

The purpose of the Application for Exemption is two-fold: (a) to determine whether the proposed research qualifies for exemption from review and continuing oversight by an Institutional Review Board; and, if so, (b) to ensure that the informed consent process protects the rights and welfare of human subjects in research. Please respond to the following questions and provide the requested documentation.

Have all investigators completed the required web-based course in the protection of human research subjects? ☒Yes ☐No

If No, see http://orrp.osu.edu/irb/training/citi.cfm for more information. EDUCATIONAL REQUIREMENTS MUST BE SATISFIED PRIOR TO SUBMITTING THE APPLICATION FOR IRB REVIEW.

Please check the categories of exemption for which you are applying. The list of categories is located at the end of this application. You may check more than one box.

EXEMPT CATEGORY: 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐ 6 ☒

<u>SCREENING QUESTIONS:</u> If you check <u>YES</u> to any of the questions below, your research is not exempt. Do not complete the exempt application. Submit an application to the appropriate Institutional Review Board for review.

Does any part of the research require that subjects be deceived?	☐ Yes	☒ No
Will research expose human subjects to discomfort or harassment beyond levels encountered in daily life?	☐ Yes	☒ No
Could disclosure of the subjects' responses outside the research reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects' financial standing, employability, or reputation?	☐ Yes	☒ No
Will fetuses, pregnant women, human *in vitro* fertilization, or individuals involuntarily confined or detained in penal institutions be subjects of the study?	☐ Yes	☒ No
For research proposed under category 2, will research involve surveys, interview procedures, or observation of public behavior with individuals under the age of 18?	☐ Yes	☒ No
For research proposed under category 4, will any of the data, documents, records, pathological specimens, or diagnostic specimens be collected or come into existence after the date you apply for exemption?	☐ Yes	☒ No
For research proposed under category 4, will any of the information obtained from data, documents, records, pathological specimens, or diagnostic specimens that come from private sources be recorded by the investigator in such a manner that subjects can be identified directly or through identifiers linked to the subjects?	☐ Yes	☒ No

---

IF YOU CHECKED <u>YES</u> TO ANY OF THE QUESTIONS ABOVE, YOUR RESEARCH IS NOT EXEMPT.

IF YOU HAVE CHECKED <u>NO</u> TO <u>ALL</u> OF THE QUESTIONS ABOVE, YOUR RESEARCH MAY BE EXEMPT. PLEASE CONTINUE WITH THE EXEMPT APPLICATION.

If you have questions about the application or review process, please contact Janet Schulte, Office of Responsible Research Practices. Phone: 688-8457 / Fax: 688-0366 / E-mail: exemptinfo@osu.edu

For purposes of this application, "research" includes the recruitment of human subjects as well as data collection and analysis. None of these research activities may begin until the investigator has received a protocol number AND has received written concurrence that the

proposed research is exempt. The "date of determination" on page one of this application is assigned by the Office of Responsible Research Practices; it indicates the date when research may begin.

Please describe your study clearly and completely, using a style of language that can easily be understood by someone who is not familiar with your research.

GENERAL QUESTIONS REGARDING THE PROPOSED RESEARCH

1. Describe the purpose of the research activity to be undertaken. Describe how it involves human subjects. Respond in the space provided here, or attach a research proposal and/or grant proposal containing the requested information.

Here is a synopsis from the attached and funded USDA proposal.
We seek to commercialize a system capable of applying a sterilizing treatment to whole shell chicken eggs. The process of sterilization was discovered and patented by researchers at OSU and commercial rights are assigned to Egg Tech, Ltd., Versailles, and EISC, Inc. of Toledo. This sterilization process has the capability of producing shell eggs that are free of the *Salmonella* enteritidis (SE) bacterium, which causes 700,000 cases of foodborne illness in the United States annually. In 1999, the U.S. Egg Safety Action Plan was signed by President Clinton as an executive order. A portion of this plan calls for a "kill step" within egg processing plants to ensure the elimination of SE. Under this plan, it is required that outbreaks of SE attributable to shell eggs be eliminated by the year 2010.

The goal of this project is for the Egg Tech producers to be in compliance with the 2010 Egg Safety Action Plan deadline by producing, processing, and marketing SE-free eggs. Our role is to conduct taste-panel sensory studies in The OSU Food Science and Technology Department's sensory evaluation lab to ensure the eggs' acceptance among consumers.

Sensory studies are employed to determine consumer acceptance of eggs. This study compares the unique sterilization treatment on fresh grocery store eggs to untreated eggs. There are no pathogens or hazards introduced beyond what consumers are exposed to routinely in the free market. In practice, the eggs we test in this study are much safer than the raw eggs commonly sold in the free market. The treatment involves use of gaseous ozone that leaves no residue within the egg. There are no additives and the use of ozone on food for human consumption is already approved by the Food and Drug Administration.

2. Provide a brief description of the subjects you plan to recruit and the criteria used in the selection process. Indicate whether subjects are 18 years of age or older.

Description: Subjects will be recruited from volunteers who consume regularly consume eggs and who are 18 years old and older. They will be recruited based on availability and willingness to participate. Most subjects are employees of OSU that are contacted via college and department email list servers.

