PHYSICAL AND MICROBIOLOGICAL PROPERTIES OF HARD-COOKED
AND PICKLED EGGS

By

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PHYSICAL AND MICROBIOLOGICAL PROPERTIES OF HARD-COOKED
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This research was conducted to evaluate physical and microbiological properties of hard-cooked and pickled eggs. Pickled eggs are peeled hard-cooked eggs immersed in a solution of vinegar and spice, which imparts a different flavor to the eggs.

The quality of the eggs, for preparation as pickled eggs, was found to be dependent upon the interior shell egg quality. A brown discoloration of hard-cooked egg albumen was found to be associated with the loss of interior shell egg quality. The ease of peeling the eggshell and the smoothness of the albumen surface improved with the age and/or increase in the pH of the egg. The delay between peeling the hard-cooked egg and using it in a food product or as a food allows for growth of microorganisms. Bacterial counts of peeled eggs held at 25°C. for four days increased from zero to 1.0×10^8 organisms per gram of egg. Storage at 5°C. did not result in a measurable amount of growth.

Five egg pickling recipes were studied in this investigation ("Red Beet", "Dill Egg", "Dark and Spicy", "Kansas Spicy" and "Sweet and Sour"), prepared from natural spices and ingredients. The egg pickling solutions required a minimal amount of heat processing for pasteurization. Microbial assay showed that the greatest contamination was from the peeled hard-cooked eggs. However, within a short time the highly acidic pickling solution destroyed a majority of the viable microorganisms. The pH of the pickling solutions was increased by the addition of alkaline hard-cooked eggs until an equilibrium pH was reached. The lower the ratio of solution to eggs the higher the pH at equilibrium. A minimum ratio of solution to egg was 1.6:1 (v/w) for an equilibrium pH of 4.0. The shift in pH of the pickling solution was similar to the dilution of acetic acid.

"Red Beet", "Dark and Spicy" and "Dill Egg" pickled eggs were subjected to a 14-week storage period at 22°C. and 4°C. and quality was assayed by a taste panel. Only "Dill Egg" maintained its quality at 22°C. storage. However, only "Red Beet" pickled eggs did not store well at 4°C. The greatest defects were discoloration of the albumen and development of off-flavor in the region of the yolk for all three pickled egg recipes.

The "Red Beet" egg pickling solution was found to be differentially absorbed by the hard-cooked egg albumen. The chalaza and the thick white did not absorb as much pigment as the thin white, and appeared lighter pink in color. This staining technique is suggested as a method to observe the relationship of the albumen layers with a minimum of distortion and destruction.

Three pickled egg recipes ("Dill Egg", "Kansas Spicy" and "Sweet and Sour") of the five recipes of pickled quail eggs were equally well accepted by taste panelists. The taste panelists indicated that these three pickled quail egg recipes are an acceptable product. The type of seasoning

spice and the color of the individual eggs were the most important criteria in the taste panelists' evaluation of the recipes.

Pickled eggs are an effective means of utilizing small and pee wee size eggs, which are under-utilized today. It is also a commercially feasible method of marketing a hard-cooked egg product.

INTRODUCTION

Over the last 25 years Florida has evidenced an increasing number of laying hens. In 1972 it reached a peak of 12,283,000 and these birds produced 2.8 billion eggs. From 1972 to 1974 the number of layers decreased slightly to 11,778,000. However, the production of eggs remained the same. Florida now is ranked seventh nationwide in egg production (Kalch and Douglas, 1974). This same level of egg production was also projected for 1974. A gross income of \$101,500,000 is expected in 1975, at the 1974 price of 43.5¢ per dozen eggs (Kalch, 1975).

About 1.2% of all eggs produced by caged layers are small and pee wee (Christmas et al., 1973). The number of small and pee wee eggs produced in Florida in 1973 was 33,672,000. The projected value of Florida small eggs would be approximately \$815,143. Small and pee wee eggs are about 3/4 the size of large eggs. The nutritional levels of the egg contents per unit weight of large and small eggs are equal. Dendy (1975) reported that the average 1974 prices for a dozen small and large size eggs were 29.05¢ and 44.10¢, respectively. The price of small eggs represented 66% of that of large size eggs. It appears that the full potential profit from small eggs is not being realized by the egg producer. If small and pee wee eggs could be used effectively for hard-cooking and subsequent pickling, the demand could be greatly increased. This could result in increasing the price received for these eggs.

This loss of profit and limited market may be reversed by the further processing of small and pee wee eggs. One such method may be pickling eggs, a gourmet product, which could retail for approximately \$1.50 for

ten eggs. The pickling solution of vinegar and spices imparts a distinct flavor and is a preservative. If a market can be developed for pickled eggs, the gross return could be \$5,050,800. This would be 6.5 times the value of the eggs as commercially marketed table eggs.

The advent of an automated egg cooker and peeler has provided a potentially enlarged market for the sale of hard-cooked eggs. Hard-cooked eggs have been used sparingly as a ready-to-eat food because of the preparation time required. Recently some distributors of hard-cooked eggs in plastic wrap for vending machines and small commercial pickled egg operations have come into existence.

The objectives of this research were to study:

- 1) The storage potential of hard-cooked eggs.
- 2) The extent and degree of color change of egg albumen associated with egg storage.
- 3) The bacterial problem of hard-cooked eggs, pickling spices and pickled eggs as they relate to product spoilage.
- 4) The consumer acceptance of pickled eggs over a three-month period to determine the color and flavor stability of the product.

LITERATURE REVIEW

Miller et al. (1960) surveyed the consumer acceptance of several methods of preparing eggs. They found that 28% of the people preferred fried eggs, 25% scrambled, 19% soft-boiled, 14% poached, 9% hard-cooked, 1% raw and 3% had no preference. The development of an automatic egg cooking and peeling machine has given the food industry the capacity to rapidly produce a large number of hard-cooked eggs (Anon., 1973). However, the survey of Miller et al. (1960) showed that hard-cooked eggs make up a small percentage of the total egg consumption. A product of hard-cooked eggs is pickled eggs, through which this method of preparation can find greater acceptance. The pickling of eggs has been practiced in the home for many years, but only limited information has been developed concerning processing and ingredient factors that could affect pickled egg quality.

The egg has maximum interior quality and monetary value as an article of food at the time it is laid. Deterioration of the egg as a dietary commodity is continuous (Romanoff and Romanoff, 1949). The opened egg is so perishable that methods for its preservation are adapted from those ordinarily used to prevent the decomposition of other foodstuffs (Romanoff and Romanoff, 1949). Historically, such compounds and techniques as low pH, spices, salt, sugar, smoking and low water activity have been used to preserve foods (Frazier, 1967). Pickling of eggs embodies all of these to preserve eggs. Preservation is not the main purpose of egg pickling today. Eggs are now available in quantity and the year-round. The novel and unique flavor that pickling imparts is the desirable attribute.

The egg is a product of the hen which is a self-contained nurturing environment for embryonic development (Romanoff and Romanoff, 1949). Birds' eggs have been used as food by human beings since antiquity. "Compared with the hen's egg, no other single food of animal origin is eaten and relished by so many peoples the world over; none is served in such a variety of ways " (Romanoff and Romanoff, 1949, p. 575). Of the three most important dietary essentials (protein, fat and carbohydrate) the egg is composed largely of the first two. Its proteins are relatively complete and are very well digested and assimilated. Eggs also are a good source of essential vitamins and minerals except that the egg contents are low in calcium and void of vitamin C (Romanoff and Romanoff, 1949).

Egg Structure .

The structure of the egg is inseparably linked to its biological potential to maintain the continuity of life (Romanoff and Romanoff, 1949). Fundamentally, the egg is comprised of a minute center of life, about which are arranged relatively enormous amounts of inanimate food substances, enclosed in a protective structure. Its structural elements are arranged with great precision, and their total organization is essential to the specific function of each part.

The yolk is the most important part of the egg. It contains the mass of nutritive material that supports embryonic development and the germ cell. It consists of 12 concentric layers of alternating yellow and white yolk material. The yolk is enclosed in a thin, pliable envelope, the vitelline or yolk membrane (Romanoff and Romanoff, 1949).

The albumen surrounds the yolk and is enclosed by the shell and membranes. It is a clear material of yellowish tint. In the albumen is the chalaze, part of the chalaziferous layer, a ropy structure of spirals

at the two poles of the yolk. The chalazae become twisted and taut, and their tension pulls the yolk nearer to the geometric center of the egg.

The whole body of the albumen is composed of four concentric layers. The chalaziferous layers makes up 2.7% of the albumen, the inner thin 16.8%, the thick 57.3% and the outer thin 23.3% (Romanoff and Romanoff, 1949).

There are two shell membranes that surround the albumen. The inner membrane is in contact with the outer thin albumen in all but the polar regions of the egg where some of the mucin fibers of the thick albumen penetrate to the membrane. The outer surface of the inner membrane is firmly cemented to the inside of the outer membrane, except in a small area usually at the blunt end of the egg. This space between the two membrane is the air cell (Romanoff and Romanoff, 1949).

The shell is a relatively smooth, hard, calcareous deposit around the outer shell membrane. The shell is composed of four structural components which include the pores and cuticle. The pores allow gas transfer between the inside and the outside. The cuticle is a proteinaceous coating that acts as a protective covering over the pores.

The percentages of the yolk, albumen and shell of the egg are dependent upon egg weight and other factors such as genetic differences, seasonal change, age of the bird, individual variation within species, intensity of laying, time of day when the egg is laid, environment, physical condition of the laying hen, and the kind and amount of feed and drugs (Romanoff and Romanoff, 1949). Since the late forties the laying hen has been improved through genetic selection. As a result the modern laying hen lays 227 eggs per year in the United States as compared to 174 in 1950 (McGregor and Stiles, 1973). As can be seen in Table 1, the yolk and shell percentages have decreased, while the albumen percentage has increased. The increased rate of production may be, in part, responsible for the difference in the

Table 1. The composition of the hen's egg

% Yolk	% Albumen	% Shell	Type	Reference
31.90	55.80	12.30	Averages	Romanoff and Romanoff, 1949
29.65	58.59 ¹	10.63	White leghorn	Marion et al., 1964
28.10	62.40	9.50	Incross breed	Gardner and Young, 1972
29.69	62.02	8.29	R.I.R.	Amer, 1972
30.44	59.20	10.36	Dandarawi	Amer, 1972

¹Separation resulted in a loss of 1.13% of the original whole egg weight; the loss is assumed to have been albumen.

percent composition of the egg contents.

The egg's structure and the unequal distribution of its chemical constituents make the egg an unstable system. The interior of the egg is imperfectly protected from the environment and is therefore exposed to external forces. For these reasons, the egg is in a continual state of readjustment, which takes place at a rate controlled largely by external factors (Romanoff and Romanoff, 1949).

Hard-Cooking of Shell Eggs

Chick and Martin (1910) found that heat coagulation of albumen is influenced by a variety of conditions. It does not occur instantaneously at normal cooking temperatures, but is a time process in which heat is the accelerator. Lowe (1955) pointed out, however, that coagulation occurs almost instantaneously at high temperatures.

Coagulation begins at about 62°C., and the albumen ceases to flow when it reaches a temperature of about 65°C. (Chick and Martin, 1910). At 70°C. the coagulum is fairly firm, but tender, and it becomes very firm at higher temperatures (Romanoff and Romanoff, 1949). Egg yolk begins to coagulate at 65°C. and ceases to flow when it reaches a temperature of about 70°C. (Romanoff and Romanoff, 1949). The coagulation reaction is endothermic; heat is absorbed. Too much heat results in over-cooking, regardless of whether the excess heat is the result of too high a temperature or exposure to heat for too long a time.

When hard-cooking eggs in the shell, the proportion of water to eggs and the size of the cooking container are essential considerations, in addition to the temperature of the water. Griswold (1962) recommended starting the cooking process with cold water and bringing it to a boil, or starting with boiling water and turning off the heat while the eggs cook.

