Consumer Acceptance, Quality, and Functionality of Heat-Ozone-Pasteurized Whole Eggs Processed with Commercial Scale Equipment

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Graduate School of The Ohio State University

By

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Abstract

Researchers at The Ohio State University developed a method of pasteurizing shelled eggs using heat and ozone that provides a 5-log reduction of *Salmonella Enteritidis* in shell eggs. Previously, consumer acceptance studies and functionality were reported for pilot scale heat-ozone-pasteurized eggs (OP). As the process nears commercialization, the scaled-up commercial process may result in different egg quality and functionality. This study focuses on consumer acceptance, physical properties, and functionality of OP processed with commercial scale equipment in comparison to untreated eggs (UT), thermally pasteurized eggs (TP) and liquid pasteurized eggs (LP). These eggs were used in broken out eggs and different egg applications such as cooked eggs, an ingredient in a raw egg recipe, and an ingredient in a baking application where egg is an important functional ingredient.

A visual perception test of raw eggs (OP, UT, and TP) was determined along with cloudiness of the thick albumen, amount of spreading of thick albumen, cloudiness of the egg yolk, height of the yolk, color of the yolk, yellowness of the egg, visual appeal of the egg yolk, visual appeal of the albumen, and visual appeal of the whole egg on a 10-point linear scale (n=98). Cooked scrambled eggs were fed to panelists in a consumer acceptance test that measured aroma, flavor, texture, visual and overall liking on a 9-point hedonic scale degree of liking test (n=98). Just-about-right (JAR) analyses with
attributes of color, moistness, and texture were conducted. Consumer acceptance tests in a Caesar dressing matrix of OP and LP eggs were conducted (n=101) using a 9-point hedonic scale degree of liking test with attributes of overall liking, liking of flavor, parmesan flavor, saltiness, thickness, mouth feel, and aftertaste. JAR analyses with attributes of flavor, saltiness, thickness, mouthcoat, and aftertaste were conducted. Consumer acceptance tests in angel food cake prepared with OP, UT, and TP were conducted (n=107) using a 9-point hedonic scale degree of liking test with attributes appearance, pores, color, overall, sweetness, and texture (springiness). JAR analysis with attributes of amount of pores, color, sweetness, and texture (springiness) was conducted. All tests were conducted in a complete balance design, serial monadic approach for all egg treatments.

In the instrumental analyses, in the uncooked eggs, OP and UT have no significant differences in their color (p>0.05). The average Haugh unit of OP was 92.7, which was significantly higher than UT, which means that OP have less spread than untreated (p<0.05) and they would be graded higher than UT. There were no significant differences in the a* value, pH, and consistency of both OP and LP Parmesan Caesar dipping sauce (p>0.05). However, OP dressing was significantly darker (lower L* value) and more yellow (higher b* value) than LP dressing (p<0.05). In the angel food cake study, OP had significantly shorter whipping time than TP, but both had significantly longer whipping time than UT (p<0.05). There were no significant differences in all texture properties and cake volume among OP, UT, and TP (p>0.05). There were some differences in color measurements such as the bottom crust of UT was less red than TP
(p<0.05) but similar with OP (p>0.05) and the top crust of UT was less red and yellow than OP and TP cakes on all egg treatments (p<0.05).

Results varied greatly in the consumer visual tests where OP were perceived to be similar to TP such as visual appeal of albumen and whole egg appeal, thick albumen spread, and yolk cloudiness (p>0.05). In measures of yolk height, yellowness and visual appeal, OP were perceived to be similar to UT (p>0.05). Moreover, in the consumer acceptance test of scrambled eggs, Parmesan Caesar dipping sauce, and angel food cake, there were no significant differences in liking among all eggs for all attributes (p>0.05). Thus it is suggested that heat-ozone-pasteurized eggs would be suitable for use in cooked eggs recipes, raw eggs recipes, and baking recipes, where egg is an important functional ingredient.
Dedication

Dedicated to

My parents (Yessi Maxkwee and Lilyanna Daud)

My brothers (Evann and Kevin Maxkwee)

My fiancé (Christopher Ingle)
Acknowledgments

I would like to sincerely give thanks to my advisor, Dr. Ken Lee for his guidance, knowledge, patience, understanding, and continuous support during my graduate studies. I have learned so much from you and I would not been able to do it without you! I would also like to thank my committee members: Dr. Yael Vodovotz and Dr. Melvin Pascall for your advice and support.

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Last, but not least, I would like to especially thank my parents, brothers, fiancé, and other family members for supporting me. Especially to my parents and fiancé who have always been there to support me.

I am blessed to have all of you in my life!
Vita

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State University

Fields of Study

Major Field:  Food Science and Technology
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</tr>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AMS</td>
<td>Agricultural Marketing Service</td>
</tr>
<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FSIS</td>
<td>Food Inspection Service</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier Transform Infrared Spectroscopy</td>
</tr>
<tr>
<td>HSD</td>
<td>Honestly Significant Difference</td>
</tr>
<tr>
<td>HU</td>
<td>Haugh Unit</td>
</tr>
<tr>
<td>JAR</td>
<td>Just About Right</td>
</tr>
<tr>
<td>LP</td>
<td>Liquid pasteurized eggs</td>
</tr>
<tr>
<td>NACMCF</td>
<td>National Advisory Committee on Microbiological Criteria for Foods</td>
</tr>
<tr>
<td>OP</td>
<td>Ozone-pasteurized eggs</td>
</tr>
<tr>
<td>OSHA</td>
<td>Occupational Safety and Health Administration</td>
</tr>
<tr>
<td>TP</td>
<td>Thermally pasteurized eggs</td>
</tr>
<tr>
<td>USDA</td>
<td>United States Department of Agriculture</td>
</tr>
<tr>
<td>UT</td>
<td>Untreated eggs</td>
</tr>
</tbody>
</table>
Chapter 1 - Literature Review

Background

Foodborne diseases, caused by consuming food that has been contaminated by pathogenic bacteria, viruses, or other organisms, are a major concern for food producers and consumers alike. According to the Centers for Disease Control and Prevention (CDC), foodborne diseases cause about 48 million cases of illness, 130,000 hospitalizations, and 3,000 deaths per year (CDC 2011). *Salmonella*, another group of bacteria that is known to cause foodborne illness (CDC 2011), causes about eleven percent of all foodborne illnesses. *Salmonella Enteritidis* has been associated with foodborne illnesses caused by egg consumption. It is estimated that there are about 200,000 illnesses attributed to *Salmonella Enteritidis* contaminated eggs annually (Schroeder and others 2005). Thus, in 2010, the Food and Drug Administration (FDA) required egg producers comply with the final Egg Safety Rule to implement preventative measures, requiring refrigeration during storage and transportation (FDA 2010). The Food Safety Inspection Service (FSIS) estimated that illnesses would be significantly decreased from 130,000 to 19,000 cases annually if all shell eggs were pasteurized for a 5-log reduction (lowering the number of organisms by 100,000 fold; commonly referred to as pasteurization (NACMCF 2005)) of *Salmonella Enteritidis* (Kamotani and others 2010). In response, researchers have developed many methods to achieve a 5-log
reduction of *Salmonella* Enteritidis such as irradiation, microwave, ultraviolet irradiation, thermal pasteurization, and heat-ozone-pasteurization of shell eggs (which will be referred hereafter as ozone-pasteurized eggs) (Tellez and others 1995; Lakins and others 2008; Davidson 2004; Perry 2010). Studies have been conducted at The Ohio State University on the quality and consumer acceptance of experimental pilot scale ozone-pasteurized eggs in comparison to pilot scale thermally pasteurized eggs. Since then a line of equipment was built and a practical, commercial-scale machine was designed and built by Ohio egg industry partners. Although the commercial-scale ozone process results in equal or better reduction in potential pathogens than the thermally pasteurized process, the impact of the scaled-up commercial process on final whole shell egg quality was unknown. The objective of this research was to investigate the consumer acceptance, physical properties, and functionality of ozone-pasteurized eggs that are produced by commercial scale equipment. This was measured against negative and positive control treatments of untreated eggs and thermally pasteurized eggs (liquid and in-shell), respectively. Several practical functional egg formulations were tried, including cooked eggs, as raw egg ingredient, and as an important ingredient in a baking application.

**Structure, nutrition, and composition of an egg**

A chicken egg weighs about 57 grams and consists mainly of yolk, white (albumen), shell membranes, and shell (USDA-AMS 2000). An egg supplies about 70 calories and contains 13 different nutrients, about 6 grams of protein, 0.4 grams of carbohydrate, and 5 grams of fat (American Egg Board 2013a). In the reproductive tract
of the female hen, the egg is generally formed in about two weeks (Stadelman and Cotterill 1973). First, the yolk is formed, then the white to surround the yolk, followed by the shell membranes and finally the outer shell (Stadelman and Cotterill 1973). Figure 1.1 shows the structure of an egg.

![Figure 1.1 - Structure of an egg (modified from Stadelman and Cotterill 1973).](image)

The yolk, which is about 31% of the whole egg, is composed mainly of proteins (ovovitellin and ovolivetin) and fats (tryglycerides, phospholipid, cholesterol, and others) (USDA-AMS 2000). The lipovitellin, livetin, and lipovitellenin in the protein part of the yolk are good surface active agents which stabilize the films around fat globules to make an emulsion (Stadelman and Cotterill 1973). The fat portion of the yolk contains
approximately 40% of oleic acid, 35% of saturated fatty acids, and the remaining portion is a mixture of other unsaturated fatty acids (Privett and others 1962). The lecithin, a phospholipid, is widely used as an emulsifier for many egg based recipes. The yolk also contains most vitamins except Vitamin C and minerals (USDA-AMS 2000). In a fertilized egg, the yolk is located in the center of the egg; however, in an egg that will be consumed by humans, the yolk moves to a peripheral location (Board and Fuller 1994). This movement is due to loss of water from the albumen to the atmosphere around egg and the migration of water from the albumen to the yolk (Board and Fuller 1994). The pH of the yolk is typically about 6, but increases to 7 during storage. The yolk is yellow-orange in color, which is mostly due to the pigment xanthophyll, but its color is influenced by the hen’s diet (USDA-AMS 2000; Li-chan and others 1995). Yellow corn or alfalfa feed will produce medium yellow yolks; while wheat or barley feed produce lighter colored yolks (American Egg Board 2013a).

A thin semi-permeable membrane called the vitelline membrane surrounds the yolk to prevent water migration. This may cause an increase in the density of the yolk (Board and Fuller 1994). As the egg ages, the water diffuses and weakens the vitelline membrane, causing the yolk appearance to be more flat, leading to a lower grade egg with a lower Haugh unit (USDA-AMS 2000).

The albumen is the next layer after the vitelline membrane. According to the United States Department of Agriculture (USDA) and Agricultural Marketing Service (AMS) Egg grading manual (2000), the albumen is approximately 60% of the total egg and is mainly composed of water (88%) and protein (11%). There are 4 layers of the
albumen. The first is the chalaziferous, which is next to the vitelline membrane and connected with the chalazae (a protein fiber that holds the yolk in its place). It makes up about 3% of the total white and is a very thin firm layer of it. Next is the inner thin layer, which surrounds the chalaziferous layer and makes up about 17% of the white. Next, a thick layer of albumen, which sticks to the shell membrane at each end of the egg, covers the inner thin albumen and yolk. This is the most abundant layer (about 57% of the white). Finally an outer thin layer lies inside of the shell membrane, comprising about 23% of the white (USDA-AMS 2000).

The albumen consists of many different types of proteins. The function of each protein is shown in Table 1.1 (Powrie 1973). The thick albumen contains about 4 times higher concentration of ovomucin than thin albumen and contributes to the viscosity of the albumen (Powrie 1973). The quantity and viscosity of the thick albumen is directly proportional to the egg’s functionality and quality grading (Stadelman 1995). Over time, the thick albumen becomes thinner due to the interaction of the ovomucin with the lysozyme as pH rises to about 9 and CO₂ is lost during storage (Stadelman and Coterrill 1973). This will affect the quality grading of the egg.
Table 1.1 - Proteins characteristics and functions in albumen of an egg (Powrie 1973; USDA-AMS 2000).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Amount in Albumen (%)</th>
<th>Characteristic and Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovaalbumin</td>
<td>54</td>
<td>Heat-stable protein during storage. In solution, it is resistant to thermal denaturation.</td>
</tr>
<tr>
<td>Conalbumin</td>
<td>13</td>
<td>More heat-sensitive than ovalbumin but more resistant to surface denaturation.</td>
</tr>
<tr>
<td>Ovomucoid</td>
<td>11</td>
<td>Heat resistant glycoprotein especially in acidic solution.</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>3.5</td>
<td>Lytic action on bacterial cell wall. Thermal inactivation depends on pH and temperature.</td>
</tr>
<tr>
<td>Globulin G3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Ovomucin</td>
<td>1.5</td>
<td>Resistant to heat alterations in solution. Good foam stabilizer and structure of thick albumen.</td>
</tr>
<tr>
<td>Avidin</td>
<td>0.05</td>
<td>Binds biotin (water soluble B-vitamin).</td>
</tr>
<tr>
<td>Others</td>
<td>1.9</td>
<td></td>
</tr>
</tbody>
</table>

Enclosing the albumen and the yolk, there is another membrane, which is not a part of the albumen, called the inner shell membrane. This membrane consists of protein fibers and is located between the albumen and the inner surface of the shell (Stadelman and Coterril 1973). The membrane consists of a thick (outer) and thin (inner) membrane. The thick membrane is attached to the shell by numerous cones and fiber associations, while the thin membrane encloses the albumen (Board and Fuller 1994; Stadelman and Coterrill 1973). Both membranes are connected except at the blunt end of the egg, where they are separated only to surround the air cell (Board and Fuller 1994). The inner shell membrane is known to be the most effective barrier to prevent bacterial penetration from the outside of the egg (Lifshitz et al. 1964).
The shell of an egg makes up 11% of the egg and is composed of three layers: the mammillary or inner layer (which encloses the thick shell membrane), the spongy layer, and the cuticle (USDA-AMS 2000). The shell has between 7-17,000 pores with a diameter of 9 to 35 µ and additional pores at the blunt end to allow gas exchange (USDA-AMS 2000; Stadelman and Coterrill 1973). These pores are small enough to prevent bacterial penetration (Stadelman and Coterrill 1973). The shells also serve as the first line of defense against bacterial contamination (American Egg Board 2013a), although the inner shell membrane is more effective. Calcium carbonate (about 94%), magnesium carbonate, calcium phosphate, and other organic matter comprise the shell of an egg (USDA-AMS 2000). The cuticle layer of the egg (made up of about 85% protein), which is also known as a bloom, is the natural coating of the shell of the egg. This cuticle layer reduces water loss and seals the pores to help prevent bacteria from penetrating the egg (American Egg Boards 2013; Board and Fuller 1994). An egg has an air cell, which is an empty area at the edge of the egg in between the shell and inner albumen. According to the USDA egg grading manual, the size of the air cell is a factor in determining the quality of the egg. As the egg is first laid, the egg has little to no air cell, but as it cools, the inner membrane separates from the outer membrane creating the air cell. Thus, as it ages, the air cell expands, which is caused from evaporation of water from the egg (USDA-AMS 2000).

The shell strength is affected by the minerals and vitamins of the hen’s diet, age and breed of the hen, freedom of disease, and a warm environment (American Egg Board 2013a; USDA-AMS 2000). The color of the shell depends on the breed of the hen. Hens
with white feathers and ear lobes will produce white-shelled eggs, while hens with red feathers and ear lobes will produce brown-shelled eggs. Brown-shelled eggs are usually more expensive than white-shelled eggs because the hens that produce the brown-eggs are slightly larger and require more food than hens that produce white-eggs (American Egg Board 2013a). Contrary to popular belief, there is no nutritional or health difference between brown and white eggs, as the only difference is the brown pigment that is the last thing the hen applies to the shell before it is laid.

**Egg grading and quality**

Whole eggs are graded based on the quality of the shell, egg white, and egg yolk, the depth of the air cell, and by a Haugh unit, which is a measurement of the correlation of the height of the thick albumen, the weight of the egg, and the internal egg temperature. In USDA approved facilities, shell eggs are graded by USDA officials based on the USDA standard (Table 1.2). The eggs are packaged in styrofoam or cardboard packaging where a USDA shield shape label with a grade (AA, A, or B) is printed on the outside packaging.

The freshness of an egg is determined by calculating the yolk index (ratio of yolk’s height to diameter). A Higher yolk index indicates fresher eggs, and therefore, a higher quality egg (Stadelman 1995). The depth of an air cell is measured by an air-cell gauge. A small air cell depth indicates higher quality eggs (USDA-AMS 2000).

A Haugh unit measurement is based on the formula $\text{HU} = 100 \log_{10} (h - 1.7w^{0.37} + 7.6)$ (Haugh 1937; USDA-AMS 2000). The measurement is taken using a Haugh meter. To measure this, an egg is cracked onto a leveled surface, and the meter is placed over
the egg with the middle pin positioned approximately in the middle of the thick albumen, avoiding the chalazae (Haugh 1937; Stadelman and Coterill 1973). A higher Haugh unit is one of the indications of a higher quality egg.

In an egg production facility, a method called candling is used to check the interior quality of the egg shell without breaking it. The candling method can be conducted by either handling the egg individually, or by flash candling, where a large amount of eggs pass over a candling area filled with high-intensity lights that make the interior of the egg visible (Board and Fuller 1994 and American Egg Board 2013a). Typical tests during candling include: air cell size, albumen condition, distinctness of yolk outline, yolk appearance, blood spots, germ development, and the presence of moldy eggs, cooked eggs, rot, and other defects (USDA-AMS 2000).

Table 1.2 Summary of USDA egg standards for shell eggs quality (modified from USDA-AMS, 2000).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Egg Shell</th>
<th>Egg white (albumen)</th>
<th>Egg yolk</th>
<th>Depth of air cell</th>
<th>Area covering and Haugh unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>Clean, unbroken, and practically normal</td>
<td>Clear, firm, stands fairly high.</td>
<td>Firm, high, and round</td>
<td>Max 1/8 inch</td>
<td>Covers moderate area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prominent chalazae</td>
<td></td>
<td></td>
<td>Haugh unit &gt;72</td>
</tr>
<tr>
<td>A</td>
<td>Clean, unbroken, and practically normal</td>
<td>Clear, reasonably firm, stands fairly high. Prominent chalazae</td>
<td>Firm and fairly high</td>
<td>Max 3/6 inch</td>
<td>Covers moderate area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Haugh unit 60 to &gt;72</td>
</tr>
<tr>
<td>B</td>
<td>Clean or slightly stained, unbroken, and abnormal</td>
<td>Minimum amount of thick white, weak, and watery. Small or absent chalazae</td>
<td>Appears enlarged and flattened</td>
<td>No limit</td>
<td>Covers wide area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Haugh unit &lt;72</td>
</tr>
</tbody>
</table>
Egg production

According to the American Egg Board, the United States produces about 75 billion eggs per year, which is about 10% of the world supply (American Egg Board 2013b). Each hen produces about 250-300 eggs per year and there are about 280 million hens in the United States. Iowa and Ohio are the top two egg producing states in the country. In April 2012, there were about 6.5 billion table eggs produced in the United States (American Egg Board 2013b).

