PROCESSING AND PRODUCTS

Relationship of Eggshell Ultrastructure and Shell Strength to the Soundness of Shell Eggs

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ABSTRACT

Factors affecting the soundness of shell eggs are of primary concern to egg processors due to substantial financial losses from cracked and leaker eggs. Ultrastructural analyses were used to examine the palisade layer width and mammillary knob layer thickness of sound, cracked, and leaker eggshells. Subjective observations were also made. There was no significant difference (P > 0.05) in the width of the palisade layer or the mammillary knob layer among sound, cracked, and leaker eggshells. The eggshell strengths of sound, cracked, and leaker eggs were evaluated using puncture force and shell thickness measurements. Sound eggshells were found to have a significantly (P < 0.05) higher mean puncture force (35.3 N) than cracked (30.4 N) and leaker (28.4 N) eggshells. The mean puncture force values for cracked and leaker eggshells were not significantly different from each other (P > 0.05). Regression and correlation analyses indicated a significant correlation (P < 0.01; r = 0.61) between palisade layer width and puncture force. The mean shell thickness values for cracked (0.36 mm) and leaker (0.35 mm) eggs were significantly lower (P < 0.05) than those of sound eggs (0.38 mm), although cracked and leaker eggshell thickness did not differ significantly (P > 0.05). The correlation coefficient between puncture force and shell thickness was significant (P < 0.01; r = 0.56), indicating a possible relationship.

(Key words: eggshell ultrastructure, eggshell strength, eggshell thickness, egg quality)

INTRODUCTION

The quality of the hen's eggshell is of primary concern to the poultry industry due to the large financial loss associated with downgraded eggs. According to Hunton (1993), losses of eggs worldwide due to damaged shells continues despite recent advances in research, quality control, and applied technology. Furthermore, the point is made that variation in shell quality may lead to Salmonella contamination. Losses of eggs in the hen house due to poor shell quality are estimated at 7.77% (Roland, 1977). Further losses due to breakage between egg collection and the consumer average 6.37%. These combined losses (14.14%), then, translate into a loss of more than $47 million when applied to production figures for 1992 (Madison and Perez, 1994). Shell breakage is dependent on both shell strength and the magnitude of the insult, and has increased with automation of the processing line (Hunton, 1993). The conditions directly affecting inherent eggshell strength have been summarized by Washburn (1982), and include bird physiology, eggshell properties, bird behavior, environment, nutrition, age and genetics. Shell formation may be determined genetically (Brah et al., 1993; Khatar et al., 1993), but it is modified environmentally (Campo and Ruano, 1993; Solomon, 1993).

The eggshell of the domestic hen has a definite shell structure consisting of six layers. The two innermost layers are the organic inner and outer shell membranes that lie in close association until they separate to form the air cell at the blunt end of the egg. The mammillary knob layer follows and is the first calcified layer deposited, with its tips embedded in the outer shell membrane. The organic mammillary cores are contained within the mammillary knobs and serve as nucleation sites during shell formation. The palisade layer forms as the mammillary knobs fuse, which results in a more uniform layer. This layer constitutes the major calcified portion of the shell and is most closely associated with shell strength. The vertical crystalline layer is the last calcified layer deposited consisting of short crystals running perpendicular to the shell membrane. The organic cuticle is the last layer deposited providing a waxy covering to protect the shell contents from environmental factors. Parsons (1982) has suggested that good shell quality is dependent upon a balanced shell architecture as well as adequate shell thickness.

Eggshell strength results from a combination of material and structural strengths. Material strength
refers to the type and association of mineral and organic shell components. Recent research in this area has led to a much greater understanding of the composition and internal structure of the eggshell (Guinotte and Nys, 1993; Hincke et al., 1993; Krampitz, 1993; Solomon, 1993). Structural strength is based on the shape, size, thickness, and distribution of the shell over the egg; however, the relationship between material and structural strength is vague (Hunton, 1993). The puncture test is one of the earliest developed methods used to assess eggshell strength (Willard and Shaw, 1909) and is still considered to be one of the most reliable (Kaminska and Skraba, 1993). The variability contributed by eggshell geometry is minimized because the puncture test measures material strength (Stevenson et al., 1981). Variation within the shell material is reduced because several measurements can be obtained per shell, and the egg need not be intact, allowing cracked and broken eggs to be tested. Shell thickness tests have been significantly correlated with direct forms of strength determination such as puncture force (Hamilton, 1982). Because shell thickness varies over the surface of the egg, and the equator has the most uniform area and yet is the weakest part of the shell, several thickness measurements are usually taken about the equator (Tyler and Geake, 1965; Hunt and Voisey, 1966).

