Physical and Microbiological Characteristics of Pickled Eggs from Japanese Quail (Coturnix coturnix japonica) of the Pharaoh Variety

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PHYSICAL AND MICROBIOLOGICAL CHARACTERISTICS OF PICKLED EGGS FROM JAPANESE QUAIL (COTURNIX COTURNIX JAPONICA) OF THE PHARAOH VARIETY

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science.
Food, Nutrition, and Culinary Sciences

by
C. Adair Hoover
May 2022

Accepted by:
Dr. Julie Northcutt, Committee Chair
Dr. Paul Dawson
Dr. Doug Smith
ABSTRACT

The quality and microbiological characteristics of quail eggs were evaluated after eggs were pickled in different vinegar brine solutions. Pickling food in vinegar is one of the oldest preservation methods; however, little research has been conducted on pickled quail eggs. Commercial quail eggs obtained from a local producer were boiled, peeled, and placed into various pickling solutions and held for 24 or 48 hours at room temperature. After the holding period, eggs were removed from the solutions, weighed, and tested for pH, water activity, texture, and color. A second experiment was conducted to determine the effects of various pickling solutions on quail egg microbiology. Eggs were boiled, peeled and then inoculated with 0.1 mL of a mixed culture containing $10^6$ to $10^8$ cells per mL of generic Escherichia coli, Listeria monocytogenes, and nalidixic acid-resistant Salmonella Typhimurium. After inoculation and a 30 minute waiting period, eggs were placed into various vinegar brine solutions or control treatments and held at room temperature for 24 hours. Pickling in accordance with food safety regulations, provides a nutritious, shelf-stable product. If pickled eggs are inadequately processed, there is a risk of Clostridium botulinum toxin, but if they are boiled too long, a rubbery, extra-firm, and undesirable texture may occur. Results revealed that weight and texture changes due to pickling did not adversely affect quality; water activity was consistent across time and treatment types; pH was below 4.6, in all components of the egg, after 24 hours in pickling brine and there was no significant change at 48 hours; the color of pickled quail egg yolks after 24 hours became lighter and less yellow, while albumen colors increased in greenness and yellowness. Analysis of microbiological data revealed that the commercial brine solutions reduced pathogenic growth below detection limits. Therefore, leading to the conclusion that a combination of thermal process and acidification did reduce pathogenic growth to safe levels.
ACKNOWLEDGMENTS

This work would not have been possible without the help of many people. To my husband, Adam, my mom Priscilla Pope, and my kids Austin, and Gabrielle, thank you for your support, humor, and love.

A big thank you to Dr. Julie Northcutt for encouraging me to pursue a graduate degree. I wouldn’t have even considered this if she hadn’t suggested it and can’t imagine a better mentor. I greatly appreciate the wealth of knowledge, patience and laughs along the way. Thank you to my committee members Dr. Paul Dawson and Dr. Doug Smith. I greatly appreciate your input and expertise.

Ten weeks of lab work and many weeks of writing, all while working full time, could not have been accomplished without the help of co-workers and fellow students. Thank you to: Belinda Cochran, and Ahmet Buyukyavuz, for help in the lab and lending your microbiology knowledge and skills; Dr. Kimberly Baker and Millie Davenport, for being beyond supportive through this process; Frances Seel, Vicki Landreth, and Chase Bailie for picking up so much extra work on my behalf; Marie Hegler and Rose Somer for helping in the lab; and Chad Carter, for sharing encouraging words throughout.
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CHAPTER ONE
INTRODUCTION

Commercial food manufacturing is a multi-billion dollar business in the U.S. According to the U.S. Bureau of Statistics, more than 36,000 commercial food manufacturing plants were in the U.S. in 2017 (USBS, 2021). The U.S. Department of Agriculture (USDA) reported that total U.S. wholesale food sales reached 76 billion dollars in 2020, and food prices continued to increase in 2021 and 2022. Manufacturing encompasses many food categories such as grains, sugars, confectionery, fruit and vegetables (raw and preserved), specialty foods, dairy products, fish and seafood, bakeries, and more. When these foods are further processed, they are considered value-added products. Among value-added foods, meats are the top commodity and largest revenue-generating category, comprising 19% of U.S. food sales per year. The next most significant revenue-generating type is termed "other foods." This category generates 14% of U.S. food sales annually and includes shell eggs, pickled vegetables, nuts, and pickled eggs (USDA, 2021).

Pickled foods are considered value-added because the process changes the products' physical state, enhancing their value, shelf-life, and convenience to consumers. Convenience plays a crucial role in consumer food demands. Consumers appreciate foods that are healthy, nutritious, easy to acquire, time-saving in preparation, and have a low price-point (USDA, 2021). Consumers have gravitated toward shelf-stable products, such as pickled foods, because they typically have a long shelf life, are affordable, and are ready to eat with no additional preparation. Data from the USDA Economic Research Service showed that in 2020, consumers spent 5% of their disposable income on food at home and another 3.6% on food away from home (USDA, 2022). Additionally, U.S. consumers, businesses, and government entities spent nearly

Food safety is paramount to the mission of the U.S. Food and Drug Administration (F.D.A.) and the USDA. There are federal regulations in place to ensure a safe food supply in the U.S. These regulations can be found under Title 21 of the Code of Federal Regulations (C.F.R.). They cover all types of food commodities, including pickled foods. Pickled eggs are subject to regulations from both F.D.A. and USDA.

F.D.A. Title 21 of the C.F.R. classifies pickled foods as "acidified foods." Acidified foods are low-acid (high pH) food products to which acid or acid food ingredients have been added to increase the safety of the product. (F.D.A., 2021). Acidified foods must have a final equilibrium pH below 4.6 (F.D.A., 2021). The process of pickling foods to be held without temperature control creates an atmosphere in which the Clostridium botulinum (C.bot) toxin can form. C.bot forms a neurotoxin that can result in death if consumed. The environment that favors the formation of this toxin is low acid (pH > 4.6), time in optimal conditions, temperature (10 - 48o C), moisture (water activity > 0.86), and anaerobic conditions (Montville, 2012). Food manufacturers producing acidified foods must follow research-based recommendations for processing time, temperature, and acidification. When these parameters are not controlled, foodborne illness outbreaks occur.

Dewey-Mattia et al. (2018) analyzed U.S. foodborne illness outbreaks occurring between 2009 and 2015. They found that foods from land animals, including eggs, caused 44% of foodborne illness outbreaks (565 outbreaks) and 52% of foodborne illnesses (13,709 illnesses). During that time, chicken eggs were implicated in 36 outbreaks and nearly 2,500 illnesses.
(Dewey-Mattia et al., 2018). The most frequent cause of foodborne illness from eggs is *Salmonella Enteritidis*; however, other *salmonellae* species such as *Typhimurium* and *Heidelberg* and other pathogens may also be present. (Schoeni et al., 1994)

Eggs are frequently implicated as the source of foodborne illness outbreaks because they have a strong association with foodborne pathogens, including generic *Listeria monocytogenes* and *Salmonella Typhimurium*. In 2020, the F.D.A. reported that approximately 79,000 foodborne illness cases occurred due to the consumption of eggs contaminated with *Salmonella Enteritidis*. In 2020, The U.S. Centers for Disease Control and Prevention (C.D.C.) and F.D.A. recalled commercial hard-cooked eggs after eight people across five states were infected with *Listeria monocytogenes*. (C.D.C., 2020) Generic *Escherichia coli* is also a microorganism of concern in eggs because it is linked to fecal contamination. These bacteria can be transferred to eggs during lay because the eggs are laid through the cloaca, the same exit point for the gastrointestinal tract. Certain serotypes of *E. coli* can cause foodborne illness when consumed, and the presence of generic *E. coli* is often linked to the existence of these more virulent strains. Tyler et al. (1953) estimated that the shells of chicken eggs contain between 7,000 and 17,000 pores, which are large enough for bacteria to become entrapped. Similarly, Tullett (1975) found that quail eggshells have 306 pores/cm2. Microorganisms on the shell surface and in the shell pores can serve as a source of foodborne illness from cross-contamination, mishandling, and inadequate preservation. This is an even bigger concern with quail table eggs, as producers typically do not wash these eggs before selling them.

As a preventative measure, commercial chicken egg processors are required, per regulation 7CFR 56.76(f)(3), to wash eggs in warm water followed by sanitizer application for surface decontamination (USDA 2012; Musgrove et al., 2008). Even with these measures, the
possibility of pathogens still exists in a manufacturing environment. Chousalkar et al. (2017) estimate that one in every 20,000 chicken eggs is contaminated with *Salmonella*. In quail farming, there is no egg wash step in processing eggs. In the U.S., F.D.A. requires refrigeration of chicken eggs designated for human consumption (at or below 7.2°C or 45°F, but above freezing) within 36 hours from the time of lay (US FDA, 2009). Commercial producers of quail table eggs typically meet this exact requirement, but they are not required. Refrigeration stops the growth of most pathogens, except psychotropic pathogens (Marth 1998).

The physical changes that occur during food preservation are an important factor in the quality of the final product. When preservation techniques are applied to food, a series of physical and chemical changes can occur that influence the quality of the final product. Some methods employ dehydration, cool or freezing temperatures, controlled or modified atmosphere storage or packaging, heat treatment, ultraviolet radiation, high-pressure processing, or acidification – all of which can affect product quality and nutrition (Farkas, 1997). Foods processed to complete sterility will likely have poor quality and reduced nutrition. Finding a balance between producing a safe product while maintaining high quality is a crucial component of any processing method. While there have been numerous studies on pickling chicken eggs, little research has been conducted on quail eggs designated for human consumption. Thus, the objectives of this research were to:

- Evaluate physical characteristics (water activity, pH, color, and texture) of pickled quail eggs held in various brine solutions for 24 or 48 hours; and

- Determine the microbiological impact of process deviations on inoculated pickled quail eggs.
CHAPTER TWO
REVIEW OF LITERATURE

Quail

Quail are a small, shy bird species in the Phasianidae family, a variety of the gallinaceous bird. There are numerous sub-species of Coturnix, but the four most common are Eurasia, Common, Japanese, Harlequin, and Button quail. Worldwide, Eurasian (also called Pharaoh) are one of the most common quail breeds raised for meat and egg production (Shanaway, 1994).

Figure 1: Newly hatched quail.
Adair Hoover, ©2022

Figure 2: Quail. Compliments of Manchester Farms

Farm-raised quail are often referred to as domesticated, but in reality, they are not. A domesticated animal is in regular contact with humans, and this contact influences the animal's behavior or environment (Piggins, 1998). In a commercial production setting, quail typically have little contact with humans and will fly away if not contained.
The classification of Pharaoh Quail in the Animal Kingdom:

<table>
<thead>
<tr>
<th>Phylum:</th>
<th>Chordata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subphylum:</td>
<td>Vertebrata</td>
</tr>
<tr>
<td>Class:</td>
<td>Avis (wings and feathers)</td>
</tr>
<tr>
<td>Order:</td>
<td>Galliformes (short beak and feet)</td>
</tr>
<tr>
<td>Suborder:</td>
<td>Galli (fowl and game birds)</td>
</tr>
<tr>
<td>Family:</td>
<td>Phasianidae</td>
</tr>
<tr>
<td>Subfamily:</td>
<td>Odontophorinaw (New World quail)</td>
</tr>
<tr>
<td>Genus:</td>
<td>Coturnix</td>
</tr>
</tbody>
</table>


Newly hatched quail weigh between 6 to 8 g. The adult males weigh between 100 to 130 g and have a maximum life span of 7 years and a mean life span of 3 to 4 years. Adult female quail weigh about 120 to 160 g. In a commercial setting, larger females are selected for egg-laying because larger layers produce bigger offspring. Female quail reach maturity and begin laying eggs at approximately 50 days of age. They typically lay one egg a day or between 280-300 eggs per year in the first year and then 150-175 in year two. The female has an average lifespan of 2 ½ to 3 years. (Makoto, Retrieved March 2022)

The first written accounts of quail date back the 12th century Japan. In the sixteenth century, quail were semi-domesticated as pets and singing birds in Japan. By the 1900s, quail were seen as viable production birds and were consumed for meat and eggs in Japan and many other parts of the world, including the United States (Lukanov, 2017). The United States attempted to launch a wild game population of quail in the 1950s by releasing more than one million quail across the country (Cain et al., 1974). This endeavor failed because many of the quail released, especially the Pharaoh quail, were true migratory birds and either flew away or were lost to predators (Cain et al., 1974). During that same period, researchers began to utilize quail for projects focused on nutrition, genetics, animal science, and human medicine. Quail are
well suited as a laboratory animal because they are small, quick to mature, robust layers, have a short lifespan, and adapt well to housing. In 1959, Padgott evaluated the use of quail for poultry genetics research and found that they had excellent potential for studying avian reproduction. According to Minvielle (2004), quail as a research animal provided much of the technical information needed to establish modern commercial production parameters. In 2008, Huss and coworkers chose quail as a test animal because of their short lifespan. They described the use of quail for research in developmental biology and human disease (Huss et al., 2008). Psychology and behavior studies have also included quail. Bolin et al. (2017) used quail to study whether nicotine induces a conditioned place preference in male Japanese quail.

Worldwide, quail continue to increase in popularity for meat and eggs. (Tolik et al., 2014). In 2019, it was estimated that more than 9.1 billion quail were produced annually worldwide, including breeding lines (Lukanov, 2019). This same author reported on the difficulties with estimating the worldwide production of quail because the statistics are not maintained, or quail are grouped with other avian species and reported as game birds (Lukanov, 2019). Other estimates have focused only on quail produced for meat and egg production, with worldwide reports of 1.4 billion annually (Katerynych et al., 2020).

