Relating Quality Characteristics of Aged Eggs and Fresh Eggs to Vitelline Membrane Strength as Determined by a Texture Analyzer

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ABSTRACT The TA-XT21 texture analyzer (TA) was used to evaluate vitelline membrane strength (VMS). Fresh and aged (1 wk at 25°C) eggs (n = 48 eggs × 2 replications) were evaluated. Fresh and aged eggs were further divided into two groups of yolk only or whole egg (with intact albumen). Yolk index, Haugh units, pH, broken-out egg weights, VMS, yolk viscosity, and scanning electron microscopy (SEM) images were evaluated. Results from the TA indicated a decrease in VMS in aged eggs compared to fresh eggs and in yolk-only eggs compared to whole eggs. The SEM images indicated a loss of structural integrity in aged eggs as compared to fresh eggs. As expected, aged eggs also had higher albumen and yolk pH, lower Haugh units, lower yolk index, and decreased viscosity compared to fresh eggs. There were no differences in broken-out egg weights or whole egg pH between fresh and aged eggs. As the yolk membrane strength increased, yolk index (r = 0.59) and Haugh units (r = 0.56) decreased, and yolk pH (r = −0.64) and albumen pH (r = −0.57) increased. The study suggests that the TA combined with the modified extrusion cell may be effective in determining VMS. In addition, yolk index, Haugh units, and yolk and albumen pH may be used to predict changes in VMS.

(Key words: vitelline membrane strength, egg quality, yolk index, Haugh units, scanning electron microscopy)

INTRODUCTION

With the increase in further processing of eggs, structural integrity of the vitelline (yolk) membrane has become an increasingly important issue for the egg-breaking industry. Today, over 753 million kg (1,660.3 million lbs) of liquid egg products are produced each year for use in food service, commercial egg products, and as ingredients in other food products (Egg Industry, 1997). In the egg-breaking operation, liquid egg products consist of liquid whole egg, egg yolk, or egg albumen. Egg albumen is a particularly effective foaming agent that is used in baking and in the preparation of other confections (Yang and Baldwin, 1995). Foaming ability of the egg albumen is dependent on the quality of albumen proteins, and slight contamination with yolk can alter protein functionality and reduce foaming properties of the egg albumen (St. John and Flor, 1931). Therefore, successful separation of the egg yolk from the albumen is extremely important, and the strength of the vitelline membrane, particularly its ability to withstand the breaking process, is a key factor in producing good quality egg albumen. New high-speed, egg-breaking equipment may increase the possibility of ruptured yolks.

Factors influencing vitelline membrane strength (VMS) are the same factors influencing albumen quality (Fromm and Lipstein, 1964). During storage, egg quality deterioration is a factor of time, temperature, humidity, and handling (Stadelman and Cotterill, 1995). As the egg ages, egg quality deteriorates and the rate of deterioration is increased with higher storage temperatures. Feeney et al. (1956) suggested that the causative mechanism, specifically, biochemical change occurring during egg storage, is responsible for the deterioration of the egg albumen as well as the yolk membrane. Moreover, egg white thinning was attributed to the loss of o-glycosidically linked carbohydrate units of the glycoprotein, ovomucin, as pH increased during egg storage (Kato et al., 1979). Similarly, Kido et al. (1976) found that degradation of the major structural glycoprotein, glycoprotein II, in the vitelline membrane was in part responsible for the loss of vitelline membrane integrity over time.

Although VMS is an important egg quality attribute, few studies have focused on improving methods for determining yolk membrane strength. Earlier methods for determining VMS included a rapid capillary technique developed from Fromm and Matrone (1962). With this tech-
nique, a 2-mm capillary tube was placed on the surface of the vitelline membrane, and a vacuum was created in the capillary tube. Vitelline membrane strength was determined by the vacuum time required to rupture the membrane. This technique was an improvement over previous methods of Munro and Robertson (1935), which required removal of the yolk prior to taking a measurement. By using the rapid capillary method, Fromm and Lipstein (1964) and Holder et al. (1968) found significant variability in VMS from different loci on the same yolk. Data from these studies indicate that the VMS is greatest at the portion of the yolk near the chalazae and is weaker near the equatorial region. Fromm and Lipstein (1964) suggested that this variability might be due to the density of the chalaziferous layer that encircles the vitelline membrane.