The extent to which each shell component influences the strength of the shell has yet to be determined. Simons (1971) reported that shell membranes have no effect on shell quality using transmission electron microscopy (TEM). However, Essary et al. (1977) found a positive correlation between eggshell breaking strength and membrane strength while determining the tensile strength of manually freed membranes. The mammillary knob layer has received attention in recent shell quality studies using the scanning electron microscope (SEM) because it is believed that abnormalities found in this layer will directly affect the stability of the rest of the eggshell. Low quality shells tend to have an irregular, uneven, or incompletely formed mammillary core arrangement (Robinson and King, 1970; King and Robinson, 1972; Bunk and Balloun, 1978). Abnormalities located in the palisade layer are often manifested as reduced thickness or increased porosity (McFarland et al., 1971; Simons, 1971). Several studies have found a relationship between increased abnormalities in the palisade layer and reduced eggshell strength (Meyer et al., 1973; Bunk and Balloun, 1978; Stevenson et al., 1981). The cuticle has been found to have a minor effect on shell strength (Belyavin and Boorman, 1980).

To further aid in the prevention of downgraded eggs due to mishandling, and to aid in the design of more automated equipment for the egg processing line, more information is needed on the relationship between eggshell structure, strength, and the incidence of breakage; however, it is difficult to assess shell quality because of the heterogeneity of the eggshell and the interplay of material and structural strengths. Therefore, ultrastructural differences in the eggshells of sound, cracked, and leaker eggs were investigated using the SEM, and used to study the relationship between eggshell strength and ultrastructure of sound, cracked, and leaker eggs. Furthermore, a combination of indirect and direct methods is often used to determine shell strength. Thus, the shell strengths of sound, cracked, and leaker eggs were determined using puncture force (direct method) and shell thickness measurements (indirect method).

**MATERIALS AND METHODS**

**Sample Procurement**

The eggs used in this study were collected at the candling station of a commercial egg packer after first being washed. For each of the following categories, 30 eggs were collected: 1) sound eggs: shell eggs with shell and shell membranes intact, 2) cracked eggs: cracked or broken shell with membranes intact, and 3) leaker eggs: both shell and shell membranes have been perforated.

The eggs were held in refrigerated storage (4°C) until evaluated. The entire study consisted of three trials (replications), using three different flocks of hens (i.e., 30 eggs per category per trial).

**Ultrastructural Analysis**

To study the ultrastructure of the eggs, 27 shells from each category of sound, cracked or leaker eggs were selected per trial based on common shell thickness. The eggs were broken-out, the shells were rinsed with tap water, and the inner shell membrane manually removed. The shell samples were then stored in a desiccator at room temperature until needed.

A scanning electron microscope (ISI-603) was used for the comparative analysis of the ultrastructure of sound, cracked, and leaker eggshells. Two orientations were examined, the radial cross section (RCS) and the inside-looking-out view (ILO).

Shell sections used for the RCS view were broken into pieces approximately 0.5 cm², mounted on an aluminum specimen mount, and gold coated for 4 min with a Polaron PSE3 sputter coating unit. The thickness of gold was approximately 28 nm. The RCS shell samples were placed in an upright position, standing at a 90° angle from the microscope stub surface. This angle allowed the shell components to be exposed in cross-section and examined. A similar procedure was used by Bunk and Balloun (1978). Two measurements were obtained from the RCS view, the width of the palisade layer (including the vertical crystalline layer and cuticle), and the thickness of the mammillary knob layer (without the shell membranes). Two shell sections were used for the RCS view per egg.
from which six measurements per parameter were obtained and averaged for the final measurement.

The preparation for the ILO view consisted of gently boiling the shell in a 10% sodium sulfide solution for 15 min to remove the organic shell membranes (Fujii and Tamura, 1970). The shells were air dried overnight at room temperature and mounted with the vertical crystal-line layer side down to expose the mammillary knob layer. The samples were gold coated as stated previously. The RCS and ILO orientations were examined at magnifications of 200x and 78x, respectively, using an accelerating voltage of 10 kV.