**Eggs**

*Chicken Eggs*

Eggs for human consumption are produced by various animal species that include but are not limited to chickens, turkeys, ducks, geese, pigeons, fish, and quail. In 2017, worldwide production of shell eggs was 76.7 million metric tons (International Egg Commission, 2018), with chicken eggs providing 93 percent of world egg production (FAOUN, 2022). In the U.S.,
The vast majority of eggs consumed are produced by chickens, likely because of their availability and familiarity to the public. In 2020, only 2.9% of shell eggs were exported, with 65.7% being sold for retail sales, 3.5% sold to foodservice, and 27.8% were sold for further processing. (United Egg producers, 2022)

The USDA National Agricultural Statistics Service (2022) estimates that there are currently 325 million chicken-laying hens producing table or market-type eggs. They produce approximately 96.9 billion table eggs each year. The U.S. National Agricultural Statistics Service estimates that 110.73 billion chicken table eggs were produced in 2021. According to the United Egg Producers (2021), the per-capita consumption of shell eggs has steadily risen from 240.4 eggs in 1998 to 286.5 eggs in 2020. During this time, egg consumption only declined one year in 2020, likely due to the SARS Cov-2 Coronavirus (COVID – 19) pandemic.

Chicken eggs are widely consumed because of their exceptional nutritional value. According to the National Institute for Health (N.I.H.), shell eggs have a perfect balance and diversity of nutrients. They are easily digested and affordable, making them an attractive commodity worldwide (Fernandez, N.I.H., 2019). Common egg preparations include scrambling, frying, poaching, and hard-cooked in the shell. Like many foods with a high nutritional value, there is often a need to preserve and extend shelf life. Standard preservation methods include dehydrating, salt curing, and pickling. Pickling has a distinct food safety advantage because an acetic acid brine not only lowers pH but also has antimicrobial characteristics. (Entani, 1998) while imparting a unique flavor on boiled eggs.

According to the United Egg Producers Association (2021), there are four categories of "further processed eggs." They are eggs produced as refrigerated liquid egg products, frozen and cooked egg products, dried egg products, and non-food byproducts. Each of the further
processing for human consumption areas has tremendous food safety protocols to contend with ensure a safe final product. For all areas of processing, the U.S. egg products inspection regulation requires that eggs for further processing be washed and sanitized before they are broken. Musgrove et al. (2008) collected egg samples from in-line egg-producing facilities. They reported that commercial washing procedures successfully removed a significant proportion of the Enterobacteriaceae species and related organisms from the eggs. The egg washing procedure requires a specific water wash temperature, a defined amount of time that the eggs can be exposed to water, and strict control of sanitizer concentrations and contact times. These controls improve the sanitary quality by reducing pathogens that might be on the shell surface.

While commercial surface washing of further processed eggs is effective, it may not eliminate all sources of shell egg contamination. As previously mentioned, bacteria can reside inside the egg's pores, between the shell and the egg membrane, and occasionally in the yolk of an uncracked egg. This can be caused by bacteria that inhabit the hen's ovary or oviduct and contaminate the yolk or albumen before lay. Fletcher and Smith (1989) studied methods of sampling boiled eggs for pathogenic activity and found large numbers of bacteria associated with the inner shell surface (membrane surfaces). For this reason, processed egg products must include a pasteurization step to eliminate salmonellae. Pasteurization time and temperatures are determined according to validated data showing the D-value required to reduce any *Salmonella* sp. by 90% (United Egg Producers Association, 20XX)

Pickled eggs rely on a multiple hurdle approach for safety, including thermal processing followed by acidification. Scientific data must support the minimum time and temperatures required to provide a 5-log reduction of existing pathogens and a product with zero *Listeria*. Validated data related to adequate acidification in all parts of the final product is necessary to
ensure that there is no opportunity for *C. bot* spores to germinate into vegetative cells and produce toxins.

**Quail eggs**

Lukanov (2017) reported that quail eggs make up about 10% of all shell eggs consumed globally. He estimated between 1.2 to 1.3 million tonnes of quail eggs are consumed each year. China is the world's largest producer of quail and quail eggs (Katerynych and Pankova, 2020). In a comprehensive study of quail origin, Chang et al. (2005) report more than 70 domestic and 20 wild quail species worldwide. The domestic variety is used for meat and egg production. The most common egg-laying quail include Japanese, Korean, and Chinese recessive feather and French white feather quail (Shanaway, 1994). Worldwide, most quail egg production occurs in East Asia and Brazil, while meat production primarily occurs in Europe, the United States, and China. Spain, China, France, Italy, Brazil, the U.S., and Japan dominate the meat and egg production. (Lukanov, 2017). According to the USDA Census of Agriculture, in 2017, there were 3,061 quail farms, with 7,367,055 quail and sales of $22,538,462 in the United States.

The popularity of quail eggs in American cuisine has risen slowly but steadily and has only started showing up on menus in the last 40-50 years. This progression started with quail eggs being a common menu item in sushi restaurants and then making their way to high-end restaurants, often served as an amuse bouche. More recently, quail eggs can be found in retail locations such as grocery stores and online retailers and occasionally in convenience stores. Quail eggs are small, so they tend to be considered a specialty food.

![Figure 3: Deviled Quail Eggs. Adair Hoover, ©2022](image-url)
Preparations include frying, boiling, deviled, pickled, and baked. They are perfectly suited for pickling because of their small, one bite-size, which appeals to consumers' need for convenience.

Quail eggs are highly nutritious and have been used in China to treat various health issues. In a 2017 study of the nutritional composition of quail meatballs and pickled eggs, Bayomy et al. describe quail eggs as a true universal panacea, listed third as a natural medicinal remedy in China, preceded by snake venom and ginseng.

Quail eggs are nutritionally superior to chicken eggs because they are richer in antioxidants, minerals, and vitamins (Liu et al., 2020). The combination of higher nutrition with the fact that quail production is simpler than other avian species (they are easy to farm and can be raised for meat and eggs) makes them a viable alternative to chicken eggs. One of the challenges to this alternative is that there is very little research specific to the food safety aspects of quail eggs. While there are few available studies on the acidification of quail eggs, there are several published articles regarding the quality of pickled quail eggs. In 1976, Angalet and coworkers evaluated customer acceptability of quail eggs pickled in five different brines. They reported that all five recipes were generally well accepted by their panelists. Bayomy et al. (2017) studied the nutritional composition of quail meatballs and pickled quail eggs. They found that they are both acceptable market products and that quail eggs are a nutritionally beneficial food. It could be tempting to treat quail eggs in the same manner as chicken eggs; however, their size and composition differences prohibit a fair comparison. Physical differences in compositions are reported in Table 1.
Table 1: Physical characteristics of Chicken Eggs compared to Quail Eggs.

<table>
<thead>
<tr>
<th>Egg Parts</th>
<th>Amount Chicken Egg</th>
<th>Amount Quail Egg</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>58</td>
<td>11</td>
<td>Gram mean weight</td>
</tr>
<tr>
<td>Albumen</td>
<td>55.8</td>
<td>59.7</td>
<td>% total weight</td>
</tr>
<tr>
<td>Yolk</td>
<td>31.9</td>
<td>32.7</td>
<td>% total weight</td>
</tr>
<tr>
<td>Egg Shell</td>
<td>12.3</td>
<td>7.4</td>
<td>% total weight</td>
</tr>
</tbody>
</table>

Sun et al. (2019) compared the egg quality and albumen properties of eggs from six different avian species. They found that quail albumen had a similar protein concentration to chicken albumen but had higher gel strength and a higher ratio of essential amino acids/total amino acids than chicken albumen. When the nutritional compositions of chicken and quail eggs are compared, quail eggs provide more calories from fat, higher monosaturated fat, lower polyunsaturated fat, and higher cholesterol (more than twice that of a chicken egg). Furthermore, quail eggs contain higher levels of Beta-carotene, some of the B-vitamins (B1, B2, B3, B12), folate, calcium, iron, phosphorus, and zinc (Table 2). For this reason, some researchers have reported that quail eggs have superior nutrition to chicken eggs. (Egg innovations book)

Data obtained from Characteristics of Egg Parts, Chemical Composition, and Nutritive Value, of Japanese Quail.

Table 2: Nutrition facts of Chicken Eggs and Quail Eggs.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount Chicken Egg</th>
<th>Amount Quail Egg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>143.00 kcal</td>
<td>158.00 kcal</td>
</tr>
<tr>
<td>Calories from Fat</td>
<td>85.59 kcal</td>
<td>99.81 kcal</td>
</tr>
<tr>
<td>Calories from Sat Fat</td>
<td>28.13 kcal</td>
<td>32.01 kcal</td>
</tr>
<tr>
<td>Protein</td>
<td>12.56 g</td>
<td>13.05 g</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>0.72 g</td>
<td>0.41 g</td>
</tr>
<tr>
<td>Total Dietary Fiber</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nutrient</td>
<td>Value</td>
<td>Value</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Total Soluble Fiber</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dietary Fiber (2016)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Soluble Fiber (2016)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>0.37 g</td>
<td>0.40 g</td>
</tr>
<tr>
<td>Added Sugar</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Monosaccharides</td>
<td>0.37 g</td>
<td>-</td>
</tr>
<tr>
<td>Disaccharides</td>
<td>0 g</td>
<td>-</td>
</tr>
<tr>
<td>Other Carbs</td>
<td>0.35 g</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Fat</td>
<td>9.51 g</td>
<td>11.09 g</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>3.13 g</td>
<td>3.56 g</td>
</tr>
<tr>
<td>Mono Fat</td>
<td>3.66 g</td>
<td>4.32 g</td>
</tr>
<tr>
<td>Poly Fat</td>
<td>1.91 g</td>
<td>1.32 g</td>
</tr>
<tr>
<td>Trans Fatty Acid</td>
<td>0.04 g</td>
<td>-</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>372.00 mg</td>
<td>844.00 mg</td>
</tr>
<tr>
<td>Water</td>
<td>76.15 g</td>
<td>74.35 g</td>
</tr>
<tr>
<td>Vitamin A – IU</td>
<td>540.00 IU</td>
<td>543.00 IU</td>
</tr>
<tr>
<td>Vitamin A – RE</td>
<td>160.75 mcg</td>
<td>157.00 mcg</td>
</tr>
<tr>
<td>Vitamin A – RAE</td>
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</tr>
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<td>Carotenoid RE</td>
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</tr>
<tr>
<td>Retinol RE</td>
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</tr>
<tr>
<td>Beta-Carotene</td>
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<td>11.00 mcg</td>
</tr>
<tr>
<td>Vitamin B1 – Thiamin</td>
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<td>0.13 mg</td>
</tr>
<tr>
<td>Vitamin B2 - Riboflavin</td>
<td>0.46 mg</td>
<td>0.79 mg</td>
</tr>
<tr>
<td>Vitamin B3 – Niacin</td>
<td>0.07 mg</td>
<td>0.15 mg</td>
</tr>
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<td>Vitamin B3 - Niacin Equiv.</td>
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<td>3.53 mg</td>
</tr>
<tr>
<td>Vitamin B6</td>
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<tr>
<td>Vitamin B12</td>
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<td>1.58 mcg</td>
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<td>Biotin</td>
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<tr>
<td>Vitamin C</td>
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<td>55.00 IU</td>
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</tr>
<tr>
<td>Vitamin E</td>
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<td>Folate</td>
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<td>66.00 mcg</td>
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<td>47.00 mcg D.F.E.</td>
<td>66.00 mcg D.F.E.</td>
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<td>Calcium</td>
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<td>0.06 mg</td>
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<tr>
<td>Fluoride</td>
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<td>Iodine</td>
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<td>3.65 mg</td>
</tr>
<tr>
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<td>Quail Egg</td>
<td>Chicken Egg</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
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<td>Magnesium</td>
<td>12.00 mg</td>
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<tr>
<td>Zinc</td>
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<td>Omega 3 Fatty Acid</td>
<td>0.11 g</td>
<td>0.04 g</td>
</tr>
<tr>
<td>Omega 6 Fatty Acid</td>
<td>1.74 g</td>
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</tr>
<tr>
<td>Choline</td>
<td>293.80 mg</td>
<td>263.40 mg</td>
</tr>
</tbody>
</table>

Comparison of quail egg to chicken egg nutrients.
Data obtained from the USDA Nutrient Database

**Pathogens Associated With Eggs**

In the U.S., it's estimated that there are 48 million cases of foodborne illness annually, causing sickness in 1 out of 6 Americans each year. These illnesses result in an estimated 128,000 hospitalizations and 3,000 deaths annually (F.D.A. 2022). According to the C.D.C. (2022), the five top pathogens linked to foodborne illness are Norovirus, *Salmonella*, *Clostridium Perfringens*, *Campylobacter*, and *Staphylococcus aureus* and cause 58%, 11%, 10%, 9% and 3% of foodborne outbreaks each year. The top pathogens likely to cause severe illness and hospitalization are *Clostridium botulinum*, *Listeria*, *Escherichia coli*, and *Vibrio*. Of these pathogens, three were selected for this research because of their association with foodborne illness in poultry and pickled eggs. It would not be uncommon to find *Salmonella*, *E. coli*, and *Listeria* resident in a farm environment and commercial processing plant. Additionally, these pathogens have a past association with foodborne outbreaks in shell eggs, poultry, and pickled foods. Additional discussion on *Salmonella*, *Listeria*, and *E. coli* is provided below.
Salmonella

*Salmonella* spp. are a motile, non-spore-forming, gram-negative, rod-shaped bacterium in the family Enterobacteriaceae and genus *Salmonella*. There are two species of *Salmonella*: *S. enterica* and *S. bongori*. Each species has multiple serotypes that together approach approximately 2500 in number. (Bad Bug Book 2012). *Salmonella* is a common pathogenic bacterium that can live in animal and human gastrointestinal tracts and is shed through feces. Humans may become infected by consuming contaminated food or water. *Salmonella* infection, termed salmonellosis, may cause significant illness, but often people with the infection have no symptoms (Mayo Clinic, 2022).