There are two types of egg production in the United States: “off-line” and “in-line.” Off-line production is where the eggs are brought to the production facility from a separate laying facility, while in-line production is where the laying, processing, packing, and shipping of the eggs all take place in one facility. Currently, most production facilities are in-line production (USDA-AMS 2000). After the eggs are collected, they are washed, sanitized, dried, and graded. The eggs are washed with water, which is at least 6.7°C warmer than the internal temperature of the eggs (USDA-AMS 2000). The temperature differences should be just large enough to meet washing standards, but not too large in order to prevent cracking or allowing contamination to penetrate into the egg (Board and Fuller 1994). If the temperature of the wash water is too cold, negative pressure can create a vacuum that allows penetration of bacteria from the wash water into the egg. The wash water has to be changed regularly (approximately every four hours) and have an iron content of less than 2 ppm (USDA-AMS 2000; USDA-AMS 2008). Studies have shown that and iron concentration of 4.8 mg/l in the wash water may allow iron and microorganisms to be deposited on the shell membranes, which accelerates rotting (Board and Fuller 1994). Only approved cleaning compounds can be used in the
wash water. Alkaline products are usually added to wash water to increase pH, where combination of high pH and high temperature may eliminate *Salmonella* from the wash water (Board and Fuller 1994). Rinse-water should have an approved sanitizer, such as chlorine or quaternary sanitizer with a concentration of 100-200 ppm (USDA-AMS 2008). It is also known that the cuticle of the egg shell is removed during the washing step, which increases the rate of carbon dioxide loss and moisture loss from the egg, and increases the possibility of bacterial penetration (USDA-AMS 2000; Board and Fuller 1994). Thus, light spray of food grade mineral oil coating is a common practice in the egg industry (USDA-AMS 2000). After the eggs are washed and sanitized, the eggs are then candled, graded, packed, and shipped to consumers, retailers, or distribution centers.

**Salmonella and eggs**

*Salmonella* is a gram-negative, rod-shaped bacteria commonly found in digestive tracts of animals such as poultry (FDA-HHS 2009; FSIS 2011). There are over 2,300 *Salmonella* serotypes, where *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most common types that cause foodborne illnesses (FSIS 2011). *Salmonella* cause about 11% of all food-borne illnesses (CDC 2011). *Salmonella* Enteritidis has been associated with foodborne illnesses caused by egg consumption. It is estimated that there are about 200,000 illnesses caused by *Salmonella* Enteritidis contaminated eggs annually (Schroeder and others 2005). The Centers for Disease Control and Prevention estimate that 1 in 20,000 eggs are internally contaminated by *Salmonella* Enteritidis (CDC 2010a), and external contamination may be greater than the number of cases of internal contamination (Musgrove and others 2005). During 2000-2005, CDC investigated that an
average of 1 in 8 sampled chickens were contaminated with *Salmonella* and out of those contaminated chickens, 1 in 20 were contaminated with *Salmonella* Enteritidis (CDC 2010a). In 2013, the Food and Drug Administration estimated that annually, approximately 79,000 cases of foodborne illnesses and 30 deaths are caused by the consumption of *Salmonella* Enteritidis contaminated eggs (FDA 2013a).

Many foodborne illnesses are associated with *Salmonella* Enteritidis caused by egg consumption (Cowden and others 1989; CDC 1996; Boetani-Mohle and others 1998; Heredia and others 2009; Laestadius and others 2012). About 80% of *Salmonella* Enteritidis outbreaks are associated with eggs or egg-containing foods (Patrick and others 2004). On June 24, 1995, twenty-six people became ill due to consumption of Caesar salad dressing (containing raw eggs) at a wedding reception in New York (CDC 1996). Increased number of illnesses caused by *Salmonella* Enteritidis in the 1980s, caused Patrick and others (2004) to investigate trends in *Salmonella* Enteritidis infection in from 1985 to 1999 and showed that 28% or 67 out of 243 foods tested were contaminated with *Salmonella* Enteritidis in the United States contained raw eggs (Patrick and others 2004). Homemade mayonnaise contains raw eggs which are associated with many *Salmonella* outbreaks in many countries such as the United States, the United Kingdom, Australia, Denmark, and Germany (Heredia and others 2009). Most recently, in 2010, a multi-state outbreak of *Salmonella* Enteritidis that caused almost 2,000 illnesses was linked with the consumption of shelled eggs and further lead to the recall of about 500 million eggs (CDC 2010b; FDA 2012).
Salmonella causes an illness called Salmonellosis. The symptoms are fever, diarrhea, abdominal cramps or pain, headache, vomiting, and nausea, where the incubation period ranges from 8-72 hours and symptoms can persist for up to a week (FSIS 2005). Elderly, infants, and immune-compromised people are more susceptible to Salmonellosis, and very small amounts of Salmonella cells can cause severe illness (FSIS 2005). Most deaths caused by Salmonella Enteritidis contaminated food mainly occurred in nursing homes and hospitals where the immune system of the residents of these establishments were already lower than normal (CDC 2010). While Salmonella is killed at temperatures >55°C (CDC 2010), most Salmonella Enteritidis contaminated food are found on dishes containing raw or under-cooked eggs such as Caesar dressing, hollandaise sauce, eggnog, sunny-side up eggs, or others (FSIS 2005; CDC 2010). A survey was conducted in 1995 that found that 51% of 1,629 people consumed raw eggs (Klontz and others 1995). Moreover, in a study involving 153 restaurants in seven states, many restaurants used unpasteurized shell eggs to prepare soft or runny fried eggs (78%), runny omelets (42%), and soft poached eggs (74%) (Lee and others 2004). Therefore, due to the high prevalence of raw egg usage in restaurants and other establishments, there is a high risk of Salmonellosis in many of these egg dishes. For this reason, it is important that pasteurized shell-eggs are processed and made safer for consumers. CDC also suggested that the use of pasteurized eggs as a substitute in dishes that needs raw eggs are needed, especially if they are prepared for high risk people in hospitals, nursing homes, or at home (CDC 2010).
**Routes of contamination**

Two pathways where *Salmonella* Enteritidis can be introduced into the eggs are by horizontal and vertical contamination (FSIS 2005). Horizontal contamination is contamination that occurs through the shell of the eggs after the eggs are laid. Vertical contamination, which is the primary route of contamination, is contamination where the *Salmonella* is introduced to the egg through the infected ovaries or oviduct tissues before the eggs are laid (FSIS 2005).

Horizontal contamination occurs after laying, either through fecal contamination of shell eggs, a contaminated nest box, or hatchery environment through the shell surface or beneath the surface of the eggs (Cox and others 2000). As the eggs are laid, the temperatures of the eggs are at the hen’s body temperature, but the air temperature is much cooler than the body temperature (Cox and others 2000), thus creating negative pressure that allows bacteria to penetrate into the eggs. Washing the eggs also enhances contamination through the shell due to the removal of the cuticle membrane, which is the egg’s first line of protection, from the egg shells (Musgrove and others 2005). Egg washing temperature is a critical factor for horizontal contamination. As previously described, warm wash water is needed to prevent negative pressure in the egg. The warm water also allows eggs to expand, which creates an outward pressure preventing bacteria to be drawn in the eggs (Board and Fuller 1994).

Vertical contamination occurs through contaminated hens. The hens can become contaminated through many factors such as contaminated feed, dust particles in the hen house, unsanitary poultry house environments, the presence of rodents and pests, and
hatcheries (where the newly laid chicks are infected from infected breeder flocks) (Mead 2005). However, according to the FSIS, not all hens produced by infected eggs are contaminated. In order for an egg to be contaminated, *Salmonella* Enteritidis must be present in the farm, it must infect more than one hen, and then the infected hens are vulnerable to produce *Salmonella* Enteritidis contaminated eggs (FSIS 2005). The eggs can become contaminated through different steps of the egg formation in the hen reproductive tract. During formation, studies have shown that the albumen and the vitelline membrane are the primary sites of contamination, but yolk contamination cannot be ruled out (Board and Fuller 1994).

Although the albumen itself has some antimicrobial defense action, such as ovotransferrin (which limits microbial growth by binding iron), the presence of *Salmonella* may still exist (Mead 2005). In the yolk, the multiplication of *Salmonella* Enteritidis is much faster than the albumen because of the rich nutrient content and the absence of antimicrobial defense. Higher storage temperature increases multiplication in the yolk, but growth ceases around 4°C (Mead 2005). Migration of *Salmonella* Enteritidis from the vitelline membrane into the yolk increases with storage temperature, dose of contamination, and the age of the eggs (Mead 2005). The age of the egg is also a factor in *Salmonella* Enteritidis growth. As the egg ages, the vitelline membrane weakens and yolk contamination is more likely to occur (Mead 2005). Thus, it is important to handle the eggs at a proper storage time and temperature (FSIS 2005). Currently in the food industry, refrigeration of eggs at 7°C during storage and transportation is mandated as part of the Egg Safety Final Rule of 2010 (FDA 2010).
Unfortunately, it is difficult to determine if an egg is contaminated (Keller and others 1995). Contaminated eggs can contain low levels of *Salmonella* Enteritidis, averaging about 10 cells per eggs (Mead 2005). The infectious dose in humans can be estimated to be as low as 10 cells (Greenwood and Hopper 1983), depending on the immune system of the person. Both routes of contamination need to be prevented, but proper handling of the eggs can help prevent horizontal contamination more than vertical contamination. Thus, vertical contamination is a much greater concern. One solution to this problem is to pasteurize all shell eggs to ensure that the *Salmonella* are killed. Although pasteurization of shell eggs is not commonly performed, FSIS recommends it. If consumers demand pasteurized shell eggs, this may reduce the illness associated with *Salmonella* Enteritidis in eggs (FSIS 2005).

**Regulations**

In the United States, many government agencies are responsible on overseeing the regulation and production practices of eggs and egg products. Egg products referred to eggs that are removed from shells for further processing into products such as: dried, frozen, or liquid eggs (with or without added ingredients) (FSIS 2011). Both the FDA and USDA regulate the safety of eggs and egg products.

Due to the number of foodborne illnesses associated with *Salmonella* Enteritidis caused by egg consumption, awareness has been raised. In 1994, the United Egg Producers developed a voluntary program for egg producers to monitor and control *Salmonella* Enteritidis called “5-star” Total Quality Assurance Food Safety Program (Egg
In 1998, the President’s Council on Food Safety was developed by President Bill Clinton to ensure safe food from production to consumption. In 1999, an egg safety action plan was proposed with the aim of eliminating *Salmonella* Enteritidis illnesses attributed to eggs by 2010 (President’s Council on Food Safety 1999). The plan was issued on July 2009 and was incorporated into 21 Code of Federal Regulation 118.8, which requires shell egg producers to have preventative measures in place in order to prevent *Salmonella* Enteritidis contamination on eggs during both storage and transportation (FDA 2013b).

Clinton’s plan later became known as the Egg Safety Final Rule and was mandated on July 9, 2010 for egg producers with 50,000 or more laying hens. On July 9, 2012, the plan became operative for producers with at least 3,000 laying hens but less than 50,000. Producers with less than 3,000 laying hens (less than 1% of eggs available in the United States) and those who sell all their eggs directly to consumers were exempt from the rule (FDA 2013b). The rule includes regulations that require pullets (young hen) monitoring, biosecurity, pest control, refrigeration, environmental sampling, egg sampling, and a recordkeeping program (FDA 2013b).

The Egg Safety Action plan mentioned that the in-shell pasteurization method was should be further developed (President’s Council of Food Safety 1999). If the shell eggs are processed with some form of treatment such as pasteurization, the egg producers do not have to comply with some other rules except for refrigeration at 7°C during storage and transportation no later than 36 hours after the eggs are laid (FDA 2010). The storage temperature and time is important to prevent the growth of *Salmonella*.
Enteritidis. FSIS, a governing body under the USDA, estimated that if all shell eggs were pasteurized for a 5-log reduction, commonly referred to as pasteurization, *Salmonella* Enteritidis illnesses would be decreased from 130,000 to 19,000 cases (FSIS 2005). Stadelman and Cotterill (1973) suggested that the risk of *Salmonella* Enteritidis contamination could be reduced by using pasteurized eggs including pasteurized shell eggs (Stadelman and Cotterill 1973).

Pasteurized shell eggs which comply with the requirements given by the USDA may have a shield-shaped “certified as pasteurized” symbol on the packaging and packaging material (USDA-AMS 2013). Only less than 0.05% of shell eggs in the United States are pasteurized (FSIS 2005). Researchers have been developing many methods to achieve a 5-log reduction of *Salmonella* Enteritidis such as irradiation, microwave treatment, UV irradiation, thermal pasteurization, and heat-ozone-pasteurization of shell eggs (Tellez and others 1995; Lakins and others 2008; Davidson 2004; Perry 2010).

**Irradiation**

Irradiation is one of the emerging methods of technology that attempts to reduce *Salmonella* in eggs. Reactive hydroxyl ions generated by low to medium doses of ionizing radiation change the microorganisms’ reproduction ability by disrupting both the cell membrane and DNA (Moy, 2005). Usually, the sources of the irradiation come from gamma rays, X-rays, or electron beams. It was found that gamma radiation of 2 kGy is effective to eliminate *Salmonella* Enteritidis from shell membranes, eggshells, (Tellez and others 1995) and yolks (Lith and others 1995) and yolks (Lith and others 1995). A 1.5 kGy of x-ray irradiation has
been effective at achieving a 4-log reduction of *Salmonella* Enteritidis in the yolks of whole shell eggs (Serrano and others 1997).

Several studies have observed that irradiation may negatively affect an egg’s quality and functionality. Several authors have observed that Haugh unit were decreased when shell eggs were irradiated at doses of about 1 kGy (Ma and others 1990; Tellez and others 1995). Haugh unit were decreased by 50 units when shell eggs were irradiated with electron beam irradiation at doses up to 4 kGy, and there was further loss after extended storage (Wong and others 2003). Several authors have observed decreases in albumen viscosity at low doses of irradiation (Pinto and others 2004). Discoloration in the egg yolks and weakening of both the eggshells and the vitelline membrane were observed in irradiated eggs (Ma and others 1986; Dvorak and others 2005). Although irradiation at a dose up to 3 kGy was approved by the FDA in 2000 to be used in shell eggs, it is not currently used commercially (FDA 2000). This is probably due to the irradiation effects on the quality and functionality of the eggs and negative consumer perception of the use of irradiation in food. Moreover, this technology has not achieved a 5-log reduction of *Salmonella* Enteritidis.

**Microwave**

Microwave pasteurization is another emerging technology method that has been used to reduce *Salmonella* Enteritidis in eggs. Microwave pasteurization uses electromagnetic waves to reduce *Salmonella* (Lakins and others 2008; Stadelman and others 1996; Erasmus and Rossouw 2007). Heat is generated by the agitation of molecules in electromagnetic field, which is caused by the interaction of microwave rays
with dielectric materials. Bacterial cells in food are destroyed as temperature in the food increases, such as during cooking (Lakins and others 2008). In one study conducted by Kozempel and others (1998), it was suggested that other theories of microwave technology include selective heating theory, electroporation, cell membrane rupture, and magnetic field coupling. The selective heating theory suggested that microwave rays cause the rapid killing of microorganisms by heating microorganisms more effectively compared to the surrounding medium. Electroporation forms pores in the microorganisms’ membrane, which causes leakage of cellular components due to the crossing of electrical potential into the membrane. Cell membrane rupture is caused by a voltage drop across the membrane. Magnetic field coupling in the microwave causes cell lysis, which is caused by interaction between the internal components of the cell and the magnetic field energy (Kozempel and others 1998).

Microwave technology has been found to be able to achieve a 2-log reduction of *Salmonella* Enteritidis in eggs. The eggs were exposed to 2.45 GHz of microwave rays for 20 seconds at 80% magnetron power and then rapidly cooled by carbon dioxide (Lakins and others 2008). According to Lakins, there were no significant differences in mineral content, Haugh unit, fatty acid profile, yolk index, pH, emulsion stability, and foaming capacity between microwave-pasteurized and regular eggs. The foaming stability and the vitelline membrane’s strength were significantly higher in the microwave pasteurized eggs, and the emulsion capacity was lower than regular eggs (Lakins and others 2009). This technology has not achieved a 5-log reduction of *Salmonella* Enteritidis.
Thermal Pasteurization (Liquid and In-shell)

Thirteen percent of 76.2 billion eggs consumed in 2009 were in the form of egg products, which are eggs removed from their shell (FSIS 2011), typically called liquid pasteurized eggs. Liquid pasteurization was first used in the United States in 1938 primarily to prolong shelf life of liquid frozen egg products (Cunningham 1986). Due to *Salmonella* outbreaks in processed egg products, FDA mandated usage of liquid pasteurization in 1966 and the Egg Safety Inspection Act of 1970 regarding egg pasteurization requirement was born (Mead 2005). The process involves heating the liquid egg below boiling to kill vegetative microbial cells (FSIS 2005). Time and temperature are important factors that need to be considered in liquid pasteurization process, but the time-temperature combinations needed to achieve complete pasteurization are near or at the condition that may cause unfavorable effects on the functionality and physical characteristics of the egg proteins (ICMSF 1980). The time and temperature combinations vary depends on the type of egg products (yolk only, whole egg, seasoned, and others). Table 1.3 shows different time and temperature combination of liquid egg products in the United States (FDA 2002b). Although widely used, liquid pasteurized eggs are incapable of being made as sunny-side up, soft boiled or hard boiled eggs.
Table 1.3 - Time and temperature requirements for conventional pasteurization of liquid egg products in the United States (FDA 2002b).

<table>
<thead>
<tr>
<th>Liquid egg product</th>
<th>Minimum temperature requirements (°C.)</th>
<th>Minimum holding time requirements (Minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumen</td>
<td>56.7</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>55.6</td>
<td>6.2</td>
</tr>
<tr>
<td>Whole egg</td>
<td>60.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Whole egg blends (less than 2 percent added non-egg ingredients)</td>
<td>61.1</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>60.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Fortified whole egg and blends (24-38 percent egg solids, 2-12 percent added non-egg ingredients)</td>
<td>62.2</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>61.1</td>
<td>6.2</td>
</tr>
<tr>
<td>Salted whole egg (with 2 percent or more salt added)</td>
<td>63.3</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>62.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Sugared whole egg (2-12 percent sugar added)</td>
<td>61.1</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>60.0</td>
<td>6.2</td>
</tr>
<tr>
<td>Plain yolk</td>
<td>61.1</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>60.0</td>
<td>6.2</td>
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<tr>
<td>Sugared yolk (2 percent or more sugar added)</td>
<td>63.3</td>
<td>3.5</td>
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</tbody>
</table>
Many researchers have conducted studies of thermal pasteurization in shell eggs using various times and temperatures in order to reach the optimal treatment to give a 5-log reduction of *Salmonella* Enteritidis without changing the quality of the eggs (Hou and others 1996; Schuman and others 1997; Brackett and others 2001; Stadelman and others 1996; Lith and others 1995). Some researchers have achieved this 5-log reduction, but the egg quality was significantly impacted (Hou and others 1996; Schuman and others 1997).