Andross (1940) reported that when shell eggs are cooked at temperatures as low as 85°C., the yolk does not completely coagulate in 30 minutes. Further, the white does not coagulate firmly in 1.5 hours when the cooking water is held at 72°C.

The dark green color around the yolk of hard-cooked eggs is caused by the formation of ferrous sulfide (Tinkler and Soar, 1920). The ring can be prevented or reduced by minimizing cooking time and immediately cooling the cooked eggs by immersion in cold, running water (Irmiter et al., 1970).

High quality eggs have yolks which remain well centered after hard-cooking and are thus more pleasing, especially when made into deviled eggs, or sliced for salads and garnishings. Albumen thinning of stored eggs results in a loss of thick white and gives the yolk more freedom of movement; the yolk may also adhere to the shell membrane or displace out of the center of the egg (Romanoff and Romanoff, 1949). As the egg ages water shifts from the albumen to the yolk, thus weakening the vitelline membrane and causing yolk rupture (Feeney et al., 1956).

Because of the many variables in the raw eggs and the different quality criteria hard-cooked eggs must meet, Irmiter et al. (1970) found it difficult to suggest a superior method of cooking. The temperature of the heating medium appeared to have more effect on the incidence of shell cracking than any other factor. The boiling water method for hard-cooking eggs resulted in fewer cracked eggs than the cold water method (Irmiter et al., 1970). These authors also reported that the boiling water method rated highest in all criteria and is the preferred method for hard-cooking eggs.

Ideally a hard-cooked egg should have the following attributes: 1) the shell does not break during cooking, 2) the shell peels off easily and does not adhere to the coagulated egg albumen, and 3) the yolk should be well centered and free from any dark ring (Irmiter et al., 1970). Several

factors can influence how well hard-cooked eggs can meet these criteria, e.g., temperature of the egg, pH of the albumen, temperature of the heating medium, length of cooking time, strength of the shell and quality of the egg.

Microbiology of the Egg

The interior of the newly-laid egg is usually free of microorganisms, chiefly because of the natural protection provided by the egg's physical structure and by the chemical composition of the albumen (Romanoff and Romanoff, 1949; Frazier, 1967; Jay, 1970). Contamination of the egg contents can occur either before the egg is laid or shortly thereafter. The shell can become contaminated by fecal matter from the hen, from contact with materials and equipment in the poultry house, by wash water if the eggs are washed, by handling and by packing material (Frazier, 1967). Molds and bacteria from these sources can grow through a moistened shell and into the egg. Since eggs usually are cooled promptly and stored at low temperatures, contamination with low temperature bacteria such as those of the genera Pseudomonas, Proteus and Achromobacter is undesirable. Gram-positive cocci and rods and coliform bacteria occur in smaller numbers on eggshells, as would anaerobes and miscellaneous bacteria as chance contaminants. Occasionally, Salmonella bacteria may be in eggs from infected hens (Frazier, 1967). However, control for S. pullorum is based on a regular testing program of breeding stock to assure freedom from this infection. Chickens are tested by a tube agglutination of whole-blood method (Siegmond, 1973). Cox et al. (1973) reported that there was no salmonellae contamination of egg meat among intact eggs from birds fed salmonellae inoculum, though salmonellae contamination of the shell surface did occur. Thus contamination of the egg with salmonellae from the hen is not likely to occur.

Species of bacteria or fungi on the exterior of the egg vary according to circumstances. The approximate composition of the flora found in one

investigation (Romanoff and Romanoff, 1949) was reported as:

Non-sporeforming rods	38%
Sporeforming rods	30
Cocci	25
Yeasts	4
Molds	3

As a result of this contamination, the egg may eventually decompose, or it may be responsible for the dissemination of disease among poultry or human beings (Romanoff and Romanoff, 1949). In light of the natural protection provided within the egg, it is one of the safest foodstuffs in man's diet. The pores in the shell are normally impervious to microbial penetration. They are filled with an organic substance (the cuticle) that, when dry, does not permit bacteria or fungi to enter. However, if the cuticle is dissolved or partially removed by abrasion, the pores are opened and microfloral invasion is possible (Romanoff and Romanoff, 1949; Fromm and Monroe, 1960). The shell membrane acts as a filter for removal of many of the microorganisms that succeed in penetrating through the pores of the shell.

Several constituents of the albumen kill bacteria before they reach the yolk with its abundance of utilizable food. The antibacterial action of the egg albumen is partially due to the inability of many bacteria to utilize native protein. The enzyme, lysozyme, hydrolyzes the mucopolysaccharides of the cell wall of both the live and dead cells. Not all bacteria are equally susceptible to the action of lysozyme. Airborne species are, in general, less resistant than those that may be isolated from the human body. Bacteria becomes less susceptible to lysozyme when they are grown in proximity to body tissue containing the substance. Although its rapidity of action increases with temperatures up to 60°C., all lytic

power disappears upon heating to temperatures higher than 70°C. (Romanoff and Romanoff, 1949). Avidin aids in inhibiting bacterial growth in egg albumen by depriving microorganisms of biotin. Those bacteria which can synthesize biotin are not affected. Ovoconalbumen in the egg albumen can completely inhibit various species of bacteria by binding or complexing iron, thus making the iron unavailable as a nutrient (Romanoff and Romanoff, 1949).

The bacteria upon surviving the defenses of the eggshell, shell membranes and albumen will reach the yolk. The yolk material, because of its nutrient content, and pH (6.8), is an excellent growth medium for most organisms. More bacteria are found in the yolk than the albumen (Jay, 1970). Once inside the yolk, bacteria grow in this nutritious medium where they produce products of protein and amino acid metabolism such as hydrogen sulfide and other foul smelling compounds.

Bacteria in eggs have been responsible in the past for many individual cases and mass outbreaks of "food poisoning" (Romanoff and Romanoff, 1949). Many species of the genus Salmonella cause "food poisoning" (Romanoff and Romanoff, 1949; Frazier, 1967; Jay, 1970). This "food poisoning" is a food infection where the actual presence and viability of the organism is essential to produce the food poisoning symptoms. Raw eggs, partially cooked eggs and especially products containing raw eggs are potentially excellent media for growth of Salmonella organisms. However, the cooking of hard-cooked eggs to a minimum of 68°C. is sufficient to destroy the Salmonella organism (Frazier, 1967). As a safeguard against salmonellae it has been recommended in the code of Federal Regulations that all liquid egg products except albumen, be pasteurized at 60°C. (140°F.) for not less than 3.5 minutes or to have been found free of salmonellae (Frazier, 1967).

Some microorganisms may be present in the egg which are resistant to

thermal treatment. Hülphers (1939) found that hens contract tuberculosis when inoculated with material from eggs infected with Mycobacterium avium when the eggs had been boiled for three minutes, whereas four minutes of boiling apparently rendered the eggs sterile. According to Löwenstein (1925), the avian tubercle bacillus remains viable in the albumen after three minutes of cooking, and in the yolk five to ten minutes.

The hard-cooking process exceeds the time and temperature required for pasteurization of egg products. As a result, the hard-cooked egg could be considered commercially sterile. However, the process of removing the shell reintroduces the microbial problem through contamination by hands, equipment and rinse water.

Microbiology of Pickled Eggs

The pickling of eggs in a vinegar solution involves the use of low pH, spices, salt, sugar and smoke derivatives to lower water activity and to provide flavoring and coloring of hard-cooked eggs.

If the microbial population of the spices is excessive, they may contaminate the product to a point where spoilage may occur (Sharf, 1966). Sporeforming bacteria in spices have been implicated in spoilage of canned foods and processed meat products (Julseth and Deibel, 1974). The potential spoilage organisms include proteolytic and thermophilic sporeformers. Krishnaswamy et al. (1971) found many spoilage and potential pathogenic microorganisms in spices from India; these included C. perfringens and E. coli.

Pickled egg products are spice-containing and therefore are suspect. Acton and Johnson (1973) reported that sporeforming bacteria present in egg pickling solutions were viable after 50 days of storage. Trongpanich and Dawson (1974) reported on the microbiological quality of hard-cooked brine-pickled duck eggs stored at 3°C. for four weeks. At the end of four

weeks there was a high number of organisms present (1.7×10^5 organisms per gram of egg).

Chung and Goepfert (1970) employed three strains of Salmonella (S. anatum, S. senftenberg and S. tennessee) to determine the minimum pH value as determined by 13 acidulants that would permit the initiation of growth of Salmonella in laboratory media. The most permissive group includes tartaric, hydrochloric and citric acids in which growth was initiated at values as low as pH 4.05. The most restrictive class was composed of adipic and pimelic acids and the short-chain volatile fatty acids, acetic and propionic. The minimum pH at which salmonellae grew in acetic acid was pH 5.40. Levine and Fellers (1940) reported that acetic and propionic acids at pH 4.9 limited growth of food spoilage microorganisms.

Salmonella typhimurium was able to survive in apple cider having a pH value less than 4 (Anon., 1975). Multiplication of the salmonellae in the cider need not occur to create a problem if the cider was heavily contaminated when it was produced. Evidence suggests that S. typhimurium can produce disease with an inoculum of only 10^4 organisms (Anon., 1975).

The potential for product spoilage or "food poisoning" is, therefore, still a possibility for pickled egg products even though the acid content is high. Microorganisms can be introduced by the egg, spices and other ingredients.

Peelability of Hard-Cooked Eggs

Hard-cooked, freshly laid eggs are difficult to peel without tearing the albumen. As an egg ages during storage, certain physical and chemical changes occur in the composition of the egg and the ease of peeling increases (Romanoff and Romanoff, 1949). It has been observed that the pH of the albumen rises from 7.0 to as high as 9.7; this change is accompanied by a breakdown in the thick albumen structure (Romanoff and Romanoff, 1949).

Swanson (1959) and Reinke and Spencer (1964) indicated that ease of peeling was related to the change in pH of the albumen. Above pH 8.6 to 8.7, they found that little or no difficulty in peeling was experienced. Similarly, Fuller and Angus (1969), in their study of the pH of both uncooked albumen and homogenized albumen from hard-cooked eggs, indicated that the "cross-over" from poor to good peeling characteristics corresponded to pH values 8.6 to 8.9 of raw egg whites. Swanson (1959) and Reinke et al. (1973) reported that freshly laid eggs could be made to peel easily if they were exposed to ammonia fumes until the pH of the albumen reached 8.7, and that stored eggs could be made to peel with difficulty if they were exposed to carbon dioxide until the pH of the albumen was lowered below 8.7.

In a study by Fry et al. (1966), albumen structure was broken down by gamma irradiation without significant pH increase. The Haugh units of the irradiated eggs were reduced to about 50% of that of non-irradiated controls. The average albumen pH value of irradiated fresh eggs was 8.39 while the control was 8.22 at 0 days storage. Both irradiated and fresh eggs peeled with difficulty, indicating that peeling ease was influenced by pH rather than albumen quality per se.

Reinke et al. (1973) suggested that peeling ease was related to pH of albumen rather than to dehydration, although a weight loss of two grams or greater might have some influence on peeling ease. Cotterill and Gardner (1956) demonstrated that low concentrations of carbon dioxide would maintain albumen quality of eggs stored at room temperature as effectively as refrigeration under normal conditions. The presence of CO_2 prevents the deterioration of ovomucin in the thick white. Almqvist and Lorenz (1932) observed that, in the presence of excess CO_2 , ovomucin fibers of the firm white contract and squeeze out a liquid solution of other proteins. Thus, in the presence of sufficient CO_2 , the ovomucin fibers are not degraded

and a pH of 8.6 or lower is maintained. Because of these factors the albumen adheres to the shell, making peeling more difficult.

Hydrogen sulfide was found by MacDonnell et al. (1951) to have a thinning effect on the albumen of eggs. Albumen of old eggs was markedly thinned by H_2S treatment and the pH values of the albumen components were decreased to those characteristic of fresh eggs. Both fresh and old eggs treated with H_2S were difficult to peel, providing further evidence that peeling quality is related to albumen pH rather than albumen thinning (Reinke et al., 1973).