The C.D.C. estimates that *Salmonella* causes 19,336 people to be hospitalized each year in the United States. The Cost of *Salmonella* illness is estimated to be $365 million, per year, just in medical costs and approximately 3.7 billion per year when medical expenses, loss of life, and lost wages are factored in. (USDA Economic Research Service, 2015). An interesting trend in *Salmonella*, reported by the C.D.C. in 2015, shows that yearly infections due to this microbe mostly stayed the same from 2008 to 2015. *Salmonella* is strongly associated with foodborne illness in shell eggs and is a pathogen of concern worldwide. Montville (2012) describes infections with egg-borne *Salmonella enterica* serovar Enteritidis infection as an ongoing pandemic. Chousalkar et al. (2017) reported 113 egg or egg product-related outbreaks from *Salmonella* in the U.S. from 2002 to 2014. In 2018 the F.D.A. recalled eggs due to a *salmonella* outbreak infecting 45 people. In February 2021, shell eggs were implicated in a foodborne outbreak in Canada with 70 laboratory-confirmed cases. Chousalkar et al. (2017) indicated that considering the risk of foodborne illness from *Salmonella* in eggs was low considering the high level of consumption.
**Listeria**

*Listeria monocytogenes* (*L. monocytogenes*) is a gram-positive, rod-shaped, facultative bacterium. There are currently 17 serotypes in the *Listeria* genus (Orsi et al., 2016). Only *L. monocytogenes* and *L. ivanovii* are pathogenic, with *L. ivanovii* mainly associated with ruminants and not humans (F.D.A., Bad Bug Book 2012; Montville 2012). *Listeria* species are commonly found in moist environments, soil, water, and decaying vegetation and animals. It is considered an environmental pathogen and can take up residence in food processing plants' drains, equipment, and air-handling systems. Once in residence, it can be tough to eliminate because it adheres to surfaces and forms biofilms in hard-to-reach places (Montville 2012; F.D.A. 2017). Moreover, as an environmental pathogen, it can serve as a source of contamination of food and other surfaces.

When consumed by humans, *Listeria monocytogenes* causes the disease listeriosis. The C.D.C. estimates that the annual number of *Listeria* cases in the U.S. is about 1,600. While that number is lower than some other foodborne pathogens, a death rate of 16% makes it a significant pathogen of concern. One of the things that makes *Listeria* unique compared to other foodborne pathogens is its ability to survive in cold temperatures. It can grow at refrigerator temperatures and tolerates temperatures as low as 1°C.

In December 2019, F.D.A. launched a multi-state investigation into a *Listeria* outbreak in commercially boiled eggs. F.D.A concluded that at least eight people across five states were infected with *Listeria* from the boiled eggs (F.D.A. 2019). These eggs were distributed under refrigeration. While these eggs were not pickled, they were hard-cooked, which is one of the hurdles in the pickling process. Thus, there is also a food safety concern relating to *Listeria* and pickled foods. *Listeria* can become acid-tolerant through acid shock due to various overlapping
mechanisms, including adaptive acid tolerance response and glutamate decarboxylase system. (Feehily et al. 2014). These systems are described further by Smith et al. (2012). In the U.S., there is a zero-tolerance level of Listeria in ready-to-eat foods F.D.A., 2008).

*Escherichia coli*

*Escherichia coli* (*E. coli*) is a large and diverse group of rod-shaped, gram-negative bacteria (F.D.A. Bad Bug Book 2012). Most *E. coli* are harmless, residing naturally in the gastrointestinal tract of humans and animals, where they are beneficial to intestinal health (F.D.A. 2019). *E. coli* is commonly found in poultry and is so common that the USDA-FSIS has established a performance standard for meat and poultry slaughter establishments (USDA 2005). In this context, it serves as an indicator organism whereby higher levels demonstrate the likelihood of fecal contamination and sanitation failure.

Some strains of *E. coli* can cause illness. *Enterohemorrhagic Escherichia coli* (EHEC) is one of six major categories of diarrheagenic *E. coli* groups and includes the serotype O157:H7, which is the predominant strain in the U.S. (Monteville, 2012). EHEC is a gram-negative, rod-shaped enterotoxin-producing bacteria that produces Shiga toxins. (F.D.A., Bad Bug Book 2012). If *E. coli* produces Shiga toxin, it only takes a minuscule amount to cause illness in humans (about 10 *E. coli* cells). *E. coli* O157:H7 was first recognized as a pathogen that caused human illness in 1983. It was initially associated with beef, but since then it has been implicated as the source of foodborne illnesses caused by contaminated leafy greens, sprouts, raw milk and cheeses, and raw beef and poultry. Additionally, *E. coli* has been linked to foodborne illness in high acid fruit juices and is under scrutiny because of the potential to survive in acidified foods. It is considered to be an indicator microorganism because it is linked to the presence of fecal material and inadequate sanitation. The F.D. A. requires detailed tests methods for detecting *E.*
*coli* in various foods, including chilled and frozen foods, bottled water, shellfish, and citrus juices, in their Bacterial Analytical Manual (B.A.M.), (F.D.A. 2020). *E. coli* 0157:H7 is a pathogen of concern in pickled eggs because it is common in poultry and associated with acid foods.

*Clostridium Botulinum*

*Clostridium Botulinum* is an anaerobic, gram-positive, spore-forming rod bacteria that produces a potent neurotoxin (Montville, 2012). This bacteria exists in two forms: vegetative cells and spores. The spores are ubiquitous in nature and serve as a protective strategy allowing the microorganism to survive heat treatments and incorrectly or minimally processed food treatments. When introduced to a specific environment, the spores will grow into vegetative cells that produce a deadly toxin. That environment is moist, low acid, low oxygen, at favorable temperatures, for a period of time. That is the exact environment that is created when canning foods if they are not adequately acidified.

Illness from the *C. botulinum* toxin is rare but important because when an infection does occur, it causes extreme illness and may cause death. The disease caused by *C. botulinum* is called botulism.

Acidified, canned foods have a past association with botulism. In 1997 there was an instance of foodborne botulism from home pickled eggs (C.D.C., 1998). After an investigation, it was determined that *C. botulinum* was in the egg yolk. Ensuring that all food components of low acid canned food reach a pH below 4.6 is the primary food safety factor in safely preparing acidified foods.
Regulation Of Acidified Food

Overall, acidified foods have a relatively safe history. However, recent outbreaks of foodborne pathogens in some high acid foods have led to scrutiny. According to the C.D.C., 34 outbreaks, 873 illnesses, and 43 hospitalizations occurred between 1990 and 2020 from the consumption of apple cider, apple juice, and orange juice – products with pH < 4.6 (CDC NORS, 2022). In 2012 Wegmans Foods Mart recalled nine foods containing boiled eggs in brine from a production lot testing positive for *Listeria monocytogenes*. Since pickled eggs are ready-to-eat, this could have caused a severe foodborne illness outbreak and possible death. Among those egg products were kosher pickled eggs. (FSN Food Safety News, 2012).

In 1973 several cases of foodborne botulism were attributed to acidified foods, and further investigation by the F.D.A. led to the addition of acidified food regulation to the low acid can food regulation (21 CFR 113 LAF) that had been implemented earlier that year. The U.S. Food and Drug Administration Code of Federal Regulations (C.F.R.), title 21, part 114, Acidified Foods, defines specific requirements for producers of acidified foods. F.D.A. defines an acidified food as low-acid foods to which acid(s) or acid food(s) are added; these foods include, but are not limited to, beans, cucumbers, cabbage, artichokes, cauliflower, puddings, peppers, tropical fruits, and fish, singly or in any combination. They have a water activity (Aw) greater than 0.85 and have a finished equilibrium pH of 4.6 or below. The average pH of quail egg albumen: 8.8 to 9.25, and the yolk: 6.25 to 6.9 (Northcutt et al., 2022) are reduced to below 4.6 during the acidification process of pickling.

The C.F.R., title 21, part 114, outlines specific recommendations and requirements for the production of acidified food, which include the following:

- Operating under current good manufacturing practices.
• Have a supervisor who has completed an FDA-recognized program for food safety in acidified foods, oversee personnel who process and package acidified foods.
• Operate under established production and process control plans, establishing and registering a scheduled process.
• Test and record the pH of the product during each batch to confirm that the pH of the final product is less than 4.6.
• Record any deviation from scheduled processes and record-keeping throughout the production process.

Before manufacturing, producers of acidified foods must have products evaluated by a qualified process control authority, must register their process and facility with F.D.A., and attend an F.D.A. Better Process Control (B.P.C.) School (C.F.R., title 21, part 114, Acidified Foods). B.P.C. School is specific to food producers of acidified and low acid canned foods and provides educational information on safe handling and processing procedures.

When it comes to poultry eggs, the regulatory authority changes depending upon the product type. Chicken eggs in the shell are regulated by F.D.A., while USDA regulates egg products. The USDA regulates quail eggs and eggs products because they are classified as game birds. Regulatory jurisdiction of quail eggs varies from state to state. In South Carolina, quail meat is regulated by the South Carolina Meat and Poultry Inspection Department; USDA regulates unprocessed quail eggs; and pickled quail eggs are regulated by SCDA (email communication, Angie Culler-Mathew, SCDA, Director, Food Safety Department)

Quail eggs do not have to meet the F.D.A. Egg Safety Rule or the USDA Egg Product Inspection Act. If sold across state lines, quail eggs must meet Food Safety Modernization Act,
Hazard-Analysis, and Risk-based Preventive Controls for Human Food. (FSMA 2021). Intrastate sales have to comply with state and local regulations.

**Pickling Process**

There are numerous methods of preserving foods, including but not limited to temperature control, pasteurization, dehydration, acidification, and the use of chemical agents. All forms act to inhibit, inactivate and prevent cross-contamination of microorganisms. Pickling is an ancient preservation technology used to preserve food and extend shelf life, typically without temperature control, dating back to the 3rd B.C. in China (Barrett 1994). This method is still a commonly used technique today. Consumers appreciate the convenience of longer shelf life and the unique flavors that pickling imparts on foods. The process involves "acidifying" a low acid food (pH above 4.6) by lowering the pH of solids with an acid brine. This is often done by adding acetic acid or vinegar (pH 2.30 – 3.20) to the low acid solids, then holding the product long enough for the acid to penetrate the solids and lower the pH. Typical foods for pickling include vegetables, fruits, and boiled eggs. Pickling can apply it to all poultry eggs, including duck, chicken, quail, and geese. It is an excellent preservation method for extending the shelf-life of boiled eggs and greatly extends the shelf life of a hard-cooked egg, which has a refrigerated shelf life of about seven days (Barbut et al. 1987). A standard method for commercially prepared pickled eggs is as follows:

1. Fill a large cooking pot with water
2. Bring water to a boil
   1. Add eggs and simmer for 11 minutes
   2. Remove eggs and place them into a water bath to cool
   3. Peel eggs and put them into a 16-ounce glass jar
4. Add desired herbs, spices, and vegetables (peppercorns, mustard seed, onions, peppers)
5. Cover with a hot acid brine (commonly 5% vinegar)
6. Thermally process for an amount of time designated by a Process Control Authority.
7. Incorporate a container pasteurization step

Food Safety of Pickling

Pickling is a very safe preservation method when performed using research-based food safety practices. It can, however, become unsafe when specific food safety hurdles are not followed (Acosta 2017). Pickling boiled eggs presents particular challenges because of the composition of the eggs compared to other foods. Ensuring that the acetic acid penetrates the albumen and completely acidifies the yolk is not as predictable as with the more homogeneous fruits and vegetables. For food safety purposes, the entire boiled egg, including the yolk, must be below 4.6 within 24 hours during a shelf-stable process, or the eggs must be refrigerated during acidification. This is of particular concern in the yolk, which has no natural defense against pathogens and has a higher fat content that can slow acidification.

There is an abundance of scientific literature related to pickling fruits and vegetables. Breidt and colleagues at the USDA-ARS Food Market Quality and Handling Research Unit have conducted numerous studies on the thermal processes required to achieve a pathogenic reduction in acidified vegetables. In 2013, they researched the development of an effective treatment for a 5-log reduction of *Escherichia coli* in refrigerated pickle products (Breidt et al., 2013). In 2014, they conducted research on the thermal processing of acidified foods with pH 4.1 to pH 4.6. Also, in 2014 they investigated the 5-log reduction times for *Escherichia coli* O157:H7, *Salmonella* enterica, or *Listeria monocytogenes* in acidified foods with pH 3.5 or 3.8 (Breidt et al., 2014). This large body of research conducted on pickling fruits and vegetables has resulted in
a thorough understanding of the processes required to produce a safe product. However, research specific to pickled eggs is more elusive.