The first pasteurized shell eggs in the market was produced by Michael’s Foods in 1996 (Mermerlstein 2001). Currently, Michael Food’s sell their pasteurized eggs to foodservice establishments under the brand name Papetti’s and to the retailers under the brand name Crystal Farms. These eggs are not available in major grocery stores in Ohio and many other states. In 1998, John Davidson has patented a method of thermal pasteurization and he has been commercially marketing this product as Davidson’s safest choice pasteurized shell eggs (Mermerlstein 2001). In the Davidson’s thermal pasteurization method, eggs are heated using hot water bath at various temperatures and times to reduce *Salmonella* Enteritidis inside the shell eggs (Davidson 2004). The method has resulted to a 5-log reduction of *Salmonella* Enteritidis in eggs (Davidson 2004). According to the patent, the Davidson process involved hot water immersion tanks with different temperature zones and holding time with a total process time of about 40 minutes. The first temperature zone was between 59.4 and 63.3°C, the second, was between 54.4 and 57°C, and the third temperature was between 57.2 and 59°C. Then, the eggs were removed from the water bath after reaching about 4.6 log reduction and air-dried. While the internal temperature of the eggs was still higher than the atmospheric
temperature, the eggs are sprayed with antibacterial fluid to prevent contamination from the surrounding air. To prevent post-contamination, the eggs are coated with sealant containing antibacterial agent. This holding section creates residual heat that will penetrate into the eggs, raising the temperature of the eggs until a possible 5-log reduction of *Salmonella* Enteritidis is reached. Then, the eggs are cooled with antibacterial spray, stamped with a red letter P, and packaged (Davidson 2004).

Care should be taken when thermal is applied to raw shell eggs because the albumin (egg white) is sensitive to heat. High temperature may denature these heat-sensitive proteins and cause irreversible damage to the eggs resulting in low quality eggs. This has led to the invention of an ozone pasteurization method where denaturation of egg protein is far less likely than thermally pasteurized.

**Ozone - pasteurization**

Ozone is an unstable three-atom oxygen (O₃) formed by recombination of available single oxygen radicals that were split from oxygen (O₂) molecules in a highly energized environment (Khadre 2001). Ozone is most commonly known as a layer in the atmosphere that protects the earth from the ultraviolet light from the sun, which can be hazardous to human health. According to the EPA (Environmental Protection Agency), while the ozone is formed naturally in the stratosphere (upper layer of the atmosphere) to protect us, the ozone at ground level is the main ingredient in smog, which causes harmful air pollution and damages crops (EPA 2003). Scientists have discovered technologies to use ozone as a disinfectant to treat both water and food, and potentially use for other applications.
Ozone usage as a disinfectant has been around for many years. In 1893, scientists in the Netherlands discovered the use of ozone as a chemical disinfectant for drinking water (Weavers 2001). Since then, researchers have investigated other applications for using ozone as a disinfectant, including algae control, odor removal from wastewater, disinfection of seawater in shellfish cleaning stations, and disinfection, mold control, and preservation in the food industry (Weavers 2001). In 2001, the FDA approved the usage of ozone in the treatment, storage, and processing of foods, including meat and poultry. Its use is permitted as an indirect food additive in either gaseous or aqueous phase, which acts as an antimicrobial agent, in accordance with current industry standards of good manufacturing practice (FDA 2001).

In general, Weavers explained that ozone is generated through a corona discharge where dry air or oxygen ($O_2$) is passed between two electrodes alienated by glass or ceramic dielectric material. The concentration of ozone generated depends on the type of incoming material (air or pure $O_2$), where pure $O_2$ leads to higher ozone concentration. Ozone is very unstable and reactive, lasting only 12 hours in its gaseous state and 20-30 minutes in pure water with a pH of 7-8. Ozone in aqueous phase is a powerful oxidizing agent and found to be an effective treatment as a disinfectant against bacteria, spores, viruses, protozoa, and fungi. It can oxidize cellular components such as proteins, nucleic acids, and enzymes, leading to cell death. Ozone does not leave behind any hazardous residues on products after processing (Weavers 2001).

Although ozone is generally recognized as safe as an antimicrobial agent in water and food, safety precautions still need to be taken when working with ozone. High
concentration of ozone (not used as a disinfectant) is blue in color, but at low concentration, ozone is not visible (Weavers 2001). Ozone has a pungent or sweet odor that at low concentrations is the smell typical of copy machines or printers. Different concentrations and time of exposure of ozone can cause different levels of toxicity in the forms of nose irritation, vision loss, inflamed lungs, and even death. Therefore, the Occupational Safety and Health Administration (OSHA) have set a limit of 0.1 ppm over an 8-hour limit, where ozone is detectable by the human nose at 0.01 ppm (Mahapatra and others 2005). An ozone environmental monitor is used to measure the level of ozone in the room and is required to be used while an ozone machine is running.

Researchers at The Ohio State University have developed a method of using heat, vacuum, and ozone to pasteurized shelled eggs (Yousef and Rodriguez-Romo, 2009). The heat-ozone-pasteurization method uses sequential application of pressurized ozone, vacuum, and immersion heating to reduce Salmonella Enteriditis in the internal and external of whole shell eggs (Perry 2010). According to Perry and others (2011), eggs are heated in an immersion of circulating water bath set to 57°C until internal temperature of the eggs reaches 56°C. Then the eggs are continued to be held in the water bath for an additional 10 minutes, are transferred into a stainless steel vessel for the ozone treatment and are exposed to 50.8 kPa vacuum prior to exposure of gaseous ozone. The gaseous ozone, which is produced from pure oxygen, is pumped into the treatment vessel until the ozone concentration reaches 160 g/m³. Ozone injection raises the pressure of the treatment vessel to ~187.5 kPa. After about 10-12 minutes, ozone injection is stopped and the eggs are held for about 60 minutes (Perry and others 2011). The ozone-pasteurization
method is able to achieve more than 6-log reduction of *Salmonella* Enteritidis in the albumen and the yolk of the eggs (Perry and Yousef 2013), thus can be called as pasteurized products.

Several studies have compared the quality and functionality of pilot-plant scaled ozone-pasteurized eggs with pilot-plant scaled thermal pasteurized eggs (Perry and others 2011; Kamotani and others 2010). The heat treatment on the process may impair the protein structure in the eggs. Analysis on protein structure during extended storage was conducted using an FTIR analysis. According to Perry and others (2011), analysis suggested that albumen protein in ozone-pasteurized eggs is less damaged compared to thermally pasteurized eggs. Thus, ozone-pasteurized eggs were more likely to resemble untreated shell eggs (Perry and others 2011). This analysis was supported by a sensory consumer acceptance study done on pilot-plant scaled ozone-pasteurized eggs in comparison to commercialized thermal pasteurized eggs and untreated eggs. One study done by Kamotani and others (2010) suggested that ozone-pasteurized eggs were perceived to be visually more appealing than thermally pasteurized eggs. When the eggs are prepared as scrambled eggs, there was no significant difference on the degree of liking among all eggs in flavor, texture, and aroma (Kamotani and others 2010).

**Egg consumption in the United States**

In 2010, the per capita consumption of eggs in the United States was about 248 eggs per person, where about 70% were shell eggs and 30% were egg products. About 60% of the eggs produced were used by consumers, 9% by the food service industry, and the remaining were converted into egg products. In 2011, about 220 million cases of shell
eggs were produced, and about 30% were further processed for food service, manufacturing, retail and export. About 57% went to retail establishments, 8% went to food service use, and 3% were exported. Eggs can be found in about 93% of household in the United States (American Egg Board 2013b). In most of the United States, white-shelled eggs are preferred over brown-shelled eggs, except for New England, where brown-shelled eggs are preferred (American Egg Board 2013a).

From a market study of 300 consumers and 100 foodservice institutions conducted in 2006 by AZG Research, it was established that flavor was the highest importance, while cost was the least importance when making food purchases. As for egg purchases, freshness, color, cost, and taste were all important. One fifth of the total respondents do not know the term “pasteurized”. More than half of the total respondents would more likely to buy food that has the term “pasteurized” on the label (AZG Research 2006). The average price of conventional white eggs in the United States was about $1.77 per dozen (Chang and others 2010). A consumer survey (n=85) of egg preferences conducted by University of Kentucky shows that over 30% of consumers are willing to purchase eggs for more than $3.00 per dozen for locally produced eggs (University of Kentucky 2011). Moreover, a study done by Rohr and others (2005) found that 80% of 449 German consumers surveyed were willing to pay 30% extra for safer eggs. The Strategy Team, LTD conducted a recent survey and focus group study on consumers’ behavior towards ozone-pasteurized eggs (Bieliauskas 2013). Their focus group study (n=29) showed most retail consumers would not buy eggs that cost $3.00 per dozen because they were not concerned about foodborne illness from eggs, and many
believed that all eggs were pasteurized or do not understand the pasteurization process. The survey, which was conducted with 200 individuals who makes decisions of purchases in restaurants, institutions, or foodservice establishments, more than 50% of the respondents, said that egg safety was their most important factor when buying eggs. Institutions had the most positive reaction (71%) towards the ozone-pasteurized eggs and they were most likely to purchase the eggs compared to other organizations (Bieliauskas 2013).

Recently the CDC advocated that retail establishments, food service establishments, and institutional settings use pasteurized in-shell eggs as a substitute to pooled eggs, or raw or undercooked shell eggs (CDC 2010a). Based on the AZG Research survey (2006), over 80% of the total respondents do not use raw eggs to make Hollandaise or Caesar dressing (which uses raw eggs in the recipe) (AZG Research 2006). Food service establishments are recommended to inform their consumers of the use of raw or undercooked eggs through brochures, flyers, and other effective forms of written communication (FDA 2002a). A survey conducted by Nelson and others (2008) of 1,630 long term care facilities found that 92% of these facilities used liquid pasteurized egg products, but only 36% used whole shell pasteurized eggs. This may be due to the different individual requirements of the egg recipes used or limited availability of whole shell pasteurized eggs.

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Chapter 2 - Consumer Acceptance Test and Physical Properties of Ozone-pasteurized Uncooked Eggs and Cooked Scrambled Eggs

Abstract

Visual perception and consumer acceptance of commercial scale ozone-pasteurized eggs was compared to thermally pasteurized and untreated eggs using a degree of liking test and a JAR analysis. Instrumental analysis of albumen turbidity, yolk color and Haugh unit were correlated to human measures. Previous reports from this lab described pilot scale ozone-pasteurized eggs so this study investigates a scale-up to commercial ozone-pasteurized eggs. All uncooked eggs were examined in a visual perception test (n=98) for these attributes: cloudiness of thick albumen, amount of spreading of thick albumen, cloudiness of egg yolk, height of yolk, color of yolk, yellowness of the egg, visual appeal of egg yolk, visual appeal of albumen, and visual appeal of the whole egg. Cooked scrambled eggs were tested for consumer acceptance (n=98) attributes: visual, aroma, flavor, texture and overall liking. Results varied greatly in visual tests where the ozone-pasteurized eggs were perceived equivalent to the thermally pasteurized eggs, such as visual appeal of albumen and whole egg, thick albumen spread, and yolk cloudiness. In measures of yolk height, yellowness and visual appeal, the ozone-pasteurized eggs were perceived equivalent to the untreated eggs. In taste tests, there were no significant differences among all eggs for visual, aroma, flavor,
texture and overall liking using scrambled eggs. From these results it appears ozone-pasteurized eggs are suitable for use in cooked applications in home or industrial settings with an added benefit of reduced risk of illness from pathogens.

Keywords: Ozone, Egg, Sensory, Acceptance, Pasteurization

Introduction

Foodborne diseases, caused by consuming food that have been contaminated by pathogenic bacteria, viruses or other organisms, are a major concern for food producers and consumers alike. According to the Centers for Disease Control and Prevention (CDC), about 3,000 Americans die per year of foodborne diseases, with nearly 130,000 hospitalized, and 48 million sick (CDC 2011). *Salmonella* Enteritidis has been associated with foodborne illnesses caused by egg consumption. It is estimated that there are about 200,000 illnesses caused by *Salmonella* Enteritidis contaminated eggs annually (Schroeder and others 2005). Thus, in 2010, FDA has mandated egg producers to comply with the final Egg Safety Rule to implement preventative measures, which requires refrigeration during storage and transportation (FDA 2010). The Food Safety Inspection Service (FSIS) estimated that illnesses would decrease if all shell eggs were pasteurized for a 5-log reduction (lowering the number of organisms by 100,000 fold; commonly referred to as pasteurization (NACMCF 2005) of *Salmonella* Enteritidis (Kamotani and others 2010). In response, researchers have developed many methods to achieve a 5-log reduction of *Salmonella* Enteritidis such as irradiation, microwave, UV, thermal
pasteurization, and heat-ozone-pasteurization of shell eggs (Tellez and others 1995; Lakins and others 2008; Davidson 2004; Perry 2010).

Ozone as a disinfectant was used since the 1800’s. In 2001, FDA approved use of ozone in the treatment, storage, and processing of foods (including meat and poultry), and as secondary food additive in gaseous or aqueous phases as an antimicrobial agent (FDA 2001). Heat-ozone pasteurization, which is referred to as ozone-pasteurization in this study, has been shown to inactivate more than 5 logs CFU/g of Salmonella Enteritidis in shell eggs (Perry and Yousef 2013).

Previous studies were conducted at The Ohio State University on the quality of pilot scale ozone-pasteurized eggs in comparison to pilot scale thermally pasteurized eggs. Analysis on protein structure during extended storage was conducted using a Fourier transform infrared (FTIR) analysis, which suggested that ozone pasteurized eggs were more likely to resemble untreated shell eggs because albumen protein in ozone-pasteurized eggs is less damaged compared to that of thermally pasteurized eggs (Perry and others 2011). According to Kamotani and others (2010), pilot scale ozone-pasteurized eggs were perceived to be visually superior to thermally pasteurized eggs in a consumer acceptance study. There was no significant difference in degree of liking among all egg variables (thermal, ozone, and untreated) in scrambled eggs (Kamotani and others 2010). Shell porosity and shell strength of the eggs were also a concern in regards to the quality of the eggs. The shell of the eggs contains about 17,000 pores with an average diameter of 9 to 35 µ which is small enough to prevent bacterial penetration (Stadelman and Coterrill 1973). Heat pasteurization may open up pores of the shells.
(Davidson 2009). No study has been conducted to investigate whether this may occur in ozone-pasteurized eggs.

Shell strength is one of the quality measurements in egg quality grading where a “practically normal” shell is a shell that has the usual shape, good texture and shape (Stadelman and Cotterill 1973). Previous study on the shell strength of pilot scale ozone-pasteurized eggs indicate that there was no significant difference in shell strength between ozone-pasteurized eggs and untreated eggs.

Since then, a practical, commercial-scale process was designed and built by Ohio egg industry partners. Although the industrial scale ozone process results in the same or better reduction in potential pathogens, the scaled-up commercial process may result in different egg quality. The hypothesis of this research was based on previous pilot-scale sensory result, where the albumen of the ozone pasteurized eggs was hypothesized to look similar to untreated eggs and less cloudy than thermally pasteurized eggs. The overall visual appeal of the ozone-pasteurized eggs was hypothesized to be similar to untreated eggs. The ozone-pasteurized eggs, thermally pasteurized eggs, and untreated eggs were hypothesized to be liked similarly in a cooked egg application. Thus, the objective of this research was to conduct sensory analyses of both the visual perception and the consumer acceptance of commercial scale ozone-pasteurized eggs in comparison to currently marketed thermally pasteurized and untreated eggs. Instrumental analyses of albumen turbidity, yolk color, Haugh unit, shell strength, and color dye test were conducted.
Materials and Methods

Eggs

Untreated and ozone-pasteurized eggs laid by hens between 32 and 45 weeks of age were obtained from local Ohio farms. Thermally pasteurized eggs were obtained from local grocery stores with the sell by date of June 18 (Julian date 110). All eggs were held at refrigerated temperature of 4°C until use. Eggs from all sources were industrially washed and sorted grade AA large eggs.

Sensory Testing

Sensory studies were performed at The Ohio State University Food Science Parker Building Sensory Analysis Laboratory (Columbus, OH, USA). In the facility, there were 10 booths that were illuminated by white fluorescent light and temperature was set at 21°C. All responses were collected using Compusense® version 5.2 software (Guelph ON, Canada) with a mouse and color LCD monitor interface used by each panelist. The test was conducted in one sitting in a complete balanced design, serial monadic approach for all three different eggs. The uncooked eggs were presented first and followed by the scrambled eggs for consumer acceptance study. Ninety-eight untrained panelists (73 female and 25 male) were recruited and presented with three uncooked eggs visual test. Over 90% of the panelists reported using eggs regularly (at least once every 2 weeks).

Visual Test

One egg from each treatment was cracked by hand into a clear 15.9 cm plate (The Kroger Co, Cincinnati, OH, USA), labeled with 3-digit randomized numbers and placed on top of a black background for the visual perception test, shown in Figure 2.1.
The panelists were instructed to individually rate the samples on a 10-point linear scale for 9 different attributes: cloudiness of the thick albumen (1=not cloudy, 10=very cloudy), amount of spreading of thick albumen (1=a little spreading, 10=a lot of spreading), cloudiness of the egg yolk (1=not cloudy, 10=very cloudy), height of the yolk (1=low, 10=high), color of the yolk (1=light, 10=dark), yellowness of the egg yolk (1=less yellow, 10=more yellow), visual appeal of the egg yolk (1=not at all appealing, 10=very appealing), visual appeal of the albumen (1=not at all appealing, 10=very appealing), visual appeal of the whole egg (1=not at all appealing, 10=very appealing).

Figure 2.1 - Presentation of visual test.
**Consumer acceptance test of uncooked and scrambled eggs**

In the consumer acceptance test, 6 eggs for each treatment were pre-mixed a day prior using a hand blender with a whisk attachment (Model #59780, Hamilton Beach, Washington, NC, USA) on high for 45 seconds. Each pre-mixed egg sample was placed into labeled Ziploc bag and stored in a commercial refrigerator (Delfield Refrigerator, Mt. Pleasant, MI, USA) under refrigeration temperature of 4°C until use. On test day, each bag of egg mix was placed into a microwave safe mixing glass bowl and mixed on high for 10 seconds. The egg mixes were cooked in a microwave (Model JES1358WL 01, General Electric, Louisville, KY, USA) for 1 minute on high at 1100 W. The eggs were then mixed on low for 10 seconds and cooked for an additional 1 minute. The eggs were cut into large pieces using a #1905 rubber spatula (Rubbermaid, Fairlawn, OH, USA) and cooked for an additional 1 minute. Finally, the eggs were cut into smaller pieces using a spatula and covered with cling wrap (Wasserstrom, Columbus, OH, USA) for 30 seconds. Temperature was taken using a handheld thermometer (Rubbermaid, Fairlawn, OH, USA) until the internal temperature reached >73.9°C. Each batch was placed in equal portion into ten 15.2 cm disposable white foam plates (Supreme Pactiv Corp., Lake Forest, IL, USA) using a 46.1 g scoop (NSF, Sheboygan, WI, USA). Filtered spring water was provided to the panelists as their palate cleanser between samples, shown in Figure 2.2. Figure 2.3 shows picture of all egg treatments. The picture is from eggs processed on February 2013, different than what was analyzed in this study.