3. **Describe how the proposed research meets the criteria for exemption from IRB review and oversight. (Refer to the criteria on the last page of this application that correspond to the category or categories you checked on the screening sheet.)**

Description: This research meets the criteria for exemption under category 6. Subjects will be asked to taste three samples of scrambled eggs and be asked to respond to questions about liking and attributes of the food. Two of the eggs will be pasteurized according to the USDA guidelines for pasteurization of eggs to eliminate pathogenic bacteria by either thermal or ozone-based methods and one will serve as an untreated control. Unpasteurized eggs are what are now sold in the grocery store and it is what consumers normally eat. By serving these ordinary eggs in a controlled setting, there is no increased risk to subjects. However, we are cooking all the eggs (scrambled) so all remaining food safety risk is eliminated by the cooking heat.
The government will recommend consuming pasteurized eggs in the FUTURE under the US Egg Safety Action Plan. This research anticipates the government's future recommendation by developing a way to pasteurize eggs. Current estimates of *salmonella* contamination of eggs varies from one in 5,000 to one in 20,000. This means if you habitually eat raw eggs or foods made from raw eggs, you could get sick (salmonellosis).
In our study there is NO raw egg consumption so there is no risk. All eggs tasted will be cooked thoroughly under clean conditions, minimizing food safety risk. Subjects with egg allergies or sensitivities will be warned to not participate.

4. **Will your subjects be recruited through schools, employers, and/or community agencies or organizations, and/or are you required to obtain permission to access data that is not publicly available? If the answer is yes, provide a letter of support from the person authorized to give you access to the subjects or to the data in question. More than one letter may be required.**

☒ **Does not apply.**
☐ **Letter(s) attached.**
☒ **Comments:**
Subjects will be recruited from the faculty, staff, and students proximate to the Parker Food Science and Technology building and surrounding areas.

5. **Describe the means you will use to obtain data. Check all boxes that apply.**

☒ **Surveys or questionnaires distributed by mail or in person. I am attaching a copy of the instrument(s).**
☐ **Surveys distributed through the Internet, through listservs, or through E-mail. I am attaching a copy of the instrument(s). Provide the Internet address:**
☐ **Interviews. I am attaching a copy of the interview questions.**
☐ **Focus groups. I am attaching a copy of the questions that will shape**

the discussion.
☐ **Observation of public behavior.**
☐ **Observation of activities in school classrooms.**
☐ **Audiotapes. I will obtain consent from the subjects to tape their responses.**
☐ **Videotapes. I will obtain consent from the subjects to tape their activities or responses.**
☐ **Review of existing records, including databases, medical records, school records, etc. I am attaching a copy of the data collection sheet. I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects. All of the information in the records to be reviewed exists as of the date of submission of this application.**
☐ **Tissue specimens. All of the specimens have already been collected and are "on the shelf." I am recording information in such a manner that subjects cannot be identified directly or through identifiers linked to the subjects.**

6. **Indicate the date when you plan to begin research, and the date when you anticipate that data analysis will be complete.**

Begin date: 8.1.08 **End date:** 8.1.09

CONFIDENTIALITY

- **Investigators are required to protect the confidentiality of the information obtained during research, unless the subjects (a) explicitly agree to be identified or quoted, and/or (b) explicitly agree to the release of material captured on audiotapes or videotapes for use in presentations or conferences.**

7. **Provide a brief description of the measures you will take to protect confidentiality. Please describe how you will protect the identity of the subjects, their responses, and any data that you obtain from private records or capture on audiotape or videotape. Describe the disposition of the data and/or the tapes once the study has been completed.**

Description: No identifying information will be collected. Individual subject responses are coded and individual responses are identified by random numbers. Demographic information such as gender and age are grouped with no possible traceback to the subject.

INFORMED CONSENT

- **In most cases, investigators are required to obtain informed consent from their subjects before collecting data. Respond to questions #8 and #9 to indicate how you will inform your subjects about the research and**

how you will obtain and document their consent.

- **Subjects must be told what they will be asked to do if they agree to participate in research, how long it will take, and how you will protect the confidentiality of the information they provide.**
- **Subjects must be told that their participation is voluntary, they can refuse to answer questions that they do not wish to answer, and they can refuse to participate or they can withdraw at any time without penalty or repercussion.**
- **With few exceptions, written consent of the child's parent(s) or guardian(s) is required if subjects are under the age of 18. In addition, children 14 years of age or older should be asked to give written assent (agreement) to participate. Children younger than 13 years of age should be asked to give verbal assent (agreement) to participate.**
- **Provide a means for subjects to contact the investigator(s) if they have questions or concerns about the research. Make it clear to the subjects that you are affiliated with The Ohio State University.**

8. What information do you plan to give to your subjects before you ask for their consent? Use a style of language that simply and clearly explains the research to your subjects. Respond in the space provided here, or attach a copy of the information you plan to provide to your subjects and/or their parents or guardians. (Note: if you use more than one method of recruitment, you may check more than one box)

☐ **Letter(s) attached. I will give each of the subjects a copy of this letter.**
☐ **I will be contacting subjects by phone or in person. I am attaching a script that contains the information I will give them.**
☐ **Does not apply. My data analysis is limited to existing records or tissue specimens.**
☒ **Response:** Information about the study will be presented on the first screen of the Compusense® data collection software (see attached questionnaire). Attached is the recruitment letter that will either be presented on paper or as an email to a list server used routinely to find potential participants.

9. How do you plan to document informed consent? Read all of the options before checking the appropriate boxes. (A sample consent form is attached to this application.)