Although several commercial processors are marketing a large portion of their hard-cooked eggs as pickled products, few publications have examined eggs' physical and chemical changes during acidification. Even fewer publications have reviewed the pickling of quail eggs. Acton and Johnson (1973) tested pH, the rate of acid penetration into chicken egg components, and the impact on bacteria recovery. They found that pH decreased rapidly in egg white and very slowly in egg yolk. Acosta et al. (2013) researched the time it took to reduce the pH in the center of yolk when pickling chicken eggs in several different acetic acid percentages and under different brine temperatures. The study showed that as the percentage of acetic acid in brines decreased, so did the acidification rate, while heat increased the time it took to acidify the center of the yolk (Acosta et al., 2013). Similar studies have not been conducted on quail eggs, and instead, information has been extrapolated from chicken eggs, which lacks validity due to differences in egg size and composition. This is noteworthy because the composition of different avian eggs significantly differs among species. Sun et al. (2019) compared the physical characteristics of the domestic chicken, duck, goose, turkey, quail, and pigeon and showed that egg weight varies from 11 g to 139 g, the proportion of yolk from 19.3 % to 37.9%, and breaking strength from 0.91 kg/cm² – 8.04 kg/cm². The penetration of acid to the center of the yolk is an important food safety factor in the pickled egg process. The composition of albumen and yolk contributes to the time it takes for the brine to reach the center of the egg. A comparison of chicken eggs to quail eggs shows the following:

Albumen percentage: chicken 62.74 ± 1.49 and quail 73.56 ± 2.10

Yolk percentage: chicken 27.52 ± 1.56 and quail 30.19 ± 2.44.
Additionally, the chicken egg is also more viscous, adhesive, and has more tackiness. At the same time, heat-induced egg albumen gel showed that the quail egg has more hardness and water holding capacity (Sun et al., 2019). These parameters contribute to the time and efficiency of acidification, and their variability emphasizes the need for studies specific to quail eggs.

Physical Characteristics

The physical changes that occur during food processing are an important factor in the quality of the final product. Quality is not a well-designed attribute. It comprises many properties or characteristics (Pathare et al., 2013). Pickling is a food preservation and processing method. F.D.A. defines processed foods as making food from one or more ingredients or synthesizing, preparing, treating, modifying, or manipulating food, including food crops or ingredients. (F.D.A., 2021). In the case of pickled eggs, processing includes boiling eggs and the addition of vinegar. These processes offer a layer of food safety but can also change the physical characteristics. When preservation techniques are applied to food, a series of physical and/or chemical changes can occur that influence the quality of the final product. Compared to their raw unprocessed state, the physical changes that occur during pickling eggs include the change in weight, pH, water activity, texture, and color. Weight can predict albumen weight and yolk weight (Ratriyanto et al., 2019). Water activity and pH are physical characteristics primarily related to food safety. Texture and color can be essential characteristics in the consumer acceptability of food.

Color

Color can impact a consumer’s assessment of quality and taste. Color is the sensation experienced by a person when radiant energy within the visible spectrum of (380-770 nm) falls
upon the eye's retina (Wrolstad, 2017). While mathematical values can be assigned to describe color, it is challenging, according to Leon et al. (2005). The appearance and color of foods are the first indicators of quality by consumers. (Acosta et al., 2011) describe the appearance of food as one of the essential sensory attributes of fresh and processed foods. The color was measured using the International Commission on Illumination (C.I.E.) method using a spectrophotometer color measurement system. To generate a more uniform color space, C.I.E. L*a*b* was developed based on the C.I.E. (x,y,z) 1931 system (Cheng et al., 2018). L* represents lightness from black (0) to white (100) on a scale of zero to 100, while a* and b* represent chromaticity with no specific numeric limits. Negative a* corresponds with green, positive a* corresponds with red, negative b* corresponds with blue and positive b* corresponds with yellow. The spectrophotometer was standardized, and then groups of albumin and yolk were measured.

*pH and Water Activity*

Pickled eggs are a product of acidifying eggs. The U.S. Food and Drug Administration Code of Federal Regulations (C.F.R.), title 21, part 114, Acidified Foods, defines specific requirements for producers of acidified foods. F.D.A. defines an acidified food as low-acid foods to which acid(s) or acid food(s) are added. They have a water activity (aw) greater than 0.85 and have a finished equilibrium pH of 4.6 or below. The average pH of quail egg albumen is 8.8 to 9.25, and the yolk is 6.25 to 6.9 (Northcutt et al., 2022). The pH and water activity were measured at 24 and 48 hours to determine whether a commercial brine compared to control brines would lower the pH below 4.6 within 24 hours and if there would be any further reduction at 48 hours. The water activity was measured to determine whether the Aw of the eggs was higher than 0.85 at 24 and 48 hours.
Texture

Texture is a sensory characteristic, and consumer preference may depend on experiencing an expected texture. The texture is an essential physical characteristic of boiled eggs. A good texture is an egg that does not exhibit a rubbery (overcooked) or gelatinous (undercooked) mouthfeel (USDA, 2021). Consumers would likely anticipate the texture of a quail egg to be similar to a chicken egg. In a comparative study of preference and compositions of different avian eggs, Akinwumi et al., (2019) found that in a sensory analysis, panelists preferred eggs from quail and exotic hens most for taste, texture, and overall acceptability. The texture was measured to determine the force to fracture and rupture strength of eggs at 24 hours and 48 hours.

There is a lack of science-based information specific to pickled quail eggs. Commercial producers of pickled eggs need research-based information on brine solutions, thermal processes, and holding times to reduce pathogens. A microbiological study, pH test, and water activity test were conducted to determine parameters to produce a safe final product that meets regulatory requirements. Additionally, quality is critical to consumer acceptance and repeat consumption of food products. Weight, color, and texture were studied to determine whether pickling quail eggs would enhance or diminish quality.

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CHAPTER THREE

PHYSICAL PROPERTIES OF HARD-COOKED PICKLED QUAIL

Abstract

The work of this chapter considers the quality characteristics of quail eggs after the pickling process. Quality is essential because consumers want safe, convenient, and high-quality foods. There are numerous studies related to the quality of pickled chicken eggs, but there is only a small body of accessible information specific to pickled quail eggs. The weight of 200 eggs was measured on raw, peeled, and boiled eggs. Four hundred pickled quail eggs were tested for texture, color, water activity, and pH in various treatments. Control treatments and commercial brine treatments included heated eggs covered with hot brine or water and unheated eggs covered with hot brine or water. The pickled quail eggs were measured after 24 and 48 hours (about two days) of pickling. The analysis found that there was a 14% reduction of weight when boiled, peeled eggs were then pickled. Texture in commercial brine solutions (hot or cold) was harder to break and less brittle than control eggs. The color was similar across treatments, but changes from 24 to 48 hours may be less appealing to consumers. The pH was reduced below 4.6 after 24 hours and did not significantly drop at 48 hours, and the water activity was consistent across treatments and hold times and was above 0.85 in all measures. This data will be a valuable addition to the body of information available for pickled quail eggs.

Introduction

The process of pickling foods is an ancient food preservation method that can be traced back to 3000 BC, when the Babylonians first used vinegar from fruit and the sap of date palms for alcohol, food, and as a pickling agent (Bourgeois & Barja 2009). Today, pickled foods are popular because of their unique flavor, texture, color, and superior shelf-life. Additionally, many
consumers purchase pickled food products because they view them as a minimally processed product that is nutritious, healthy, and convenient (Acosta et al., 2013; Behera et al., 2020). Among the commercially available pickled foods, eggs present a unique challenge because they are not as homogeneous as fruits and vegetables. Numerous studies have investigated the sensory and quality aspects of hard-cooked, pickled eggs, but most have focused on the more popular chicken egg (Acton and Johnson, 1973; Essary and Georgiades, 1979; Acton, 1981; McCready, 1974). These investigations have demonstrated that the parameters that have the most significant influence on the acidification of pickled eggs are the type and concentration of acid, brine composition, egg-to-brine ratio, egg temperature, pickling brine temperature, storage time, and temperature of the finished product, (Acton and Johnson, 1973; Essary and Georgiades, 1979; Acton, 1981; Usaga et al., 2017).

While the most common acidulant for pickling food is acetic acid, some foods have been pickled with citric acid (Fischer et al., 1985) and lactic acid (Eze et al., 2018). Previous research has demonstrated that the acidification process for pickled eggs occurs much faster (less time to achieve a pH of 4.6 or lower) with a higher concentration of acetic acid in the brine (Acosta et al., 2014). Other researchers showed that the brine recipe could affect the acidification rate as non-acid ingredients such as spices can react with the acidulant, reducing its concentration and causing either a higher equilibrium pH or a slower acidification process (Acton and Johnson, 1973). The effect of these other ingredients on the acidification process should be accounted for to ensure that the egg achieves pH 4.6 or lower as required by the Code of Federal Regulations, Title 21 Part 114 (Acton and Johnson, 1973; Usaga et al., 2017).

The process of pickling foods to be held without temperature control may create an atmosphere in which *Clostridium botulinum* (*C. bot*) grows and produces botulinum toxin. *C. bot*
toxin attacks the autonomic nervous system blocking the production of acetylcholine and affecting muscle contraction, including muscles needed for breathing. The environment that favors the formation of C. bot toxin is low acid (pH > 4.6), time in optimal conditions, temperature (10ºC - 48ºC), moisture (water activity > 0.86), and anaerobic conditions (Montville, 2012). Food manufacturers of acidified foods must follow research-based recommendations for processing time, temperature, and acidification, to create a safe product. Federal regulations include specifications to ensure that acidification will inhibit or inactivate C. bot. Specifically, Title 21 of the Code of Federal Regulation (C.F.R.) defines pickled foods or “acidified foods” as low-acid (high pH) food products to which acid or acid food ingredients have been added to improve the safety of the final product (F.D.A., 2021). According to this regulation, acidified foods must have a water activity above 0.85 and a final equilibrium pH equal to or below 4.6 (F.D.A. 2021). This means that the acidification process must reduce the quail egg's pH of all parts (albumen and yolk). The average pH of the quail egg albumen (pH 8.8 to 9.25) and yolk (pH 6.25 to 6.9; Buyukavuz et al., 2022) must be reduced during the pickling process to pH 4.6 or less within 24 hours of acidification to achieve a safe product.

Acton (1981) reported that the pickling of hard-cooked eggs on a commercial scale constituted a major change in the traditional product's characteristics. Thus, studies evaluating the quality of pickled eggs may be of value for establishing parameters that influence consumer preference. Since most consumers make their purchase decisions based on appearance, evidence of discoloration on any food is often associated with reduced quality and questionable product safety (Barrett et al., 2010). Brine formulations can significantly affect the color of pickled quail eggs and consumer acceptability. Gunathilaka et al. (2021) evaluated consumer preference for quail eggs pickled in seven different brine solutions. They found that the color and tenderness of
the albumen varied depending on the brine solution and that one brine had better consumer acceptability. When the pH of a food is altered, it affects color and texture (Andrés-Bello et al., 2013). In addition, when eggs are hard-cooked, the process affects egg color and texture. In some cases, an unappealing greenish-black discoloration of the yolk can occur during hard cooking. This happens if ferrous sulfide is produced at the interface of the yolk and albumen by the reaction of iron from the yolk and hydrogen sulfide from the albumen (Baker et al., 1967). The effect of pH on the yolk had a definite impact on blackening at the interface of the yolk and albumen in a study by Baker et al., (1967). Because quality attributes have an enormous impact on repeat purchases and marketing, the present study was conducted to determine the impact of the pickling process on the quality attributes of quail eggs. Specifically, the effect of various brine solutions on the weight, pH, water activity, texture, and color of pickled quail eggs was determined.

**Materials And Methods**

Four hundred eggs were collected from a commercial quail farm within two days of lay. Eggs were inspected, sorted, and packaged for retail sale by the producer, including removing any obvious damaged or cracked eggs. Before packaging, the producer sprayed the eggs with a dilute solution of 45 to 90 ppm of quaternary ammonium chloride (ChemStation ChemSan0300). Eggs were packaged and held for 1-2 days at 4°C until they were transported to the laboratory. Eggs were stored under refrigeration for less than 24 hours at the laboratory before being analyzed. During each of the two replications, eggs were removed from refrigeration and held at room temperature for approximately one hour. Eggs were individually weighed raw in the shell and then boiled by placing 20 eggs at a time in a metal strainer. The metal strainer was completely submerged in a container of 100°C boiling water. Eggs were boiled for 4 minutes,
removed from the boiling water, and immediately submerged in ice water for 5 minutes. After cooling, eggs were peeled using a Vevor Electric quail egg peeler machine, 500G/H. Peeled eggs were inspected, and those with imperfections were discarded. Eggs were sorted into groups of 20 eggs per group. One group of eggs was placed into a single 473 mL (16 ounce) glass jar, and one of the liquid treatments, as noted in Table 3, was added to the jar.

Table 3: Control or brine solutions used to pickle hard-cooked Japanese quail eggs.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Code</th>
<th>Solution Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Hot</td>
<td>CH</td>
<td>Boiled eggs were placed in a pot of water at 70°C and held for 5 minutes. They were then removed, dried, placed in jars, and covered with 237 ml of 70°C water.</td>
</tr>
<tr>
<td>Control Cold</td>
<td>CC</td>
<td>Unheated eggs are placed in jars and covered with 237 ml of 70°C hot water.</td>
</tr>
<tr>
<td>Brine Hot</td>
<td>B.H.</td>
<td>Boiled eggs were placed in a pot of water at 70°C and held for 5 minutes. They were then removed, dried, placed in jars, and covered with 237 ml of 70°C Commercial brine.</td>
</tr>
<tr>
<td>Brine Cold</td>
<td>BC</td>
<td>Boiled eggs were placed in jars and covered with 237 ml of 70°C Commercial brine.</td>
</tr>
</tbody>
</table>

Two jars of each treatment were prepared during each replication. One jar of each treatment was held at room temperature for 24 hours, and a second jar was held for 48 hours. After 24 or 48 hours, eggs were removed from the jars, weighed, and tested for texture, color, water activity, and pH.