Panelists were instructed to individually rate the samples for degree of liking on a 9-point hedonic scale, where 9= like extremely,8=like very much, 7=like moderately,
6=like slightly, 5=neither like or dislike, 4=dislike slightly, 3=dislike moderately, 
2=dislike very much, and 1=dislike extremely. The attributes tested were visual, aroma, 
flavor, texture, and overall liking. Panelists were instructed to use a 5-point category Just- 
about-right (JAR) scale to rate the amount of scrambled egg color (5=way too dark, 
4=slightly too much, 3=just about right, 2=slightly too little, 1=way too light), moistness (5=way too moist, 4=slightly too much, 3=just about right, 2=slightly too little, 1=way too dry) and texture (5=way too tough, 4=slightly too much, 3=just about right, 
2=slightly too little, 1=way too tender). Finally, demographic questions were asked of 
each panelist (age, gender, egg usage, and race).

Figure 2.2 -Presentation of scrambled egg on taste test.
Instrumental Analysis

Instrumental analyses were conducted on ozone-pasteurized and untreated eggs only. Albumen turbidity measurements were based on 12 replicates. Haugh unit and yolk color measurements were based on 10 replicates.

Albumen turbidity measurement was performed by separating the albumen from the yolk using an egg separator (MSC International, Montreal, Canada). Three ml of the albumen were pipetted into disposable cuvettes. Absorbance was measured at 600 nm with a Shimadzu UV-Vis 2450 spectrophotometer (Kyoto, Japan) and samples were read against a blank reference of distilled water (Perry and others 2011). Twelve measurements were taken and a standard deviation was calculated.
Haugh unit (HU) were measured using a HU meter (Mattox & Moore, Indianapolis, IN, USA) and is based on the formula HU = 100 log₁₀ (h - 1.7w^{0.37} + 7.6) (Perry and others 2011). Egg weight of 680 g per dozen was set manually. Eggs were cracked onto a leveled surface; the meter was placed over the egg with the middle pin positioned approximately in the middle of the thick albumen, avoiding the chalazae. Measurements from the dial were recorded when the middle pin touched the albumen. Averages from 2 different measurements were taken in two different areas of the albumen. Ten eggs were measured and standard deviation was obtained.

The yolk color was measured using a Colorquest XE Hunter Lab colorimeter (Reston, VA, USA) with Illuminant D65/2 and 2.54 cm diameter viewing aperture (adapted from Pihlsgard and others 2007). Yolk was separated from the albumen. Ten ml of yolk was placed into a cuvette. Ten measurements of L*, a*, and b* value were recorded.

The porosity of the egg was tested using Easter egg dye to investigate whether the dye will penetrate into the eggs. One tablespoon of water and a tablet of the dye were placed in a cup. After the tablet was dissolved, 125 ml of water was added. Hardboiled eggs (one for each treatment) were immersed in the dye for 5 minutes and 4 days.

The shell strength study was conducted only on the untreated and ozone-pasteurized eggs at 21°C. The test was conducted using Universal Testing Machine (Instron Corp., Norwood, MA, U.S.A.). An extension test (applied with a 35 mm diameter probe and an egg based with a square plate with a hole in the center to balance the egg) was used with a speed of 50 mm/min resulting force (N) required to achieve a
compressive extension of 16 mm distance. The instrument software (Bluehill® 2 version 2.17, Instron Corp., Norwood, MA, U.S.A.) was used to automatically record the measurements. Maximum force was used to determine the first crack of the shell while measurements of average forces after the first peak, which will be referred as secondary crack, was used to measure shattering of the eggs.

Measurements of the shell strength were on ozone-pasteurized eggs and untreated eggs with ten replicates from each treatment. This determined shell strength after storage of eggs prior to the ozone-pasteurization process. Measurements for ozone-pasteurized and untreated eggs were obtained at 0, 6, and 12 storage days. On 0 day of storage, the shell strength of 7 batches of the ozone-pasteurized eggs, where each batch was placed in different racks in the ozone-pasteurization machine were measured to determine differences in shell strength of eggs pasteurized on different racks. On day 6 and day 12, there were 2 batches of ozone-pasteurized eggs measured. Batches were identified with letters and numbers such as: “O-6.1” means ozone on day 6 of batch 1, “U-6.1” means untreated eggs on day 6 of batch 1.

Statistical analysis

Significant differences within consumer acceptance tests were determined using repeated measures ANOVA and Tukey’s HSD post hoc analysis at p < 0.05, generated from Compusense software. Significant difference analysis on instrumental analyses was performed using independent T-test and ANOVA with Tukey’s HSD post hoc analysis at p<0.05, generated from SPSS Statistics version 16.0 software (SPSS Inc., Chicago, Ill).
**Results and Discussion**

*Visual test*

Cloudiness of the albumen can be a visual indicator of egg freshness. Fresher eggs are cloudier than older eggs because carbon dioxide inside eggs escapes over time, thus creating albumen that is more translucent (American Egg Board 2010). As shown in Figure 2.4, the thick albumen of the ozone-pasteurized eggs was significantly cloudier than thermally pasteurized eggs, which was significantly cloudier than the untreated eggs (p < 0.05). This means that the hypothesis is rejected. This result is supported in the instrumental analysis. As shown in Table 2.1, albumen turbidity of the ozone-pasteurized eggs was significantly higher than the untreated egg (p < 0.05) by spectrophotometric analysis. In this case, turbidity is a measure of cloudiness of the albumen, which means that the ozone-pasteurized eggs were significantly cloudier than the untreated eggs. In Perry’s (2011) pilot scale study, although albumen turbidity from ozone-pasteurized eggs was significantly higher than that of untreated eggs, the ozone-pasteurized eggs were significantly less turbid than thermally pasteurized eggs (Perry and others 2011), showing slightly different results than both consumer tests. This is probably due to a different thermal pasteurization technique from the Perry (2011) study and thermally pasteurized eggs purchased from a local store, thus a controlled comparison cannot be made.
Table 2.1 - Physical properties of ozone-pasteurized and of untreated eggs.

Haugh unit is expressed as $HU = 100 \log_{10} (h - 1.7w^{0.37} + 7.6)$. Tristimulus colorimeter measures of value, hue and chroma as $L^*, a^*, b^*$ respectively. Turbidity measured by optical density.

Mean ± standard error of mean. Within each row, same superscript on each mean indicates no significant difference at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th>Replicates</th>
<th>Ozone-pasteurized egg</th>
<th>Untreated egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haugh Unit, HU</td>
<td>10</td>
<td>92.2 ± 1.22$^a$</td>
<td>85.6 ± 2.70$^b$</td>
</tr>
<tr>
<td>L*</td>
<td>10</td>
<td>62.4 ± 0.368$^a$</td>
<td>61.7 ± 0.442$^a$</td>
</tr>
<tr>
<td>a*</td>
<td>10</td>
<td>12.2 ± 0.286$^a$</td>
<td>12.2 ± 0.227$^a$</td>
</tr>
<tr>
<td>b*</td>
<td>10</td>
<td>57.5 ± 0.341$^a$</td>
<td>57.1 ± 0.601$^a$</td>
</tr>
<tr>
<td>Turbidity</td>
<td>12</td>
<td>0.321 ± 0.0388$^a$</td>
<td>0.0182 ± 0.00701$^b$</td>
</tr>
</tbody>
</table>

One egg-grading criterion is the spreading of the thick albumen. Eggs with the least spreading of the thick albumen are graded higher than those with more spread (American Egg Board 2010). The spreading of the thermally pasteurized eggs’ thick albumen was not significantly different when compared to ozone-pasteurized and untreated eggs. Ozone-pasteurized and untreated eggs were significantly different from each other, where ozone-pasteurized eggs are perceived to have more spreading (mean rating of 4.65) than untreated eggs (mean rating of 3.80 in Figure 2.4). Our previous pilot scale study shows a different result. Kamotani showed that there is no significant difference between the ozone-pasteurized and untreated eggs, but thermally pasteurized
eggs were perceived to have significantly less spread than the others (Kamotani and others 2010). Instrumental analysis shows a different result in regards to Haugh unit. A Haugh unit is a measure of the height of the thick albumen; because spreading of the albumen is directly correlated with the height, a higher Haugh unit value suggests thicker albumen and less spread. Eggs that have greater than 72.0 Haugh unit would be graded as grade AA (USDA-AMS 2000). Table 2.1 shows that the average Haugh unit measurement of the ozone-pasteurized eggs was 92.2, which was significantly higher than that of the untreated eggs (85.63). This suggests that ozone-pasteurized eggs are of higher quality. Regardless, both untreated and ozone-pasteurized eggs qualify as grade AA.

Similar statistical results as the spreading of the thick albumen occurred in the measure of yolk color. Ozone-pasteurized eggs were perceived by consumers to be darker in color than untreated eggs by less than one mean rating scale (a 6.18 mean rating score compare to 5.65 mean rating score), but all three eggs were perceived to have no significance difference in their yellowness, which is shown in a spider plot (Figure 2.5). In the Kamotani study (2010), there were no significant differences across all eggs color lightness but the thermally pasteurized eggs were perceived to be less yellow than the others and there was no significant difference between ozone-pasteurized and untreated eggs (Kamotani and others 2010). In comparison to the instrumental analysis data shown in Table 2.1, ozone-pasteurized eggs and untreated eggs have no significant differences in their color, which includes lightness and yellowness.
Figure 2.4 - Visual attribute ratings by 98 consumer panelists on a linear 10 point scale for ozone-pasteurized, thermally pasteurized, and untreated eggs. Descriptive anchors are listed in Table 2.2.

a,b,c Within each group of three contiguous bars a different superscript indicates a significant difference at p>0.05. Error bars indicate standard error of mean.
Figure 2.5 - Spider diagram illustrating the visual attribute fingerprint of ozone-pasteurized, thermally pasteurized, and untreated raw eggs (n=98). Descriptive anchors are listed in Table 2.2.
Table 2.2 - Visual attributes and endpoint anchors used by consumer panelists for the results shown in Figure 2.4 and Figure 2.5. All attributes were rated by each panelist on a ten point linear scale labeled with these descriptive endpoints.

<table>
<thead>
<tr>
<th>Visual Attribute</th>
<th>Descriptive endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 = Minimum rating</td>
</tr>
<tr>
<td>Thick albumen cloudiness</td>
<td>Not cloudy</td>
</tr>
<tr>
<td>Thick albumen spreading</td>
<td>A little spreading</td>
</tr>
<tr>
<td>Yolk cloudiness</td>
<td>Not cloudy</td>
</tr>
<tr>
<td>Yolk height</td>
<td>Low</td>
</tr>
<tr>
<td>Yolk color</td>
<td>Light</td>
</tr>
<tr>
<td>Yolk yellowness</td>
<td>Less yellow</td>
</tr>
<tr>
<td>Yolk visual appeal</td>
<td>Not at all appealing</td>
</tr>
<tr>
<td>Albumen visual appeal</td>
<td>Not at all appealing</td>
</tr>
<tr>
<td>Whole egg visual appeal</td>
<td>Not at all appealing</td>
</tr>
</tbody>
</table>

In the cloudiness of the egg yolk, the ozone-pasteurized eggs and thermally pasteurized eggs were not significantly different from each other, but were perceived to be significantly cloudier than untreated eggs. In comparison to our previous pilot-plant study, ozone-pasteurized eggs were rated to be significantly different from thermal and untreated (Kamotani and others 2010). This suggests that commercial ozone-pasteurized eggs are better than thermally pasteurized eggs in terms of yolk cloudiness, which is an improvement from the pilot scale. Moreover, as shown in Figure 2.4, all eggs were rated below 4 on the intensity scale of one being not cloudy and 10 being very cloudy. Therefore, yolk cloudiness should not be a major consumer acceptance concern.

Height of egg yolk is another criterion for egg grading. Yolk that is round and upstanding is graded AA or A, while yolk that is enlarged and flattened is graded B.
(American Egg Board 2010). This can be measured using yolk index, which is a measure of the height of the yolk divided by the width of the yolk. In the visual test shown in Figure 2.4, there was no significant difference \( (p > 0.05) \) in the height of the yolk among ozone, thermal, and untreated eggs. Conversely, in the Kamotani and others (2010) study, ozone-pasteurized eggs were perceived to have shorter yolk when compared to other treatments (Kamotani and others 2010). This shows improvement of the yolk quality since the current study shows that ozone-pasteurized eggs are perceived equivalent to thermally pasteurized and untreated eggs. Yolk index of the ozone-pasteurized eggs were shown to be not significantly different than untreated eggs between two to eight weeks of storage (Perry and others 2011). Moreover, all eggs met grade AA standards.

Regardless of the different perceptions on each of the yolk attributes, the visual appeal of the yolk was perceived to be no different among all egg treatments with the rating of about 7 in the intensity scaling, where 10 was very appealing (Figure 2.4). In the visual appeal of the albumen and whole egg, the ozone-pasteurized eggs and thermally pasteurized eggs were not significantly different from each other, but were perceived to be less appealing than the untreated eggs, thus the hypothesis is rejected. Moreover, the ozone-pasteurized egg is perceived equivalent to thermally pasteurized eggs in regards to the visual appeal of the albumen and whole egg.

**Taste tests**

While visual testing produced variable results among the attributes of ozone-pasteurized, thermally pasteurized, and untreated eggs, taste tests using scrambled eggs did not. As shown in Figure 2.6 the scrambled egg taste test results suggest that all three
scrambled egg samples made with ozone-pasteurized, thermally pasteurized, and untreated eggs were liked similarly with a rating of 6 (like slightly) for all attributes (visual, aroma, texture, flavor, and overall liking). This showed no significant difference across all samples in all attributes, which supports the hypothesis. The scrambled eggs were rated to be liked slightly, probably because they were served unseasoned. Seasoning the scrambled eggs may have increased liking, but would have introduced variability among treatments. These results are parallel with a pilot scale study conducted with the same design (Kamotani and others 2010). In a separate study of a focus group consisting of five chefs, scrambled eggs prepared with ozone-pasteurized eggs were deemed equivalent or better to untreated eggs. In the focus group, the chefs observed that the ozone-pasteurized egg yolk tasted very good and had an appealing bright yellow color (Heavenridge 2013).
Figure 2.6 - Degree of liking attribute ratings of ozone-pasteurized, thermally pasteurized, and untreated scrambled eggs (n=98).

Attributes were evaluated for degree liking on a 9-point hedonic scale, where 9= like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like or dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, and 1=dislike extremely. Error bars indicate standard error of mean.

There were no significant differences at p>0.05 for the degree of liking of all attribute tested.
Figure 2.7 - Frequency distribution of consumer scores (n=98) on Just-about-right (JAR) scales of scrambled eggs by treatment for (a) Color, (b) Moistness, (c) Texture.

### (a) Color

<table>
<thead>
<tr>
<th>Percentage of Responses</th>
<th>Ozone</th>
<th>Thermal</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Way too Dark</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Slightly too dark</td>
<td>6%</td>
<td>5%</td>
<td>6%</td>
</tr>
<tr>
<td>Just about right</td>
<td>85%</td>
<td>85%</td>
<td>81%</td>
</tr>
<tr>
<td>Slightly too light</td>
<td>8%</td>
<td>9%</td>
<td>12%</td>
</tr>
<tr>
<td>Way too light</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

### (b) Moistness

<table>
<thead>
<tr>
<th>Percentage of Responses</th>
<th>Ozone</th>
<th>Thermal</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Way too moist</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Slightly too moist</td>
<td>3%</td>
<td>3%</td>
<td>2%</td>
</tr>
<tr>
<td>Just about right</td>
<td>39%</td>
<td>35%</td>
<td>47%</td>
</tr>
<tr>
<td>Slightly too dry</td>
<td>45%</td>
<td>49%</td>
<td>45%</td>
</tr>
<tr>
<td>Way too Dry</td>
<td>13%</td>
<td>13%</td>
<td>6%</td>
</tr>
</tbody>
</table>

### (c) Texture

<table>
<thead>
<tr>
<th>Percentage of Responses</th>
<th>Ozone</th>
<th>Thermal</th>
<th>Untreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Way too tender</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Slightly too tender</td>
<td>3%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Just about right</td>
<td>44%</td>
<td>40%</td>
<td>50%</td>
</tr>
<tr>
<td>Slightly too tough</td>
<td>43%</td>
<td>47%</td>
<td>43%</td>
</tr>
<tr>
<td>Way too tough</td>
<td>10%</td>
<td>11%</td>
<td>5%</td>
</tr>
</tbody>
</table>
For the Just-about-right (JAR) questions in Figure 2.7, More than 80% of panelists perceived all eggs’ color to be just about right. This is an improvement from Kamotani’s pilot-plant study where, the ozone-pasteurized eggs were perceived to be slightly too light (Kamotani and others 2010). Furthermore, when panelists were asked to rate the amount of moistness on a JAR scale, thermally pasteurized eggs were rated as slightly drier than the other samples. In the attribute of moistness, both thermally and ozone-pasteurized eggs received more “way too dry” ratings than untreated eggs. When panelists were asked to rate eggs in regards to the egg texture on the JAR scale, thermally pasteurized eggs were rated as slightly tougher than the other samples, while untreated eggs received the most just about right ratings in the texture attribute among all eggs. Thermally pasteurized eggs had the highest number of responses in the “slightly” and “much too dry” category. In correlation with the liking results, the dryness and toughness were most likely due to the method of cooking, which was microwave oven. The results are similar to our prior work where there were no differences in JAR ratings for moistness and texture among treatments. Both methods of pasteurization of eggs were perceived to be slightly too dry and too tough (Kamotani and others 2010).

Shell strength and color dye test

When eggs were dyed using an Easter egg dye for 5 minutes, shown in Figure 2.8, the shell of the untreated eggs absorbed the color well, while the thermally pasteurized eggs have a slightly more intense color than the untreated. The ozone-pasteurized eggs did not absorb the color well, resulting in a blotchy shell and faint color. On the third day, the untreated eggs have a more intense color compared to the egg soaked at 5 minutes.
Ozone-pasteurized eggs have more color than the egg soaked at 5 minutes but still less intense than the untreated eggs. When the shells were peeled, the dye did not penetrate through the shells of all cooked eggs.

![Image of dyed eggs](image)

**Figure 2.8** - Hardboiled uncracked shell eggs dyed with Easter egg dye.

In the shell strength study, prior to using this method to measure the shell crack and shattering, empirical measurements of measuring the diameter of the egg cracks were used. After multiple experiments, a systematic method was developed using the instrument and settings described. The development of this method involved multiple trials using different settings, probe attachments, and texture analyzer machine model
TAXT 2 (Stable Micro System, Surrey, UK). The current method gave the least variance among replicates.

Shown in Table 2.8, there was no significant difference on the first crack and secondary crack in the ozone-pasteurized eggs regardless of storage days and batches (p>0.05). First crack measurement was measured to determine whether the ozone-pasteurized eggs have the same strength with untreated eggs. Secondary crack measurement was measured to determine whether the ozone-pasteurized eggs shatters (the crumbling or falling apart of the shells after being cracked.). Similarly, there was no significant difference on the first crack and secondary crack in the untreated eggs regardless of storage days and batches (p>0.05). There was no significant difference on the first crack, which signify the amount of force required to crack the eggs, between both treatments (p>0.05). However, it required significantly more force to shatter the untreated eggs compared to the ozone-pasteurized eggs (p<0.05). Thus, it is suggested that the untreated eggs are more resistant to shatter than the ozone-pasteurized eggs. The ozone-pasteurized eggs inner membrane was observed to not adhere to the shell as much as the untreated eggs, while the shells of the untreated eggs are more cohesive to the inner membrane, and easily removed by hand peeling. This may be a contributing factor to why the shell on the ozone-pasteurized eggs shattered more than the untreated eggs, because there was no support from the inner membrane.
### Table 2.3- Amount of force required to crack and shatter eggs based on treatments (ozone-pasteurized and untreated) and storage days (n=10).