**Puncture Force**

Puncture force was determined using an Instron Universal Testing Machine fitted with a micropunch and die. The micropunch and die hold diameters used were 0.5 and 0.8 mm, respectively. High carbon steel and stainless steel were used to construct the micropunches for Trial 1. The strength of the two materials is similar; however, the size of the micropunch was such that a small variation in the metal used for construction had a pronounced effect on performance. The values from the high carbon steel punch were more variable and not consistent with those obtained from the stainless steel punch. Thus, the stainless steel punch was used to eliminate variability due to material type. A constant cross-head speed of 10 mm/min was used. Values are reported in units of force (Newtons).

The eggs were removed from refrigerated storage (4°C) approximately 8 h before testing to permit the eggs to reach ambient temperature. The eggs were then broken-out and shells rinsed with tap water. Three sections of eggshell ranging from 7 to 9 cm² were taken from the equatorial region of the shell. The shell pieces were tested while moist with membranes intact to simulate actual processing conditions. Thirty eggs were tested per run after they were randomly selected and arranged in an alternating sequence of sound, cracked, and leaker eggs. The average of three readings represented the puncture force on one eggshell.

**Shell Thickness**

Eggshell thickness was determined using an Ames Thickness Gauge 25 ME after puncture force measurements were taken. Two thickness measurements were taken per shell section in close proximity to the location of each puncture site. Therefore, the final thickness measurement for an eggshell represented the mean value of six measurements. Measurements were obtained from the equatorial region of the shell while moist with membranes intact, as stated previously.

**Statistical Analysis**

Analysis of variance was performed on all data using the General Linear Models procedure of SAS® (SAS Institute, 1993). The experimental treatments included sound eggs, cracked eggs and leaker eggs. Three replications were performed (n = 27 eggs per treatment per replication). The main effects that were tested included puncture force, shell thickness, palisade layer width, mammillary knob layer width, and mammillary knob density. Mean differences were evaluated for significance using the Waller-Duncan test and the residual MSE as the error term. Pearson’s correlation coefficient between puncture force and shell thickness was also used.

**RESULTS AND DISCUSSION**

The production profiles for the three flocks used for this study were obtained. The flock used for Trial 2 had more medium and large eggs and may have been younger than the flocks used for Trials 1 and 3, which had more large, extra-large, and jumbo eggs. Consequently, Trial 2 had a higher percentage of sound eggs (96.2%) than Trials 1 (90.9%) and 3 (87.9%), because larger eggs tend to have thinner shells and are more prone to damage.

Favorable characteristics of a sound egg include 1) well rounded mammillary knobs that are in close association with fibers of the organic shell membranes, 2) uniform width of mammillary knobs at the basal cap and from the organic membranes to the palisade layer, and 3) a nonporous palisade layer of sufficient thickness.

The palisade layer constitutes the major calcified component of the eggshell and is considered that portion of the shell most closely associated with shell strength. The intrinsic strength of the palisade layer is dependent upon the stability of the mammillary knob layer, the degree to which the calcite columnar structures interdigitate and the density of calcium carbonate present (King and Robinson, 1972; Meyer et al., 1973; Bunk and Balloun, 1978).

There was no significant difference (P > 0.05) in the palisade layer width among sound, cracked, or leaker eggs (Table 1). A decrease in the width of the palisade layer will weaken the shell because of a decrease in calcified shell material. Microporosity in the palisade layer was an observed structural characteristic indicative of low quality eggshells. Microporosity in the palisade layer reduces the strength of the eggshell due to a decrease in the density of shell material. A reduction in shell thickness and microporosity of the palisade layer may be influenced by several factors such as insufficient calcium and vitamin D in the diet, increase in ambient temperature and relative humidity of the environment, genetics, age of the hen, and residual pesticides in the feed (McFarland et al., 1971; Hamilton et al., 1979; Washburn, 1982).

Slanted or incomplete calcite columns running throughout the palisade layer, which may weaken the shell even in the presence of sufficient shell material,
TABLE 1. Palisade layer widths, mammillary knob layer thickness values, puncture force, and shell thickness values for sound, cracked, and leaker eggshells

<table>
<thead>
<tr>
<th>Egg type</th>
<th>Palisade layer width (μm)</th>
<th>Mammillary knob layer width (μm)</th>
<th>Puncture force (N)</th>
<th>Eggshell thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sound</td>
<td>337 ± 31.7</td>
<td>54 ± 16</td>
<td>35.3 ± 5.9a</td>
<td>0.38 ± 2.2a</td>
</tr>
<tr>
<td>Cracked</td>
<td>316 ± 40.3</td>
<td>58 ± 13</td>
<td>30.4 ± 6.2b</td>
<td>0.36 ± 2.7b</td>
</tr>
<tr>
<td>Leaker</td>
<td>303 ± 55.3</td>
<td>58 ± 13</td>
<td>28.4 ± 8.2b</td>
<td>0.35 ± 3.7b</td>
</tr>
</tbody>
</table>