Texture
The force to fracture (kg) and brittleness (mm) were measured on individual eggs using a T.A. X.T. plus texture analyzer affixed with a 5 kg load cell. Eggs measuring approximately 7 cm by 6 cm were placed on their side in the center of a stationary platform (10 cm x 10 cm). A uniaxial compression was applied at a 75% strain (Figure 5). The texture analyzer was set to a 10 g trigger force with a 2mm/second crosshead speed. The force at which the pickled eggs failed under compression (force to fracture) and brittleness were measured.

After measuring the texture of all the individual eggs from one jar, the eggs were separated into four groups, each consisting of five eggs. The egg whites and egg yolks from each group were then separated for additional testing.

Color

The color of quail egg whites and yolks was measured using a handheld HunterLab A60-1014-085 manual version 1.2 Spectrocolorimeter. Before measuring color, the spectrophotometer was standardized using a black glass light trap and a standard white tile covered with clear plastic wrap. The clear plastic wrap minimized product adherence and contamination of the device during readings. The spectrocolorimeter was set to take three readings for lightness (L*), redness (+a*), and yellowness (+b*) and then record the average of these three readings. One reading (representing the average of three) was recorded for egg whites and yolks for each pooled sample (4 readings/jar for each replication).
Water Activity

Water activity (Aw) of egg whites or yolks was tested as a true duplicate using two separate Rotronics Water Activity meters and chambers. The water activity meters were attached to a water bath to control sample temperature at 25°C. Before recording Aw, the machine was allowed to equilibrate for 30 minutes. Egg white or yolks were sealed in the chambers for an additional 30 minutes before readings were recorded.

pH

The pH of each yolk and albumen sample was measured directly on the blended samples using two individual Accumet, model 10 pH meters. The samples included albumen and yolks from pooled samples, blended with 5 times their weight with distilled water (Acton et al. 1972)

Statistical Analyses

The data analysis for this thesis was generated using JMP® Pro Version 16.1.0 Copyright © 2020-2021 S.A.S. Institute Inc. Cary, NC, U.S.A. Data were analyzed for statistical significance. Data was tested across treatment means for statistical significance (P< 0.05) using a continuous response versus categorical variable (ANOVA). For groups with a statistical difference of (P > 0.05), means were compared using the Tukey method to compare specific groups' means differences.

Results And Discussion

Boiling of quail eggs did not affect egg weight (13.1 to 13.4 g). The eggshell and adhering membranes contributed nearly 12% of the boiled egg weight (1.6 grams). After boiling and peeling, boiled eggs weighed approximately 11.9 grams (Table 4). Furthermore, pickling reduced the weight of boiled eggs by 14%. The boiling process denatured the proteins in the egg,
allowing amino acids to interact and form a three-dimensional network entrapped and excluded water. The heat changes the structure of the protein by transforming the protein from an ordered state to an unordered state without the rupture of covalent bonds (Gossett et al., 1984).

When the texture of hard-cooked eggs was measured, eggs stored in commercial brine solutions (hot or cold) were harder to break (7.0 kg fracture force) and less brittle (17.9-18.0 mm) than control eggs (5.5 to 5.6 kg fracture force; 18.3 to 18.4 mm brittleness, (Table 6). A typical curve for force to fracture and brittleness is shown in (Figure 6). Ball and Saffores (1973) reported similar findings on the shear strength of pickled chicken eggs stored in 0, 2.5%, or 4.7% salt and either 50% or 100% vinegar. These researchers reported a noticeable toughening of egg whites with increasing salt levels regardless of the vinegar concentration (Ball and Saffores, 1973). Wang and Damodaran (1991) suggested that exposed functional groups on heated proteins react with ions, and the interaction affects the texture. According to Gharbi and Labbafi (2018), there are four sulphhydryl groups on egg white proteins that can become exposed during processing and form disulfide bonds that increase the gel strength. Moreover, salts used during pickling modify the osmotic pressure, causing water to migrate from eggs which produces a firm gel structure (Yang et al., 2019)

Egg color was measured after 24 hours and 48 hours in brine and control treatments. When egg yolk color was compared among treatments after 24 hours of holding, there was no difference in lightness (L*), redness/greenness (a*), or yellowness/blueness (b*) in yolks. The color of egg yolks held for 48 hours followed a similar pattern. (Table 5). However, when egg yolk color was compared within a treatment after 24 hours to yolks held for 48 hours, lightness increased while redness and yellowness decreased. Egg yolks primarily get their color from xanthophylls, which are lipophilic carotenoids. When raw eggs age, osmotic differences between
the egg white and yolk cause moisture to migrate from the white to the yolk (Chi, 1998), and a similar phenomenon may be occurring with pickled eggs.

Albumen color was less consistent than yolk color. When color was compared across treatments but within a holding time (either 24 or 48 hour), there was no significant difference in albumen lightness. Greenness (-a*) was higher in albumen from hot control treatments compared to albumen in commercial brine treatments (Table 5). Albumen yellowness in eggs held in commercial brine treatments was more consistent, but that was not the case for the albumen yellowness in the control treatments. Comparing the albumen color at 24 and 48 hours revealed that at 48 hours, there was a decrease in lightness for cold brine and hot control brine eggs and an increase in greenness for all treatments. Albumen yellowness also increased in all treatments when comparing holding times, except for the hot control treatment.

During pickling, the pH of the egg yolks decreased to pH 3.75 to 3.99 when stored in the commercial brine solutions (Table 7). These values were approximately three pH units lower than the control yolks (pH 6.3 to 7.2). Yolk pH continued to decrease in eggs with longer holding times across all treatments (Table 7). From 24 to 48 hours of storage, the pH of control egg yolks decreased by approximately 0.6 to 2.0 pH units, while the pH of egg yolks stored in commercial brine solutions decreased by 0.15 to 0.22 units with longer holding times. Albumen pH was 2.4 to 4.3 pH units lower for eggs stored in commercial brine solutions compared to albumen of control eggs. Albumen pH stayed close to the same (0.04 units difference) or continued to decrease (0.12 units lower) for commercial brines with longer holding times. Control albumen pH decreased by 1.7 to 1.95 pH units over time. As mentioned previously, this is likely due to differences (or lack thereof) in osmotic pressure where control eggs pick up water and brined eggs release water (Sullivan et al., 2013). Sullivan et al. (2013) also indicated that acetic acid
penetrated egg white very quickly during pickling, while longer holding times were required to reach equilibrium pH for yolks. The water activity was consistent across control and brine treatments and hold times and was above 0.85 in all data (Table 7).

Overall, data collected during this study showed that pickling quail eggs for 24 hours slightly reduced the weight of eggs in commercial brines and remained nearly the same in control treatments; egg yolks were consistent among treatments, but lightness, redness, and yellowness decreased from 24 to 48 hours; albumen were mostly consistent among treatments, with an increase in greenness and yellowness from 24 – 48 hours; rupture strength was higher in commercial brine compared to controls and control treatments were more brittle than commercial brine treatments; water activity was mostly consistent with no values below 0.85; pH was significantly lower in brine treatments compared to control treatments, and while it did continue to decrease between 24 and 48 hours in brine treatments, the difference wasn't notable. Consumer preference for a darker, yellower yolk may lead to the reduced appeal of overall egg colors held for 48 hours compared to 24 hours; the rupture strength and brittleness indicate higher quality expectations for pickled eggs than eggs in water treatments. From a food safety and regulatory perspective, pH was well below 4.6, which is critical to a safe final product that is acidified and shelf-stable. Water activity remained above 0.85, a regulatory standard for acidified foods.

**Conclusion**

Pickling is an ancient and common method of preserving foods that imparts a unique flavor and appealing texture to foods. Pickled quail eggs are rising in popularity in the United States, and the need for data on physical characteristics was addressed in this study. Results revealed that the weight and texture changes due to pickling did not have an adverse effect on
quality. The pH and water activity tested at 24 and 48 hours showed that the water activity was mostly consistent across time and treatment types and remained above 0.85, a regulatory requirement for acidified foods. P.H. data showed that pickling for 24-hour was sufficient to acidify all parts of the quail eggs to a level below 4.6, and in fact, the pH was below 4.0 in all the commercial brine variations. The color was tested, and while treatment types had a minimum difference, the color of pickled quail egg yolks after 24 hours became lighter and less yellow. At the same time, albumen colors increased in greenness and yellowness. These color changes would most likely be undesirable to consumers.

**Future Research Recommendations**

Pickled foods commonly include ingredients in addition to low acid food and vinegar. Future works may address the change in physical characteristics when ingredients such as garlic, chili peppers, and spices are added to the pickled food.

**References**

Table 4: Effect of boiling and pickling on quail (Coturnix coturnix japonica) egg weight.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Temperature of eggs at pickling</th>
<th>Temperature of brine at pickling</th>
<th>Weight (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw egg²</td>
<td>N.A.</td>
<td>N.A.</td>
<td>NA</td>
<td>13.1&lt;sup&gt;ab&lt;/sup&gt; ± 0.13</td>
</tr>
<tr>
<td>Boiled eggs&lt;sup&gt;2&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>13.4&lt;sup&gt;a&lt;/sup&gt; ± 0.13</td>
</tr>
<tr>
<td>Peeled eggs&lt;sup&gt;3&lt;/sup&gt;</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>11.6&lt;sup&gt;b&lt;/sup&gt; ± 0.6</td>
</tr>
<tr>
<td>Egg shells</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>1.26 ± 0.25</td>
</tr>
<tr>
<td>Unheated eggs in commercial brine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>BC</td>
<td>approximately 18°C</td>
<td>70°C</td>
<td>10.1&lt;sup&gt;ab&lt;/sup&gt; ± 3.2</td>
</tr>
<tr>
<td>Warm eggs in commercial brine&lt;sup&gt;4&lt;/sup&gt;</td>
<td>B.H.</td>
<td>approximately 94°C</td>
<td>70°C</td>
<td>10.0&lt;sup&gt;ab&lt;/sup&gt; ± 4.6</td>
</tr>
<tr>
<td>Control Unheated eggs in water&lt;sup&gt;5&lt;/sup&gt;</td>
<td>CC</td>
<td>approximately 18°C</td>
<td>70°C</td>
<td>11.8&lt;sup&gt;ab&lt;/sup&gt; ± 7.7</td>
</tr>
<tr>
<td>Control Heated eggs in hot water&lt;sup&gt;5&lt;/sup&gt;</td>
<td>C.H.</td>
<td>approximately 94°C</td>
<td>70°C</td>
<td>11.6&lt;sup&gt;ab&lt;/sup&gt; ± 9.1</td>
</tr>
</tbody>
</table>
1BC refers to unheated eggs treated with 70°C commercial brine; CC refers to albumen samples from heated eggs (~ 94°C) treated with 70°C water; B.H. refers to heated eggs (~ 94°C) treated with 70°C commercial brine; C.H. refers to albumen samples from heated eggs (~ 94°C) treated with 70°C water.

2N = 205.

3Peeled eggs in 20 egg pooled samples, N=10.

4Eggs in commercial brine, 20 egg pooled samples, N =20.

5Control eggs in water, 20 egg pooled samples, N= 2.

Table 5: Effect of boiling and pickling on quail (Coturnix coturnix japonica) egg objective color (C.I.E. L*, a*, b*)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Lightness (L*)</th>
<th>Redness (+a*)</th>
<th>Greenness (-a*)</th>
<th>Yellowness (+b*)</th>
<th>Blueness (-b*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 = black</td>
<td>100 = white</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24 hours</td>
<td>48 hours</td>
<td>24 hours</td>
<td>48 hours</td>
<td>24 hours</td>
</tr>
</tbody>
</table>

**Yolks**

<table>
<thead>
<tr>
<th></th>
<th>Lightness (L*)</th>
<th>Redness (+a*)</th>
<th>Greenness (-a*)</th>
<th>Yellowness (+b*)</th>
<th>Blueness (-b*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YBC</td>
<td>85.8 ±2.1</td>
<td>92.9 ±2.1</td>
<td>0.8 ±0.4</td>
<td>0.3 ±0.4</td>
<td>33.7 ±1.9</td>
</tr>
<tr>
<td>CYC</td>
<td>85.8 ±6.9</td>
<td>91.9 ±7.6</td>
<td>1.1 ±0.2</td>
<td>0.3 ±0.2</td>
<td>32.1 ±1.6</td>
</tr>
<tr>
<td>YBH</td>
<td>88.2 ±1.7</td>
<td>92.2 ±1.6</td>
<td>1.0 ±0.3</td>
<td>0.04 ±0.2</td>
<td>34.0 ±1.5</td>
</tr>
<tr>
<td>CYH</td>
<td>88.9 ±1.5</td>
<td>95.5 ±1.5</td>
<td>1.3 ±0.2</td>
<td>0.4 ±0.2</td>
<td>32.2 ±1.2</td>
</tr>
</tbody>
</table>

**White**

<table>
<thead>
<tr>
<th></th>
<th>Lightness (L*)</th>
<th>Redness (+a*)</th>
<th>Greenness (-a*)</th>
<th>Yellowness (+b*)</th>
<th>Blueness (-b*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>88.6 ±2.5</td>
<td>84.5 ±2.5</td>
<td>-4.3 ±0.2</td>
<td>-3.7 ±0.2</td>
<td>2.5 ±1.2</td>
</tr>
<tr>
<td>CWC</td>
<td>80.9 ±3.5</td>
<td>83.6 ±3.5</td>
<td>-3.9 ±0.2</td>
<td>-3.4 ±0.2</td>
<td>2.0 ±0.8</td>
</tr>
<tr>
<td>WBH</td>
<td>85.9 ±1.6</td>
<td>87.1 ±1.6</td>
<td>-4.3 ±0.1</td>
<td>-4.0 ±0.1</td>
<td>2.6 ±0.6</td>
</tr>
<tr>
<td>CWH</td>
<td>83.8 ±8.6</td>
<td>68.7 ±8.3</td>
<td>-3.4 ±0.2</td>
<td>-3.4 ±0.2</td>
<td>-0.5 ±1.1</td>
</tr>
</tbody>
</table>

1*L (lightness ranges from 0 (absence of light) to 100 (pure white); a* ranges from +a *(redness) to –a (greenness); b* ranges from +b (yellowness) to -b (blueness).