*a,b* Mean ± standard error of mean. Within each row, same superscript on each mean indicates no significant difference at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Days</th>
<th>Treatments</th>
<th>Batch #</th>
<th>First Crack (N)</th>
<th>Secondary Crack (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>a,b</td>
<td>a,b</td>
</tr>
<tr>
<td>Day 0</td>
<td>Ozone</td>
<td>1</td>
<td>31.7±2.29</td>
<td>7.46±0.827</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>2</td>
<td>38.2±2.18</td>
<td>5.53±0.307</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>3</td>
<td>34.1±1.74</td>
<td>6.47±0.585</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>4</td>
<td>34.1±1.52</td>
<td>6.99±0.469</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>5</td>
<td>36.1±1.74</td>
<td>5.73±0.377</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>7</td>
<td>35.9±1.98</td>
<td>7.27±0.590</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>9</td>
<td>35.0±2.36</td>
<td>6.83±0.735</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>2</td>
<td>39.5±2.55</td>
<td>10.8±0.102</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>7</td>
<td>36.8±2.59</td>
<td>11.1±0.947</td>
</tr>
<tr>
<td>Day 6</td>
<td>Ozone</td>
<td>1</td>
<td>32.0±1.01</td>
<td>5.76±0.390</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>2</td>
<td>34.9±1.51</td>
<td>6.10±0.417</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>1</td>
<td>35.4±0.999</td>
<td>12.7±0.471</td>
</tr>
<tr>
<td>Day 12</td>
<td>Ozone</td>
<td>1</td>
<td>33.4±1.25</td>
<td>6.90±0.416</td>
</tr>
<tr>
<td></td>
<td>Ozone</td>
<td>2</td>
<td>34.2±1.27</td>
<td>6.99±0.344</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>1</td>
<td>38.0±1.70</td>
<td>12.9±0.660</td>
</tr>
</tbody>
</table>
A solution to keep the shell intact and reduce shattering was waxing the eggs shell, which is performed in the laboratory of the inventor, Professor Ahmed Yousef. The shell strength measurements were performed using the same shell strength measurement method (Figure 2.9).

Ozone-pasteurized eggs were dipped in a wax coating (paraffin and bees waxes) to determine if either coating affects the shattering of the ozone-pasteurized eggs. Two batches of ozone-pasteurized eggs were measured to determine if there was any effect of batch variability. Eight measurements of the first and secondary crack from each treatments were measured, except for ozone bees waxed 2, which only have 7 measurements due to cracked eggs prior to measurement.

There were no significant differences on the first crack among all treatments (p>0.05). This means that the wax coating had no effect on the amount of force required to crack the eggs. On the secondary crack, there were no significant differences between unwaxed ozone-pasteurized eggs and paraffin waxed ozone-pasteurized eggs (p>0.05), but paraffin waxed ozone-pasteurized eggs required significantly less force to shatter the egg shells compared to bees waxed ozone-pasteurized eggs (p<0.05). Untreated eggs were not significantly different than paraffin waxed ozone-pasteurized eggs (p>0.05), but bees waxed ozone-pasteurized eggs required significantly less force to shatter the eggs compared to untreated eggs (p<0.005). Thus, ozone-pasteurized eggs coated with bees wax resulted in more intact shells compared to paraffin or unwaxed eggs. Based on visual observation, when the eggs were cracked on the 90 degree edge of a hard table, the paraffin wax fell off the shell, which risks having wax fall into dishes prepared with these
eggs. In contrast, the bees wax on the eggs stayed together. The bees waxed appeared more yellow compared to other treatments. Coating the eggs helped reduce shattering. Further work needs to be conducted on other types of waxes or different coatings to minimize shattering of the shell.

Figure 2.9 - Amount of force required to crack and shatter eggs of waxed ozone-pasteurized eggs and unwaxed untreated eggs (n=8 for all treatments; except ozone bees waxed 2, where n=7)).

Within each bar of the same color, a different superscript indicates a significant difference at p>0.05. Error bars indicate standard error of mean.
Conclusion

In visual tests ozone-pasteurized eggs were rated as equivalent to thermally pasteurized eggs. In some cases, including height, yellowness, and visual appeal of the yolk, ozone-pasteurized eggs were perceived equivalent to untreated eggs. In the taste test, there were no significant differences on liking score among all eggs for the overall visual, aroma, flavor, texture, and overall liking using scrambled eggs. Sensory test results indicate yolk quality has improved over previous pilot scale studies, where the yolk quality of the commercially ozone-pasteurized eggs is perceived equivalent to that of thermally pasteurized eggs. Ozone-pasteurized eggs had an average of 92.2 Haugh unit, to qualify as grade AA. This shell strength study shows that further work may apply different types and amounts of coating to the ozone-pasteurized eggs. It is suggested that ozone-pasteurized eggs, which have a significant benefit of reduced risk of Salmonella Enteritidis contamination, would be appropriate for in-home or institutional cooked egg recipes.

References


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Perry JJ. 2010. Ozone based treatments for inactivation of *Salmonella enterica* serovar Enteritidis in shell eggs [Dissertation]. Columbus, OH: The Ohio State University. 200 p. Available from The Ohio State University, Columbus, OH.


Chapter 3 - Efficacy and Consumer Acceptance of Two Types of Pasteurized Whole Shell Eggs in a Recipe Requiring Uncooked Eggs

Abstract

Prepared foods requiring fresh raw egg as an ingredient may be hazardous, thus pasteurization is required. Innovative ways to pasteurize whole shell eggs are available and were evaluated for impact on a raw egg recipe for consumer acceptance and functional properties. Two types of Parmesan Caesar dipping sauce were prepared with heat-ozone-pasteurized eggs processed with commercial scale equipment (which is referred as ozone-pasteurized eggs) and liquid pasteurized whole eggs. A 9-point hedonic scale degree of liking test was completed with attributes of overall liking, liking of flavor, parmesan flavor, saltiness, thickness, mouth feel, and aftertaste. Just-about-right (JAR) analysis with attributes of amount of flavor, saltiness, thickness, mouthcoat, and aftertaste were conducted. Instrumental analyses of color, pH, and consistency were performed. There were no significant differences in the a* value, pH, and consistency among all treatments. Ozone-pasteurized dressing was significantly darker (lower L* value) and more yellow (higher b* value) than liquid-pasteurized dressing (p < 0.05). From the JAR analysis, most panelists perceived both dressings to be just about right in all attributes. From the penalty analysis, the thicknesses of ozone-pasteurized dressings were perceived to be too thick with a significant aftertaste from the liquid-pasteurized dressing. There
was no significant difference (p > 0.05) between both dressings in all attributes in the
degree of liking test (n=101). These results suggest whole shell ozone-pasteurized eggs
are a safe and suitable alternative to raw eggs for use in a Parmesan Caesar dipping sauce
or other type of recipe containing this raw ingredient in home or industrial settings.

Keywords: Ozone, Egg, Sensory, Acceptance, Pasteurization

Introduction

Salmonella Enteritidis has been associated with foodborne illnesses caused by egg
consumption. It is estimated that there are about 200,000 illnesses of Salmonella
Enteritidis contaminated eggs annually (Schroeder and others 2005). According to the
Centers for Disease Control and Prevention, about 3,000 Americans die per year of
foodborne diseases, nearly 130,000 are hospitalized, and 48 million who get sick (CDC
2011). In 2010, about 1,939 illnesses were associated with a multistate outbreak of
Salmonella Enteritidis, which was linked with the consumption of shell eggs (CDC
2010). On June 24, 1995, twenty-six people became ill due to consumption of Caesar
salad dressing in a wedding party in New York. Egg was the contaminated ingredient in
the dressing (CDC 1996). Data collected from 1985-1999 shown 28% or 67 out of 243
foods tested were contaminated with Salmonella Enteritidis in the United States
contained raw eggs and Caesar salad dressing is among those foods (Patrick and others
2004). Homemade mayonnaise contains raw eggs, which are associated with many
Salmonella outbreaks in different countries such as the United States, the United
Kingdom, Australia, Denmark, and Germany (Heredia and others 2009). Since raw egg
yolk consumption is linked to *Salmonella* outbreaks, an alternative is needed for recipes that need raw eggs.

The outbreaks noted here were mainly associated with homemade dressing or mayonnaise. Commercially produced, undiluted mayonnaise or salad dressing will not support the survival of *Salmonella* spp. due to the amount of acid level in these products (Smittle 1977). However, consumers or foodservice industries such as hospitals, restaurants, and institutions, do not routinely check the pH or the acid level in the formulated mayonnaise or salad dressings. Thus, it is important that the eggs used in those recipes be pasteurized. FDA recommended that pasteurized eggs be used when preparing food containing raw or undercooked eggs. Foodservice industries must inform their consumers regarding the use of raw or undercooked eggs through brochures, flyers, and other effective written communication (FDA 2002).

Different ingredients such as succinylated whey concentrate, whey protein concentrate, modified cornstarch, have been used in salad dressing or mayonnaise as egg substitutes and no significant differences in consumer acceptability were documented (Herald and others 2009; Thompson and Reniers 1982). Consumers and food service industries typically use liquid pasteurized eggs and dried egg products in their recipes that use raw eggs (FSIS 2011). Although liquid pasteurized eggs are able to produce good quality salad dressing, consumers would have to buy two different type of eggs (in-shell and liquid) to produce variety types of food such as mayonnaise (using liquid eggs) and sunny side up egg (using in-shell eggs). Thus, it is more convenient for consumers to use in-shell pasteurized eggs to be used in different applications.
In 2010, Egg Safety Rule, which is mandated by the FDA, requires egg producers to implement preventative measures to prevent foodborne illnesses. This rule has led to development of many pasteurization techniques for shell eggs. Researchers have been developing many methods to achieve a 5-log reduction (to achieve pasteurization) of *Salmonella* Enteritidis in shell eggs such as irradiation, microwave, UV, thermal pasteurization, and heat-ozone-pasteurization of (Tellez and others 1995; Lakins and others 2008; Davidson 2004; Perry 2010).

Studies have been conducted at The Ohio State University on the technology of using heat and ozone combination to pasteurize shell eggs. Ozone usage as a disinfectant has been employed since the 1800s. In 1893, scientists in the Netherlands discovered the use of ozone as a chemical disinfectant in drinking water (Weavers 2001). In 2001, FDA approved use of ozone in the treatment, storage, processing of foods (including meat and poultry), and as secondary food additive in gaseous or aqueous phases, functioning as an antimicrobial agent, in accordance with current industry standards of good manufacturing practices (FDA 2001). Previous study in this laboratory has shown that heat-ozone-pasteurized eggs, which will subsequently be referred to ozone-pasteurized eggs, are liked similarly than thermally pasteurized and untreated eggs in a consumer acceptability test using scrambled eggs. Thus, this study is conducted to investigate the consumer acceptability of ozone-pasteurized eggs in a mayonnaise or Caesar dressing matrix. A Caesar dressing matrix, which will be called Parmesan Caesar dipping sauce due to its thickness, made with ozone-pasteurized eggs and liquid pasteurized eggs were used in this study. It was hypothesized that both sauces made with ozone-pasteurized and liquid
pasteurized eggs would be liked similarly. Thus, the objective of this study is to conduct consumer acceptance test and functionality of food formulated with ozone-pasteurized eggs, such as Parmesan Caesar dipping sauce in comparison to that made with liquid pasteurized eggs to understand whether ozone-pasteurized eggs are an acceptable alternative in a recipe that uses raw eggs. Instrumental analyses of pH, color, and consistency will be conducted to evaluate the physical properties.

**Materials and Methods**

*Consumer Acceptance Test*

The sauces were made two days before test day and both ozone-pasteurized (OP) and liquid pasteurized (LP) sauces were made using the same recipe shown in Table 3.1. Frozen liquid pasteurized eggs (Gordon Food Service, Grand Rapids, MI) were obtained from a local grocery store and thawed under refrigeration temperature (4°C) until needed. All ingredients except vegetable oil were blended in a Waring blender (Torrington, CT) for 10 seconds. Vegetable oil was added and the emulsion was blended for 50 seconds. The sauces were placed in closed plastic containers and stored in a walk-in refrigerator (Bally Refrigerated Boxes, Inc, Morehead City, NC) (4°C) overnight. Then, about 21 g of the cooled dressings was scooped into a 57 g plastic cup with lid (Gordon Food Service, Grand Rapids, MI) labeled with 3-digit randomized numbers. The portioned cups were stored in a walk-in refrigerator (4°C) overnight. A 10 cm fresh celery stick was placed on top of a white napkin next to the dressing cup as a vehicle for the sauces.
Table 3.1 - Formula of Parmesan Caesar dipping sauce.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Source</th>
<th>Amount (% by wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ozone-pasteurized whole shell eggs*</td>
<td>Local Ohio farms</td>
<td>21</td>
</tr>
<tr>
<td>Frozen liquid pasteurized eggs*</td>
<td>Gordon Food Service (Grand Rapids, MI)</td>
<td>21</td>
</tr>
<tr>
<td>Grated parmesan cheese</td>
<td>The Kroger Co. (Cincinnati, OH)</td>
<td>18</td>
</tr>
<tr>
<td>Lemon juice</td>
<td>The Kroger Co. (Cincinnati, OH)</td>
<td>12</td>
</tr>
<tr>
<td>Minced garlic in oil</td>
<td>The Kroger Co. (Cincinnati, OH)</td>
<td>4.2</td>
</tr>
<tr>
<td>Worcestershire sauce</td>
<td>The Kroger Co. (Cincinnati, OH)</td>
<td>1.9</td>
</tr>
<tr>
<td>Salt</td>
<td>The Kroger Co. (Cincinnati, OH)</td>
<td>1.2</td>
</tr>
<tr>
<td>Pepper</td>
<td>The Kroger Co. (Cincinnati, OH)</td>
<td>0.69</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>The Kroger Co. (Cincinnati, OH)</td>
<td>41</td>
</tr>
</tbody>
</table>

*Ozone-pasteurized eggs grade AA large laid by hens between 32 and 45 weeks of age. Frozen liquid pasteurized eggs were obtained 2 days before test day. All eggs were held at refrigerated temperature of 4°C until use.

The consumer acceptance tests were performed at The Ohio State University Food Science Parker Building Sensory Analysis Laboratory (Columbus, OH). In the facility, there were 10 booths that were illuminated by white fluorescent light and temperature was set at 21°C. All answers were collected using Compusense® version 5.2 software (Guelph ON, Canada) with a mouse and color LCD monitor interface used by each panelist. The test was conducted in one sitting in a complete balanced design, serial monadic approach for both salad dressings. Filtered spring water and unsalted crackers (The Kroger Co., Cincinnati, OH) were used to cleanse pallet in between samples in the taste test. Figure 3.1 is the sauce presentation and Figure 3.2 compares the appearance of two sauces.
Figure 3.1 – Sample presentation of Parmesan Caesar dipping sauce.

Figure 3.2 – Liquid-pasteurized and ozone-pasteurized Parmesan Caesar dipping sauces.

One-hundred-one untrained panelists (83 female and 18 male) were recruited and were instructed to individually rate the samples for degree of liking on a 9-point hedonic scale, where 9= like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=like a little, 4=indifferent, 3=dislike a little, 2=dislike moderately, 1=dislike very much, and 0=dislike extremely.
5=neither like or dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, and 1=dislike extremely. The attributes tested were overall liking, liking of flavor, Parmesan flavor, saltiness, thickness, mouth feel, and aftertaste. The panelists were instructed to rate the amount of flavor, saltiness, thickness, mouth coat, and aftertaste on a 5-point category just about right scale (5=much too much, 4=slightly too much, 3=just about right, 2=slightly too little, 1=much too little). Finally, demographic questions were asked of each panelist (age, gender, egg usage per week- not in baking, and race).

**Instrumental Analysis**

Instrumental analyses of pH, color, and consistency were conducted on the sauces. Five replicates of pH measurement were conducted using pH meter (Model 430, Corning, NY). Five replicates of value, hue, and chroma, represented as L*, a*, b* respectively were measured using a handheld colorimeter (Model C-300, Konica Minolta, Tokyo, Japan). Sauce consistency was measured on four replicates using a Bostwick consistometer (CSC Scientific Company Inc, Fairfax, VA) at 9°C for 30 seconds.

**Statistical analysis**

Significant difference analysis on the degree of liking test and JAR score were performed using repeated measures ANOVA and Tukey’s HSD post hoc analysis at p<0.05 generated from Compusense software. Penalty score of JAR analysis was conducted using Microsoft Excel 2007(Microsoft, Redmond, WA). Significant difference
analysis on the instrumental analysis was performed using independent T-test generated from SPSS Statistics version 16.0 software (SPSS Inc., Chicago, Ill).

**Results and Discussion**

There was no significant difference in the consistency of both sauces (p > 0.05), as shown in Table 3.3. In color measurement, L* indicates the lightness of the color, (0 = black and 100 = white), while a positive a* value is red, and a* negative value is green. A positive b* value is yellow and a negative b* value is blue. There was no significant difference in the a* value of both sauces (p > 0.05). OP sauce was significantly darker (lower L* value) and more yellow (higher b* value) than LP sauce (p < 0.05), shown in Table 3.2.

There was no significant difference on the pH of both sauces (p > 0.05). Smittle (2000) stated that according to a survey conducted by The Association for Dressings and Sauces members, the maximum pH for commercialized salad dressings in the United States is 4.4 (Smittle 2000). In the United States, *Salmonella* is not a concern in commercialized dressings if the dressings are made with acetic acid with a minimum pH of 4.7 and titratable acidity of 0.40% of the acetic acid in the water phase. If the eggs that are used in the dressings are contaminated with *Salmonella*, the acetic acid in the dressings will rapidly kill the *Salmonella* (Smittle 2000). However, in a restaurant or homemade setting, pH meter is not used to check the acidity level of the acetic acid (vinegar) and the pH of the dressings. Some restaurants do not use vinegar and instead use lemon juice for flavor and acidity. Thus these conditions may allow the survival of *Salmonella* if the eggs are contaminated. Therefore, using pasteurized eggs, such as
ozone-pasteurized eggs, and storage at refrigeration temperature are imperative to control the growth of *Salmonella* in home-made dressings. Shown in Figure 3.3, consumers have accepted ozone-pasteurized eggs to be used in the Parmesan Caesar dipping sauce, where there was no significant difference compared to the sauce made with liquid pasteurized eggs, which is currently used in restaurants to substitute raw eggs in dressing recipes.

Table 3.2– Instrumental analyses of ozone-pasteurized and liquid-pasteurized Parmesan Caesar dipping sauce.