Reinke et al. (1973) found that all fresh eggs containing old egg contents and all old eggs containing old egg contents peeled easily. Old eggs containing fresh egg contents and fresh eggs containing fresh egg contents peeled with varying degrees of difficulty. It appeared that pH of the transferred albumen was the influencing factor on peeling quality as the transfer of old egg contents of high pH resulted in an easy-to-peel egg, while the reverse was true of the transfer of fresh egg contents of low pH. Cotterill et al. (1959) indicated that fresh egg white is strongly buffered in the regions of pH 6.4 and pH 10.3, and that the buffering capacity is minimal at pH 8.3. The pH of the transferred albumen could modify the pH of the adhering albumen and membrane and affect peeling quality.

Low viscosity values for adhering albumen may be responsible in part for determining the peeling quality, since a less viscous albumen may not adhere as strongly to the membrane as a more viscous albumen. Reinke et al. (1973) noted an inverse relationship to exist between pH and viscosity of adhering albumen. A high pH was usually accompanied by a low viscosity reading. Conrad and Scott (1939) showed that a breakdown of thick gel structure occurred after escape of CO_2 , concurrently with increased pH, and

possibly the extension or change in elasticity of the ovomucin fibers. The integrity of the ovomucin-lysozyme complex has been reported to be dependent on pH (Cotterill and Winter, 1955). Consequently, when CO_2 was added to eggs, thus lowering the albumen pH, the ovomucin fibers of the adhering albumen are possibly contracted or at least reverted to their original elasticity, and resulted in lower peelability.

Froning et al. (1960) reported that, as CO_2 in the storage atmosphere was increased, there was a marked decrease in the percentage of ovomucin in the outer thin white and a marked increase in the percentage of ovomucin in the thick white during storage. Brooks and Hale (1961), however, concluded that ovomucin chains are linked together, such as by assuming an ovomucin-lysozyme complex cross-linked into a network.

One of the main differences between fresh and old eggs was the deterioration of the adhering albumen layer adjacent to the membrane. Albumen from the small end of a hard-cooked egg contained fiber-like structures which stained dark purple, indicating the presence of mucin. The adhering albumen had some structural material present which accounted for its viscosity being slightly higher than that of thin albumen. This fiber-like material was not visible after aging or NH_3 treatment (Reinke et al., 1973).

An additional difference between fresh and old eggs was the appearance of a dark staining border between the inner shell membrane and the adhering albumen. The border was wider and/or darker in old eggs and contained mucin. Fuller and Angus (1969), in their gross examination of shell membranes, also observed that the membranes from eggs which peeled cleanly were compact and tore easily from both the shell and egg white. They found that the membranes from eggs which peeled poorly were not compact, but could only be torn in layers and adhered firmly to the shell.

It is possible that when pH of eggs is low, ovomucin fibers (in albumen) may protrude into the membrane, while ovomucin fibers (in the membrane) may extend into the albumen, to form the narrow border observed in fresh eggs. However, when pH of eggs is high, these fibers may be affected in such a way that they pull apart, forming the wide border observed in old eggs (Reinke et al., 1973).

Hard et al. (1963b), in their study of methods of preserving interior egg quality, noted that even after eight weeks of storage at 0°C. some difficulty in peeling was experienced if eggs were coated with oil or silicone grease or if maintained in an atmosphere containing 95% carbon dioxide. Britton and Hale (1972) found that fresh and old eggs which were oiled when fresh had increased peeling times and that oiled eggs had greater peeling damage.

Fuller and Angus (1969) indicated that peeling properties of hard-cooked eggs could be altered by the addition of 1%, 5% and 10% NaCl or CaCl_2 to the cooking medium. These treatments did not, however, affect the peeling quality of eggs collected within eight hours of oviposition. Addition of NaCl to the cooking medium of eggs stored at 10 to 13°C. for 24 hours enhanced peeling properties, while the addition of CaCl_2 made peeling more difficult. The effect of addition of NaCl disappeared by the second day of storage of uncooked shell eggs.

Spencer and Tryhnew (1973) reported that, if hard-cooked eggs are not aged sufficiently before cooking, storage after cooking will not remedy the problem of difficulty in peeling.

The research of these authors indicates that the ideally suited egg for successful hard-cooking is one which has an albumen pH above 8.7 and a firm albumen to keep the yolk centered. In order for such an egg to have a pH above 8.7, it should have been stored a sufficient length of time to

allow enough CO_2 to escape. Fresh eggs should not be oiled or stored in a CO_2 atmosphere because this inhibits the rise of albumen pH to 8.7, thus impairing good peelability.

Color and Flavor of Hard-Cooked and Pickled Eggs

Dodge et al. (1965), Baker and Darfler (1969) and Schnell et al. (1969) reported that hard-cooked eggs developed a brown discoloration of the albumen due to the Maillard reaction. The longer the eggs were cooked the browner the albumen became. Dodge et al. (1965) found that ultraviolet irradiation was a factor in discoloration. Baker and Darfler (1969) reported that, as the pH of the albumen was increased, the amount of color increased. Color increased gradually up to a pH of 8.5 and then increased rapidly.

The Maillard reaction (Meyer, 1968) involves the reducing groups of glucose or similar sugars and the amino groups of protein. This reaction results in the development of the brown color. McWeeny et al. (1969) found that the Maillard reaction does not begin until "free" SO_2 has almost disappeared from the system. A pH of 5.6 and above inhibits the Maillard reaction. No inhibition of the Maillard reaction occurs at pH 4.3 or below.

Sherwood (1959) reviewed shell egg quality and concluded that pink albumen and yolk color are dependent upon the hen's diet and that watery albumen may be the result of chemicals or illness.

Flavor is one of the most important factors influencing acceptability of eggs (Hard et al., 1963a; Romanoff and Romanoff, 1949). Taste panel evaluations indicate that richness, mustiness and astringency of the yolk are masked or diluted by the presence of egg white. Adjectives used to describe eggs which were rated as acceptable in the study by Hard et al. (1963a) were "fresh", "mild" and "sweet". Koehler and Jacobson (1966)

reported musty and earthy qualities in stored eggs, and less "sulfury" and "hydrolyzed protein" flavor in the yolks from stored than from fresh eggs. Miller et al. (1960) found that customers did not distinguish between the flavor of eggs of different ages. Although taste panels differentiated between flavor of newly laid and stored eggs (Dawson et al., 1956), consumers may be accustomed to variation and may not respond to small differences in flavor.

Koehler and Jacobson (1966) and McCannion et al. (1934) showed that no significant differences in flavor were associated with the color of the egg yolk, although dark-colored yolks tended to be rated as slightly objectionable. Palmer (1972) reported that eggs stored near odorous substances can absorb odors and exhibit flavor changes. Banwart et al. (1957) reported that an off-flavor in stored oiled eggs after soft-cooking was more detectable in the white than in the yolk.

A bitter off-flavor of hard-cooked eggs develops with the increase of the Maillard reaction (Baker and Darfler, 1969). Arroyo and Lillard (1970) screened several sulfur-containing amino acids in reaction with glucose. They found that when the amino acid, cysteine, complexed with glucose, an overcooked egg odor developed; this was attributed to the presence of H_2S . However, when methionine complexed with glucose, a boiled potato odor developed.

McCready (1973) reported that flavor scores were highest when egg and pickling solution temperatures were at least 65°C. at the time eggs and pickling solutions were combined, and when aging was conducted at a storage temperature of 24°C. Panel scores indicated that a pickling solution of 24°C. or less and a storage (aging) temperature of 3°C. inhibited pickled egg flavor development during a 24-hour test period.

Physical Properties of Pickled Eggs

Acetic acid (CH_3COOH) is used as a fixative in histology and as a food preservative (Humason, 1962). The rate of acid penetration in hard-cooked eggs was judged by Acton and Johnson (1973) to be determined by the rate of diffusion of acetic acid into the egg white and by the initial acid strength of the pickling solution. They indicated that the pH of eggs pickled in 3 or 5% acetic acid vinegar solutions required six days to equilibrate with the pH of the pickling solution. Ball and Saffores (1973) reported the equilibrated pH to range from 4.0 to 5.1 after six to seven days of storage.

Ball and Saffores (1973) reported that eggs lost 4 to 12% of their weight during pickling. Eggs pickled in weaker acid solutions lost more weight. They posutlated that the egg white was losing moisture to the pickling solution and the yolk. A noticeable toughening of egg white occurred during pickling (Ball and Saffores, 1973). Initial forces required to shear cooked egg white averaged 0.6 kg./g. and the force to shear pickled egg white ranged up to 1.6 kg./g. The addition of salt to pickling solutions increased the magnitude of weight loss, changes in egg white solids and tended to increase toughness, but did not affect the equilibrated pH (Ball and Saffores, 1973).

Cunningham and Cotterill (1964) reported that native egg albumen protein components had an isoelectric point of pH 4.0 which is also the point of maximum viscosity. In the study of Ball and Saffores (1973), equilibrated pH's were close to or lower than the isoelectric point of egg white proteins. Eggs exhibited minimal values for volume and water holding capacity when the albumen proteins were at their isoelectric points. The loss of moisture and toughening of the pickled egg white could result from proteins assuming these minimal physical characteristics. The process could be syneresis, the

loss of moisture from a gel by contraction of the gel.

Panel evaluations conducted by McCready (1973) indicated significant differences between the tenderness of eggs pickled in solutions containing zero to 40% sugar. Eggs pickled in solutions containing 45 to 60% sugar were rated significantly lower in tenderness. The shear values of pickled eggs, reported as p.s.i./g. of egg, were significantly greater than the shear values of non-pickled, hard-cooked eggs. Sugar concentrations of 25% or greater significantly decreased the tenderness of pickled eggs as indicated by shear values. Eggs pickled in solutions containing no sugar had weight losses ranging from 6.2 to 9.0%, and additional losses in weight occurred as the percentage of sugar increased and the pH values became greater than 5.0. Adding salt to pickling solutions created osmotic forces that result in further dehydration of pickled egg white (Ball and Saffores, 1973).

Ball and Saffores (1973) reported that turbidity of the pickling solution may result from suspension of pickling spices or yolk material from exposed yolks. Splitting of egg white that exposes yolks may be the result of combined forces of syneresis in egg white and expansion of yolk as it takes up moisture. These same authors also reported that off-centered yolks in eggs with weak albumen also contribute to splitting of the egg white.

Frazier (1967) has reported that oxidation of acetic acid in vinegar to CO_2 and H_2O can be brought about by the acetic acid bacteria themselves during the vinegar-making process if there is a shortage of alcohol or an excessive amount of aeration. This may also occur in a finished product to reduce the acidity. Further, ferrous iron may be oxidized to the ferric form and combine with tannins, phosphate or protein to produce haze in the

acetic acid. Cloudiness also may be caused by salts or tin or copper. Iron acting upon tannin or oxidase may be responsible for darkening of vinegar (Frazier, 1967). It is speculated that potential contamination and/or pickling ingredients may also influence the quality of the pickling solution causing discoloration, change in acid strength, and development of off-flavor.

CHAPTER I

STORAGE STABILITY OF HARD-COOKED EGGS

Numerous investigators have reported on the factors involved in the preparation and pickling of hard-cooked eggs. The majority of this work has dealt with either the investigation of peeling attributed (Fuller and Angus, 1969) or the use of various pickling solutions on hard-cooked eggs (Ball and Saffores, 1973; Cunningham et al., 1970; Maurer, 1972; and McCready, 1973). A few of these investigations have also been on the organoleptic acceptability of the product after varying storage periods. Spencer and Tryhnew (1973) found that storage of hard-cooked shell eggs at 1.1°C. for up to 21 days resulted in lower taste panel scores after only one week of storage and that, after 21 days storage, serious off-flavors were detected by panel members. Britton and Hale (1972) found that bacterial counts of hard-cooked eggs were initially quite low; however, they considered the eggs spoiled after 10 days at 4°C.

In their report dealing with the pH and rate of acid penetration in eggs undergoing the pickling process, Acton and Johnson (1973) found that eggs pickled in a 3% acetic acid pickling solution were bacteriologically safe.