2YBC refers to yolk samples from unheated eggs treated with 70°C commercial brine; CYC refers to yolk from unheated eggs treated with 70°C water; Y.B.H. refers to yolk samples from heated eggs (~ 94°C) treated with 70°C commercial brine; C.Y.H. refers to yolk from heated eggs (~ 94°C) treated with 70°C water heated to 70°C water; WBC refers to albumen samples from unheated eggs treated with 70°C commercial brine; C.W.C. refers to albumen samples from unheated eggs treated with 70°C water; W.B.H. refers to albumen samples from heated eggs (~ 94°C) treated with 70°C commercial brine; C.W.H. refers to albumen samples from heated eggs (~ 94°C) treated with 70°C water.

3Standard deviations across treatment times.

a-c Means with different subscripts are different at (P ≤ 0.05) across columns of yolks and albumen treatments.

x-y Means with different subscripts are different at (P ≤ 0.05) for treatment rows at 24 and 48 hours.
Table 6: Effect of boiling and pickling on quail (Coturnix coturnix japonica) egg texture.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Rupture Strength</th>
<th>Britteness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Force (kg)</td>
<td>mm</td>
</tr>
<tr>
<td>Commercial Brine Cold BC</td>
<td>7.0 ± 0.15</td>
<td>17.86 ± 0.099</td>
</tr>
<tr>
<td>Control Cold CC</td>
<td>5.6 ± 0.17</td>
<td>18.43 ± 0.10</td>
</tr>
<tr>
<td>Commercial Brine Hot B.H.</td>
<td>7.0 ± 0.13</td>
<td>17.98 ± 0.08</td>
</tr>
<tr>
<td>Control Hot CH</td>
<td>5.5 ± 0.19</td>
<td>18.29 ± 0.11</td>
</tr>
</tbody>
</table>

B.C. refers to unheated eggs treated with 70°C commercial brine, CC refers to albumen samples from heated eggs (~ 94°C) treated with 70°C water, B.H. refers to heated eggs (~ 94°C) treated with 70°C commercial brine, C.H. refers to albumen samples from heated eggs (~ 94°C) treated with 70°C water.

a-c Mean with different subscripts are different at (P ≤ 0.05) across treatment columns.

Table 7: Effect of boiling and pickling on quail (Coturnix coturnix japonica) egg pH and water activity

<table>
<thead>
<tr>
<th></th>
<th>pH and water activity</th>
<th>ALL REPLICATIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>YOLK</td>
<td>ALBUMEN</td>
</tr>
<tr>
<td></td>
<td>24 hour pH</td>
<td>48 hour pH</td>
</tr>
<tr>
<td>CC</td>
<td>6.8 ± 0.10</td>
<td>6.3 ± 0.1</td>
</tr>
<tr>
<td>CH</td>
<td>7.2 ± 0.10</td>
<td>6.4 ± 0.1</td>
</tr>
<tr>
<td>BC</td>
<td>3.9 ± 0.08</td>
<td>3.7 ± 0.07</td>
</tr>
<tr>
<td>BH</td>
<td>3.9 ± 0.08</td>
<td>3.8 ± 0.07</td>
</tr>
</tbody>
</table>

BC refers to unheated eggs treated with 70°C commercial brine, CC refers to albumen samples from heated eggs (~ 94°C) treated with 70°C water, BH refers to heated eggs (~ 94°C) treated with 70°C commercial brine, CH refers to albumen samples from heated eggs (~ 94°C) treated with 70°C water.

a-b Means with different subscripts are different at (P ≤ 0.05) across egg part and treatment columns.

x-y Means in a row within the same egg component (yolk or albumen) with different superscripts are significantly different P < 0.05.”
Figure 6: Typical Rupture Strength and Brittleness. Force/time

Rupture Strength and Brittleness for cold-filled eggs in commercial brine. The analyzer was set to a 10 g trigger force, with a 2mm/second crosshead speed 75% strain.


"Assessment of different pickling solutions on quality characteristics of pickled quail


(accessed March 12, 2022).


CHAPTER FOUR
MICROBIOLOGY

Abstract

The quality and microbiological characteristics of quail eggs were evaluated after eggs were pickled in different vinegar brine solutions. Pickling food in vinegar is one of the oldest methods of preservation, dating back to 3000 BC when the Babylonians were using vinegar from fruit and the sap of date palms for alcohol, food, and a pickling agent (Bourgeois & Barja 2009). Little research has been conducted on pickled quail eggs. Commercial quail eggs obtained from a local producer were boiled and peeled and then inoculated with 0.1 mL of a mixed culture containing $10^6$ to $10^8$ cells per mL of generic *Escherichia coli*, *Listeria monocytogenes*, and nalidixic acid-resistant *Salmonella Typhimurium*. After inoculation and a 30-minute waiting period, eggs were placed into various vinegar brine solutions or control treatments and held at room temperature for 24 hours. Results indicated that commercial brine solutions reduced pathogens. Overall, the results of the present study indicate that when a commercial brine solution (or various components: vinegar, sugar, or salt) is used for pickling quail eggs, the process will significantly reduce ($\geq 99\%$ reduction) pathogenic microorganisms that are in or on the surfaces of eggs from horizontal or vertical transmission.

Introduction

According to the U.S. Food and Drug Administration (FDA), foods classified as low-acid (high pH) must be processed and handled according to the U.S. F.D.A. Code of Federal Regulation, Title 21, Part 114. (F.D.A. 2021). Specifically, these foods termed acidified must be produced using basic hygiene practices outlined in current Good Manufacturing Practices. Additionally, manufacturers of acidified foods must have their process procedures validated by a
qualified process authority (as defined by the regulation), who offers scientific evidence that the process conditions, when performed correctly, will achieve a 5-log reduction in pathogenic bacteria. Documentation of the critical values for each production batch and documentation of process deviations and corrective actions are also required. Per regulation, pickled foods, such as pickled quail eggs, are considered acidified foods, and therefore these requirements apply. Federal laws for acidified foods are designed to eliminate pathogenic and spoilage microorganisms and specifically target the prevention of clostridium botulinum which can produce botulinum toxin. The botulinum toxin causes severe illness and sometimes death when consumed by humans. The seriousness of this microorganism makes it a primary pathogen of concern in acidified foods.

During the pickling process, several factors are critical to the safety of the final pickled product. The amount of acid, thermal process, pH, and holding time required for the product to achieve a pH below 4.6 (in all parts of the food) contribute to a safe final product. FDA regulation requires that a pH less than 4.6 be reached in every component of the food within 24 hours (Black et al., 2015). In the case of pickled eggs, the pH must penetrate and lower the pH of the albumin and center of the yolk within 24 hours. Several scientific studies have evaluated the conditions for achieving complete acidification of chicken eggs within 24 hours (Acton 1973; Richard et al. 2011; Acosta 2013). Still, there is very little accessible information on the conditions necessary to achieve this in quail eggs.

The purpose of the microbiology study for this project is to determine whether a specific commercial brine will sufficiently reduce or destroy pathogens that might be present.
Materials And Methods

Quail eggs were inoculated by placing 20 boiled eggs into a sterile metal strainer and submerging them into a large beaker containing the cocktail solution of mixed culture prepared as previously described. The eggs were thoroughly covered and remained in the solution for 30 minutes. After 30 minutes, the eggs were removed from the inoculum solution, allowed to air dry for five minutes, and added to 473.2 ml jars for treatments (Table 9).

A subset of raw, unshelled eggs was inoculated by injecting 100 μL of inoculum directly into the center of the egg using a 28-gauge sterile needle. This was conducted to evaluate the effects of egg processing on horizontal contamination. The injection site was sealed with Loctite® super glue and allowed to dry for 5 minutes. Inoculated eggs were placed in a metal strainer and completely submerged in boiling water at 100°C for 4 minutes. After boiling, eggs were transferred to an ice water bath for 5 minutes and then peeled using a Vevor Electric quail egg peeler machine, 500G/H. Peeled eggs were inspected as previously described, and only eggs with no visible defects were used in the experiment. Twenty eggs were then placed into a 473.2 ml glass jar. Two hundred thirty-seven milliliters of 70°C brine was added to the jar, leaving 1.27 cm of headspace. A flat metal lid with a metal screw band was placed on the jar and tightened. The jar was then inverted for three minutes.

Treatments were prepared as previously described. Jars containing eggs and pickling brines were held at room temperature for 24 hours. After the holding period, eggs were removed from the pickling brine, prepared for plating, and brines were tested for pH.

During the preliminary study and replications one and two, 270 quail eggs were obtained from a flock commercial flock of Pharoah quail Coturnix coturnix that were approximately 48 weeks old at the time of collection. Eggs were inspected, sorted, and packaged for retail sale by
the producer, including removing any obvious damaged or cracked eggs. Before packaging the eggs in cartons, the commercial producer sprays the eggshells immediately after lay with 1 ml of quaternary ammonium chloride per gallon of water. The eggs are then inspected, sorted, and packaged. They were then held at 4°C for 1-2 days until they were picked up and transported to the laboratory. Eggs were stored under refrigeration for less than 24 hours at the laboratory before beginning the research.

_Egg Processing_

Eggs were removed from refrigeration and held at room temperature for approximately one hour. All eggs from one replication were boiled, cooled, peeled, and then sorted into groups of 20 eggs per group. Only eggs deemed free from tears, cracks, or imperfections were used. The eggs were then divided into groups of 20 eggs. Each group of 20 eggs was placed in a metal strainer and completely submerged in boiling water at 100°C for 4 minutes. After boiling, eggs were submerged in an ice water bath for 5 minutes and peeled using an electric quail egg peeler. Peeled eggs were inspected for surface tears, cracks, and imperfections, and only the eggs with no visible deformities were retained. Six groups of eggs were placed in Ziploc bags and held overnight in a refrigerator at 5°C. The next day, eggs were removed from refrigerated storage, allowed to warm to room temperature for one hour, and then placed into various brine or control treatments.

_Treatments_

Pickling brines were prepared by heating the combined ingredients to 70°C, holding that temperature until adding the brine to the jars containing eggs (20 eggs/jar). Treatments, detailed in Table 8, consisted of a commercial pickling brine, sugar only brine, salt only brine, vinegar only, tap water (control), and no liquid (control).
Table 8: Description of pickling brine solutions used for hard-cooked Japanese quail eggs.

<table>
<thead>
<tr>
<th>Brine Type</th>
<th>Distilled Vinegar (5%) acidity (mL)</th>
<th>Granulated White Sugar (g)</th>
<th>Pickling Salt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial Brine</td>
<td>236.588</td>
<td>3.59</td>
<td>10.28</td>
</tr>
<tr>
<td>Sugar Brine</td>
<td>236.588</td>
<td>3.59</td>
<td></td>
</tr>
<tr>
<td>Salt Brine</td>
<td>236.588</td>
<td>10.28</td>
<td></td>
</tr>
<tr>
<td>Vinegar</td>
<td>236.588</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>236.588</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Treatment Preparations

Table 9: Brine or control treatments used for pickling hard-cooked Japanese quail eggs.

<table>
<thead>
<tr>
<th>Control</th>
<th>C.N.</th>
<th>Twenty inoculated eggs were placed into a 473.2 ml glass jar. A flat metal lid with a metal screw band was placed on jars and tightened. The jars were then inverted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>C.W.</td>
<td>Twenty inoculated eggs were placed into a 473.2 ml glass jar. Two hundred thirty-seven milliliters of 70°C tap water was added to the jar, leaving 1.27 cm of headspace. A flat metal lid with a metal screw band were placed on jars and tightened. The jars were then inverted.</td>
</tr>
<tr>
<td>Control</td>
<td>CMIN</td>
<td>No treatment added. Eggs were plated after 30 minutes of inoculation. Twenty inoculated eggs were separated into (5) 4 egg samples. Each sample was weighed and then added to stomacher filter bags. Sterile phosphate buffer solution (0.1% PBS) was added to the stomacher bags, in a quantity of milliliters equal to the gram weight of the sample. The egg mixture was stomached, by hand, for one minute. Serial dilutions were prepared from the rinsate using true duplicates, and duplicates were plated onto Brilliant Green Sulfa (B.G.S.) with nalidixic acid for Salmonella growth, PalCam with antimicrobial supplement (P.A.L.) for Listeria growth, and 3M E. coli Petrifilm for E. coli growth. After plating, the B.G. Sulfa and P.A.L. plates were inverted. Inverted B.G.S. and 3M Petrifilm were incubated at 37°C, and the P.A.L. was incubated at 30°C for 48 hours.</td>
</tr>
</tbody>
</table>
## Control M.B.C.
Twenty inoculated eggs were placed into a 473.2 ml glass jar. Two hundred thirty-seven milliliters of 70°C commercial brine was added to the jar, leaving 1.27 cm of headspace. A flat metal lid with a metal screw band was placed on jar and tightened. The jar was then inverted.