<table>
<thead>
<tr>
<th>Physical properties</th>
<th>Replicates</th>
<th>Ozone</th>
<th>Liquid-Pasteurized</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>5</td>
<td>77.4 ± 0.360a</td>
<td>80.5± 0.100b</td>
</tr>
<tr>
<td>a*</td>
<td>5</td>
<td>-1.15 ± 0.140a</td>
<td>-1.13 ± 0.260a</td>
</tr>
<tr>
<td>b*</td>
<td>5</td>
<td>25.2 ± 0.480a</td>
<td>22.6 ± 0.170b</td>
</tr>
<tr>
<td>pH</td>
<td>5</td>
<td>4.09 ± 0.0242a</td>
<td>4.07 ± 0.0396a</td>
</tr>
<tr>
<td>Consistency</td>
<td>4</td>
<td>7.9± 0.36a</td>
<td>8.6 ± 0.31a</td>
</tr>
</tbody>
</table>

\(^1\)Tristimulus colorimeter measures of value, hue and chroma as L*, a*,b* respectively. Bostwick consistometer measures consistency at 30 seconds, data presented in centimeters.  
\(^a,b\) Mean ± standard error of mean. Within each row, same superscript on each mean indicates no significant difference at p > 0.05.

*Taste Test*

Panelists were surveyed regarding their typical egg usage; 25% of the panelists used 3 eggs per week, while 14% used 4 - 5 eggs per week. When asked about dipping sauce used most often, 40% of the panelists used ranch, 26% used Italian and 17% used Caesar. The panelists were representative of OSU population with 35% being 56-65 years
old, 21% being 46-55 years old, 10% being 36-45, 15% being 24-35 and 16% being 21-25 years old.

Despite some differences in the physical properties as shown in Table 3.3, both OP and LP sauces were liked similarly with an average rating of 6.4 (like slightly) for all attributes overall, flavor, parmesan flavor, saltiness, thickness, mouthfeel, and aftertaste liking (Figure 3.3). This indicates no significant difference in the liking score across all samples in all attributes, which supports the hypothesis ($p > 0.05$).

The JAR scale was used in this study to determine reasons for any preference or rejection of products by asking consumers additional questions about the product attributes (Meilgaard and others 1999). For the Just-about-right (JAR) questions (Figure 3.4), about 60% of panelists perceived the amount of flavor, parmesan flavor, saltiness, and thickness to be just about right for both sauces. When panelists were asked to rate the amount of mouthcoat on the JAR scale, OP sauce were rated slightly too much (18%) compared to the LP sauce (8%). In the aftertaste attribute, 74% of panelists perceived the aftertaste of OP sauce to be just about right, compared to 69% of panelists on the LP sauce.
Penalty score analysis was conducted for individual panelist JAR scores for each attribute. This is used to show which attributes should be modified to improve product acceptability. Adjustment needs to be made in the “too low” or “too high” category if the penalty score in that category is ≥ 0.5 or ≤ -0.5 (Meilgaard and others 1999). Thus, using the penalty score analysis (Table 3.4), LP sauce received ≥0.5 score of penalty score on the “too high” category in the aftertaste attribute, which suggests that there was too much aftertaste in the LP sauce. This result is similar to JAR percentage frequency rating, where 22% of panelists perceived the LP sauce to have a slightly too much aftertaste compared to the OP sauce (20% panelists). The differences in both penalty score and
JAR percentage frequency rating is not disproportionate from the just about right rating. The result was supported with the degree of liking test where there was no significant different in liking score between both sauces in the aftertaste attribute. Some differences occurred between the JAR percentage frequency rating and penalty analysis result in the thickness attribute. In the JAR percentage frequency rating, about 61% of panelists perceived the thickness of the OP sauce to be just about right and 63% of panelists perceived the LP sauce to be just about right. However, the penalty score analysis showed that OP sauce obtained ≥ 0.5 score in the “too high” category, which suggests that the sauce was too thick. The Bostwick measurement shows that there was no significant difference in the consistency, which can be correlated to the thickness of the sauces (Table 3.3). Regardless, OP and LP sauces were not significantly different in regards to their thickness in the degree of liking scale and OP sauce received a higher mean score of liking at 6.5 compared to LP sauce at 6.4.

Table 3.3– Penalty analysis for JAR scale of Parmesan Caesar dipping sauce (n=101) Red color text show those adjustment needs to be made in those category.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Ozone-pasteurized</th>
<th>Liquid-pasteurized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Too low</td>
<td>Too high</td>
</tr>
<tr>
<td>Flavor</td>
<td>0.28</td>
<td>0.19</td>
</tr>
<tr>
<td>Parmesan Flavor</td>
<td>0.37</td>
<td>0.26</td>
</tr>
<tr>
<td>Saltiness</td>
<td>0.084</td>
<td>0.41</td>
</tr>
<tr>
<td>Thickness</td>
<td>0.037</td>
<td>0.70</td>
</tr>
<tr>
<td>Mouthfeel</td>
<td>0.052</td>
<td>0.19</td>
</tr>
<tr>
<td>Aftertaste</td>
<td>0.079</td>
<td>0.40</td>
</tr>
</tbody>
</table>
Conclusions

Although there were some differences in the physical properties between both sauces, such as the L* value and b* value, consumers liked both sauces equally for all attributes tested. From the JAR analysis, most panelists perceived both sauces to be just about right in all attributes. Penalty analysis suggested that OP sauce was too thick and the aftertaste of the LP sauce was too much. However, there was no significant difference on the liking for both sauces in all attributes. Further research is needed in testing the performance of ozone-pasteurized eggs in different raw egg recipes and different cooked applications, such as baking recipes. It is suggested that ozone-pasteurized eggs, which have an added benefit of reducing the risk of Salmonella Enteritidis contamination compared to raw eggs would be a safe and suitable alternative to raw shell eggs for use in home-made Parmesan Caesar dipping sauce or other types of recipes that use raw eggs.

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Perry JJ. 2010. Ozone based treatments for inactivation of Salmonella enterica serovar Enteritidis in shell eggs [Dissertation]. Columbus, OH : The Ohio State University.200 p. Available from The Ohio State University, Columbus, OH.


Chapter 4 - Consumer Acceptance of Raw or Pasteurized Eggs in a Food where Egg is an Important Functional Ingredient

Abstract
A consumer acceptance test of a food requiring egg as a functional ingredient was conducted. Angel food cake was selected for its dependence upon the egg ingredient for emulsification or its foam forming function. Cake prepared with commercially ozone-pasteurized eggs (OP), thermally pasteurized eggs (TP) and untreated eggs (UT) were compared. A 9-point hedonic scale degree of liking test was completed with attributes appearance, pores, color, overall, sweetness, and texture (springiness). Just-about-right (JAR) analyses with attributes of amount of pores, color, sweetness, and texture (springiness) were conducted. Instrumental analysis included color, pore formation, whipping time of egg whites, and cake volume. OP exhibited significantly shorter whipping time than TP and both OP and TP had significantly longer whipping time than UT (p<0.05). There was no significant difference in all texture properties and cake volume (p>0.05). There was no significant difference in colors except the bottom crust of UT had significantly less red color than TP (p <0.05), but were not different than OP (p >0.05). The top crusts of UT were less red and less yellow than OP and TP (p <0.05).

The pore size of UT appeared smaller than TP and OP, but UT and TP were not significantly different (p>0.05). Just-about-right (JAR) and penalty score analysis results indicates that springiness of all cakes rated just about right, while attributes such as pores, color, and sweetness results varied. For example: in the penalty analysis, all cakes were
rated as just about right, while in the JAR result, only TP and OP were rated as just about right. There was no significant difference (p > 0.05) among all cakes in the degree of liking test (n=107). OP has faster whip times than TP but longer than UT and OP is suitable for use in angel food cake in home or industrial settings.

Keywords: Ozone, Egg, Sensory, Acceptance, Pasteurization

**Introduction**

*Salmonella* Enteritidis has been associated with foodborne illnesses caused by egg consumption (Cowden and others 1989; CDC 1996; Mohle-Boetani and others 1998; Heredia and others 2009; Laestadius and others 2012). It is estimated that there are about 200,000 illnesses of *Salmonella* Enteritidis contaminated eggs annually (Schroeder and others 2005). Thus, in 2010, FDA has mandated egg producers comply with the final Egg Safety Rule to implement safety measures, requiring refrigeration during storage and transportation (FDA 2010). This rule has led to a development of many pasteurization techniques for shell eggs. Researchers have been developing many methods to achieve a 5-log reduction (to achieve pasteurization) of *Salmonella* Enteritidis in shell eggs such as irradiation, microwave, UV, thermal pasteurization, and heat-ozone-pasteurization (Tellez and others 1995; Lakins and others 2008; Davidson 2004; Perry 2010).

The Ohio State University have been conducting studies on the technology of using heat and ozone combination to pasteurize shell eggs, which will be referred to ozone-pasteurized eggs. Previous studies in this laboratory have been conducted on the quality, functionality, and consumer acceptance study in scrambled eggs and Caesar
dressing. No study has been made on ozone-pasteurized eggs in baking applications, where egg is an important functional ingredient.

Egg whites mainly consist of protein, which is denatured by heat. The protein is used to disperse large volumes of air into the batter while whipping and stabilize the uniformly air cells during baking (Arunepanlop and others 1996). The free sulfhydryl groups in the egg protein are highly susceptible to cross-linking when they are denatured (Wilderjans and others 2013). The cross-linking is important to trap the air while mixing to create foam, which is an important element for baked goods. Due to the heat in the ozone-pasteurization method, the foaming property of the egg white is measured. Angel food cake, which consists of sugar, flour, and egg whites, is widely used as a model system to test egg functionality. The structure of the foam in angel food cake is a main factor to obtain desire volume and stability of the cake (Ziedler, 2002).

Other studies have used other types of eggs or egg products in angel food cakes to investigate the functionality of the eggs. Irradiated frozen egg whites were compared with heat pasteurized frozen egg products on the cake volume of angel food cakes and no significant difference was found (Ma and others 1993). Increased whipping time and decreased foam stability were detected in irradiated liquid and frozen egg whites (Ma and others 1993). The functionality of thermally pasteurized eggs is controversial. The ability to foam decreased and whip time increased and whip volume decreased (Cunningham 1995; Schuman and others 1997; ICMSF 1980), but another study showed that the foaming ability and stability in thermal pasteurized eggs increased because of the
unfolding of the protein and the increase in surface hydrophobicity (Hou and others 1996).

Thus, angel food cake is used to measure the functional properties of ozone-pasteurized eggs (OP), thermally pasteurized shell eggs (TP) and untreated eggs (UT). Based on preliminary work, it was hypothesized that cakes made with OP, TP, and UT would be liked similarly. It was hypothesized that UT would be a better functional ingredient compared to OP, but OP would be a better functional ingredient compared to UT. The objective of this study was to evaluate consumer acceptance of egg functional properties using angel food cake made with OP, TP, and UT. Instrumental analyses of color, pore size, whipping time of egg whites, and cake volume evaluated physical properties.

Materials and Methods

Consumer Acceptance Test

Angel food cakes made with untreated eggs, thermally pasteurized eggs, and ozone-pasteurized eggs were baked two days before the sensory test. The sensory tests were performed in two baking days with 4 replicates per treatment per day. All cakes were made using same recipe shown in Table 4.1. Egg whites were separated from the yolks. Ozone-pasteurized and thermally pasteurized eggs required 13 eggs to yield 372 g due to the stickiness of the egg white to the vitelline membrane, while untreated eggs only required 12 eggs. The eggs were at about 4°C when used. Egg white temperature did not significantly influence angel food cake volume, but it is suggested to use eggs under 4°C temperature to reduce potential micro growth (Oldham and others 2000).
Confectioner sugar, flour, and salt were sifted one time using a stainless steel flour sifter (Baker’s Secret, World Kitchen, Rosemont, IL). The egg whites and cream of tartar were whipped using an Ultra Power Kitchen Aid stand up mixer (Model KSH90PSWH, St. Joseph, MI, USA) with a stainless steel mixing bowl (Kitchen Aid Model K45, St. Joseph, MI, USA) and a whisk attachment (Kitchen Aid Model K45WW, St. Joseph, MI, USA) at speed 6 until the foam reached medium peak. Cream of tartar was added to lower the pH and increase foam stability (Oldham and others 2000). The mixing time, which is indicated in Table 4.2, varies for each treatment. After the medium peak was formed, sugar was added at the rate of one tablespoon at a time until stiff peak was formed, which took 1 minute and 30 seconds at speed 6. Then, vanilla was added and mixed at speed 6 for 3 seconds. The flour mixtures were added at about 32 grams at a time and folded using a large spatula. Then, the batter was placed into a non-stick angel food cake pan with feet (Chicago Metallic, Lincolnshire, IL). A butter knife was used to cut through the batter to remove air pockets. All batters were baked at 177°C for 46 minutes in conventional ovens. After 22nd minutes, each pan was turned halfway to ensure uniform heat was transferred into all sides of the pan. There were three ovens used to bake the cakes, a double oven (Model FGEF306TMF Frigidaire gallery, Augusta, GA), which has two levels of individual oven: upper level (Oven A) and lower oven (Oven B), and a single oven (Oven C) (Model WOS92EC7AS Whirlpool Gold Series, Benton Harbor, MI). Two pans were placed in the oven at one time to produce more cakes in one day. In order to reduce variability, cakes from each treatment were baked in every oven,
shown in Table 4.3. After baking is completed, the pans were inverted and cooled overnight in a 21°C room.

In the morning of the sensory test day, all cakes were taken out from the pans using a butter knife. Physical measurements of cake volume and color were obtained (discussed in the “Instrumental Analysis” section). Cakes were sliced and cut using a 23 cm electric knife (Black&Decker, Toroson, Maryland) on top of a gridded 45 cm x 30 cm cutting mat (Scrappin-Gear, Multi-Craft Imports, Inc, Ottawa, ON, Canada). The mat was used to aid in obtaining uniform size of cakes. Four slices (25 mm thick) from the middle of each cake were obtained for visual test. Each slice of cake was placed in a clear Ziploc bag (Gordon Food Service, Grand Rapids, MI) and labeled with a 3-digit randomized numbers. Then, the rest of the cake were cut into a 25 mm cube and placed into a Ziploc bag (Gordon Food Service, Grand Rapids, MI) until the time of the sensory test. Two pieces of the 25 mm cube cakes were placed in a 56.9 g plastic cup (Gordon Food Service, Grand Rapids, MI) labeled with 3-digit randomized numbers.

The consumer acceptance test was performed at The Ohio State University Food Science Parker Building Sensory Analysis Laboratory (Columbus, OH). In the facility, there were 10 booths that were illuminated by white fluorescent light and temperature was set at 21°C. All answers were collected using Compusense® version 5.2 software (Guelph ON, Canada) with a mouse and color LCD monitor interface used by each panelist. The test was conducted in two sittings in a complete balanced design, serial monadic approach for all cakes. First, sliced cakes were presented for visual test, then two pieces of 25 mm cubes were presented for taste test. Filtered spring water was used
to cleanse pallet in between samples. Figure 4.1 shows a picture of the sample presentation for the visual test and Figure 4.2 shows a picture of the sample presentation for the taste test.

Table 4.1 - Formula of angel food cake

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Source</th>
<th>Amount (% by wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg white*</td>
<td>*</td>
<td>42</td>
</tr>
<tr>
<td>Confectioner’s sugar</td>
<td>Giant Eagle (Pittsburgh, PA)</td>
<td>18</td>
</tr>
<tr>
<td>All-purpose flour</td>
<td>Giant Eagle (Pittsburgh, PA)</td>
<td>15</td>
</tr>
<tr>
<td>Cream of tartar</td>
<td>Private Selection (Cincinnati, OH)</td>
<td>1</td>
</tr>
<tr>
<td>Pure vanilla extract</td>
<td>Giant Eagle (Pittsburgh, PA)</td>
<td>1</td>
</tr>
<tr>
<td>Salt</td>
<td>Morton (Chicago, IL)</td>
<td>0.1</td>
</tr>
<tr>
<td>Sugar</td>
<td>Valutime (Skokie, IL)</td>
<td>23</td>
</tr>
</tbody>
</table>

*OP and UT eggs grade AA large laid by hens between 32 and 45 weeks of age were obtained from local Ohio farms. TP eggs were obtained from local grocery stores 5 days before test day. All eggs were held at refrigerated temperature of 4°C until use.

Table 4.2 - Whipping time of egg white until medium peak prior to sugar addition.

<table>
<thead>
<tr>
<th>Whip time (minutes)</th>
<th>Ozone (n=5)</th>
<th>Thermal (n=5)</th>
<th>Untreated (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.70±0.200b</td>
<td>10.3±0.515c</td>
<td>2.50±0.000a</td>
</tr>
</tbody>
</table>

Mean ± standard error of mean. Within each row, same superscript on each mean indicates no significant difference at p > 0.05.

Table 4.3- Combination of cake location in ovens to reduce oven variability.

<table>
<thead>
<tr>
<th>Oven</th>
<th>Day 1</th>
<th>Day 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Ozone</td>
<td>Untreated</td>
</tr>
<tr>
<td>B</td>
<td>Untreated</td>
<td>Thermal</td>
</tr>
<tr>
<td>C</td>
<td>Thermal</td>
<td>Ozone</td>
</tr>
</tbody>
</table>
Figure 4.1 – Sample presentation for visual test of angel food cakes.

Figure 4.2 – Sample presentation for taste test of angel food cakes.

One-hundred seven untrained panelists (74 female and 26 male) were recruited and were instructed to individually rate the samples for degree of liking on a 9-point hedonic scale, where 9= like extremely, 8=like very much, 7=like moderately, 6=like slightly, 5=neither like or dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, and 1=dislike extremely. For the visual test, the attributes were appearance liking, air bubbles liking, and color liking. For the taste test, the attributes were overall liking,
sweetness liking, texture (springiness) liking. The panelists were instructed to rate the amount of the attributes on a 5-point category just about right scale for air bubbles (5=way too much, 4=slightly too much, 3=just about right, 2=slightly too little, 1=way too little), color (5=way too dark, 4=slightly too dark, 3=just about right, 2=slightly too light, 1=way too light), sweetness (5=way too much, 4=slightly too much, 3=just about right, 2=slightly too little, 1=way too little), and texture (springiness) (5=way too light, 4=slightly too light, 3=just about right, 2=slightly too dense, 1=way too dense).

Comments were asked at the end of visual and taste test. Finally, demographic questions were asked of each panelist (age, gender, egg usage per week- not in baking, and race, type of angel food cake you eat, reasons of why don’t you make homemade cake, and reservations of using eggs in general).

**Instrumental Analysis**

Instrumental analyses of cake volume, color, texture and pore measurements were conducted on the cakes. In the instrumental analysis section, “air bubbles” terms are described as “pores” to better explain 2 dimensional images instead of “air bubbles” that may be misinterpreted as 3D images. Cake volume and color measurements were obtained in the morning before the sensory panel. Four replicates (one from each cake) of cake volume measurements were conducted using American Association of Cereal Chemists (AACC) method 10-15 (AACC 2000). Twenty replicates (5 from each cake per treatment) of value, hue, and chroma, represented as L*,a*,b* respectively of the inner cake, top crust, side crust, and bottom crust were measured using a handheld colorimeter (Model C-300, Konica Minolta, Tokyo, Japan).
Texture characteristic measurements were obtained within 24 hours of the sensory panel. Twenty replicates (5 from each cake per treatment) of hardness and springiness were measured using a Universal Testing Machine (Instron Corp., Norwood, MA, U.S.A.) and the AACC method 74-09 (AACC 2000). The same samples that were used for the taste test (25 mm cube) were used for the Instron measurement. A compression method (applied with a 35 mm diameter probe) with a speed of 100 mm/min resulting force (N) required for 40% compression was used. The instrument software (Bluehill® 2 version 2.17, Instron Corp., Norwood, MA, U.S.A.) was used to automatically record the measurements. Definition and explanation of texture characteristics from the TPA curve used in the Instron machine was adapted from Meullenet and others (1998). Hardness, which was the maximum load that was applied to the cake during first compression, mimics the force required for human to bite the cake completely when the cake was placed between molars. Springiness, which was the ratio of the time of contact with the cake during second compression to the time of contact during first compression, indicates the degree where the cake returns to its original shape after fractional compression between tongue and mouth ceiling (Meullenet and others 1998).