The investigation reported herein was undertaken to examine the effect of storage at two different temperatures (5 and 25°C.) on the bacteriological quality of both peeled and unpeeled hard-cooked eggs.

Experimental Procedure

Egg Source

Medium size eggs were collected from Babcock B-300 Leghorn hens at the University of Florida Poultry Science Department. All eggs were washed and graded on an Aquamagic egg processing machine (National Poultry Equipment Co., Renton, Wash.) containing a detergent-sanitizer Egclor (Sanfax Chemicals, Atlanta, Ga.) in accordance with the manufacturer's specifications. The washed eggs were placed into clean molded pulpboard filler flats and held in a cooler at 13°C. for four days. All eggs used in these investigations were obtained from laying flocks maintained on a uniform diet.

Hard-Cooking and Peeling Procedure

A total of 320 eggs were placed in a steam-jacketed kettle and completely covered with cold water which was brought to a boil. The heat was then reduced, and the eggs were simmered for 15 minutes. The steam was turned off and the eggs were cooled under running tap water. Prior to peeling each batch of eggs or after any interruption of the peeling procedure, all personnel washed their hands using a hand sanitizer rinse, Klenzade Mikroklene DF (25 p.p.m., Economics Laboratory, Inc., St. Paul, Minn.).

Storage Conditions

Half of the hard-cooked eggs were peeled and the remainder were left in the shell. Peeled and shell hard-cooked eggs were then placed into sterile one-pint Mason jars (three eggs/jar). An equal number of jars containing peeled and shell hard-cooked eggs were placed in storage at 5 and 25°C. The higher temperature was selected since it roughly corresponds to the room temperature at which hard-cooked eggs are often stored in commercial establishments.

Bacteriological Sampling Procedure

Three eggs from each of the four storage conditions were blended individually at each sampling. Each egg was aseptically placed in a sterile blender jar and a 1:10 dilution prepared using 0.1% peptone diluent and blending for two minutes. Subsequent serial dilutions were prepared by transferring 11 ml. aliquots to 99 ml. 0.1% peptone blanks. Samples were plated in quadruplicate into Plate Count Agar (Difco) and duplicate plates of appropriate dilutions were incubated at 22 and 35°C., respectively.

Results and Discussion

Bacteriological Investigations

Microbiological data obtained from peeled and shell hard-cooked eggs stored at 25°C. over a period of 24 days are shown in Figure 1. Each point on the figure represents an average of plate counts from the three eggs from one jar sampled individually at a particular time. Visible sliminess was noted on the peeled eggs after four days storage, and discrete bacterial colonies were observed on the sixth day of storage.

This sliminess and subsequent colony formation corresponded to populations of approximately 1.0×10^7 organisms/g. Discoloration on shell hard-cooked eggs were noted only after 13 days storage. Bacterial numbers leveled off after the tenth day of storage and remained at about 1.3×10^8 organisms/g. for the balance of the storage period at 25°C.

Microbiological data from storage of peeled and shell hard-cooked eggs at 5°C. are shown in Figure 2. Bacterial development was not evident on peeled hard-cooked eggs stored at 5°C. until after 21 days of storage. Shell hard-cooked eggs displayed sporadic counts over the storage period. At no time during the 24-day storage period was there any visible slime or colony formation on either group of eggs.

Figure 1. Plate counts of peeled and shell hard-cooked eggs stored at 25°C.

Figure 2. Plate counts of peeled and shell hard-cooked eggs stored at 5°C.

Total counts on eggs prior to hard-cooking ranged from 0 to 30 organisms/g. (approximately 1500 organisms/egg). The low counts observed on eggs immediately after cooking indicate that most bacterial problems encountered with hard-cooked eggs would be directly related to post-cooking contamination. Sources of such contamination would be improperly cleaned equipment, poor hand washing and poor quality wash water supply. Efforts were made in this study to use conditions that were not too far removed from what might be expected in commercial practices. However, some of the variables encountered in this study would be eliminated if automatic cooking, peeling and packaging becomes widely available (Anon., 1973).

A source of bacteria that contributed to the microflora of the hard-cooked eggs in this study was the water used in the actual processing of the eggs. Plate counts of cooking water sampled immediately after cooking showed no viable bacteria present in a 10 ml. undiluted sample; however, rinse water used during the cooling, peeling and rinsing procedures showed a count of 20 organisms each, in two 10 ml. samples.

Peeled hard-cooked eggs stored at 5°C. did not support as much bacterial growth as did shell hard-cooked eggs. This might be explained by the presence of a higher bacterial load on the surface of shell-hard-cooked eggs. This may have been the result of a more thorough rinsing of the peeled hard-cooked eggs during preparation. It is also possible that the growth of microorganisms was somewhat restricted at 5°C. Position of the eggs in the jar (top, middle or bottom) did not have an effect on bacterial growth. Counts obtained at incubation temperatures of 22 and 35°C. were not significantly different, indicating that bacteria on these eggs were capable of growth at either incubation temperature.

CHAPTER II

THERMAL DESTRUCTION OF MICROORGANISMS IN EGG PICKLING SOLUTIONS

The advent of an automatic egg cooking and peeling machine has given the food industry the capacity to rapidly produce a large number of hard-cooked eggs (Anon., 1973). One of the potential products is pickled eggs. Cunningham et al. (1970) and Maurer (1972) reported procedures and recipes for pickling eggs in the home. However, only limited information is available concerning commercial processing and ingredient factors which may affect the final quality of pickled eggs.

When used in egg pickling solutions, spices enhance both flavor and appearance. However, various researchers have shown that if the microbiological population of the spice is excessive, the organisms may contaminate the product, and result in a spoilage problem or a health hazard. Cunningham et al. (1970) and Maurer (1972) have suggested that heating a pickling solution to boiling and then simmering it for five minutes would result in sterilization of the pickling solution. This concept should be refined for application of the pickling process to commercial processing.

Acton and Johnson (1973) reported that the acid strength of pickling solutions was decreased by 20 to 23% by the addition of spices to the vinegar solution. They hypothesized that this was probably due to the absorption and neutralization of acetic acid (vinegar) by the pickling spice ingredients. They also demonstrated that acid penetration into the alkaline hard-cooked egg can shift the pH upward in the egg pickling solution.

The toxic effects on microorganisms that can be observed at unfavorable

pH values are not a direct result of high hydrogen ion and hydroxide ion concentration (Stanier et al., 1963). Undissociated molecules of acidic and basic substances can penetrate into cells much more readily than the corresponding ions. At low pH values, weak acids, which exist to a considerable extent in undissociated form, can enter cells and damage them by changing the internal pH, whereas strong acids cannot. In slightly acid environments, a weak acid (acetic acid) is toxic to microorganisms, whereas a strong acid, HCl, has relatively little effect on these organisms. The toxicity of acetic acid disappears under neutral or alkaline conditions, where the molecule is almost completely ionized (Stanier et al., 1963).

The objectives of this investigation were: 1) to determine if microorganisms could survive in the high acid environment of pickling solutions and 2) to determine the appropriate heating time for sterilization of egg pickling solutions.

Experimental Procedure

Spices and pickling ingredients were purchased at local retail markets. Each of these materials was assayed for microbial content as described in the Recommended Methods for the Microbiological Examination of Foods (Sharf, 1966).

Five recipes for egg pickling solutions (Maurer, 1972) (Table 2) were evaluated as to the time required to effect thermal destruction of the microorganisms present. Each solution was heated in a one-liter beaker on a Corning electric hot plate with a magnetic stirrer. Each pickling solution was brought to boiling and then simmered for ten minutes. Aliquots of an appropriate volume of pickling solution were drawn at zero time, eight minutes, 16 minutes, boiling (25 minutes) and every two minutes thereafter for ten minutes.

Table 2. Egg pickling recipes (for one quart of pickled eggs)¹

1) Red Beet Eggs	4) Kansas Spicy Eggs
1 cup red beet juice	1 1/2 cups apple cider
1 cup cider vinegar ²	1 cup white vinegar
1 teaspoon brown sugar	2 teaspoons salt
a few small canned red beets	1 teaspoon mixed pickling spice
	1 clove peeled garlic
2) Dilled Eggs	1/2 sliced onion
1 1/2 cups white vinegar	1/2 teaspoon mustard seed
1 cup water	5) Dark and Spicy Eggs
3/4 teaspoon dill seed	1 1/2 cups cider vinegar
1/4 teaspoon white pepper	1/2 cup water
3 teaspoons salt	1 tablespoon dark brown sugar
1/4 teaspoon mustard seed	2 teaspoons granulated sugar
1/2 teaspoon onion juice	1 teaspoon mixed pickling spice
1/2 teaspoon minced garlic	1/4 teaspoon liquid smoke or
3) Sweet and Sour Eggs	hickory smoke salt
1 1/2 cups apple cider	
1/2 cup cider vinegar	
1 package (12 oz.) red cinnamon candy	
1 tablespoon mixed pickling spice	
2 teaspoons salt	
1 teaspoon garlic salt	

¹Maurer, 1972.²Changed from 1/2 cup to 1 cup.

Two methods for assaying the presence of viable microorganisms were used. In trial 1, the procedure of Sharf (1966) was used. Ten ml. of pickling solution was divided equally among three petri dishes and poured with approximately 25 ml. of Plate Count Agar (Difco). The plates were incubated at 32°C. for 48 hours and then examined to determine the total number of microorganisms present. Each heating trial was replicated twice.

An ideal transfer aliquot of pickling solution was sought for use in trial 2. It was necessary to increase the environmental pH by reducing the acidity of the agar to a level which would support microbial growth. White vinegar was used because of its high acidity (pH 2.6). Aliquots of vinegar (3.0 ml., 1.0 ml., 0.5 ml. and 0.1 ml.) were pipetted into 25 ml. of Plate Count Agar and the pH then determined. The 0.1 ml. aliquot was selected for subsequent use in microbial evaluations of the pickling solutions.

In trial 2, a 0.1 ml. aliquot of pickling solution was placed in a petri dish and poured with 25 ml. of Plate Count Agar. Three petri dishes were prepared at each sampling time. The same incubation and replication procedure as in trial 1 was used.

The criteria for thermal destruction time was established as the heating time necessary for development of no viable growth after 48 hours at 32°C., on all three pour plates of the same sampling period.

The pH of all the solutions was measured by a Corning pH meter at each step of the process to detect changes in pH as a result of the addition of an ingredient. The pH of the agar was also measured when the pickling solution was added to determine that the agar's pH (final pH 7.0 at 25°C.) was not sufficiently altered to inhibit bacterial growth.

Results and Discussion

The division of ten ml. of pickling solution equally among three

petri dishes, in trial 1, resulted in no observable microbial growth. The pickling solutions ranged in pH from 2.8 to 3.5 (Table 3). The pH of these pour plates was measured and found to be less than 4.5, too low for effectively monitoring the extent of microbial destruction. Thus, this method, discussed by Sharf (1966), was not appropriate for assaying microbial destruction in egg pickling solutions.

The volume of egg pickling solution, which would be suitable to enable detection of viable bacterial growth was determined to be 0.1 ml. This aliquot is the smallest practical transferable amount. Only the 0.1 ml. aliquot, yielding an agar pH of about 6.8, was considered acceptable for use in trial 2. The environment of the agar, with its pH approaching neutrality through dilution, and its limited buffering capacity, was sufficiently adjusted to support growth, if any viable microorganisms were present.

The egg pickling solutions varied in amount of heat needed to reach total destruction of the microorganisms present (Figure 3). No egg pickling solution required a longer heating time for total destruction of the bacteria than that necessary to reach boiling. The "Dark and Spicy" egg pickling solution took as little as 16 minutes for total microbial destruction while the other solutions took as long as 25 minutes. The "Red Beet" egg pickling solution was found to have too few organisms to plot a thermal destruction curve. The difference in heating time required to kill microorganisms apparently is dependent upon the thermal protection that ingredients may give the microorganisms and the number of microorganisms introduced by the various spices. The standard aerobic plate counts for each spice and ingredient used in the egg pickling solutions are presented in table 4.