☐ **The subjects are 18 years of age or older. Before collecting data, I will ask them to sign a written consent form. I am attaching a copy of the consent form.**
☐ **The subjects are 18 years of age or older. Before collecting data, I will ask them to give verbal consent to participate in this research study.**
☐ **The subjects are 18 years of age or older. I am distributing a survey or questionnaire to the subjects. They can choose whether or not they want to respond. I am requesting a waiver of written consent.**
☐ **The subjects are <u>under</u> the age of 18. I am attaching a copy of the**

consent form that I will use to obtain consent from their parents or guardians and assent (agreement) from subjects who are 14 years of age or older.

☐ **Some of the subjects are 18 years of age or older, and some are younger than 18. I have checked more than one box above to reflect the methods I will use to document informed consent.**

☐ **Does not apply. My data analysis is limited to existing records or tissue specimens.**

☒ **Other. Please explain and provide justification for your request.** Data will be collected via Compusense® software where subjects will indicate their ratings of the samples. Prior to making these ratings, a screen will describe the study and ask panelists to indicate informed consent by clicking "Yes, I consent to participate."

☐ **Comments:**

Recruitment Letter – for distribution by email and flyer

THE OHIO STATE UNIVERSITY

Department of Food Science and Technology
110 Parker Food Science and Technology Building
2015 Fyffe Road
Columbus, OH 43210

Phone 614-292-6281
FAX 614-292-0218

PARTICIPANTS NEEDED

Ken Lee, Principal Investigator

Date: TBA
Time: TBA
Place: Parker 122 (Sensory Booths), 2015 Fyffe Road building 64.

Your help is sought to evaluate scrambled eggs. All eggs are from local groceries and thoroughly cooked under clean conditions so the food is wholesome and there is minimal food safety risk. Anyone allergic to eggs, anyone who does not eat eggs or anyone under the age of 18 may not participate.

In this study, you would get three samples of cooked eggs each on a small plate, one at a time. You will judge each sample for appearance, aroma, texture and flavor using easily understood prompts from our computer display. You will answer a few demographic questions such as gender and age, but your identity remains confidential.

Your answers go directly into a computer using a mouse and keyboard. This will likely take 20 minutes but there is no time limit. Your participation is voluntary and you may refuse to answer any question, but missing replies will not tabulate and your session will end. Your responses are not linked to your identity. You must answer all questions in order to qualify for a $5.00 gift certificate to the world renowned OSU Dairy Store (just across the hall from our test in Parker).

If you have questions you may contact Ken Lee at lee.133@osu.edu, 614-292-6281 or Setsuko Kamotani at kamotani.2@osu.edu, 614-247-7135. Everyone working on this project is affiliated with The Ohio State University and this work is supported by a federal grant.

Questionnaire:

Welcome to Sensory Testing!
Ken Lee, Principal Investigator

This study is designed to look at consumer perception of cooked eggs. In this study, you will be presented with three samples each on a small tray and you will be asked to assess them. At the end, you will be asked a few demographic questions such as gender and age.

Your answers will be entered directly into the computer using the mouse and keyboard. This has been estimated to take less than 20 minutes but you may take as long as you need. Your responses will in no way be linked to your identity and you will be compensated with a Dairy Store gift certificate at the end. If you have any questions, please feel free to ask the attendant at any time.

If you wish to participate, please read the following statement and indicate your consent to participate.

INFORMED CONSENT STATEMENT

I understand the purpose, procedures and time requirements of this study. This study compares common grocery store eggs to pasteurized eggs. All eggs are thoroughly cooked so there is minimal food safety risk. All questions have been answered to my satisfaction. I may withdraw at any time without penalty or compensation. I am 18 years of age or older and regularly consume eggs. I give my consent to participate.

I have read the **INFORMED CONSENT STATEMENT** on the previous screen, and in accordance, I voluntarily give my consent to participate by marking **"YES"** below. (click "Display Instructions" if you wish to read the statement again.

❍ YES
❍ NO

Please rinse your mouth with water.
Now you will **LOOK AT** the first sample **263** and answer the following questions

Question # 1 – Sample 263
Please rate your **OVERALL LIKING OF THE EGG APPEARANCE** of this sample on the following scale.

Dislike Extremely	Dislike very Much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Question # 2 – Sample 263
Please rate your **OVERALL LIKING OF THE EGG AROMA** of this sample on the following scale.

Dislike Extremely	Dislike very Much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Question # 3 – Sample 263

Now, **TASTE** this sample of scrambled eggs and answer the following questions.

Please rate your **OVERALL LIKING OF THE EGG FLAVOR** of this sample on the following scale.

Dislike Extremely	Dislike very Much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Question # 4 – Sample 263
Please rate your **OVERALL LIKING OF THE EGG TEXTURE** of this sample on the following scale.

Dislike Extremely	Dislike very Much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Question # 5 – Sample 263

Continue **TASTING** this sample of scrambled eggs and answer the following questions.

Please click on **EACH** box to answer **EACH** question.

Rate the **EGG COLOR**

Way too Dark	Slightly too Dark	Just About Right	Slightly too Light	Way too Light
1	2	3	4	5

Rate the **EGG MOISTNESS**

Way too Dry	Slightly too Dry	Just About Right	Slightly too Watery	Way too Watery
1	2	3	4	5

Rate the **EGG TEXTURE**

Way too Tough	Slightly too Tough	Just About Right	Slightly too Tender	Way too Tender
1	2	3	4	5

Question # 6 - Sample ______

Continue **TASTING** this sample of scrambled eggs and answer the following question.

Please check only **ONE BOX.**

Please rate your **OVERALL LIKING OF THIS EGG SAMPLE**

Dislike Extremely	Dislike very Much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely
1	2	3	4	5	6	7	8	9

Please feel free to comment on the eggs you have

JUST TASTED

by pulling out your keyboard and typing in your comments.