## Control V
Vinegar Only Brine. Twenty inoculated eggs were placed into a 473.2 ml glass jar. Two hundred thirty-seven milliliters of 70°C brine was added to the jar, leaving 1.27 cm of headspace. A flat metal lid with a metal screw band was placed on jar and tightened. The jar was then inverted for a minimum of 3 minutes.

## Control S.H.
Vinegar and Sugar Brine: 20 inoculated eggs were placed into a 473.2 ml glass jar. Two hundred thirty-seven milliliters of 70°C brine was added to the jar. The eggs were covered with 70°C Vinegar and Sugar Brine, leaving 1.27 cm of headspace. A flat metal lid with a metal screw band was placed on jar and tightened. The jar was then inverted for a minimum of three minutes.

## Control S.L.T.
Vinegar and Salt Brine: 20 inoculated eggs were placed into a 473.2 ml glass jar. Two hundred thirty-seven milliliters of 70°C brine was added to the jar, leaving 1.27 cm of headspace. A flat metal lid with a metal screw band was placed on jar and tightened. The jar was then inverted for a minimum of three minutes.

## Experiment

### Inoculation

Frozen cultures of generic *E. coli, Listeria monocytogenes, Salmonella Typhimurium* and were thawed at room temperature, and loopfuls of each culture were added individually to separate tubes of sterile tryptic soy broth. Tubes of inoculant were incubated at 30°C for *Listeria monocytogenes*, and at 37°C for *E. coli* and *Salmonella*, for 48 hours. After incubations, cells were centrifuged (20°C, 4081 rpm, 5 min), washed twice with 0.1% sterile phosphate buffered saline, and then resuspended in sterile 0.1% phosphate buffered saline to prepare the inoculum.

The optical density of the suspension of each microorganism was measured at 600 nm using a spectrometer, to estimate cell count and then later confirmed by direct plate counts. When the target O.D.₆₀₀nm reading of approximately 0.45 was obtained for each suspension of bacteria,
equal volumes of each solution of bacteria was combined to prepare the mixed culture inoculum. The inoculation was prepared to achieve between 106 to 108 cells per ml of each microorganism. Quail eggs were inoculated with 100 microliters per egg of the inoculant, which was a mixed culture of generic *E. coli, Listeria monocytogenes, Salmonella Typhimurium*. *Salmonella* was tested during preparation to confirm resistance to 200 ppm nalidixic acid.

Serial dilutions of the final inoculum were prepared and plated onto selective media to get the actual cell counts. The inoculum was prepared fresh for each replication on the day that it was used to inoculate eggs. Serial dilutions of the mixed culture inoculum were prepared and plated onto Brilliant Green Sulfa (B.G.S.) with nalidixic acid for *Salmonella Typhimurium*, 3M *E. coli/Coliform* Petrifilm for *E. coli*, and PalCam with antimicrobial supplement (P.A.L.) for *Listeria monocytogenes*. They were incubated for 48 hours at 30°C for *Listeria monocytogenes* and at 37°C for *E. coli* and *Salmonella*, and colonies were counted.
Figure 7: Experiment Flow Diagram

- Commercial quail eggs 1-2 days post lay
- Transported packaged eggs with ice packs
- Refrigerated storage @ 4°C <24 hours
- Removed from refrigeration 1 hour before processing
- Boiled in 100°C water for 4 minutes, cooled in ice water for 5 minutes, peeled with a mechanical peeler
- Egg inspection, placed in zip lock bags and stored @ 4°C for less than 24 hours
- Removed from refrigeration 1 hour before processing
  - Only perfect eggs selected
- Inoculated and placed into treatments, described in Table 8

| Hot water brine | No brine | Hot commercial brine | Vinegar brine | Vinegar and sugar brine | Vinegar and salt brine | No treatment |
Microbiological Analyses

On the day of testing, jars of pickled eggs were opened and the liquid covering the eggs was poured into separate 400 ml beakers. Eggs without brine were weighed on a per jar basis (20 eggs per jar) and were then separated into five groups of 4 eggs per group (N=5 per jar). Each group of 4 eggs was re-weighed, and then sterile phosphate buffer saline (0.1%) solution was added 1:1 (w/v) to the eggs in a sterile filter stomacher bag. The egg-saline mixture was macerated on the outside of the bag by hand for one minute. Serial dilutions were prepared from the mixture using true duplicates dilutions. Duplicate dilutions were plated on Brilliant Green Sulfa (B.G.S.) agar containing 200 ppm nalidixic acid for Salmonella enumeration, PalCam agar with PalCam antimicrobial supplement (P.A.L.) for Listeria enumeration, and 3M E. coli/Coliform Petrifilm™ for E. coli and coliform enumeration. For petrifilm plates, one mL of each dilution was placed on the media and spread. One hundred microliters of each serial dilution was added to B.G.S. or P.A.L. plates and spread using a sterile loop. After drying, plates were inverted and incubated at either 30 C (Listeria) or 37 C (E. coli/coliform and Salmonella) for 48 hours before counting colony-forming units. Colony-forming units (CFU) were converted to log_{10} cfu per gram of egg. Statistical analyses were performed on CFU and log transformation numbers.

Statistical Analyses

The data analysis for this thesis was generated using JMP® Pro Version 16.1.0 Copyright © 2020-2021 S.A.S. Institute Inc. Cary, NC, U.S.A. Data were analyzed for statistical significance. Data was tested across treatment means for statistical significance (P< 0.05) using a continuous response versus categorical variable (ANOVA). For groups with a statistical
difference of \((P > 0.05)\), means were compared using the Tukey method to compare specific groups' means differences.

**Results And Discussion**

**Preliminary Study**

A preliminary study was conducted on quail eggs using a drop and spread inoculation method. The drop spread inoculation was not feasible for inoculating large numbers of eggs, so a submersion inoculation was used after the preliminary study. During the preliminary research, an additional treatment involved inoculation and recovery of microorganisms after two division cycles (30 minutes) to determine initial levels of *Salmonella Typhimurium* (3.5 log cfu/g), *E. coli* (4.6 log cfu/g), *coliforms* (4.6 log cfu/g), and *Listeria monocytogenes* (3.5 log cfu/g) on eggs (Table 10). Other control treatments involved holding the inoculated eggs for 24 hours at room temperature in jars with or without water to determine the effect of various brine solutions on cell counts. When the numbers of bacteria recovered from eggs in the brine treatments were compared to numbers recovered from eggs stored in the water control treatments, generic *E. coli*, *Listeria monocytogenes*, *Salmonella Typhimurium* were reduced by ≥ 99% (reductions of 2 to 4 log cfu/g). Since all brine treatments reduced the numbers of bacteria to levels below the detection limit for direct plating, it was impossible to determine if one brine solution was substantially better than the others at eliminating bacteria (Table 10). In the absence of a brine solution or water, counts continued to increase for all microorganisms achieving levels ≥ 7.9 log cfu/g (Table 10). When boiled eggs were inoculated internally, *Salmonella Typhimurium*, *E. coli*, coliforms, and *Listeria monocytogenes* remained high. They were similar to control treatments, and an additional experiment was conducted to further investigate the impact of brine solutions on quail eggs.
Experiment

After the preliminary study, an additional experiment consisting of two replications was conducted to determine the microbial growth of pathogens on pickled eggs after 24 hours in treatment solutions. When eggs were inoculated and tested 30 minutes later, levels of *Salmonella* recovered from eggs were between 3.6 to 3.7 log cfu/g. Eggs pickled with a water brine had lower *Salmonella* numbers (3.9 to 7.1 log cfu/g) than eggs with no solution, which were TNTC. *Salmonella* growth in water brine was expected as pathogens may survive in low nutrient water (Liu et al. 2018). The key factor governing the growth of all organisms is nutrient availability, and there were likely enough egg nutrients dispersed in water, as evidenced by turbid appearance, to allow for *Salmonella* growth. When eggs were treated with various vinegar brine solutions, very low levels of *Salmonella* were detected, and these levels were all below the limit of detection for direct plate counting (< 1.5 log cfu/g).

Similar findings were observed with *E. coli*, *coli*form, and *Listeria*, where low numbers of bacteria were recovered from eggs pickled with commercial vinegar, sugar, or salt brines. *Listeria* recovered from eggs 30 minutes after inoculation varied from 2.7 to 4.0 log CFU/mL, and levels remained between 3.3 to 3.7 log cfu/g on eggs stored in tap water. Francis and Beirne (1998) reported that survival of *Listeria* inoculant was diminished when inoculated in a mixed culture, possibly due to competition for or depletion of specific nutrients. A comparison of individual pathogen performance indicates that *E. coli* had the most robust growth on pickled eggs in control treatments. *E.coli* grows well in pH greater than 4.4 and has been shown to be acid tolerant. Exposure to acid ingredients has been shown to increase heat tolerance in *E. coli* (Lee, 2004). *Listeria*, however, is not acid-tolerant but can grow in small numbers in an acidic environment due to stress hardening in stressful environments. These factors could explain the
higher microbial growth in *E. coli* and lower microbial growth in *Listeria* samples, Konstantinos et al., (2003). Overall, when the numbers of bacteria recovered from eggs in the brine treatments were compared to numbers recovered from eggs stored in the water control treatments, *Salmonella Typhimurium, E. coli, coliforms, and Listeria monocytogenes* were reduced by $\geq 99\%$ (reductions of 2 to 4 log cfu/g). Since all brine treatments reduced the numbers of bacteria to levels below the limit of detection for direct plating, it was impossible to determine if one brine solution was significantly better than the others at eliminating bacteria (Table 11).

An additional test evaluated the effects of hard cooking and pickling on eggs that may have been contaminated through a vertical transmission route. The mixed culture of *Salmonella Typhimurium, E. coli, and Listeria monocytogenes* was injected into the center of the raw eggs shell. These eggs were then boiled, peeled, and inoculated again on the egg surface. Pathogen growth was below the detectable limit (less than 30 cfu/gram). This suggests that implementing "hurdle technology," specifically combining a thermal and pickling process, will reduce *Salmonella* that might be transferred from a *Salmonella*-positive hen and from environmental contaminants. Overall, the results of the present study indicate that when a commercial brine solution (or various components: vinegar, sugar, or salt) is used for pickling quail eggs, the process will significantly reduce ($\geq 99\%$ reduction) pathogenic microorganisms that are in or on the surfaces of eggs from horizontal or vertical transmission. Under the conditions of the present study, the author could not confirm the numbers of inoculated pathogens were not detectable. Additional research is needed to validate the pickling process for quail eggs.

**Conclusion**

This chapter focused on the microbiological effects of pickling quail eggs. Eggs were boiled, peeled, and then placed in various commercial brine solutions. The quail eggs were held
in glass jars for 24 hours to determine whether the pickling process would reduce or eliminate pathogens: generic *Escherichia coli*, *Listeria monocytogenes*, and nalidixic acid-resistant *Salmonella Typhimurium*. After 24 hours, inoculated eggs were removed from treatments, stomached, plated onto media, and incubated for 48 hours. After incubation, colony-forming units were counted, and data were recorded and statistically analyzed. It was determined that the commercial brine solutions reduced pathogenic growth below detection limits. Therefore, leading to the conclusion that a combination of thermal process and acidification did reduce pathogenic growth to safe levels.

**Future Research Recommendations**

Previous research has suggested that different combinations of spices may interact with acetic acid and alter the acidification process during pickling. Commercial pickled quail eggs are available in various flavors, including but not limited to Cajun and jalapeno. It is suggested that microbiological studies include adding these ingredients with boiled eggs before pickling and then evaluating the acidification process's effect.

**THESIS CONCLUSIONS**

The research for this thesis was undertaken to add to the body of knowledge on pickled quail eggs. Pickling is an ancient and common method of preserving foods and, when done according to regulations, can provide a nutritious, shelf-stable product that must be refrigerated after opening. Care must be taken during the process to ensure a safe product with desirable texture and flavor. If pickled eggs are inadequately processed, there is the risk of *Clostridium botulinum* toxin, but if they are boiled too long, there is the risk of rubbery, extra-firm, and undesirable texture. In many Asian countries, pickled quail eggs are refrigerated, while in the U.S., it is recommended that pickled quail eggs be refrigerated after being opened. Lack of
knowledge on the impact of the pickling process on the quality and microbiological safety of quail eggs was the rationale for conducting the studies presented in this thesis.

During the first study, quality characteristics were evaluated. Results revealed that the weight and texture changes due to pickling did not have an adverse effect on quality. The pH and water activity were tested at 24 and 48 hours, and data showed that the water activity was primarily consistent across time and treatment types and remained about 0.85, which is a regulatory requirement for acidified foods. P.H. data showed that pickling for 24-hour was sufficient to acidify all parts of the quail eggs to a level below 4.6, and in fact, the pH was below 4.0 in all the commercial brine variations. The color was tested, and while treatment types had a minimum difference, the color of pickled quail egg yolks after 24 hours became lighter and less yellow, while albumen colors increased in greenness and yellowness. These color changes would most likely be undesirable to consumers.