Table 3. Acidity (pH) of the pickling solution

Recipe	pH
Red Beet Egg	3.55
Dill Egg	2.80
Sweet and Sour	3.27
Kansas Spicy	3.10
Dark and Spicy	3.15

Figure 3. Thermal destruction curve for egg pickling solutions.

Table 4. Microbiological content of each spice and ingredient

Ingredient	Microorganisms
Liquid	Number per ml.
Cider vinegar	0
White vinegar	0
Tap water	10
Red beet juice	450
Apple cider	0
Solids	Number per g.
Brown sugar	10
Dill seed	180
White pepper	120,000
Salt	712
Mustard seed	160
Red cinnamon candy	10
Mixed pickling spice	10
Garlic salt	700
Sugar	30
Smoke salt	250
Yellow onion	120
Minced garlic	21,000

The reported heating sequence of Cunningham et al. (1970) and Maurer (1972) is more than adequate to sterilize the egg pickling solutions used in these investigations. Sporeforming bacteria were expected to be able to grow when the environmental conditions were suitable for growth. Even with the agar at a pH of 6.8, an optimal pH for microbial growth, very few organisms grew. It is apparent that the combination of heat and acid was enough to destroy spores as well as vegetable cells.

Several other observations can be reported as a result of this investigation. The decrease in the acid strength of the pickling medium due to the addition of spices observed by Acton and Johnson (1973) was observed in the "Dill Egg" pickling solution as the spices were added. In the other four egg pickling recipes the addition of spices tended to decrease the pH and increase the acid strength. The thermal treatment did not affect the pH of the egg pickling solutions. The pH values of the egg pickling solutions were the same before and after heating. As with other foods, thermal processing is useful in minimizing the effect of microbiological contamination.

CHAPTER III

MICROBIAL GROWTH IN PICKLED EGGS

The preparation of pickled eggs involves the use of vinegar, seasoning and heat treatment, not unlike the procedure for preparing dill pickles. The principles used in commercial dill pickle processing may relate to egg pickling. A unique similarity of the two is a rapid increase in the pH of the pickling solution. If the increase in pH exceeds 4.0 microbial problems may result.

Monroe et al. (1969) recommended that the internal product temperature of dill pickles should be 165°F. (73.5°C.) with a 15-minute holding period and an equilibrated acidity sufficient to maintain a brine pH of 4.0 and below to assure protection from spoilage. Bell et al. (1972) found that a 95% equilibrium would occur in 50 hours for fresh packaged pickles (40% brine to 60% pickles). These authors found that in a commercial operation little acid penetration occurs before the heating starts.

Acton and Johnson (1973) reported the presence of 540 sporeforming bacteria per ml. of egg pickling solutions after 50 days of storage as opposed to half this number of organisms present at 20 days of storage. Low microbial counts are achieved in commercial dill pickling by low pH and thermal processing.

The "Dill Egg" recipe has been found earlier to contribute the greatest number of microorganisms and was studied in this investigation. The objectives were three-fold: 1) to determine whether microorganisms in the pickling solution can multiply during storage, 2) to determine

whether sterilizing the egg pickling solution was necessary for safe pickled eggs and 3) to determine the percent equilibrium for pickled eggs during short-term storage.

Experimental Procedure

Large and medium size eggs were collected from Babcock B-300 Leghorn hens. All eggs were cleaned as in Chapter 1, placed into clean molded pulpboard filler flats and held in a cooler at 18.5°C. and 75% relative humidity for one week prior to hard-cooking. The eggs were all hard-cooked by the cold water method (Irmiter et al., 1970).

Microbiological Study

Microbiological assay was conducted on the eggs and the pickling solutions in all experimental groups following the fore-mentioned sampling pattern. The egg or eggs were assayed as in Chapter 1, and the solution was assayed as described in Chapter 2.

Experiment 1. A total of ten large size eggs (average weight 56 grams) were collected. Each peeled egg (average weight 50 grams) was placed into a one-pint Mason jar. The "Dill Egg" pickling solution was heated to boiling and simmered five minutes. Approximately 150 ml. of hot (80°C.) pickling solution was poured over the egg in each jar. The ratio of pickling solution to egg was 3:1 (v:w). The ten jars, each containing one eggs, were stored at 25°C. Two jars were selected at random and the egg and solution sampled for microbiological analysis at 0, 1, 2, 48, and 168 hours of storage.

Experiment 2. A total of 24 medium size eggs (average weight 49 grams) were collected. Two peeled eggs (average weight 44 grams each) were placed into a one-pint Mason jar. Approximately 176 ml. of hot (80°C.) pickling solution was poured over the eggs in six of the jars and an equal amount of

unheated pickling solution was poured over the eggs in each of the remaining six jars. The 12 jars of pickled eggs were stored at 25°C. The ratio of pickling solution to egg in each jar was 2:1 (v:w). One jar from each treatment of "Dill Egg" pickling solution was selected at random and sampled for microbiological analysis of both eggs and solution at 0, 4, 8, 12, 30, and 52 hours of storage.

Experiment 3. The procedure of experiment 1 was duplicated for a total of 14 medium size eggs. Two jars were selected at random and sampled for microbiological analysis following 0, 1, 2, 3, 4, 24, and 48 hours of storage.

Experiment 4. A total of 12 medium size eggs were collected. Each peeled egg was placed into a one-pint Mason jar. The "Dill Egg" pickling solution was prepared but unheated. Approximately 132 ml. of the pickling solution was poured over the egg in each jar. The ratio of pickling solution to egg in each jar was 3:1 (v:w). The 12 jars of pickled eggs were stored at 25°C. Two jars were selected at random and sampled for microbiological analysis following 0, 4, 8, 24, 48, and 168 hours of storage.

Titration for Acidity

The liquid portion of the pickled "Dill Egg" was evaluated for acetic acid content. The acid content was measured (weight/volume) by titrating a 10 ml. sample with a 0.1000 N NaOH solution to pH 7.5 as measured with a Sargent Model LS, glass electrode, pH meter. Acid titration curves were recorded for two trials of "Dill Egg" and of 5% w/w acetic acid (vinegar) at several dilutions.

Trial 1. Eight large size eggs were collected and prepared as in experiment 1 for the microbiological study. The eight jars of pickled eggs were stored at 25°C. One jar was selected at random and a ten ml. aliquot

sampled following 0, 1, 2.5, 5, 10, 24, 50, and 168 hours of storage. A single sample was removed from each jar and a jar was sampled only once.

Trial 2. Twenty-four medium size eggs were hard-cooked and peeled. Three peeled eggs were placed into a one-pint Mason jar. The "Dill Egg" pickling solution was heated to boiling and simmered five minutes. Approximately 211 ml. of hot (80°C). pickling solution was poured over the eggs in each jar. The ratio of pickling solution to egg in each jar was 1.6:1 (v:w). The eight jars were stored and sampled as in trial 1.

The percent equilibrium was calculated as the percent difference between the titrated volume of 0.1000 N NaOH of the time-sample from 0 time as compared to the volume difference that existed between the 168 hour sample and 0 time.

$$\text{Percent equilibrium} = \frac{\text{Volume (at 0 time - sample-time)}}{\text{Volume (at 0 time - 168 hours)}} \times 100$$

The percent equilibrium was plotted linearly on the Y-axis and the log of the time, in hours, on the X-axis. A least squares line was plotted and the point at which it intersected 100 percent equilibrium indicated the time required to reach equilibrium.

Results and Discussion

Microbial Viability

Regardless of the procedure for the preparation of pickled eggs with "Dill Egg" Solution, there was a marked reduction in the number of viable microorganisms. The data of the four experiments were combined into two tables. Table 5 is a composite of microbial counts of eggs and solutions for the heated "Dill Egg" solutions and Table 6 is the composite for the unheated "Dill Egg" solutions.

The number of eggs per jar or the different solution to egg ratio did

Table 5. Viability of microorganisms present in "Dill Egg" from all experiments in which the pickling solution was heated

Time	Eggs	$\bar{X}$	Obs.	Solution	$\bar{X}$	Obs.
0 hr.	70, 310, 378, 270, 129, 54	202	(6)	70, 0, 0, 0	18	(4)
1 hr.	150, 240, 6, 0	99	(4)	50, 90, 0, 0	35	(4)
2 hr.	140, 230, 0, 0	93	(4)	0, 0, 3, 0	1	(4)
3 hr.	0, 0	0	(2)	0, 0	0	(2)
4 hr.	0, 0, 0, 0	0	(4)	0, 3, 0	1	(3)
8 hr.	0, 3	2	(2)	0	0	(1)
12 hr.	3, 6	5	(2)	0	0	(1)
24 hr.	0, 0	0	(2)	0, 0	0	(2)
30 hr.	0, 0	0	(2)	0	0	(1)
48 hr.	3, 3, 0, 0	2	(4)	0, 0, 0, 0	0	(4)
52 hr.	18, 3	11	(2)	7	7	(1)
168 hr.	0, 0	0	(2)	0, 0	0	(2)

Table 6. Viability of microorganisms present in "Dill Egg" from all experiments in which the pickling solution was not heated

Time	Eggs	$\bar{X}$	Obs.	Solution	$\bar{X}$	Obs.
0 hr.	378, 270, 6, 3	164	(4)	28	28	(1)
4 hr.	6, 3, 0, 3	3	(4)	23, 70, 23	39	(3)
8 hr.	3, 3, 21, 3	8	(4)	10, 10, 3	8	(3)
12 hr.	27, 0	14	(2)	27	27	(1)
24 hr.	6, 6	6	(2)	3, 20	12	(2)
30 hr.	0, 3	2	(2)	43	43	(1)
48 hr.	9, 0	4	(2)	13, 13	13	(2)
52 hr.	6, 12	9	(2)	20	20	(1)
168 hr.	9, 6	8	(2)	37, 7	22	(2)

not appear to have any effect upon the number of viable microorganisms present in the pickling solution. The unheated "Dill Egg" solution appeared to maintain a stable number of viable microorganisms. However, the solution either destroyed or diluted the microorganisms present on the peeled eggs. As would be expected, heating the pickling solution greatly reduced the number of viable microorganisms.

Acidity

The change in pH and acetic acid concentration during the first 50 hours appeared to be one of dilution. Acton and Johnson (1973) and Ball and Saffores (1973) stated that the acetic acid migrated into the albumen of the hard-cooked egg and water migrated away from the albumen to the yolk and the pickling solution. The titration curves for "Dill Egg" pickling solutions (3:1) and (1.6:1) are plotted in Figures 4 and 5, respectively, and are very similar to the curves for the several dilutions of 5% w/w acetic acid (Figure 6).

The egg albumen did not act as a buffer to dampen the inflection point during titration. However, the alkaline groups of the albumen did reduce the number of hydronium ions in the solution. These two physical (dilution) and chemical (neutralization) effects are evidenced by an increase in pH and reduction in volume of sodium hydroxide required to reach a pH of 7.5.

The lower the ratio of solution to egg the higher the pH was raised in the pickled egg solution. The ratio (1.6:1) exceeded an equilibrium pH of 4.0. This is undesirable if the recommendation of Monroe et al. (1969) is to be followed. However, when the percent equilibrium was plotted in Figure 7, the 1.6:1 ratio required 80 hours to reach 95% equilibrium and the 3:1 ratio took 70 hours. The six to seven days time to reach equilibrium presented by Acton and Johnson (1973) and Ball and Saffores (1973) is of

Figure 4. Titration curve of "Dill Egg" solution (3:1) stored at 25°C.

Figure 5. Titration curve of "Dill Egg" solution (1.6:1) stored at 2.5°C.

Figure 6. Titration curve of 5% w/w acetic acid and dilutions of acetic acid

Figure 7. Acetic acid equilibrium curve for "Dill Egg" solutions

questionable application. The plotting of Acton and Johnson's data (Figure 8), according to the procedure of Bell et al. (1972), yielded a 100% equilibrium after 20 hours. Therefore, the data of Acton and Johnson (1973) and that of this investigation are in agreement on the time required for 95% acid equilibrium. This time ranged between 20 and 80 hours depending upon the solution to egg ratio.