Thank you!

____

____

____

<present a new tray containing the SECOND sample of cooked egg for evaluation. Repeat the questions above>

Please rinse your mouth with water.
Now you will **LOOK AT** sample **854** and answer the following questions.

<present a new tray containing the THIRD sample of cooked egg for evaluation. Repeat the questions above>

Please rinse your mouth with water.
Now you will **LOOK AT** sample **506** and answer the following questions.

Please **ANSWER** the following demographic questions.

Question # 7.

Please indicate **HOW OFTEN YOU EAT ANY TYPE OF EGGS** by clicking on **ONE BOX** below

- ❍ Once a day
- ❍ Once a week
- ❍ Once every two weeks
- ❍ Once a month
- ❍ Once every 6 months
- ❍ Rarely (less than once every 6 months)
- ❍ Never

Question # 8.

Please indicate your **GENDER** by clicking on **ONE BOX** below

- ❍ FEMALE
- ❍ MALE

Question # 9.

Please indicate your **YOUR AGE CATEGORY** by clicking on **ONE BOX** below

- ❍ 18 - 20 years
- ❍ 21 - 25 years
- ❍ 26 - 35 years
- ❍ 36 - 45 years
- ❍ 46 - 55 years
- ❍ 56 - 65 years
- ❍ Over 65 years

Question # 10.

Please indicate your **ETHNICITY** by clicking on **ONE BOX** below

- ❍ American Indian
- ❍ Asian, or Pacific Islander
- ❍ Black, not of Hispanic origin
- ❍ Hispanic
- ❍ White, not of Hispanic origin
- ❍ Other
- ❍ Prefer not to disclose

Question # 11.

Please indicate if **YOU HAVE ANY PREVIOUS KNOWLEDGE OF EGG PROCESSING AT OSU** by clicking on **ONE BOX** below

- ❍ YES
- ❍ NO

APPENDIX D

PANELISTS' COMMENTS FROM VISUAL DISCRIMINATION TEST

Table D.1: Panelists' comments on visual attributes of control eggs.

531- Control		
Positive	Negative	Other
SAMLE 531 IS THE MOST APPEALING	531's fetus part doesn't look good	sample 531 got a large bubble on it during the test
Egg 531 looks the best	the white stuff in the whites of 531 and 753 makes me think the eggs are not fresh.	sample 531 & 753 look about the same to me
531 Looks like an eggs in the commericals while the other look like the eggs I find in the store - runny and more cloudy.	531 - not good, what happened to the white? major loss of viscosity	531 seems to be the most different of the three
look much better and much more appetizing.	531 looks old with the egg white so thin	
i preferd yellowish eggs so number 531 looks better for me also i think it has a good amount of yolk, in the other hand i dont like at all the white pat so i woud like to have some egg without that part	531 is typical of a very old egg - no separation between thick or thin albumen, very thin and clear albumen	
the 531 egg yolk is vey appealing, but there's so little thick part.	#531 is especially thin	
	The cloudiness of 531 is unappealing,	
	i preferd yellowish eggs so number 531 looks better for me also i think it has a good amount of yolk, in the other hand i dont like at all the white pat so i woud like to have some egg without that part	
	753 & 531 look older, aged, held warm	
	the 531 egg yolk is vey appealing, but there's so little thick part.	
	THE '531 ' EGGS SEEMS TO HAVE LARGER SIZE COMPARE TO THE OTHER TWO. HOWEVER, IT LOOKS LESS APPLEALING.	

Table D.2: Panelists' comments on visual attributes of ozone-treated eggs.

753-Ozone		
Positive	Negative	Other
They all looked pretty good if i had to choose which one to eat, i'd choose 753	SAMPLE 753 LOOKS OLD AND WATERY	sample 531 & 753 look about the same to me
Overall specs of 753 more appealing and easy on the eye.	the white stuff in the whites of 531 and 753 makes me think the eggs are not fresh.	
753 - looks like a standard fresh egg, no problems	753 & 531 look older, aged, held warm	
753 looks the best and most delicous		
Sample 753 looks more fresh than sample 531, and then 181, may be more attractive		
The egg 753 is the best.		
look much better and much more appetizing.		
Egg 753 was the most appealing because it appeared to be fresher.		
THE 753 HAS THE MOST PROMISING APPEARENCE		

Table D.3: Panelists' comments on visual attributes of thermal treated eggs.

181- Thermal		
Positive	Negative	Other
sample 181 appears to more appealing as compared to the other two as it has the thickest consistancey while the other two samples appear to spread out	SAMPLE 181 LOOKS SIMILAR TO 531 BUT WITH A MORE CLOUDY WHITE PORTION	sample 181 appears to be different from the other two,
181 looks very fresh	181 doesn't seem natural	
Overall #181 is the most appealing because of the absence of white.	181 - there appears to be a little separation within the yolk (sedimentation), lots of white on the yolk membrane, large yolk	
#181 was yellow enough and the yold intact or compact enough to make it more appealing.	181 is less appealing bc the egg white is a little cloudier.	
I believe egg 181 is the best overall	181 yolk is freakishly large	
181 is most appealing	#181 seems to have a very large yolk	
	the yolk on 181 does not looktoo good, i do not like the whiteness of it	

Table D.4: Panelists' general comments on visual attributes of control, ozone-treated, and thermal treated eggs.