Study two focused on the microbiological effects of pickling quail eggs. Eggs were boiled, peeled, and then placed in various commercial brine solutions. The quail eggs were held in glass jars for 24 hours to determine whether the pickling process would reduce or eliminate pathogens: generic Escherichia coli, Listeria monocytogenes, and nalidixic acid-resistant Salmonella Typhimurium. After 24 hours, inoculated eggs were removed from treatments, stomached, plated onto media, and incubated for 48 hours. After incubation, colony-forming units were counted, and data was recorded and statistically analyzed. It was determined that the commercial brine solutions reduced pathogenic growth below detection limits. Therefore, leading to the conclusion that a combination of thermal process and acidification did reduce pathogenic growth to safe levels.
Future Research Recommendations

Previous research has suggested that different combinations of spices may interact with acetic acid and alter the acidification process during pickling. Additionally, other types of packaging will also affect the shelf-life of pickled quail eggs, although they are currently packed primarily in glass jars or traditional aluminum cans. Since commercial pickled quail eggs are available in a variety of flavors, including but not limited to Cajun, jalapeno.

References


Francis, Gillian A., and David O'Beirne. "Effects of storage atmosphere on Listeria


http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.496.875&rep=rep1&type=pdf


(accessed March 12, 2022.)

Table 10: Populations of microorganisms recovered from inoculated Japanese quail eggs (Coturnix coturnix japacona) before or after hard cooking and pickling in various solutions.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Jar</th>
<th>Eggs (gram weight)</th>
<th>Log$_{10}$cfu/g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRELIMINARY REPLICATION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Salmonella Typhimurium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>CMIN</td>
<td>233.51</td>
<td>$3.5^b \pm 0.03$</td>
</tr>
<tr>
<td>Control 2</td>
<td>CW</td>
<td>233.53</td>
<td>$3.4^b \pm 0.03$</td>
</tr>
<tr>
<td>Control 3</td>
<td>CN</td>
<td>229.70</td>
<td>$&gt;4.5^a \pm 0.03$</td>
</tr>
<tr>
<td>Commercial Brine 4</td>
<td>MBC</td>
<td>229.70</td>
<td>$&lt;1.5^e$</td>
</tr>
<tr>
<td>Vinegar Brine 5</td>
<td>V</td>
<td>247.54</td>
<td>$&lt;1.5^e$</td>
</tr>
<tr>
<td>Sugar Brine 6</td>
<td>SH</td>
<td>228.28</td>
<td>$&lt;1.5^e$</td>
</tr>
<tr>
<td>Salt Brine 7</td>
<td>SLT</td>
<td>230.66</td>
<td>$&lt;1.5^e$</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>CMIN</td>
<td>233.51</td>
<td>$4.6^a \pm 0.08$</td>
</tr>
<tr>
<td>Control 2</td>
<td>CW</td>
<td>233.53</td>
<td>$4.7^b \pm 0.08$</td>
</tr>
<tr>
<td>Control 3</td>
<td>CN</td>
<td>229.70</td>
<td>$&gt;5.5^a \pm 0.08$</td>
</tr>
<tr>
<td>Commercial Brine 4</td>
<td>MBC</td>
<td>229.70</td>
<td>$&lt;1.4^e$</td>
</tr>
<tr>
<td>Vinegar Brine 5</td>
<td>V</td>
<td>247.54</td>
<td>$&lt;1.4^e$</td>
</tr>
<tr>
<td>Sugar Brine 6</td>
<td>SH</td>
<td>228.28</td>
<td>$&lt;1.4^e$</td>
</tr>
<tr>
<td>Salt Brine 7</td>
<td>SLT</td>
<td>230.66</td>
<td>$&lt;1.4^e$</td>
</tr>
<tr>
<td><strong>Coliforms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
<td>CMIN</td>
<td>233.51</td>
<td>$4.6^a \pm 0.04$</td>
</tr>
<tr>
<td>Control 2</td>
<td>CW</td>
<td>233.53</td>
<td>$4.7^a \pm 0.04$</td>
</tr>
<tr>
<td>Control 3</td>
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<td>229.70</td>
<td>$\sim$</td>
</tr>
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<td>MBC</td>
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<td>$&lt;1.4^b$</td>
</tr>
<tr>
<td>Vinegar Brine 5</td>
<td>V</td>
<td>247.54</td>
<td>$&lt;1.4^b$</td>
</tr>
<tr>
<td>Sugar Brine 6</td>
<td>SH</td>
<td>228.28</td>
<td>$&lt;1.4^b$</td>
</tr>
<tr>
<td>Salt Brine 7</td>
<td>SLT</td>
<td>230.66</td>
<td>$&lt;1.4^b$</td>
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<tr>
<td><strong>Listeria monocytogenes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control 1</td>
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<td>$0.6^e \pm 0.8$</td>
</tr>
<tr>
<td>Control 2</td>
<td>CW</td>
<td>233.53</td>
<td>$3.5^b \pm 0.8$</td>
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<td>Control 3</td>
<td>CN</td>
<td>229.70</td>
<td>$&gt;4.2^a$</td>
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<tr>
<td>Commercial Brine 4</td>
<td>MBC</td>
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<td>$&lt;1.4^e$</td>
</tr>
<tr>
<td>Vinegar Brine 5</td>
<td>V</td>
<td>247.54</td>
<td>$&lt;1.4^e$</td>
</tr>
<tr>
<td>Sugar Brine 6</td>
<td>SH</td>
<td>228.28</td>
<td>$&lt;1.4^e$</td>
</tr>
<tr>
<td>Salt Brine 7</td>
<td>SLT</td>
<td>230.66</td>
<td>$&lt;1.4^e$</td>
</tr>
</tbody>
</table>

Japanese quail variety was Pharaoh.
Five samples of eggs from each jar were tested using a pool of four eggs. Counts were reported per gram of egg (N=5/jar).

Treatments were: CMIN refers to eggs tested 30 min after inoculations. C.W. represents inoculated eggs pickled in tap water. C.N. represents eggs stored in jar with no liquid. M.B.C. refers to cold eggs pickled in a commercial brine. V refers to unheated eggs pickled in vinegar only. S.H. refers to cold eggs pickled in sugar and vinegar. S.L.T. refers to cold eggs pickled in salt and vinegar.

Gram weight of eggs per jar after pickling. Each jar contained 20 quail eggs.

Means within a genus and species of bacteria with different subscripts in a column are significantly different (P ≤ 0.05). Statistical analyses on samples with TNTC were assumed to be >9.0 log_{10} cfu/g

Table 11: Populations of microorganisms recovered from inoculated Japanese quail eggs (Coturnix coturnix japacona) before or after hard cooking and pickling in various solutions.

<table>
<thead>
<tr>
<th>TREATMENT²</th>
<th>Jar</th>
<th>REPLICATION ONE</th>
<th>REPLICATION TWO</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Weight of eggs (g)</td>
<td>Log_{10} cfu/g</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Salmonella Typhimurium</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>1</td>
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<td>3.7b±0.9</td>
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<td>Control</td>
<td>2</td>
<td>CW   230.29</td>
<td>3.9b±0.9</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>CN   229.48</td>
<td>&gt;4.5a</td>
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<td>Commercial Brine</td>
<td>4</td>
<td>MBC 220.54</td>
<td>1.6c±0.2</td>
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<tr>
<td>Vinegar Brine</td>
<td>5</td>
<td>V    230.29</td>
<td>&lt;1.5c</td>
</tr>
<tr>
<td>Sugar Brine</td>
<td>6</td>
<td>SH   228.87</td>
<td>&lt;1.5c</td>
</tr>
<tr>
<td>Salt Brine</td>
<td>7</td>
<td>SLT  223.13</td>
<td>&lt;1.5c</td>
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<tr>
<td>Escherichia coli</td>
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<td></td>
<td></td>
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<tr>
<td>Control</td>
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<td>CMIN 227.47</td>
<td>4.7b±0.1</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>CW   230.29</td>
<td>5.2a±0.1</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>CN   229.48</td>
<td>&gt;5.5a</td>
</tr>
<tr>
<td>Commercial Brine</td>
<td>4</td>
<td>MBC 220.54</td>
<td>&lt;1.4c</td>
</tr>
<tr>
<td>Vinegar Brine</td>
<td>5</td>
<td>V    230.29</td>
<td>&lt;1.4c</td>
</tr>
<tr>
<td>Sugar Brine</td>
<td>6</td>
<td>SH   228.87</td>
<td>&lt;1.4c</td>
</tr>
<tr>
<td>Salt Brine</td>
<td>7</td>
<td>SLT  223.13</td>
<td>&lt;1.4c</td>
</tr>
<tr>
<td>Coliforms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td>CMIN 227.47</td>
<td>4.5b±0.14</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>CW   230.29</td>
<td>5.1b±0.14</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>CN   229.48</td>
<td>&gt;5.5a</td>
</tr>
<tr>
<td>Commercial Brine</td>
<td>4</td>
<td>MBC 220.54</td>
<td>&lt;1.4c</td>
</tr>
<tr>
<td>Vinegar Brine</td>
<td>5</td>
<td>V    230.29</td>
<td>&lt;1.4c</td>
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<tr>
<td>Sugar Brine</td>
<td>6</td>
<td>SH   228.87</td>
<td>&lt;1.4c</td>
</tr>
<tr>
<td>Salt Brine</td>
<td>7</td>
<td>SLT  223.13</td>
<td>&lt;1.4</td>
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</table>
**Listeria monocytogenes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CMIN</th>
<th>CW</th>
<th>CN</th>
<th>MBC</th>
<th>V</th>
<th>SH</th>
<th>SLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 1</td>
<td>227.47</td>
<td>230.29</td>
<td>229.48</td>
<td>220.54</td>
<td>230.29</td>
<td>228.87</td>
<td>223.13</td>
</tr>
<tr>
<td>Control 2</td>
<td>226.58</td>
<td>233.51</td>
<td>226.83</td>
<td>220.56</td>
<td>232.50</td>
<td>222.42</td>
<td>226.2</td>
</tr>
<tr>
<td>Commercial Brine</td>
<td>226.58</td>
<td>233.51</td>
<td>226.83</td>
<td>220.56</td>
<td>232.50</td>
<td>222.42</td>
<td>226.2</td>
</tr>
<tr>
<td>Vinegar Brine</td>
<td>226.58</td>
<td>233.51</td>
<td>226.83</td>
<td>220.56</td>
<td>232.50</td>
<td>222.42</td>
<td>226.2</td>
</tr>
<tr>
<td>Sugar Brine</td>
<td>226.58</td>
<td>233.51</td>
<td>226.83</td>
<td>220.56</td>
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<td>226.2</td>
</tr>
<tr>
<td>Salt Brine</td>
<td>226.58</td>
<td>233.51</td>
<td>226.83</td>
<td>220.56</td>
<td>232.50</td>
<td>222.42</td>
<td>226.2</td>
</tr>
</tbody>
</table>

1. Japanese quail variety was Pharaoh.
2. Five samples of eggs from each jar were tested using a pool of four eggs. Counts were reported per gram of egg (N=5/jar).
3. Treatments were: CMIN refers to eggs tested 30 min after inoculations. C.W. represents inoculated eggs pickled in tap water. C.N. represents eggs stored in jar with no liquid. M.B.C. refers to cold eggs pickled in a commercial brine. V refers to unheated eggs pickled in vinegar only. S.H. refers to cold eggs pickled in sugar and vinegar. S.L.T. refers to cold eggs pickled in salt and vinegar.
5. Means within a genus and species of bacteria with different subscripts in a column are significantly different (P ≤ 0.05). Statistical analyses on samples with TNTC were assumed to be > 9.0 log₁₀ cfu/g

Table 12: Populations of microorganisms recovered from Japanese quail eggs (Coturnix coturnix japonica) that were inoculated in the center of the egg before or after hard cooking and pickling in a commercial brine solution.

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Eggs (gram weight)</th>
<th>Salmonella Typhimurium</th>
<th>Escherichia coli</th>
<th>Coliforms</th>
<th>Listeria Monocytogenes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injected egg in commercial brine</td>
<td>IMB</td>
<td>3.4 ±0.5</td>
<td>4.4 ±0.05</td>
<td>4.6 ±0.05</td>
<td>3.1b ±0.06</td>
</tr>
<tr>
<td>Preliminary Replication</td>
<td>2</td>
<td>237.43</td>
<td>1.5 ±0.5</td>
<td>&lt;1.4c</td>
<td>&lt;1.4c</td>
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<tr>
<td>Replication One</td>
<td>3</td>
<td>231.3</td>
<td>&lt;1.4c</td>
<td>&lt;1.4c</td>
<td>&lt;1.4c</td>
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<tr>
<td>Replication Two</td>
<td>3</td>
<td>231.3</td>
<td>&lt;1.4c</td>
<td>&lt;1.4c</td>
<td>&lt;1.4c</td>
</tr>
</tbody>
</table>

Five samples of eggs from each jar were tested using a pool of four eggs. Counts were reported per gram of egg (N=5/jar).
1. Japanese quail variety was Pharaoh.
2. Preliminary study was performed before experiments one and two. Treatment: inoculant was injected in the center of boiled eggs and then surface inoculation in a dropwise method.
3. Treatment: inoculant was injected in the center of raw eggs, then eggs were boiled peeled and surface inoculated by submerging pooled eggs into inoculant.