The equilibrium pH attained in dill pickles as a result of the degree of acid penetration reported by Bell et al. (1972) was the same in pickled eggs in this investigation. The adjustment of pH of the pickling solution was dependent upon time and the ratio of solution to eggs (v/w). A ratio of 1.6:1 was found to yield an equilibrated pH of 4.1, which could develop into a microbial problem if the product is improperly handled or pH rises to a level better suited for microbial growth.

Figure 8. Acetic acid equilibrium curve for pickled egg solution plotted from Acton and Johnson's (1973) data

CHAPTER IV

THE EFFECT OF STORAGE TIME OF SHELL EGGS ON DISCOLORATION OF EGG ALBUMEN IN HARD-COOKED EGGS

Baker and Darfler (1969) and Schnell et al. (1969) reported a brown discoloration of the albumen of hard-cooked eggs which was due to the Maillard reaction. The longer the eggs were cooked the browner the albumen would become in addition to the green ring formation around the yolk.

This investigation was undertaken to determine if the length of storage of refrigerated day-old shell eggs would develop the brown discoloration of the hard-cooked egg albumen following conventional cooking.

Experimental Procedure

Egg Source

Medium size eggs were collected from Babcock B-300 Leghorn hens. All eggs were washed as in Chapter I and placed into clean molded pulpboard filler flats and held in a cooler at 18.5 °C. and 75% relative humidity from one day to 12 weeks. Three replications were conducted with different laying diets and lengths of shell egg storage.

Replication 1. Eggs were collected at random from a laying flock maintained on a uniform laying diet. A total of 100 medium size eggs were collected. Ten eggs were sampled at random for evaluation of albumen discoloration following 1, 2, 3, 4, 5, 6, 7, 8, 10, and 12 weeks of storage.

Replication 2. This replication was conducted the same way as

replication 1, except only 90 medium size eggs were collected. Ten eggs were sampled at random for evaluation of albumen discoloration following 1, 2, 3, 4, 5, 6, 8, 10, and 12 weeks of storage.

Replication 3. Eggs were collected at random from several laying flocks. A total of 100 medium size eggs were collected. Ten eggs were sampled at random for evaluation of albumen discoloration following 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 weeks of storage.

Interior Egg Quality

A total of 180 medium size eggs were collected at random from several flocks of laying hens and stored at 18.5°C. and 75% relative humidity. One hundred of these eggs were broken out for Haugh unit scores and 80 of these eggs were candled for air cell depth (Anon., 1972). Ten eggs were sampled at random each week for Haugh unit score and eight eggs were sampled each week for air cell depth measurements. The storage time was identical to that in replication 3.

Hard-Cooking

Ten eggs from each sampling period were placed large end up in cold water, hard-cooked by the cold water method (Irmiter et al., 1970), peeled and sliced with a commercially available egg slicer perpendicular to the long axis. The slices were wrapped in Handi-wrap^R to prevent moisture loss prior to being examined for albumen discoloration.

COLOR-EYE Procedure

An albumen slice containing no yolk and a diameter greater than 25 mm. was placed in the viewing port of an IDL COLOR-EYE^R (Kellmorgen Corporation, Color Systems Division, Attleboro, Mass.). The COLOR-EYE values were converted to dominant wavelength, excitation purity and luminosity which represent a mathematical description of the egg albumen color, thus eliminating

possible biases of human judgement. The procedure of Fry and Damron (1971) was used to obtain values for dominant wavelength, excitation purity and luminosity from the COLOR-EYE readings.

Statistics

The analysis of variance was calculated for the pooled data for each variable (dominant wavelength, luminosity, excitation purity and Haugh units) across storage treatments by using the Statistical Analysis System (Service, 1972). The data were plotted and the least squares line was obtained (Freund, 1967). A correlation coefficient was computed (Freund, 1967) to determine the degree to which variables vary together or a measure of the intensity of their association.

Results and Discussion

A darkening of hard-cooked egg albumen was observed as storage time of the uncooked eggs increased. The albumen did not look brown as suggested by Baker and Darfler (1969) but appeared as a gray-brown.

The dominant wavelength increased significantly from an average of 570 nm. to 572 nm. during 12 weeks of storage (Figure 9). The luminosity values changed significantly by decreasing from 76.5 to 75.5 during 12 weeks (Figure 10). The excitation purity (Figure 11) changed significantly at the 0.01 level. The dominant wavelength, luminosity and excitation purity were all highly and equally correlated (+.88) with storage time. The increase in dominant wavelength and decrease in luminosity indicated that the egg became darker with storage. Further, the slight increase in excitation purity indicated greater color intensity.

The non-uniform fed laying flock yielded an albumen discoloration similar to that of the uniformly fed flock. The longer the eggs were stored under this condition (18.5°C. and 75% relative humidity), the more

Figure 9. Dominant wavelength of albumen from eggs hard-cooked after storage at 18.5°C.

Figure 10. Luminosity intensity of albumen from eggs hard-cooked after storage at 18.5°C.

Figure 11. Excitation purity of albumen from eggs hard-cooked after storage at 18.5°C.

off-white (gray-brown) the albumen became.

The interior quality (Table 7) reflects the degree of deterioration undergone by the shell eggs in this storage condition. The interior quality of eggs as measured by Haugh units decreased in value linearly as the storage time increased (Figure 12) with a correlation coefficient of 0.82. The air cell depth increased as the storage time increased (Table 7). The discoloration of egg albumen appears to be reciprocal to the deterioration of the interior quality of the shell egg.

The eggs for Haugh unit scores and air cell depth were collected from a flock of birds with an unknown rate of production and age of lay. The Haugh unit measurements of the first group of eggs were made after one week of storage. Fry et al. (1965) reported that the decline in Haugh unit scores during the first seven days is by far the larger than the second and third seven-day periods for eggs stored at 56°F. (13.5°C) and 65% relative humidity. The loss of CO₂, increase in pH and water loss during the first seven days of storage cause weakening of the albumen which results in a reduction of Haugh unit scores. A range of 10.9 to 13.5% loss in Haugh unit score was observed in the first seven days (Fry et al., 1965). If the average percent Haugh unit score loss of 12.2% was used, the initial Haugh unit score for these eggs would have been 64.4 (USDA Grade A). This is still lower than desirable for an initial Haugh unit score. The initial Haugh unit score may have been lowered by the warm summer temperatures. Wilcox and Wilson (1962) reported that birds after eight months of production had an initial Haugh unit score of 69. This does not differ much from the 64.4 Haugh unit score extrapolated from the seven-day Haugh unit score in this investigation.

It is apparent from this research that as the interior quality of the shell egg decreases it is accompanied by a discoloration of the hard-cooked

Table 7. Interior quality change of shell eggs at selected lengths of storage.

Storage time (weeks)	Albumen		Air Cell	
	Haugh units	USDA quality	Depth (in.) ²	USDA quality
1	56.7	B	1/8	AA
2	60.3	A	1/8	AA
3	46.4	B	3/16	A
4	48.1	B	3/16	A
5	53.9 ¹	B	1/4	B
6	43.9 ²	B	1/4	B
7	50.7	B	1/4	B
8	37.0 ¹	B	1/4	B
9	41.0 ¹	B	1/4	B
10	39.4 ¹	B	5/16	B

¹Average of ten eggs²Average of eight eggs

Figure 12. Haugh unit score of eggs stored at 18.5°C.

egg albumen. Since a pH of 8.7 was achieved after one week of storage and discoloration increased gradually throughout the storage period, the conclusion by Baker and Darfler (1969), that the increase in pH was the cause of hard-cooked egg white discoloration, may not be valid. However, significant discoloration did not occur until after six or more weeks of storage. This development may affect the consumer's acceptance of hard-cooked egg products prepared from eggs held in storage or of low USDA quality.

CHAPTER V

QUALITY AND ACCEPTABILITY OF PICKLED CHICKEN EGGS

Various researchers have evaluated pickled hard-cooked chicken eggs (Cunningham et al., 1970; Maurer, 1972; and McCready, 1973) and brine-pickled duck eggs (Trongpanich and Dawson, 1974). Two of the pickled egg recipes suggested by Maurer (1972), "Red Beet" and "Dark and Spicy", contribute a characteristic color as well as flavor to the pickled hard-cooked eggs. Red beet juice is used in the "Red Beet" recipe. The red beet (Beta vulgaris) is a rich source of red pigments. The class of colored substances containing both the betacyanins (red pigments) and betaxanthin (yellow pigments) are termed as betalains (Von Elbe and Maing, 1973). The betacyanins found in beets include betanin (the major pigment), isobetanin, prebetanin, isoprebetanin and the betaxanthins, vulgaxanthin I and vulgaxanthin II. These pigments are water soluble and naturally occur as zwitterions (Von Elbe and Maing, 1973). In the "Dark and Spicy" recipe the brown surface color is contributed by all the ingredients.

This study was conducted to evaluate the acceptability of chicken eggs pickled in three pickling solutions and stored for a period of three months at two storage temperatures (4°C. and 22°C.).

Experimental Procedure

Eggs were collected from Babcock B-300 Leghorn hens maintained in laying cages with wire floors. All eggs were washed as in Chapter I and placed into clean molded pulpboard filler flats and held in a cooler at 18.5°C. and 75% relative humidity for one week prior to hard-cooking.

A total of 324 large size eggs were collected. The eggs were hard-cooked by the cold-water method (Limiter et al., 1970) and peeled. Three eggs were placed into clean one-pint Mason jars, for a total of 108 jars.

Three egg pickling solutions ("Dill Egg", "Red Beet" and "Dark and Spicy"; Table 2), as described by Maurer (1972), were prepared by heating to boiling. Enough hot solution (80°C.) was then poured over the eggs for a ratio of pickling solution to egg of 1.6:1 (v/w). Eighteen jars of pickled eggs from each recipe were stored at room temperature (22°C.) and 18 jars were stored at refrigerated temperature (4°C.).

Taste Panel

One jar of each of the three pickled egg recipes was randomly removed from each storage temperature after 1, 2, 6, 10, and 14 weeks of storage and presented to a taste panel. Each storage period was replicated three times on three consecutive days. Eighteen control eggs were prepared for each recipe five days prior to each taste panel evaluation. Preparation of the control eggs was identical to that of the treatment eggs and they were stored at 22°C. In the evaluation at one week of storage the control and treatment eggs were from the same preparation.

The paired-comparison test suggested by Dawson et al. (1964) was used to select the different sample. The three eggs of each recipe and storage treatment were sliced into sixths longitudinally as were an equal number of control pickled eggs. Individuals from the Poultry Science Department were previously exposed to tasting pickled eggs and as a result were considered to be experienced panelists. The panelists sampled six pairs of eggs each day with one pair compared at a time. The eggs were served at room temperature (22°C) on a nine-inch plate with a treatment slice placed on the extreme right and the control on the extreme left. The panelists were asked to evaluate

separately whether the treatment egg was the same as or different than the control egg in color, taste and overall quality. If a difference was detected, then the panelist was asked to say whether the treatment egg was better or worse than the control. A minimum of three to 10 paired-comparisons was necessary for the binomial-distribution statistics (Dawson et al., 1964).

The number of panelists available each day varied from six to 12. Each panelist was separated from others by dividers. Water was provided between samples. No attempt was made to alter the normal room lighting (Fluorescent).

Statistics

The binomial-distribution compared the fraction of panelists who reported no difference at week one with the fraction of panelists who reported no difference at each of the other four storage times. A two sided z statistic was used to determine if there was any significant difference relating change in quality to storage time. A significant difference was present between the treatment and control when the rejection probability of 0.05 was reached. The null hypothesis of $H_0: p_1 = p_2$ was used in this investigation. The test statistic

$$z = \frac{\hat{p}_1 - \hat{p}_2}{\sqrt{\frac{\hat{p}\hat{q}}{n_1} + \frac{\hat{p}\hat{q}}{n_2}}}$$

for binomial comparisons (Mendenhall, 1971) was used to calculate the significant difference.