General
However, the other look fine too. If I did not have 531 as omparison, i would not think ay egg looks out of the ordinary
not much difference for me
I would eat any of these eggs (after being cooked, of course :)
They are pretty similar
I don't usually look at eggs so intently, but if you look long enough the stringy white albumin is very unappetizing.
You don't really make note of the heighth of the yolk or how spread out it is during a cursory look
they all look very similar in appearance
I dont think the cloudiness of the egg its too crucial for the appeal of the egg. I personally think that the egg it's not appealing when there are little pieces of ... ? floating arond the egg white. That reminds me,that those are rests of an unborn chicken.
All 3 egg samples looked normal and I would eat them all.
If I were to crack open any of these eggs at home, I probably would not notice a difference.
Given the prior questions I would say the thinckness of the albumin the the major difference between the eggs
The yellowness of all samples is enough
otherwise all 3 eggs are very acceptable from a consumer standpoint
DOES THE LIGHT AND THE EGG POSITION HAVE EFFECT ON HOW THE EGG APPEARENCE?
the color of the light may have slight influence on the judge of the egg.
To some extent the looks of each sample is affected by the light/glare and the amount of light that hits the sample. I tried not to let that influence my ratings, but that's hard to do.
THE EGGS LOOK DIFFERENLY FROM ONE ANOTHER
The egg don't look difference a lot one of another. The lighting probably affect the color of the eggs.
All the eggs look normal on th visual appearance and the only diference just in th egg whites.
Seems tasty enough
the embryo is discusting to me
Overall the eggs look very appealing. The biggest difference that I see among them is the runniness/spreading of the yolk - one egg obviously has less spreading. All of the yolks look great.
nice eggs!
each egg is ery different

APPENDIX E

PANELISTS' COMMENTS FROM CONSUMER AFFECTIVE TEST

Table E.1: Panelists' comments on color of control, ozone-treated, and thermal treated scrambled eggs.

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
THE COLOR WAS PERFECT	Color and visual texture made it look drier than it was	good color	THE COLOR WAS TOO LIGHT	good color	pale
good color	pale.	Color is ok	color looks like scrambled eggs w/milk added.	Color and aroma were nice	The egg is too shiny
the slightly lighter color actually gave the illusion of being more natural.	but the color is slightly too light.	Some of the egg was darker than the rest nd some was what I consider the right color of scrambled eggs.	they may have been a little lighter than the previous eggs, also.	nice color	too yellow
this egg has an appealing color, both on the 'outside' of each clump, as well as when i cut it open. uniform throughout.	The egg appears too yellow and shiny.	liked the color though.	the color of this egg looks a liitle bit dark.	Sample 148 has a great color	the appearance is not so good, too yellow and agglomerated
nice color to it	too yellow color	The yellowness is good,		NICE COLOR BUT NOT SURE IF IT'S ARTIFICIAL	more color but a litte bit too much
even though they had the right texture and color.	too yellow	more acceptable yellow color,			color and taste to be slightly manufactured
		very desirable color			

Continued

Table E.1 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
		but it had a slightly lighter color that was good,			
		color in my sample very uniform. certainly not a bad thing ; merey an observation			

Table E.2: Panelists' comments on taste of control, ozone-treated, and thermal treated scrambled eggs.

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
The taste is the best thing about this egg.	NEEDS MORE FLAVOR	BUT TASTE WAS GOOD	TASTE WAS SLIGHTLY BLAND. NEEDS SALT	BEST TASTE SO FAR. MAPLE? EXCELLENT TASTE OVERALL	has a weird aftertaste that i can't really describe
The taste, despite lacking in other ways has a a better resolution of flavor	slightly bland flavor	good flavor,	lOOKED GOOD BUT TASTED ONLY OK	Overall flavor was good	This sample seemed too bland.
overall good flavor	Kinda bland, not a lot of flavour	TASTE IS NORMAL	Have a strong egg flavor	Taste was good	TASTELESS
OVERALL THE SAMPLE TASTED PRETTY WELL. IT COULD HAVE A LITTLE MORE FLAVOR OR BE A LITTLE WARMER	BUT NOT ALOT OF FLAVOR	overall flavor not bad	NO FLAVOR	It's not the same as the ones I make but it was very tasty.	It has a funny flavor... like burned protein or something like that... the flavor affected my likeness of the product
It was a good tasting egg. Tastes like an egg you would get at a Denny's Buffet.	more pepper and salt	flavor wasn't terrible	Seemed to have an even stronger metallic off flavor than previous sample (362?)	this sample tasted a lttle dryer than the first but stll tasted pretty good	almost plastic-like flavor is not pleasing

Continued

Table E.2 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
Intermediate egg flavor and odor between the previous two samples	poor flavor as well	This one tastes like scrambled eggs that you can get at a Petro Truck Stop. It's not bad but I have had better.	sample seems to have a lightly unfavorable after-taste.	tastes like normal scrambled eggs	dIDN'T HAVE MUH TASTE
This egg tastes well, but like previous two samples still seems bland to me. I think my problem is that I am very used to seasoning my scrmbled eggs with pepper,salt, herbs, etc.	At the end has a funny after taste... I just felt it like 15 seconds after I swalowed the egg	Very tasty. I would like to have some salt, though.	It is just too plain.	It tastes ok	Rather bland taste
good overall flavor. not too sulfury and a slight buttery flavor.	less egg flavor tasted more like 'fake eggs'	It has good flavor and a nice after taste.	has a dry mouthfeel.	taste and flavor is good	Tasted more bland than other samples.
taste good and common food.	It tasted a little salty or metallic?	This sample had a very good flavor	flavor was not very strong	TASE WAS GOD AS WELL.	These eggs tasted as if they had an extra (unpleasant) flavor added to them