Cake crumb images were obtained by scanning sliced cakes using a Canon MX850 flatbed scanner (Melville, NY, USA) with a method adapted from Tlapale-Vladivia and others (2010). Brightness and contrast of the images were not changed per the scanner default parameter. Images were saved as jpeg files with a 300 DPI resolution in grey scale color. A ruler was scanned next to the cake for scale calibration purposes. Using ImageJ software (Maryland, USA), images were cropped into 1260x1260 pixels to
zoom into the cake slices. Scale was calibrated using the ruler for mm measurements. A 25x25 mm field of view was placed in the center of each cake. The image was duplicated and manually threshold. The threshold intensity for grey scale polarization into binary images was set at 180. Particle size was analyzed to obtain the area of the pores. Each cake treatments were measured in four replicates.

**Statistical analysis**

Significant difference analysis on the degree of liking test and JAR score were performed using repeated measures ANOVA and Tukey’s HSD post hoc analysis at p < 0.05 generated from Compusense software. Penalty score of JAR analysis was conducted using Microsoft Excel 2007 (Microsoft, Redmond, WA). Significant difference analysis on the instrumental analysis was performed using independent one way ANOVA and nested ANOVA at p < 0.05 generated from SPSS Statistics version 16.0 software (SPSS Inc., Chicago, Ill).

**Results and Discussion**

**Instrumental analysis**

There was no significant difference in all of the texture attributes as shown in Table 4.4. This is interesting because TP were thought to be harder or denser than UT due to the denaturation of the egg protein that caused less air to expand. Moreover, in the consumer acceptance test, shown in Figure 4.5, there was no significant difference in the liking of the texture for all treatments.

In the color measurement, L* indicates the lightness of the color, (0 = black and 100 = white), while a positive a* value is red, and a* negative value is green. A positive
b* value is yellow and a negative b* value is blue. There was no significant difference in all of the colors measurement except for the bottom a* value, top a* value, and top b* value. Indicated in table 4.4, the bottom crust UT had significantly less red color than TP (p <0.05), but were not significantly different than OP (p >0.05). The top crusts of UT were less red and less yellow than OP and TP (p <0.05). Despite the differences on some of the cake colors, indicated in Figure 4.3, OP had the highest liking rating of 6.7 on colors compared to the other treatments, but there was no significant different on the liking of the cake colors among all three treatments (p >0.05).

<table>
<thead>
<tr>
<th></th>
<th>Ozone (n=20)</th>
<th>Thermal (n=20)</th>
<th>Untreated (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner L*</td>
<td>80.2 ± 0.290a</td>
<td>79.6 ± 0.307a</td>
<td>80.1 ± 0.414a</td>
</tr>
<tr>
<td>Inner a*</td>
<td>-2.00 ± 0.0566a</td>
<td>-1.99 ± 0.0536a</td>
<td>-1.99 ± 0.0329a</td>
</tr>
<tr>
<td>Inner b*</td>
<td>12.4 ± 0.122a</td>
<td>12.3 ± 0.105a</td>
<td>12.0 ± 0.149a</td>
</tr>
<tr>
<td>Bottom L*</td>
<td>49.3 ± 1.68a</td>
<td>49.4 ± 1.323a</td>
<td>50.6 ± 1.23a</td>
</tr>
<tr>
<td>Bottom a*</td>
<td>16.2 ± 0.459ab</td>
<td>16.6± 0.342a</td>
<td>15.4 ± 0.232b</td>
</tr>
<tr>
<td>Bottom b*</td>
<td>39.4 ± 0.412a</td>
<td>38.7 ± 0.799a</td>
<td>38.2 ± 0.443a</td>
</tr>
<tr>
<td>Side L*</td>
<td>56.6 ± 0.909a</td>
<td>54.5 ± 1.07a</td>
<td>54.3 ± 0.805a</td>
</tr>
<tr>
<td>Side a*</td>
<td>14.0 ± 0.427a</td>
<td>14.9 ± 0.406a</td>
<td>15.0 ± 0.335a</td>
</tr>
<tr>
<td>Side b*</td>
<td>39.8 ± 0.534a</td>
<td>40.2 ± 0.506a</td>
<td>39.2 ± 0.365a</td>
</tr>
<tr>
<td>Top L*</td>
<td>68.9 ± 0.524a</td>
<td>66.4 ± 0.541a</td>
<td>68.6 ± 1.56a</td>
</tr>
<tr>
<td>Top a*</td>
<td>8.19 ± 0.434a</td>
<td>9.50 ± 0.199c</td>
<td>6.96 ± 0.222b</td>
</tr>
<tr>
<td>Top b*</td>
<td>31.6 ± 0.517a</td>
<td>31.7 ± 0.189a</td>
<td>28.7 ± 0.382b</td>
</tr>
<tr>
<td>Springiness</td>
<td>7.35 ± 0.074a</td>
<td>7.34 ± 0.0795a</td>
<td>7.32 ± 0.0636a</td>
</tr>
<tr>
<td>Hardness</td>
<td>1.63 ± 0.0735a</td>
<td>1.54 ± 0.0604a</td>
<td>1.62 ± 0.111a</td>
</tr>
</tbody>
</table>
Indicated in Table 4.2, the whipping time for all treatments was significantly different. UT eggs took significantly shorter time than OP and TP eggs, while TP eggs took the longest. This may be caused by the globulin proteins, which are sensitive to heat. Egg whites consisted of more than 40 different proteins (Mine and others 2002). Different proteins have different functions and degree of heat sensitivity, such as the globulin fractions which are a good foaming agent and are heat labile (Powrie and Nakai 1986). Both OP and TP were treated with heat during the egg production process, where TP eggs received only heat treatment compared to OP with combination of heat and ozone, acquiring less heat than the thermal only process. This may explain the significantly shorter whipping time of OP than TP (p<0.05), which is an advantage over the TP in baking applications and supported the hypothesis. Perry and others (2011) reported heat-ozone and heat only pasteurization treatment in shell eggs took longer to create foam compared to untreated eggs.

Although the whipping times of the cakes were significantly different, the volume of the cake indicated in Table 4.5, was not significantly different among all treatments (p>0.05). The ovomucin protein in the egg white may cause this. Ovomucin, a glycoprotein, is a good foam stabilizer and is resistant to heat (Powrie and Nakai 1986; Mine 1995). Interactions of different egg white proteins such as lysozyme-ovomucin, which are both heat stable, also increase foam stability (Weijers and others 2006). After reaching the stiff peak foam formation, the stability of the foam is not an issue while it is baked because the ovomucin, which is responsible for foam stability is heat resistance. This may be a reason why the volume of the cake is not significantly different.
While making the cake batters, both OP and TP batters felt thinner than UT batters, which create more uniform top crust on the pan, shown in Figure 4.3. Shown in Figure 4.4, the pores on both OP and TP appeared larger than UT. This may be due to the ability of the pores on OP and TP to expand more than UT, which may explain the cake volume of both TP and OP would reach the same volume although the batter viscosity were thinner than UT.

Scanned images of the cakes in Figure 4.4 showed that UT appeared to have smaller pores but UT had some very large pores compared to other treatments. TP and OP appeared to have a uniform pore size but appeared to be larger than the pore size of UT. The pores size was measured using the median because the data for the pores size measurements were skewed. Statistical analysis (Table 4.5) shows that the pore size of UT were significantly smaller than TP (p<0.05), while the other two pairs of the treatments were not significantly different (p>0.05). This is a surprising result, because by inspection UT appeared different than OP.
Table 4.5 – Cake volume of angel food cake (in cm$^3$) and air pores size (mm$^2$) made with ozone pasteurized eggs (OP), thermally pasteurized eggs (TP), and untreated eggs (UT).

<table>
<thead>
<tr>
<th>Physical properties</th>
<th>Units and measurements</th>
<th>OP (n=4)</th>
<th>TP (n=4)</th>
<th>UT (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cake volume</td>
<td>cm$^3$, mean</td>
<td>2691±77.3$^a$</td>
<td>2549±15.3$^a$</td>
<td>2540 x10$^3$±39.4$^a$</td>
</tr>
<tr>
<td>Pores size</td>
<td>mm$^2$, median</td>
<td>0.071±0.014$^{ab}$</td>
<td>0.10±0.010$^b$</td>
<td>0.041±0.0095$^a$</td>
</tr>
</tbody>
</table>

$^{ab}$ Within each row, same superscript on each number indicates no significant difference at p > 0.05.

Figure 4.3- Angel food cakes made with ozone-pasteurized eggs (OP), thermally pasteurized eggs (TP), and untreated eggs (UT).
Figure 4.4- Magnified 1.6x images of angel food cakes made with ozone-pasteurized eggs (OP), thermally pasteurized eggs (TP), and untreated eggs (UT). Four replicates of each treatment were taken, shown in four images per treatments. Images were taken from the center of sliced cakes.
Consumer acceptance test

Panelists were surveyed regarding their typical egg usage; 24% of the panelists used 2 eggs per week, while 13% used 4 eggs per week. The panelists were representative of OSU population with 39% being 56-65 years old, 6% being 46-55 years old, 3% being 36-45, 20% being 26-35 and 19% being 21-25 years old.

Despite some differences in the physical properties as shown in Table 4.4 and 4.7, cakes were liked similarly with an average rating of 6.6 (like slightly) for all attributes: appearance, air bubbles, color, overall, sweetness, and texture (springiness) (Figure 4.5). This indicates no significant difference in the liking score across all samples in all attributes (p > 0.05), which supported the hypothesis.

JAR scale was used to determine reasons for any preference or rejection of products by asking consumers additional questions about the product attributes (Meilgaard and others 1999). Penalty score analysis was conducted for individual panelist JAR scores by each attribute, shown in Table 4.6. The penalty scores show which attributes can be modified to improve product acceptability. According to Meilgaard and others (1999) adjustment needs to be made in the “too low” or “too high” category if the penalty score in that category is ≥ 0.5 or ≤ -0.5.

In the Just-about-right (JAR) analysis, about 70% of panelists perceived the amount of air bubbles on the TP to be just about right, while 47% of panelists perceived the amount of air bubbles on OP to be just about right. When penalty score analysis was conducted, all cakes were perceived to be just about right.
Figure 4.5- Degree of liking attributes ratings of angel food cakes made with ozone-pasteurized eggs, thermally pasteurized eggs, and untreated eggs. There were no significant differences at p>0.05 for the degree of liking of all attribute tested. (n=107). Error bars indicate standard error of mean.

When panelists were asked to rate the amount of color on the JAR scale, 75% of the panelists rated the TP to be just about right, while 67% of the panelists rated the UT and 65% rated the OP to be just about right. Cake slices, including the top, side, and bottom crusts were given to the panelists when evaluating the color. This may influence the decision of the panelists regarding their color JAR score. Instrumental measures with a colorimeter indicate the top crust of UT were less red and less yellow than OP and TP, with no significant differences on the lightness of the cakes, where light and dark are the JAR scale description for color. Despite these differences, consumer liking of the color of the cakes were not significantly different among all treatments.
Differences occurred between the JAR% frequency rating and penalty analysis result in the sweetness attribute. In the JAR% frequency rating, about 63% of panelists perceived the sweetness of the UT to be just about right, 45% of panelists perceived the TP to be just about right and 56% of panelists perceived the TP to be just about right, indicating that TP were perceived to be less sweet than the others. However, the penalty score analysis showed that both OP and TP obtained ≥ 0.5 score in the “too low” category, suggesting both cakes were not sweet enough and UT to be just about right. Similarly with other attributes, there was no significant different in liking score among all cakes in the sweetness attribute.

In the springiness attribute, all cakes were rated similarly as just about right at about 60% of the panelists perceived all cakes to be just about right, which is supported by the penalty score, and about 27% of the panelists perceived all cakes to be slightly too dense.

Table 4.6– Penalty analysis for JAR scale of angel food cake made with untreated eggs (UT), thermally pasteurized eggs (TP), and ozone-pasteurized eggs (OP). Red color text show those adjustment needs to be made in those category.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Untreated</th>
<th></th>
<th>Thermal</th>
<th></th>
<th>Ozone</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Too low</td>
<td>Too high</td>
<td>Too low</td>
<td>Too high</td>
<td>Too low</td>
<td>Too high</td>
</tr>
<tr>
<td>Amount of air</td>
<td>0.43</td>
<td>-0.94</td>
<td>0.33</td>
<td>-0.71</td>
<td>0.20</td>
<td>-1.6</td>
</tr>
<tr>
<td>bubbles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Color</td>
<td>0.56</td>
<td>-0.61</td>
<td>0.23</td>
<td>-0.69</td>
<td>0.23</td>
<td>-0.91</td>
</tr>
<tr>
<td>Sweetness</td>
<td>0.35</td>
<td>-1.0</td>
<td>0.92</td>
<td>-1.1</td>
<td>0.65</td>
<td>-1.1</td>
</tr>
<tr>
<td>Springiness</td>
<td>0.14</td>
<td>-1.8</td>
<td>0.31</td>
<td>-1.3</td>
<td>0.34</td>
<td>-1.8</td>
</tr>
</tbody>
</table>
Demographic results show 74% of the panelists eat store bought cakes while only 19% of the panelists eat homemade cakes, where the top reason of not making home-made cake was that it takes too much time. This may be the reason of the liking score for all attributes were less than 7 (like moderately). Panelists were used to eating store bought cakes, which may be sweeter and less dense. Some of the comments from the panelists indicated that the cake were like white bread or pound cake. Demographic questionnaire indicated that 87% of the panelists have no reservations on using eggs in general and 9% of them indicated that they reserve using eggs when it is raw or if the recipe calls for raw eggs.

**Conclusions**

Although there were some differences in the physical properties between the cakes, such as the top crust color, the pore sizes, and the whipping time of the eggs, consumers liked all cakes equally for all attributes tested. Most important, OP produce equivalent physical properties (pores size, volume, and inner crumb colors) to UT. Although OP required longer whipping time than UT, TP took significantly longer than both eggs. Thus, OP are better in terms of whipping time and emulsification properties compared to TP. OP have an added benefit of reducing the risk of *Salmonella Enteritidis* contamination compared to UT and are suitable for use in applications where foaming and emulsification of the egg are important.

**References**


Perry JJ. 2010. Ozone based treatments for inactivation of *Salmonella* enterica serovar Enteritidis in shell eggs [Dissertation]. Columbus, OH : The Ohio State University. 200 p. Available from The Ohio State University, Columbus, OH.


Conclusion

Whole shell eggs remain one of the few animal products consumed directly from the farm raw and unpasteurized. The increasing scrutiny of foodborne illnesses has properly focused attention on improved egg safety. An emerging technology such as heat-ozone-pasteurization is a promising method to ensure safer eggs with similar qualities as untreated eggs.

Consumer acceptance studies and functionality have been previously done in pilot scale heat-ozone-pasteurized eggs, demonstrating its potential as a commercially viable alternative to raw eggs or thermally processed eggs. With a major investment by farmers and processors in design, construction and operation of commercial scale heat-ozone processing, it is important to determine consumer acceptance, quality, and functionality of these eggs, as compared to untreated eggs and thermal-only eggs now available in the marketplace.

Chapter 2 evaluated the quality and consumer acceptance of untreated, thermally pasteurized, and heat-ozone pasteurized eggs, based on visual characteristics of uncooked eggs and sensory characteristics of scrambled eggs. In some cases, height, yellowness, and visual appeal of the yolk, heat-ozone-pasteurized eggs were perceived equivalent to untreated eggs. In the taste test, all eggs were liked similarly. The shell strength study shows that the shell of the heat-ozone-pasteurized eggs were as strong as untreated eggs.
but the heat-ozone-pasteurized eggs shatters more than untreated eggs. Preliminary work indicates coating of the shell promises to improve the egg and further work needs to be conducted on applying different types and amounts of coating in the heat-ozone-pasteurized eggs.

Chapter 3 examined the physical properties and consumer acceptance of Caesar dressing made with heat-ozone-pasteurized eggs and liquid pasteurized eggs. There were no significant differences in the pH and the consistency of both sauces. Although there were some differences in the physical properties (lightness and yellowness) between both sauces, consumers liked both sauces equally for all attributes tested.

Chapter 4 evaluated the functionality (such as foaming, color, pore size, whipping time) of heat-ozone pasteurized eggs as an ingredient in an angel food cake. Although there were some differences in the physical properties between the cakes, such as the top crust color, the pore sizes, and the whipping time of the eggs, consumers liked all cakes equally. Cake made with heat-ozone-pasteurized eggs produce equivalent physical properties (pores size, volume, and inner crumb colors) to cake made with untreated eggs. Heat-ozone-pasteurized eggs were better in terms of shorter whipping time compared to thermally pasteurized eggs.

Based on these findings, heat-ozone-pasteurized eggs would be suitable for use in cooking applications (scrambled eggs), raw egg recipes (used in Caesar dressing), and as an important functional ingredient. Moreover, the consumer panel results in this study show that ozone-pasteurized eggs were liked similarly, as compared to pasteurized eggs and untreated eggs.
This study shows that using heat and ozone combination in shell eggs provides a pasteurized egg with acceptable sensory properties when consumed in a traditional egg recipe (scrambled), when used in a raw egg recipe, and when used as a functional ingredient in baking. The scale-up of the heat and ozone process from pilot scale machinery processing three dozen eggs per batch to commercial scale machinery processing more than 10,000 eggs per batch yielded new egg quality challenges such as shattering, albumen leakage, cracking and process control. These de novo quality issues were solvable with proprietary adjustments in the process and equipment design. Future work may include optimizing the shell strength of the eggs and continued consumer acceptance studies to validate the optimized shell eggs. As consumers and regulatory agencies demand safer foods in the market, there is great potential to satisfy this need, particularly where any risk prohibits raw eggs, such as high risk populations in hospitals, nursing homes, or foodservice operations.
List of References


AZG Research. 2006. SE-free egg study. Market study report of AZG Research, Bowling Green, OH prepared for Eggtech Ltd.

Bieliauskas H. 2013. Personal communication on proprietary survey and focus group research completed on December 2012.


CDC. 2011. CDC estimates of foodborne illness in the United States, Atlanta: Centers for Disease Control and Prevention, Available from:


Heavenridge JR, 2013. Personal communication from a proprietary chef panel and focus group.


Perry JJ. 2010. Ozone based treatments for inactivation of Salmonella enterica serovar Enteritidis in shell eggs[Dissertation]:The Ohio State University.p 200.Available from The Ohio State University, Columbus, OH.