Results and Discussion

Hard-cooked eggs immersed in "Red Beet" pickling solution for seven days at 22°C. developed a bright red-violet color in the albumen and the yolk.

This was an appealing color to many and may be associated with holiday use. The "Red Beet" eggs stored at 4°C. had only slightly less color development than those stored at 22°C. The "Dill Egg" pickled eggs had essentially no color change. The "Dark and Spicy" eggs had a brownish color at the exterior surface of the albumen but little or no penetration of the color into the egg.

The taste panelists noticed a marked difference after two weeks of storage in color, taste and overall quality of the "Red Beet" pickled eggs stored at both 22°C. and 4°C. (Table 8). Visually the color changed from a bright purple-red in the fresh solution to a murky brown. The pigment absorbed by the hard-cooked egg discolored but not as rapidly as in the solution. This difference was a result of color fading from red to pink in the albumen. The red color of the solution progressively turned brown in the presence of hard-cooked eggs subsequent to two weeks of storage at 22°C. It was observed that if the egg was removed after one week of storage the light red color of the solution remained stable for more than one month at 22°C. The presence of hard-cooked eggs affected the stability of the betalain pigment, possibly in much the same way as previously reported for red beets per se and in other food products.

Von Elbe and Maing (1973) observed that when gels were formed with betalains the resulting color was lightest at pH 5.0 as compared to pH 2.5 and 8.5. Storage for 16 days resulted in fading of the color in all gels with those at pH 5.0 being least affected (Von Elbe and Maing, 1973). In the range of pH 3.5 to 7.0 the spectrum of betalain had a maximum absorption at 537 and 538 nm. (Von Elbe and Maing, 1973). Studies have shown that the stability of betalain is greatest at pH 5.0 and the pigment degrades following first-order kinetics. The half-life at 25°C. for betalain in a model system at pH 5.0 has been calculated as $1,150 \pm 100$ minutes (Von Elbe

Table 8. Taste panelists' evaluation of "Red Beet" pickled eggs stored for 14 weeks at 4°C. and 22°C.¹

Attribute	Storage (weeks)				
	1	2	6	10	14
4°C. Color	.66	.10**	.13**	.03**	.22**
Taste	.63	.30**	.22**	.17**	.37*
Overall	.63	.40	.19**	.07**	.44
22°C. Color	.57	.00**	.00**	---	---
Taste	.43	.20	.25	---	---
Overall	.54	.23**	.16**	---	---
Number of panelists	35	30	32	29	27

¹Expressed as the fraction of panelists who reported no difference between the control and the treatment eggs.

*Significant difference at the 0.05 level.

**Significant difference at the 0.01 level.

et al., 1974b). Betalain, like other natural pigments, is subject to degradation by air and light. Therefore, products containing betanin must be protected against long exposures to air and light. Darkening or browning of beet products by oxidation both before and after processing has been reported by Von Elbe et al. (1974b). Livingston et al. (1954) studied the role of trace metal contamination in discoloration of beet purée and reported that both iron and copper accelerate darkening.

The panelists reported that after six weeks of storage at 4°C. "Red Beet" eggs were significantly different in color and overall quality (Table 8). The "Red Beet" eggs stored at 22°C. for 10 weeks were removed from testing when the solution and eggs were brown throughout (Figure 13) and had an objectionable straw-like or stale odor. The eggs stored at 4°C. were presented to the taste panelists and received significantly different scores for color, taste and overall quality. The panelists commented that at 14 weeks of storage at 4°C. the discoloration and off-flavor was developed to a lesser degree than at 10 weeks of storage. A possible explanation for this result is that the eggs stored for 1, 2, 3, 6, and 10 weeks were prepared on the same day. The eggs stored for 14 weeks were prepared on another day. The eggs were from the same flock but were collected one month apart. Also, the availability of the same supply of canned red beets was not constant. It is conceivable that the egg source, brand of canned red beets and other unidentified procedural changes could cause this reduction in deterioration of the "Red Beet" pickled eggs stored at 4°C.

Color deterioration of the "Red Beet" eggs first occurred in the solution, followed by the darkening and loss of red color at the surface of the eggs, a fading of the red color in the albumen to pink and a slow darkening inward of the yolk. Von Elbe et al. (1974a) indicated that color changes of sausage colored with betanin are very small during storage. They pointed

Figure 13. Discoloration of "Red Beet" pickled eggs stored for 10 weeks at 4°C. and 22°C. (Left: 5 days at 22°C.; Middle: 10 weeks at 4°C.; Right: 10 weeks at 22°C.).

out that a protective effect of betalain in protein foods exists and is adequate to permit use of betalains as food colorants.

The evidence of this investigation showed that the albumen was the last portion of the jar of "Red Beet" pickled eggs that darkened, which may be due to the protective action of proteins. The absence of eggs in the pickling solution prevented the continued discoloration of the red beet pigment. The low pH of the pickling solution did not prevent the occurrence of fading or darkening when eggs were present in the solution.

"Dill Egg" was free of significant change in quality during this investigation, at both storage temperatures, with exception of taste between the treatment eggs and control after two weeks storage at 22°C. (Table 9). There was no significant color change observed for "Dill Egg" during this investigation in either the pickling solution or the eggs.

"Dark and Spicy" pickled eggs stored at 22°C. for six weeks were found to be significantly different in taste and overall quality (Table 10). After ten weeks of storage the "Dark and Spicy" eggs stored at 4°C. were significantly different only in color. However, after six weeks of storage at 22°C. differences were found in color, taste and overall quality (Table 10); taste panel evaluations indicated that a further decline in quality occurred as storage time increased to 14 weeks.

The discoloration in the "Dark and Spicy" eggs did not appear to be one of penetration of the color of the solution (Figure 14) but may be related to the discoloration observed in other egg products. The bitter and stale off-flavor observed in the yolk of these eggs may also be related to flavor deterioration in dehydrated egg products.

Dodge et al. (1965) and Baker and Darfler (1969) reported that hard-cooked eggs developed a brown discoloration of the albumen due to the Maillard reaction. In addition to the glucose-protein reaction resulting

Table 9. Taste panelists' evaluation of "Dill Egg" pickled eggs stored for 14 weeks at 4°C. and 22°C.¹

	Attribute	Storage (weeks)				
		1	2	6	10	14
4°C.	Color	.71	.77	.91	.66	.78
	Taste	.49	.67	.59	.55	.63
	Overall	.49	.67	.66	.45	.70
22°C.	Color	.80	.83	.84	.62	.70
	Taste	.66	.33**	.28**	.28**	.37*
	Overall	.66	.50	.50	.38*	.52
Number of panelists		35	30	32	29	27

¹Expressed as the fraction of panelists who reported no difference between the control and the treatment eggs.

*Significant difference at the 0.05 level.

**Significant difference at the 0.01 level.

Table 10. Taste panelists' evaluation of "Dark and Spicy" pickled eggs stored for 14 weeks at 4°C. and 22°C.¹

	Attribute	Storage (weeks)				
		1	2	6	10	14
4°C.	Color	.63	.70	.41	.38*	.48
	Taste	.60	.57	.55	.73	.45
	Overall	.54	.53	.59	.59	.52
22°C.	Color	.71	.77	.44*	.28**	.18**
	Taste	.69	.47	.41*	.45*	.33*
	Overall	.71	.47*	.50*	.41*	.33**
Number of panelists		35	30	32	29	27

¹Expressed as the fraction of panelists who reported no difference between the control and the treatment eggs.

*Significant difference at the 0.05 level.

**Significant difference at the 0.01 level.

Figure 14. Discoloration of "Dark and Spicy" pickled eggs stored 10 weeks at 4°C. and 22°C. (Left: 5 days at 22°C.; Middle: 10 weeks at 4°C.; Right: 10 weeks at 22°C.).

in a brown color, researchers have presented evidence that some deteriorative changes occurring in dried whole eggs and yolks are independent of the glucose-protein reaction (Hill and Sebring, 1973). The results of testing led these authors to suggest that the reaction was between a cephalin amino group and aldehydes. Fevold et al. (1946) and Boggs and Fevold (1946) presented evidence that the major changes resulting in loss of palatability takes place in the fatty constituents of the egg. Whole egg tends to develop stale or stored flavors during storage, which in part might be due to the lipid oxidation (Kwon and Norgaard, 1966). Kline et al. (1951a, b) pointed out that glucose is the reactive aldehyde involved in the cephalin amine-aldehyde reaction. The changes which occurred in the phospholipid fraction of stored whole egg powder were essentially eliminated by the removal of glucose from the liquid before drying. The glucose-cephalin reaction is involved in off-flavor development.

Changes in flavor and odor during storage of dehydrated eggs is quite noticeable. Flavor stability of whole egg powder can be improved to a certain extent by acidifying the liquid to a pH of 5.5 before drying. This inhibits the browning reaction involving the glucose and protein but does not stop it completely (Berquist, 1973). Boggs and Fevold (1946) reported that excessive acidity is detrimental to the flavor of dried eggs.

The pickling process is somewhat similar to dehydration in that the egg loses about 5% of its weight. The Maillard reaction is preferentially selected for by a low pH (4.3) and an absence of SO_2 (McKeeny et al., 1969). As a result the potential for albumen discoloration and off-flavor is present.

As a result of this investigation, it has been observed that the egg pickling recipe significantly affects the shelf-life potential of pickled hard-cooked eggs. The "Red Beet" pickled eggs did not have a reasonable shelf-life at these two storage conditions and may not be practical for

commercial use.

The shelf-life of "Dill Egg" and "Dark and Spicy" eggs could be successfully extended by refrigeration at 4°C. for 14 weeks. The "Dill Egg" pickled egg recipe could have the greatest commercial potential due to its resistance to color and flavor deterioration at 4°C.

CHAPTER VI

STRUCTURE AND MICRO-STRUCTURE OF HARD-COOKED EGGS

Many researchers have studied the internal structure of the egg. Romanoff (1943) described the gross structure of the albumen of a newly laid chicken egg. Almquist and Lorenz (1932), Cole (1938) and Conrad and Scott (1939) observed ovomucin fibers in thick egg white. Schaible et al, (1935) demonstrated a means of revealing stratified layers within the thick egg white by breaking a fresh egg into distilled water and slitting the envelope of thick white. After a time, ovomucin and some globulins are precipitated on the edges of the cut surface showing approximately six laminations regularly spaced about one mm. apart. The thick white is not a homogeneous gel but is composed of bands and layers (Almquist and Lorenz, 1932; Moran and Hale, 1936). Scott and Huang (1941) reported that the fibers are laid down, not as disconnected small fibers, but rather as sheets or layers with a mesh or sieve-like appearance.

It has been observed in earlier investigations of "Red Beet" pickled eggs that the albumen was differentially stained pink. The red beet (Beta vulgaris) is a rich source of red colored, water soluble pigments.

The objectives of this investigation were two-fold: 1) to determine if this differential penetration of red beet pigment was based upon the chemical composition of the albumen layers and 2) to demonstrate the presence of stratification in the thick albumen of hard-cooked chicken eggs.

Experimental Procedure

Medium and large size eggs from Babcock B-300 Leghorn hens were collected. The eggs were washed and stored as in Chapter I for one week prior to cooking. All the eggs were hard-cooked by the cold water method (Irmiter et al., 1970). Three peeled eggs were placed into a one-pint Mason jar. Approximately 211 ml. of hot (80°C.) "Red Beet" pickling solution were poured over the eggs in each jar. The ratio of pickling solution to egg in each jar was 1.6:1 (v/w). The jars of "Red Beet" pickled eggs were stored at 4°C. and 22°C. for various lengths of time.

The eggs were sliced into nine sections, three mm. thick, yielding eight profiles of each egg. The banding observed on each slice was traced on clear acetate. The tracings were xeroxed, each section cut out and then grouped as to the three major albumen layers, outer thin, thick and inner thin, and the paper weighed to obtain an approximate percentage of each albumen layer.