Measurements within a column with no common superscript differ significantly (P > 0.05). Each value is the mean ± standard deviation; n = 27 for palisade layer width and mammillary knob layer width, n = 88 to 89 for puncture force and eggshell thickness.

were observed in the RCS view of leaker eggshells. A decrease in shell strength may occur due to poorly interlocking calcite columns. Bunk and Balloun (1978) suggested that the degree to which adjacent columns interlock (interdigitate) may dictate the amount of intrinsic strength in the palisade layer. They further hypothesized that slanted or incomplete calcite columns may be created due to a wave in the nucleation plane on the outer shell membrane during shell formation. This condition would form uneven levels on nucleation sites, giving rise to disorganized, poorly interlocking calcite columns, and a decrease in overall shell strength.

There was no significant difference (P > 0.05) in the mean mammillary knob layer thicknesses among sound, cracked, and leaker eggshells (Table 1). The role of the mammillary knob layer relative to shell quality has received much attention (King and Robinson, 1972; Bunk and Balloun, 1978; van Toledo et al., 1982). Prior to research with the SEM, the general consensus was that a thick shell was the only prerequisite for a high quality eggshell. However, examination of the ultrastructure of this layer and its role in the initial calcification of the shell has led researchers to hypothesize that if the mammillary knob layer is deposited as an organized, stable structure, it will provide a firm foundation for the formation of the ensuring shell components, namely the palisade layer. If the mammillary knob layer is unorganized and unstable, the strength of the palisade layer will be compromised.

Meyer et al. (1973) used SEM and breaking strength to describe shell quality. They found a decrease in total shell width and palisade layer width as shell quality decreased. The width of the mammillary knob layer remained fairly constant as shell thickness decreased. Therefore, it was concluded that shell strength was directly proportional to the thickness and concentration of the palisade layer. The effect of the mammillary knob layer on overall shell quality may not be influenced by the thickness but rather the structure and concentration of shell material of each individual mammillary knob. Perhaps the configuration rather than the thickness of this layer contributes more to shell quality.

The frequency of low quality characteristics most commonly found in the palisade and mammillary knob

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TABLE 2. Frequency of occurrence (percentage) of low quality ultrastructural characteristics of eggshells as viewed from the scanning electron microscope

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Sound (%)</th>
<th>Cracked (%)</th>
<th>Leaker (%)</th>
<th>Combined total for all eggs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microporous PL</td>
<td>30^2</td>
<td>41</td>
<td>59</td>
<td>43</td>
</tr>
<tr>
<td>Varying width MK</td>
<td>22</td>
<td>22</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>Pointed MK</td>
<td>26</td>
<td>18</td>
<td>26</td>
<td>23</td>
</tr>
<tr>
<td>Micro porous MK</td>
<td>15</td>
<td>33</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Membrane bodies</td>
<td>15</td>
<td>22</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Interrumillary cleft</td>
<td>7</td>
<td>15</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>Multinucleated MK</td>
<td>7</td>
<td>26</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>Incomplete MK</td>
<td>0</td>
<td>11</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>Fissures</td>
<td>4</td>
<td>4</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>Slanted columns PL</td>
<td>7</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Depressions</td>
<td>4</td>
<td>4</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Limited contact between MK</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>and OSM</td>
<td>7</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Total defects, no^5</td>
<td>39</td>
<td>56</td>
<td>67</td>
<td>162</td>
</tr>
</tbody>
</table>

^1PL = palisade layer.
^2n = 27 for each egg type of sound, cracked, and leaker egg.
^3MK = mammillary knob.
^4OSM = outer shell membrane.
^5Total number of defects observed in the 27 eggs of each egg type studied.
layers are shown in Table 2. Microporosity of the palisade layer was the most common defect detected with 43% of all of the eggs examined showing some degree of this condition. The frequency of this defect increased as the shell quality decreased. If the microporosity frequencies of both the palisade layer and mammillary knobs are combined, 65% of the eggs examined show evidence of this aberration. This microporosity of the palisade and mammary layers suggests that incomplete calcification, leading to porosity throughout the eggshell, is the most common factor in low quality eggshells. Other low quality characteristics that increase in frequency with decreasing shell quality were membrane bodies, intermammillary clefts, incomplete mammillary knobs, fissures, and depressions.