Continued

Table E.2 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
There was no significant flavor to the egg, neither good nor bad	TOO LESS TASTE	had a great egg flavor	if some salt added, it will taste better.	flavor was about that of a natural scrambled egg	however it was bland tasting
and semed to have a better flavor.	they didn't have as much flavor as previous samples.	a little more flavor than 148, so id say it was better than 148 about the same as the other sample in regards to taste.	not much flavor	can hardly find any difference on flavor compared to the first sample.	flavorless, too plain(bland)
REALLY ENJOYED THE TASTE,	Not a great deal of flvor	for some reason these tasted better than the ones before. but they seemed to have more flavor.	had a little bit of an aftertaste		There is a slight bitter taste
tasted ok,	lacking taste	better taste and texture	Slight after taste that was different than the other eggs.		this sample also seems somewhat bland to me
Thse eggs were also pretty good with slightly improved taste.	This sample seemed little too dry and not as tasty as the first one.	better taste and texture	bland		These eggs were a little too bland for my taste.
I really liked the tasteof these eggs.	Flavor was obstructed by the texture.	they are ok, homemade taste	but the taste was not as good.		They seemed to lack taste

Continued

Table E.2 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
Mild flavor is ok - not exceptional or unappealing.	tastes somewhat unnatural, but when the taste combined with texture is somewhat dissapointing. it looks better then it tastes.	They seemed to have more flavor	it is also fairly tasteless. while i'm more pre-occuped by the poor texture, it takes me a moment to realize all i'm sensing is the texture and there is no taste. very bland. while the appearance looks nice, the rest of the taste and texture is dissapointing		Rather bland
	it has a strange flavor.	but it tastes slightly better	IT IS TOO PLAIN, HAS NO SALT		the smell and flavor is not so good as the other 2 may due to too much moisture
	The flavor of this sample is not as noticable. Tastes neutral, not as eggy.	the taste and texture are the best. there is not much that is objectional.	They deffinitely had very little taste		the flavor is blank. should add some salt or other sauce
	but it did have a strong flavor, although it was not entirely egg.	the flavor and texture were good	but it could have had a stronger egg flavor.		Eggs without any flavor
	The was a very very faint foul aftertaste, not enought to dissaude me from eating it, but not as good as egg one.	Good flavor!	i'm not going to lie, this tasted bad!		THERE WAS ALMOST A BURNT TASTE O THE EGGS. NOT SURE EXACTLY, BUT THE FLAVOR WAS A LITTLE OFF.

Continued

Table E.2 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
	but the taste is bland.		bland and dry to taste		favor had a slight maple taste that was unexpected
	and the flavor was a little less then what I would expect		basically there is barely any taste in this egg sample. it would be better if some salts can be added.		has very off flavor and taste, almost burned flavor. no taste of egg at all. tasted as though the eggs had been cooked too long
	and sort of bland but not bad				had a weird flavor
	the taste was bland				Bland
	These eggs lacked flavor.				had an odd after taste
	no flavor				had a flavor not like egg mixed in with the egg flavor
	it tastes undercooked! yuck				eggs had an after taste which I didn't care for
	I do no like this flavor very much.				ONE TONE NOTE
					the taste was slightly amiss.

Continued

Table E.2 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
					taste seems rather bland. I've gladly eaten worse, but only on an empty stomach in the middle of the wilderness
					a little blander than I would have liked
					IT WILL BE BETTER WITH MORE FLAVORS
					the taste was not bad, however even with some water in-between, it could be something observable.
					there was a very odd flavor to this egg
					these had another flavor, maybe from the oil/fat used to cook the eggs -- my first thought was butter?

Continued

Table E.2 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
					Somehow the flavor didn't have the staying power. Not a bad taste at all, just not as strong as first thought.
					bland to taste and n appeal to the eye
					there was an off flavor as the first flavor noticed, it went away, but very noticeable
					the flavor is a little light

Table E.3: Panelists' comments on aroma of control, ozone-treated, and thermal treated scrambled eggs.

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
Intermediate egg flavor and odor between the previous two samples	looks very tasty, but smells not as good	does not smell like raw eggs,	THE SMELL OF THIS EGG MADE ME NOT WANT TO TASTE IT,	Color and aroma were nice	aroma not as strong,
the aroma is perfect besides it tastes good!	no egg smell	The aroma was very nice, though.	a little bit less aromatic than the 1st sample, which can be ignored.	The smell is good	FIRST I SMELL THIS EGG I FELT A BIT FARM SMELL. EXCEPT SMELL OTHER THINKS ARE OK.
Odor was not bad	although the egg samle did not have as much of an aroma to it		I really didn't like the smell when sniffing closely, but who really smells their freshly cooked eggs after eating?	does not have raw egg smell	THE SMELL OF THE 148 WAS A LITTLE SOUR
smells more like raw eggs,	THE FLAVOR AND AROMA AREN'T VERY STRONG, BUT ARE MORE AGREEABLE THAN THE PREVIOUS SAMPLE.		there is no aroma at all	The egg smell was not strong which was good	There is no egg odor
Smell and apperance are slightly better than 148,	but lacked the general yummy scent of a good egg.		the smell is a little strong		the smell and flavor is not so good as the other 2 may due to too much moisture

Continued

Table E.3 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
					smelled funky
					there was a distinct odor to this sample, but was that 'egg smell?'