Results and Discussion

The color differentiation observed was well defined and seemed to outline the three major albumen layers (Figure 15). The percentages obtained from the weighed paper portions were similar to the ratios given by Brooks and Hale (1959) for inner thin, thick and outer thin albumen layers (Table 11) obtained using the "screen" technique described by Holst and Almquist (1931). Romanoff (1943) found that the albumen of the newly-laid chicken egg is differentiated into four layers with distinct chemical and physical properties. The ratio of the albumen layers (Table 11) confirmed the assumption that the difference in chemical composition of the albumen layers was related to the uptake of betalain pigment. The boundary (or interface) between the outer thin and thick albumen, as seen by color

Figure 15. The three major albumen layers differentially stained with red beet juice with laminations visible in the thick albumen.

Table 11. Proportion of albumen layers

Layer	% Albumen		
	Mean ¹	Range ¹	Range ²
Outer thin	29.6	24.0 - 32.5	13.9 - 38.0
Thick	43.2	36.0 - 48.0	35.4 - 54.0
Inner thin	27.3	22.0 - 33.0	18.1 - 39.0

¹Experimental (n = 6).²Brooks and Hale, 1959.

differentiation, could be torn with a little force (Figure 15). Separation was also possible between the colored boundary between thick and inner thin. The physical separation along the boundary between the various albumen layers was most easily accomplished in the pointed region of the hard-cooked egg and became more increasingly difficult as the blunt end of the egg was approached. The thick albumen was the only layer observed to have a series of light and dark pink colored layers or laminations (Figure 15). A separation of the individual layers was possible and even separations within a single lamination. This was also easily accomplished in the pointed end of the egg. This agreed with data obtained by Scott and Huang (1941) that indicated that the mucin is laid down, not as disconnected small fibers, but rather as sheets or layers. The inner thin and outer thin were not composed of layers but peeled away in chunks. This was due to the lower amount of ovomucin in the thin whites (Brooks and Hale, 1961).

Baker and Stadelman (1957) related that housewives complained about a large chalaza as a hinderance in cooking . The chalaza showed up well in the cross sections of "Red Beet" pickled eggs because it did not take up the betalain pigment as intensely as the surrounding albumen and appeared lighter in color. The thick albumen exhibited laminations as reported by several researchers. The layers were composed of two chemically different materials; one was light in color (due to ovomucin fibers) and one was dark in color (due to a small concentration of ovomucin fibers). It is interpreted that the ovomucin fibers in the thick albumen and chalaza are compact bundles which prohibit the absorption of betalin pigments.

As can be seen in Figure 15, the inner thin albumen did not encircle the yolk but was concentrated in the pointed end of the egg. The thick albumen was likewise concentrated in the pointed end of the egg. The majority of the laminations were present in the pointed end, with the widest

band of lamination occurring there, and thinned as it wrapped around the yolk to the blunt end. The individual layer of lamination also thinned from a width of 1.0 mm. to an unmeasurable width. Regardless of the position of the egg during hard-cooking, the inner thin albumen was not found in the blunt end of the egg. The research conducted by Romanoff (1943) indicated that the inner thin albumen is the second layer, surrounding the yolk and it enwraps the inner most chalaziferous layer. The thick albumen surrounds the inner thin albumen and constitutes the third and concentric layer of albumen (Romanoff, 1943). These findings are contradictory to what was observed in this investigation.

The chalaza was rarely found in the polar ends of the egg but off to one of the sides, regardless of the cooking position.

The "Red Beet" pickled eggs stored at 4°C. required a month for the color to penetrate and equilibrate sufficiently, in order to observe differentiation clearly. The low storage temperature may have slowed the rate of penetration of the betalain pigment, permitting a more discernible difference. The "Red Beet" eggs stored at 22°C. were red-purple after five days of storage and the entire albumen was colored to the same degree, which made for poor differentiation. After one week of storage at 22°C., the red color began to degrade and for a time improved contrast was observed.

The differential staining possible with the use of red beet juice is a potential technique to demonstrate the position of the several albumen layers and their relationship to the egg as a whole. This technique has the added advantage of measuring the percentage of the three albumen layers, which is usually done by the "pipette" method (Romanoff, 1943). and the "screen" method of Holst and Almquist (1931).

CHAPTER VII

ACCEPTABILITY OF PICKLED QUAIL EGGS

Various researchers have evaluated hard-cooked chickens eggs in pickling solutions (Cunningham et al., 1970; Maurer, 1972; McCready, 1973) and brine-pickled duck eggs (Trongpanich and Dawson, 1974).

This study was conducted to evaluate the acceptability of quail eggs pickled in five egg pickling solutions.

Experimental Procedure

Eggs were collected from Bobwhite quail housed in wire floor cages. The eggs were placed on clean molded pulpboard filler flats and held in a cooler at 18.5°C. and 75-80% relative humidity for one week.

The eggs were hard-cooked by the cold water method (Irmiter et al., 1970), peeled and placed in clean one-quart Mason jars, 40 eggs per jar. Five egg pickling solution recipes (Table 2) developed by Maurer (1972) were used in this investigation. The hot solutions (80°C.) were poured over the eggs and the jars were sealed. The cooled jars were then held in a refrigerator (5°C.) for one week.

The design and analysis procedures for taste panels outlined by Street and Carroll (1972) were used in this investigation. The participants in this consumer acceptance taste panel of pickled quail eggs were members of groups whose meetings were held in Gainesville, Florida. They were either from poultry interest groups or homemakers clubs. The participants were asked to take part in this taste panel during their coffee breaks and the procedure was very informal. The participants were asked to express their

feelings (consumer acceptance) about pickled quail eggs from each recipe. This was done by scoring the egg product on a seven-point hedonic scale: excellent (+3), very good (+2), good (+1), average (0), poor (-1), very poor (-2), and terrible (-3). A frequency distribution was constructed from this information.

Consumer acceptance scoring was performed on eggs from all five recipes in four trials, representing each of the four groups of participants: trial 1 (16 individuals), trial 2 (14 individuals), trial 3 (35 individuals) and trial 4 (64 individuals). The consumer acceptance data were pooled yielding a total of 129 panelists. As can be seen the groups of participants were not of the same size. Some people refused to participate and others sampled less than five recipes. The results of a panelist were used only if all five recipes were scored. The panelists by and large were men; however, records as to the ratio of men to women were not maintained.

A criterion of acceptability was established as the sum of the panelists scoring the pickled quail egg product in the excellent (+3), very good (+2) and good (+1) categories. Products accepted by more than 70% of the panelists were judged to be of such acceptability that a potential market for pickled quail eggs may exist. Those below 70% would not be of sufficient acceptability to warrant processing or marketing of that product.

Results and Discussion

Three recipes for pickling solutions were found to meet the criterion of acceptability of 70%; "Kansas Spicy" was scored in the top three categories by 74% of the panelists and "Sweet and Sour" and "Dill Egg" by 71% each (Figure 16). "Red Beet" was moderately acceptable with 67% and "Dark and Spicy" was least acceptable at 57%.

The majority of those who tasted the pickled eggs demonstrated discriminatory judgement when tasting. A total of 103 panelists (or 80%)

Figure 16. Frequency distribution of consumer acceptance of five pickled egg recipes

selected and scored the recipes that they individually found acceptable and others which were unacceptable. Only 26 panelists (or 20%) found all the recipes to their liking. This was based on the individual scoring of all recipes with either an excellent (+3), very good (+2) or good (+1). Not one of the panelists gave eggs from all of the recipes an average score (0); likewise, no panelists scored the eggs of all five recipes in the combined unacceptable categories of poor (-1), very poor (-2) and terrible (-3).

The difference in acceptance between individual recipes was possible due to the type of seasoning spice and the color of the pickled eggs. It could be a possibility that if someone did not like or was unaccustomed to either a certain spice or color, the recipe would be rated low. If the panelist favored a certain spice or color the recipe would be rated high. Additional factors affecting the acceptance scores might have been the age, sex, social background, health or the panelist, a panelist's like or dislike for eggs, and the time of day at which the taste panels were conducted. It is felt, however, that the data reported herein indicate that pickled quail eggs are an acceptable market product.

CONCLUSION

The effect of microorganisms on hard-cooked and pickled eggs was observed to be minimal in this investigation when appropriate storage temperatures were utilized. Deterioration of color and flavor of pickled eggs was the greatest factor in reduction of shelf-life and loss of taste panel acceptance.

The quality of the eggs, for preparation as pickled eggs, is dependent upon the interior shell egg quality. A brown discoloration of hard-cooked egg albumen was found to be associated with the loss of interior shell egg quality. The ease of peeling the eggshell and the smoothness of the albumen surface improved with the age and/or increase in the pH of the egg. The delay between peeling the hard-cooked egg and using it in a food product or as a food allows for growth of microorganisms. Peeled eggs held at 25°C. for four days increased from zero to 1.0×10^8 organisms per gram of egg. Storage at 5°C. did not result in a measurable amount of growth.

Five egg pickling recipes were studied in this investigation ("Red Beet", "Dill Egg", "Dark and Spicy", "Kansas Spicy" and "Sweet and Sour"), prepared from natural spices and ingredients. The egg pickling solutions required a minimal amount of heat processing for pasteurization. Microbial assay showed that the greatest contamination was from the peeled hard-cooked eggs. However, within a short time the highly acidic pickling solution destroyed a majority of the viable microorganisms. The pH of the pickling solutions was increased by the addition of alkaline hard-cooked eggs until

an equilibrium pH was reached. The lower the ratio of solution to eggs the higher the pH at equilibrium. A minimum ratio of solution to egg would be 1.6:1 (v/w) for an equilibrium pH of 4.0. The shift in pH of the pickling solution was similar to the dilution of acetic acid.

"Red Beet", "Dark and Spicy" and "Dill Egg" pickled eggs were subject to a 14-week storage at 22°C. and 4°C. and quality was assayed by a taste panel. Only "Dill Egg" maintained its quality at 22°C. storage. However, only "Red Beet" pickled eggs did not store well at 4°C. The greatest defects were discoloration of the albumen and development of off-flavor in the region of the yolk.

The "Red Beet" egg pickling solution was found to be differentially absorbed by the hard-cooked egg albumen. The chalaza and the thick white did not absorb as much pigment as the thin white, and appeared lighter pink in color. This staining technique may be used as a method to observe the relation of the albumen layers with a minimum of distortion and destruction.

Three pickled egg recipes ("Dill Egg", "Kansas Spicy" and "Sweet and Sour") of the five recipes of pickled quail eggs were equally well accepted by taste panelists. This indicated that pickled quail eggs are an acceptable market product. The acceptance of an individual pickled egg recipe was due to the type of seasoning spice and the color of the pickled eggs.

Pickled eggs are an effective means of utilizing small and pee wee size eggs, which are under-utilized today. It is also a feasible method of marketing commercially a hard-cooked egg product.

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BIOGRAPHICAL SKETCH

Stevan Alex Angalet was born August 13, 1947, in New Brunswick, New Jersey. He attended local schools and was graduated from Franklin High School, Somerset, New Jersey, in June, 1965. Four years later he received a Bachelor of Science degree with a major in Food Science from Rutgers University. In 1969, he entered the Graduate School of the University of Florida. He worked as a graduate research assistant in the Department of Food Science and received his Master of Science degree in 1971.

During 1971-72 Mr. Angalet served on active duty with the United States Army as a First Lieutenant in the Quartermaster Corps. Upon completion of his active duty assignment he returned to the University of Florida to pursue the Doctor of Philosophy degree in Animal Science (Poultry Products Technology). He was appointed a graduate research assistant in the Department of Poultry Science.

The author has been actively involved in various clubs and honorary fraternities while attending the University of Florida. He has served as an officer in the Food Science Club and a member of the Poultry Science Club. He was elected to membership in the honorary fraternities of Alpha Zeta, Phi Sigma and Gamma Sigma Delta. He is also a member of two professional organizations, the Institute of Food Technologists and the Poultry Science Association.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Jack L. Fry, Chairman
Professor of Poultry Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James L. Oblinger, Co-Chairman
Assistant Professor of Food Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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Professor (Retired) of Agriculture
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Robert H. Harms
Professor of Poultry Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ramon C. Littell
Assistant Professor of Statistics

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1975

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