The total frequency of defects for sound (39%), cracked (56%), and leaker (67%) eggs increased as shell quality decreased (Table 2). The greatest increase in frequency of detection occurred between sound and cracked eggshells (i.e., 44% increase), whereas only a 20% increase was observed between cracked and leaker eggs. This result suggests that the difference in shell quality between sound and cracked eggshells is greater than that between cracked and leaker eggs.

In some areas of low quality shells, mammillary knobs were present with varying widths. This variability may inhibit the association of the mammillary knob layer and the outer shell membrane resulting in an unstable structure. Pointed mammillary knobs may compromise shell strength in the same manner as mammillary knobs that vary in width (King and Robinson, 1972). Microporosity of mammillary knobs causes uneven growth of the mammillary knobs and the subsequent calcite columns, and is responsible for the irregular shape of mammillary knobs and thinness in low quality eggshells. Membrane bodies are rounded calcified deposits on the outer shell membrane that may represent incompletely formed mammillary knobs. These calcified mammillary remnants may provide an unstable base for the palisade layer because they are not wide enough to support a calcite column. Membrane bodies may also decrease the eggshell strength due to the lack of formation of a calcite column that normally accompanies a mammillary knob. Intermammillary clefts, in which the separation between two individual mammillary knobs is wider than in other areas of the shell, may create weak points allowing fracture to occur more easily. These clefts may serve as natural lines of fracture or as initiation points for fracture to occur.

Areas of the shell where several mammillary knobs are fused together may give rise to calcite columns that are unequal in width. This inequality of width may interfere with the way the two columns interdigitate, leading to a weaker palisade layer. Fissures pervading the mammillary knob layer of low quality eggshells were detected frequently. This condition weakens the eggshell because it may interfere with the way in which the calcite columns interdigitate in the palisade layer. Depressions noted in the mammillary knob layer, where the membranes are removed, may reduce shell strength by creating a wave in the nucleation plane. Mammillary knobs that are not situated on the same level on the outer shell membrane may give rise to unequal, poorly interlocking calcite columns. This inequality of columns again may decrease the strength of the palisade layer by reducing the degree to which the columns interdigitate. Limited association between the outer shell membrane and the mammillary knob layer may weaken the shell in that a loose association between these two shell components may create an unstable foundation for the deposition of the rest of the shell material during formation. King and Robinson (1972) reported that in weak and thin eggshells, the mammillary knobs were frequently not attached to the outer shell membrane fibers.

As seen in Table 1, the force required to puncture shells decreased with shell quality, that is, from sound to leaker eggs. Sound eggs had significantly (P < 0.05) higher puncture force values (35.3 N) than cracked (30.4 N) and leaker (28.4 N) eggs. No significant difference (P > 0.05) was found between the puncture force measurements of cracked and leaker eggs. The calcified portion of the eggshell, primarily the palisade layer, is believed to make the greatest contribution to shell strength (Meyer et al., 1973). Regression analysis indicated a significant correlation (P < 0.01, r = 0.61) between palisade layer width and puncture force. This relationship suggests that the concentration of shell material and thickness of the palisade layer have an influence on shell quality. The width of the palisade layer was the only shell component that decreased with decreased shell quality. Therefore, when the shell fails due to a decrease in shell thickness, the shell component responsible is likely the palisade layer. A decrease in the concentration of shell material may contribute to the incidence of downgraded eggs but is probably not the sole factor in the production of low quality eggshells. Factors such as shell structure (King and Robinson, 1972; Bunk and Balloun, 1978) and handling during processing (Washburn, 1982; Reynnells, 1983) are also important in explaining the incidence of downgraded eggs such as cracked and leaker eggs.

The puncture force distribution is illustrated in Figure 1, and shows a decrease in shell strength with shell quality. The sound egg category appeared to have the highest puncture force values in the higher region of the scale. Sound eggs follow a normal curve distribution with the largest number of eggs having a puncture force of around 33.9 N. The cracked egg category also showed a normal curve with a maximum at 28.1 N, just below that for sound eggs. The leaker egg category appeared to have two group maxima and a more scattered distribution. The eggs of the first group (16.5 N) may have been influenced by a reduction in the quality of shell material. The group constituting the second curve...
in this egg type was closer to that of the sound egg maximum (31.0 N). The leaker eggs associated with this group appeared to have a higher average shell quality, therefore, they may have been damaged due to mishandling during collection or at the packing plant.