Appendix A: Ballot and Consent Form of Uncooked and Scrambled Egg Consumer Acceptance Test
This study is designed to look at consumer perception of raw un-cooked eggs and cooked eggs. In the raw-uncooked eggs study, you will be presented with three samples, one at a 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. On the cooked eggs, you will be presented with three samples, one at a time 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 a mouse and keyboard. This has been estimated to take about 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 $10 Target Gift Card. 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

You will receive samples of EGGS one-at-a time, Evaluate (LOOKING AT ONLY) by rating each sample

Please, LOOK ONLY at these EGGS before you answer the first question.

PLEASE DO NOT EAT ANY OF THESE EGGS!

Question #1
LOOK AT all the samples and mark your score on the line scale for EACH sample.
Rate the THICK PART OF THE EGG WHITE FOR:

Please rate CLOUDINESS OF THE THICK PART OF THE WHITE on the following scale.

Not Cloudy                      Very Cloudy
Sample _____ I-------------------I-------------------I-------------------I-------------------I

Question #2

Please rate AMOUNT OF SPREADING OF THE WHITE AT THE THICKHEST PART on the following scale.

A Little Spreading               A Lot of Spreading
Sample _____ I-------------------I-------------------I-------------------I-------------------I

Question #3

LOOK AT all the samples and mark your score on the line scale for EACH sample.
Rate the THE EGG YOLK

Please rate CLOUDINESS OF THE EGG YOLK on the following scale.

Not Cloudy                      Very Cloudy
Sample ______ I-------------------I-------------------I-------------------I-------------------I

Question #4

Please rate HEIGHT OF THE YOLK on the following scale

Low                                High
Sample _____ I-------------------I-------------------I-------------------I-------------------I

Question #5

Please rate COLOR OF THE YOLK on the following scale.
Question #6

Please rate **YELLOWNESS OF THE EGG YOLK** on the following scale.

<table>
<thead>
<tr>
<th>Less Yellow</th>
<th>More Yellow</th>
</tr>
</thead>
</table>

Sample _____

Question #7

Please rate **AMOUNT OF SPREADING OF THE YOLK** on the following scale.

<table>
<thead>
<tr>
<th>A Little Spreading</th>
<th>A Lot of Spreading</th>
</tr>
</thead>
</table>

Sample _____

Question #8

**LOOK AT** all the samples and mark your score on the line scale for EACH sample.

**Rate the VISUAL APPEAL FOR:**

Please rate **THE VISUAL APPEAL OF THE EGG YOLK** on the following scale.

<table>
<thead>
<tr>
<th>Not at All Appealing</th>
<th>Very Appealing</th>
</tr>
</thead>
</table>

Sample _____

Question #9

Please rate **THE VISUAL APPEAL OF THE EGG WHITE** on the following scale.

<table>
<thead>
<tr>
<th>Not at All Appealing</th>
<th>Very Appealing</th>
</tr>
</thead>
</table>

Sample _____

Question #10
Please rate THE VISUAL APPEAL OF THE WHOLE EGG on the following scale.

Not at All Appealing | Very Appealing
Sample _____ I-----------------I-----------------I-----------------I-----------------I-----------------I

REPEAT WITH REMAINING SAMPLE
-----------------------------------------------------------------------------------------------BREAK-----------------------------------------------------------------------------------------------

Now you will receive samples of SCRAMBLED EGGS one-at-a time. Please follow the instructions below.

Please, LOOK ONLY at these EGGS before you answer the first question.

DO NOT TASTE YET!

Please click THE GRAY BOX TO ANSWER.

Sample _____

Rate your OVERALL VISUAL LIKING OF THE APPEARANCE OF THE EGGS

<table>
<thead>
<tr>
<th>dislike extremely</th>
<th>dislike very much</th>
<th>dislike moderately</th>
<th>dislike slightly</th>
<th>neither</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like very much</th>
<th>like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

Take a drink of water BEFORE each sample.
Please **TAKE AT LEAST ONE BITE OF THE EGGS**

**BEFORE** you answer this question for **OVERALL LIKING**?

(click the **GRAY BOX**)

Rate the EGGS for your **OVERALL LIKING**

<table>
<thead>
<tr>
<th>dislike extremely</th>
<th>dislike very much</th>
<th>dislike moderately</th>
<th>dislike slightly</th>
<th>neither</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like very much</th>
<th>like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

Please click **THE GRAY BOX** then one box per question.

Rate your **LIKING OF THE EGG AROMA**

<table>
<thead>
<tr>
<th>dislike extremely</th>
<th>dislike very much</th>
<th>dislike moderately</th>
<th>dislike slightly</th>
<th>neither</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like very much</th>
<th>like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

Rate your **LIKING OF THE EGG FLAVOR**

<table>
<thead>
<tr>
<th>dislike extremely</th>
<th>dislike very much</th>
<th>dislike moderately</th>
<th>dislike slightly</th>
<th>neither</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like very much</th>
<th>like extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
</tr>
</tbody>
</table>

123
Rate your LIKING OF THE EGG TEXTURE

[Scale: dislike extremely, dislike very much, dislike moderately, dislike slightly, neither like nor dislike, like slightly, like moderately, like very much, like extremely]

Continue tasting the EGGS and answer the following questions.

Please click THE GRAY BOX to see the scale then CLICK ON one per question.

Rate the AMOUNT OF SCRAMBLED EGG COLOR

[Scale: Way too Little, Slightly too Little, Just About Right, Slightly too Much, Way too Much]

Describe the AMOUNT OF SCRAMBLED EGG MOISTNESS

[Scale: Way too Dry, Slightly Dry, Just About Right, Slightly Moist, Way too Moist]
Describe the SCRAMBLED EGG TEXTURE

Way too Tough  Slightly Tough  Just About Right  Slightly too Tender  Way too Tender

1  2  3  4  5

Sample _____ (Continue with balance of the samples.)

Please ANSWER the following demographic questions.

Please choose one box below to indicate about HOW OFTEN DO YOU EAT/USE EGGS? (NOT IN BAKING)

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

Please choose one box below to indicate your GENDER.

☐ Male  ☐ Female

Please choose one box below to indicate your AGE CATEGORY.

☐ 18 – 20 years  ☐ 21 – 25 years
☐ 26 – 35 years  ☐ 36 – 45 years  ☐ 46 – 55 years
☐ 56 – 65 years  ☐ Over 65 years

Please choose one box below to indicate your ETHNICITY.
☐ American Indian
☐ Asian, or Pacific Islander
☐ Black, not of Hispanic origin
☐ Hispanic
☐ White, not of Hispanic origin
☐ Other
☐ Prefer not to disclose
Appendix B: Ballot and Consent Form of Parmesan Caesar Dipping Sauce Consumer Acceptance Test
Questionnaire: Parmesan Caesar Dipping Sauce
Welcome to Sensory Testing!
Melody Leidheiser, Sensory Evaluation Program Coordinator

This study is designed to look at consumer perception of Parmesan Caesar Dipping Sauce. In this study, you will be presented with two samples, one at a time in 2 ounce soufflé cups. The sauce will be kept chilled and served not to exceed 50 degrees Fahrenheit. You will be presented with a stick of celery to dip into the sauce for assessing. 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 about 15 minutes but you may take as long as you need. Your responses will in no way be linked to your identity. You will be compensated with a $15 Target Gift Card for your participation. If you have any questions, please feel free to ask the attendant at any time.

(This sauce contains eggs and dairy, but no fish, nuts nor gluten.)

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

INFORMED CONSENT STATEMENT

I have been informed of 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 loss of compensation. I am 18 years of age or older. I freely and voluntarily give my consent to participate.

You may contact the Principal Investigator, Ken Lee.133 with any questions or concerns (292.7797). For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows.8 in the Office of Responsible Research Practices at 1-800-678-6251.

This research is supported by the State of Ohio Third Frontier Wright Projects competitive research program and by Egg Tech Ltd.

I have read the INFORMED CONSENT STATEMENT 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

You will receive 2 samples of PARMESAN CAESAR DIPPING SAUCE one-at-a time, Evaluate (BY TASTING WITH A CELERY STICK) each sample

TASTE, DIPPING YOUR CELERY STICK INTO THE SAUCE
before you answer the first question.

Sample ______

Question #1 Please take at least 2 bites of celery & sauce then answer the following:

Rate your OVERALL LIKING OF THE PARMESAN CAESAR DIPPING SAUCE

<table>
<thead>
<tr>
<th>dislike extremely</th>
<th>dislike very much</th>
<th>dislike moderately</th>
<th>dislike slightly</th>
<th>neither</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like very much</th>
<th>like extremely</th>
</tr>
</thead>
</table>

1 2 3 4 5 6 7 8 9

Rate your LIKING OF THE DIPPING SAUCE FLAVOR

<table>
<thead>
<tr>
<th>dislike extremely</th>
<th>dislike very much</th>
<th>dislike moderately</th>
<th>dislike slightly</th>
<th>neither</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like very much</th>
<th>like extremely</th>
</tr>
</thead>
</table>

1 2 3 4 5 6 7 8 9

Rate your LIKING OF THE DIPPING SAUCE PARMESAN FLAVOR

<table>
<thead>
<tr>
<th>dislike extremely</th>
<th>dislike very much</th>
<th>dislike moderately</th>
<th>dislike slightly</th>
<th>neither</th>
<th>like slightly</th>
<th>like moderately</th>
<th>like very much</th>
<th>like extremely</th>
</tr>
</thead>
</table>

1 2 3 4 5 6 7 8 9
Rate your **LIKING OF THE DIPPING SAUCE SALTINESS**

dislike extremely  dislike very much  dislike moderately  dislike slightly  neither dislike nor like  slightly dislike  like moderately  like slightly  like much  like extremely

[ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]

Rate your **LIKING OF THE DIPPING SAUCE THICKNESS**

dislike extremely  dislike very much  dislike moderately  dislike slightly  neither dislike nor like  slightly dislike  like moderately  like slightly  like much  like extremely

[ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]

Rate your **LIKING OF THE DIPPING SAUCE MOUTHFEEL (how it coats your tongue)**

dislike extremely  dislike very much  dislike moderately  dislike slightly  neither dislike nor like  slightly dislike  like moderately  like slightly  like much  like extremely

[ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ] [ ]

Rate your **LIKING OF THE DIPPING SAUCE AFTERTASTE (if any)**

130
Continue tasting the PARMESAN CAESAR DIPPING SAUCE and answer the following questions.

Please click THE GRAY BOX to see the scale then CLICK ON one per question.

Rate the **AMOUNT OF FLAVOR**

Way too Little  Slightly too Little  Just About Right  Slightly too Much  Way too Much

Rate the **AMOUNT OF PARMESAN FLAVOR**

Way too Little  Slightly too Little  Just About Right  Slightly too Much  Way too Much

Rate the **AMOUNT OF SALTINESS**
Rate the **AMOUNT OF THICKNESS**

<table>
<thead>
<tr>
<th>Way too Thin</th>
<th>Slightly too Thin</th>
<th>Just About Right</th>
<th>Slightly too Thick</th>
<th>Way too Thick</th>
</tr>
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</table>

| 1 | 2 | 3 | 4 | 5 |

Rate the **AMOUNT OF MOUTHCOAT** (how much it coats your tongue)

<table>
<thead>
<tr>
<th>Way too Little</th>
<th>Slightly too Little</th>
<th>Just About Right</th>
<th>Slightly too Much</th>
<th>Way too Much</th>
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</thead>
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</table>

| 1 | 2 | 3 | 4 | 5 |

Rate the **AMOUNT OF AFTERTASTE** (if any)

<table>
<thead>
<tr>
<th>Way too Little</th>
<th>Slightly too Little</th>
<th>Just About Right</th>
<th>Slightly too Much</th>
<th>Way too Much</th>
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</tbody>
</table>

| 1 | 2 | 3 | 4 | 5 |

Please feel free to type a brief comment on the Parmesan Caesar Dipping Sauce.
Sample ________ (Continue with the 2nd sample.)

DEMOGRAPHICS:
Please ANSWER the following demographic questions.

Please choose one box below to indicate about **HOW MANY EGGS DO YOU EAT/USE EGGS per week? (NOT IN BAKING)**

<p>| | |</p>
<table>
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<tr>
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<tbody>
<tr>
<td>□</td>
<td>0</td>
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<tr>
<td>□</td>
<td>1</td>
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<td>□</td>
<td>2</td>
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<tr>
<td>□</td>
<td>3</td>
</tr>
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<td>□</td>
<td>4</td>
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<td>5</td>
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<td>□</td>
<td>6</td>
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<td>7</td>
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<td>8</td>
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<td>□</td>
<td>9</td>
</tr>
<tr>
<td>□</td>
<td>10</td>
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</table>

Please choose one box below to indicate which **SALAD DRESSING** you use most often.

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>□</td>
<td>Caesar</td>
</tr>
<tr>
<td>□</td>
<td>Garlic</td>
</tr>
<tr>
<td>□</td>
<td>Honey Mustard</td>
</tr>
<tr>
<td>□</td>
<td>Italian</td>
</tr>
<tr>
<td>□</td>
<td>Ranch</td>
</tr>
</tbody>
</table>

Please choose one box below to indicate your **GENDER**.

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>□</td>
<td>Male</td>
</tr>
<tr>
<td>□</td>
<td>Female</td>
</tr>
</tbody>
</table>

Please choose one box below to indicate your **AGE CATEGORY**.

<p>| | |</p>
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<tbody>
<tr>
<td>□</td>
<td>18 – 20 years</td>
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<tr>
<td>□</td>
<td>21 – 25 years</td>
</tr>
<tr>
<td>□</td>
<td>26 – 35 years</td>
</tr>
<tr>
<td>□</td>
<td>36 – 45 years</td>
</tr>
<tr>
<td>□</td>
<td>46 – 55 years</td>
</tr>
<tr>
<td>□</td>
<td>56 – 65 years</td>
</tr>
<tr>
<td>□</td>
<td>Over 65 years</td>
</tr>
</tbody>
</table>
Please choose one box below to indicate your **ETHNICITY**.

- [ ] American Indian
- [ ] Asian, or Pacific Islander
- [ ] Black, not of Hispanic origin
- [ ] Hispanic
- [ ] White, not of Hispanic origin
- [ ] Other
- [ ] Prefer not to disclose
Appendix C: Ballot and Consent Form of Angel Food Cake Consumer Acceptance Test
Questionnaire: Angel Food Cake

Welcome to Sensory Testing!
Melody Leidheiser, Sensory Evaluation Program Coordinator

This study is designed to look at consumer perception of Angel Food Cake. In this study, you will be presented with three samples, one at a time in 3 ounce soufflé cups. All three cakes will be baked in the same oven under the same conditions then cut into 1” for cubes assessing. 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 about 15 minutes but you may take as long as you need. Your responses will in no way be linked to your identity. You will be compensated with a $15 Target Gift Card for your participation. If you have any questions, please feel free to ask the attendant at any time.

(This cake contains eggs and gluten, but no fish, nuts nor dairy.)

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

INFORMED CONSENT STATEMENT

I have been informed of 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 loss of compensation. I am 18 years of age or older. I freely and voluntarily give my consent to participate. You may contact the Principal Investigator, Ken Lee.133 with any questions or concerns (292.7797). For questions about your rights as a participant in this study or to discuss other study-related concerns or complaints with someone who is not part of the research team, you may contact Ms. Sandra Meadows.8 in the Office of Responsible Research Practices at 1-800-678-6251.

This research is supported by the State of Ohio Third Frontier Wright Projects competitive research program and by Egg Tech Ltd.

I have read the INFORMED CONSENT STATEMENT 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

You will receive 3 samples of ANGEL FOOD CAKE one-at-a-time, Please TASTE this sample - then ANSWER the questions.

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making sure to save some for the remaining questions,

Rate your LIKING OF THE ANGEL FOOD CAKE FLAVOR

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

Rate your LIKING OF THE ANGEL FOOD CAKE SWEETNESS

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

Rate your LIKING OF THE ANGEL FOOD CAKE TEXTURE (SPRINGINESS)

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

Continue tasting the ANGEL FOOD CAKE and answer the following questions.

Please click THE GRAY BOX to see the scale then CLICK ON one per question.
Rate the **AMOUNT OF FLAVOR**

<table>
<thead>
<tr>
<th>Way too Little</th>
<th>Slightly too Little</th>
<th>Just About Right</th>
<th>Slightly too Much</th>
<th>Way too Much</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Rate the **AMOUNT OF SWEETNESS**

<table>
<thead>
<tr>
<th>Way too Little</th>
<th>Slightly too Little</th>
<th>Just About Right</th>
<th>Slightly too Much</th>
<th>Way too Much</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Describe the **TEXTURE (SPRINGINESS)**

<table>
<thead>
<tr>
<th>Way too Light</th>
<th>Slightly too Light</th>
<th>Just About Right</th>
<th>Slightly too Dense</th>
<th>Way too Dense</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

**NEXT** you will receive 3 sample **SLICES** of **ANGEL FOOD CAKE** one-at-a-time,

Please **LOOK ONLY** at the sample you answer then **ANSWER** the questions.

Sample ______

Please look at the SLICE OF CAKE then answer the following:
Rate your **OVERALL LIKING OF THE APPEARANCE OF THE ANGEL FOOD CAKE**

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

Rate your **LIKING OF THE AMOUNT OF AIR BUBBLES ON ANGEL FOOD CAKE SURFACE**

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

Rate your **LIKING OF THE ANGEL FOOD CAKE COLOR**

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

Continue **LOOKING** at the **SLICE** of Cake
Rate the **AMOUNT OF COLOR**

<table>
<thead>
<tr>
<th>Way too Light</th>
<th>Slightly too Light</th>
<th>Just About Right</th>
<th>Slightly too Dark</th>
<th>Way too Dark</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Describe the **AMOUNT OF AIR BUBBLES**

<table>
<thead>
<tr>
<th>Way too Light</th>
<th>Slightly too Light</th>
<th>Just About Right</th>
<th>Slightly too Dark</th>
<th>Way too Dark</th>
</tr>
</thead>
<tbody>
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<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Please feel free to type a brief comment about the Angel Food Cake.

______________________________________________________________________

Sample ______ (Continue with the 2\textsuperscript{nd} then 3\textsuperscript{rd} sample.)

**DEMOGRAPHICS:**

Please **ANSWER** the following demographic questions.

Please choose one box below to indicate about **HOW MANY EGGS DO YOU EAT/USE EGGS per week? (NOT IN BAKING)**

- 0
- 1
- 2
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
Please choose one box below to indicate which type of Angel Food Cake you eat most often.

- [ ] Home Made
- [ ] Other Made
- [ ] Store Bought
- [ ] Don’t Know

If you DO NOT make your own ANGEL FOOD CAKE AT HOME, why not?

- [ ] Takes too much time
- [ ] Don’t enjoy baking
- [ ] Don’t have supplies I would need
- [ ] Store bought is better
- [ ] I DO make my own!

Do you have any reservations about cooking with EGGS?

- [ ] Yes
- [ ] No
- [ ] Sometimes
- [ ] Only when they are raw

Please feel free to tell us about your egg concerns if you have them.
Comment: ____________________________________________

Please choose one box below to indicate your GENDER.

- [ ] Male
- [ ] Female

Please choose one box below to indicate your AGE CATEGORY.

- [ ] 18 – 20 years
- [ ] 21 – 25 years
- [ ] 26 – 35 years
- [ ] 36 – 45 years
- [ ] 46 – 55 years
- [ ] 56 – 65 years
- [ ] Over 65 years

Please choose one box below to indicate your ETHNICITY.

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