Table E.4: Panelists' comments on moistness of control, ozone-treated, and thermal treated scrambled eggs.

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
TEXTURE AND MOISTNSS WAS GREAT	Poor texture an moistness	good consistency and isn't slimey	SLIGHTLY DRY	The moisture and texture is just right	MOISTURE WAS SLIGHTLY DRY
Moistness seemed just about right without eggs being undercooked.	Color and visual texture made it look drier than it was	this sample was slightly more moist than the first one	slightly dry	this egg has a good moisture content	DRY
looks watery. moist.	it was kind of dry, but I like dry eggs.	the moisture level seemed to be almost perfect	but still seemed a little to dry to me.		eggs were dry
compare to the last egg sample (O) this egg was more moist	This sample just seemed dry and too tough.	it was rather moist	What I didn't like is the dryness of the egg		A LITTLE BIT WATERY
Slightly more moist than previous sample.	NOT BAD LITTLE DRY	good texture and moisture	DRY		they seemed overly moist
but had a nice moistness and texture.	,too dry,microoven cook?		IS DRY FOR ME		this sample tasted a lttle dryer than the first but stll tasted pretty good
	Not as moist as i would like eggs that I would normally eat!		I like my scrambled eggs a little on the moist side and fluffier.These were a little too dense.		Dry
	This sample seemed little too dry and not as tasty as the first one.		a little dry		were a little dry

Continued

Table E.4 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
	BUT IT WAS JUST A TOUCH DRY. not enough to ruin it, though.		very dry,		the smell and flavor is not so good as the other 2 may due to too much moisture
	Main problem was the texture. The texture was too dry and slightly rubbery with a noticeable graininess or biscuit quality.		Way too dry		I like my eggs a little less dry than that, but its ok
	The texture seems 'layered' and dry in some places.		BETTER TEXTURE THAN OTHER TWO, STILL DRY AND SPONGY		but a little dry
	I probably would have preferred to have them a little bit more moist overall, with a slightly more fluffy texture.		Texture too dry and grainy		a little bit too dry
	I liked them, but they were a little stiff and slightly dry.		too dry, and almost rough in the mouth. I don't enjoy the mouth feel of this egg.		they were too dry and rough
	A LITTLE BIT TOO DRY		they were a little dryer		drier than the previous sample
	too watery (at least by looking)		These were a little rubbery - could use a little more liquid in them.		Too dry

Continued

Table E.4 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
			sample was crumbly and thus harder to eat, I left the 'pan scrapings' behind		It was slightly too dry
			bland and dry to taste		A little dryer than those I make at home though.
			the sample was a bit dry, and so the pieces were broken into small pieces.		The egg was a little dry
					the texture was slightly crumbly, and may be correlated with slightly dry
					a little too dry (overcooked?)

Table E.5: Panelists' comments on texture of control, ozone-treated, and thermal treated scrambled eggs.

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
I LIKED THE TEXTURE	the texture was too chewy.	TEXTURE WAS BETTER.	rougher texture	TEXTURE WAS OK.	slightly tougher texture
good texture	DRIER	TEXTURE WAS BETTER THAN THE FIRST,	it has like a rubbery texture.	I prefer firm eggs, so ok with firmness.	Texture was tough
TEXTURE AND MOISTNSS WAS GREAT	Poor texture	taste and texture better than first	eggs were slightly tough, some parts were tougher than others.	THE EGGS WERE VERY SIMILAR IN TEXTURE	Texture a little bit rubbery
Texture also seemed appropriate without eggs being overcooked	This sample was less tough than the first one but the difference was not significant enough that the scale provided would let me rate them	THE EGG WAS A LITTLE MORE FINE TEXTURED WHICH I LIKED	Slightly too firm	not as rubbery as the first sample	A LITTLE BIT WATERY
I like this one much better than the first, especially for the texture of this one since it's much better than the first one	Color and visual texture made it look drier than it was	good consistency and isn't slimey	THE TEXTURE IS LIKE A LITTLE BIT STANGE FOR AN EGG	although the texture was appropriate	A little rubbery
right texture and color.	very tough	the visual sight and texture is a little bit better than the 1st sample.	the texture looks rubbery.	very good texture, and look	rubbery tough

Continued

Table E.5 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
BUT TEXTURE IS GOOD	but the texture was a little to tough	better taste and texture	This sample is too tender	TEXTURE WAS GREAT	these eggs seemed a bit more rubbery. i did not like the texture.
not runny at all ; seems to have been cooked to perfect firmness.	the egg seemed to require more chewing then the eggs I make at home, though any difference would not be as noticable if it were sered on a sandwich	better taste and texture	a bit rubbery.	texture better than previous two	Chewy
The firmness was nice though..	but the texture seems a little off.	the taste and texture are the best. there is not much that is objectional.	tough.	it was more tender	litle tough. Fluffier is better
	The texture seemed strange	The texture was good	BETTER TEXTURE THAN OTHER TWO, STILL DRY AND SPONGY		when i chew it, it leaves small pieces of egg in my mouth. i don't like that.
	This sample just seemed dry and too tough.	was a little softer in texture which was better than the other samples.	but the texture is too tough		but the texture is not satified yet.
	texture is very dry	the flavor and texture were good	Texture too dry and grainy, egg was somewhat tough; beyond rubbery. Need to make them more fluffy and less dense.		tough.