The shells used for puncture force evaluation were tested with membranes attached, and thus some of the resistance to puncture may have been due to this organic component of the shell. Solomon (1993) has found that bonding between the shell membranes and the seed crystals from the saturated calcium solution is crucial to the formation of a sound shell. However, the extent to which the organic shell membranes influence shell quality is still vague and needs additional research (Parsons, 1982).

A conical perforation of the eggshell was created during the puncture test. Stevenson et al. (1981) reported a similar finding when testing eggshell strength using the puncture force method. They concluded that during the puncture test the eggshell behaves as a brittle material with fracture resulting from tensile stress. Prior to this study, it was thought that the eggshell fractured due to shear stress during the puncture test (Clark and Acree, 1974; Hunt et al., 1977; Voisey et al., 1979).

The shell thickness test was used to give an indication of the structural strength of the eggshell. Shell thickness, measured at the equator of the egg, decreased with shell quality in sound, cracked and leaker eggs (Table 1). Significant differences (P < 0.05) were found between sound (0.38 mm) and cracked (0.36 mm) eggs and between sound and leaker (0.35 mm) eggs, but not between cracked and leaker eggs.

The shell thickness distribution of sound, cracked, and leaker eggs for the total study is shown in Figure 2. The sound egg distribution had the narrowest range of values with a peak at 0.39 mm. The distribution curve for cracked eggs covered a wider range than sound eggs; with the increase in distribution occurring at the lower end of the curve. The distribution for leaker eggs appeared to pervade the entire length of the graph with a maximum comparable to the curve for cracked eggs. This grouping was more scattered, with representation in almost every area of the graph.

A significant (P < 0.01) correlation coefficient (0.56) was found between puncture force and shell thickness. According to Hamilton (1982) and Hunton (1993), a moderate relationship usually exists between indirect and direct methods used to assess shell strength. A correlation coefficient as high as −0.90 was noted between nondestructive deformation and compression fracture force; however, most correlation coefficients were below 0.80 (Hamilton, 1982). It was suggested that, considering the low correlations found between shell thickness and other direct methods of assessing shell strength such as puncture force, other mechanisms may contribute to shell quality such as aberrations in shell structure that may create an imbalance of shell components and, hence, compromise shell quality. Therefore, in order to understand, determine, or predict the incidence of shell breakage at the processing plants, it is imperative to understand the ultrastructure of the eggshell and any structural anomalies that may cause inferior eggshell strength and breakage. Most of the aberrations and structural defects observed during the ultrastructural studies of these experiments are considered to compromise shell quality. Thus, the incidence and frequency of these anomalies in sound, cracked, or leaker eggshells yield valuable information for future study of the incidence of eggshell breakage in the processing plants.
Thompson et al. (1985) and Thompson and Hamilton (1986) studied the relationship between laboratory measurements of shell quality and actual breakage during commercial processing. In both studies they found differences in shell quality measurements of cracked and sound eggs; however, the laboratory values did not predict actual rates of breakage.

Results of these ultrastructural analyses indicated that there was no significant difference (P > 0.05) in the width of the palisade layer or the mammillary knob layer of sound, cracked, and leaker eggshells. However, there was a significant correlation (P < 0.01) between palisade layer width and puncture force indicating that the shell component responsible for eggshell failure due to a decrease in shell thickness may be the palisade layer. The results of this study also indicate that the force required to puncture shells and the thickness of the shell decreased with shell quality, that is, from sound to leaker eggs. Sound eggs had higher puncture force values than cracked and leaker eggs, suggesting that sound eggs may have better quality shell material. The laying conditions directly affecting eggshell strength have been summarized by Washburn (1982). These include: 1) bird physiology, 2) eggshell properties, 3) bird behavior, 4) environment, 5) nutrition, 6) age, and 7) genetics. The puncture test may best describe the quality of shell material in the palisade layer because the calcified palisade layer is believed to make the greatest contribution to shell strength. Further research is needed in this area to evaluate the contribution of the individual eggshell layers to overall eggshell strength, and methods to increase material strength of these layers. Though a decrease in the amount of shell material may contribute to the incidence of downgraded eggs it may not be the sole factor in the production of low quality eggshells, which would also include shell structure and handling practices during processing.

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