Continued

Table E.5 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
	Main problem was the texture. The texture was too dry and slightly rubbery with a noticeable graininess or biscuit quality.	texture is good, mouth feel is good.	and had a harder texture.		Also, too tough
	too stiff and almost cardboard like in texture.		The textue is the main complaint.		also the crumbliness appearnace of presentation remindedme a bit of animal droppings
	tough texture,		These were a little rubbery - could use a little more liquid in them.		they were too dry and rough
	I would like these more if they had more of a fluffy appearance and texture.		The texture seems a little off		tough; does not have creamy texture
	The texture seems 'layered' and dry in some places.		this sample was TOUGH -- I had to stab it with the fork, and bent the plastic tines in the process		way too tough

Continued

Table E.5 continued

Control		Thermal		Ozone	
Positive	Negative	Positive	Negative	Positive	Negative
	I liked them, but they were a little stiff and slightly dry.				RUBBERY IN TEXTURE
	This egg was tougher then the last (148)				but is not fluffy enough for my tastes. The eggs are dense and slightly tough
	a little tough				this particular sample seemed slightly 'heavier' than the previous two.
	Also seemed to have been microwaved which affects the texture.				the texture was slightly crumbly, and may be correlated with slightly dry
	Egg texture is very rubbery - I prefer eggs with a more tender texture and bite.				texture is not so good as previous sample.

Table E.6: Panelists' general comments on control, ozone-treated, and thermal treated scrambled eggs.

General		
Control	Thermal	Ozone
GOOD EXAMPLE OF UNSEASONED EGG.	was an improvement over the first sample	more salt or pepper
nOT MUCH DIFFERENT FROM THE FIRST	Great	NOT BAD
they just seemed like normal scrambled eggs to me.	needs salt and pepper	These are over cooked.
looks very tasty, but smells not as good	lOOKED GOOD BUT TASTED ONLY OK	overall good
overall, not too bad	i'm really neutral on this one. Would use this egg as an ingredient in a cake or something rather than serve it alone.	Lage curd size
These eggs were just a little bit off from being great great eggs.	overall appeal not very appetizing	not as good as the second sample
It may improve if the slice is thinner	Not much different from the first 2	very good texture, and look
only problem is the visual sight is less attractive:)	pretty good.	I THINK I LIKED THIS EGG THE BEST. THAT OR THE FIRST ONE.
Overall, I like this one	Eggs seemed a little overcooked	I liked this sample the best.
average scambled egg	the egg clumped together much like home cooked eggs. maintained the expected density as well	I like this egg more than previous sample (148)
THIS SAMPLE IS ALSO FLUFFIER THAN THE PREVIOUS	there was a hair in my sample so that immediately made my opinion skewed.	I think this sample is really good
typical of scrambled egg.	liked it .	Like it!
it has a nice fluff appearance	the scrambled eggs were ust about perfect in all regards	REAL GOOD WELL DONE
they were very plain and ordinary. there was nothing special about them,	it was not really scrambled!!!	shape is the best among the 3 samples
NO DIFFERENCEE FROM EGGS I WOULD PEPARE AT HOME	They are some very good eggs. They will taste better with some salt and pepper though.	My overall would have been the highest but I always eat pepper on my eggs so I do not enjoy eggs as much without the pepper.
this sample is ok	I think the egg ws pretty good.	not too bad

Continued

Table E.6 continued

Control	Thermal	Ozone
They looked pretty good	NOT SURE HOW TO SAY BAD TRY AGAIN	i did not like these eggs at all---the worst out of the three.
Hardly seemed like an egg!	the visual sight and texture is a little bit better than the 1st sample.	the appearance is not so good, too yellow and agglomerated
This egg seemed a little greasy,	I liked this sample the most. It was fluffy, ooked like the scarmbled eggs I would eat	It looked like it had been cooked too long
I would like these more if they had more of a fluffy appearance and texture.	not an egg i would like to eat in the morning	not semblance to real eggs.
PRETTY GOOD.	All samples are within acceptable rangeof cooked, scrambled eggs.	Pretty good overall,
Thse eggs were also pretty good with slightly improved taste.	it seems like a 'fake' egg	This egg seemed like your average 'down on the farm egg' which I like very much
Smell and apperance are slightly better than 148,	I found that the eggs were better when they first came out than they were after a few minutes.	This sample of eggs was overall good. But if these were served ata restaurant I would be satisified.
if I was to recieve these eggs at a restaurant I would be very satisfied.	The egg was pretty good over all,	These eggs were good, better than I get at the fast food restaurant.
These eggs were very similar to the first sample.	These eggs were very good	NOT BAD.
I THINK THIS EGGS IS BETTER THAN 148	This egg looks the same to me	This sample was far better in every category when compared to the first one
THE FIRST ONE IS BETTER THAN THIS ONE	my favorite so far	bland to taste and n appeal to the eye
These probably would be better with some salt.	However, if I was to have received these eggs at a restaurant, I'd be a little disappointed.	
inaccurately rated visual part of this test, but as the first trial can also say 'neither like nor dislike' since it may be considered my baseline	This sample was also good,	
Seemed as if yolks only were used.	Over all I think this was the better of the three samples. It just needed a little salt for my tastes.	

Continued

Table E.6 continued

Control	Thermal	Ozone
This sample was characteristically nearly identical to the second one, and thus was much improved fom the first sample.	I STILL PREFER THE FIRST ONE	
good overall	seemed overcooked	
	O.k., but not great. Seemed much like the first sample. Microwaved!	
	not really appeling to the eye since, the presentation is blah!	