Another factor influencing VMS is egg temperature. By using the vacuum-time method, membrane strength was observed to be higher at lower yolk temperatures. Ngoka et al. (1983) found that yolk temperature also influenced VMS. Specifically, their study indicated compression strength was increased at lower yolk temperatures with a back-extrusion food cell with an Instron to measure membrane-breaking strength. These researchers suggested that the compression head of the Instron made contact with the yolk surface and applied pressure over a larger area of the yolk surface compared to the vacuum-time method, which only makes contact with a very small portion of the yolk. As a result, it was thought that the Instron method would better simulate an impact of an egg on a skillet or the impact received in an egg-breaking operation. The purpose of this study was to characterize egg attributes as a result of aging and to evaluate the texture analyzer (TA; TA-XT21) in determining VMS of fresh and aged eggs. The TA is similar to the Instron but is more commonly used to evaluate texture attributes of foods. A modified extrusion food cell was developed to further facilitate the application of force over a larger surface area of the yolk compared to traditional vacuum-time methods of measurement (Figure 1).

MATERIALS AND METHODS

A total of 96 eggs were analyzed. Eggs were collected from 32-wk-old Single Comb White Leghorns (Hyline-W 36 strain). Treatments consisted of fresh eggs (n = 24 per replication) and eggs aged 2 wk at room temperature (n = 24 per replication). In each of two replications, fresh eggs (laid within past 24 h) and aged eggs were further divided into groups of yolk only or yolk with intact albumen. Previous studies have indicated that the chalaziferous layer encircling the yolk adds to the membrane strength (Fromm and Lipstein, 1964). One of the objectives was to determine if removal of the chalaziferous layer and the remaining adhering egg albumen altered VMS measurements.

Prior to measuring VMS, egg weight, yolk index, and Haugh units were analyzed. Yolk index, a ratio of yolk height to width, was analyzed and calculated according to Funk (1948). Haugh units, a mathematical relationship between the egg weight and albumen, were measured according to Haugh (1937). The VMS (g) was measured using the TA. The TA was equipped with a modified-extrusion food cell and a 5-kg tension-compression load cell. An extrusion food cell was specifically designed to fit the compression heads of the TA. The modified extrusion cell consisted of a 5.4 × 4.06-cm (length × width) cylinder mounted on a 8.89 × 10.16-cm (length × width) aluminum base (Figure 1). Blunted, 0.02-cm open slates cut 0.32 cm apart covered the entire surface of the cylinder bottom. Compared to previous methods, this design allowed for pressure to be applied over a greater surface area of the yolk membrane prior to rupturing. Whole eggs with intact albumens were placed directly onto the center of the food cell prior to measuring. Alternatively, prior to analysis of yolk-only eggs, egg albumen was removed using an egg separator. The remaining adhering albumen was removed by rolling the yolk on a damp paper towel. Individual eggs were then placed in the center of the extrusion cell, and force (g) required to rupture the vitelline membrane was determined. All eggs were maintained at room temperature (25 C) to prevent any variation in measurements caused by differences in egg temperature.

A pH meter was utilized to determine yolk pH and albumen pH, and a Zahn Cup-type Viscosimeter was used to analyze the viscosity of fresh and aged eggs. A thermometer was inserted into the bracket holes of the viscosimeter while determining yolk viscosity. Viscosity was expressed in Zahn seconds (i.e., time in seconds re-
required for 44 mL egg yolk to flow through the Visco-

Scanning electron microscopy (SEM) of the vitelline membrane was also performed on fresh and aged eggs. An egg separator was used to separate (fresh or aged) egg yolk from the albumen. The egg yolk was rolled on a wet paper towel to remove bound albumen and the chalaziferous membrane. The blastoderm on the surface of the egg yolk was located and marked with a small amount of a tracking dye (0.02% bromophenol blue, 50% glycerol, and 50% stacking gel buffer 1:8). By using clippers, a portion of the vitelline membrane opposite from the blastoderm was removed. The membrane was washed exhaustively with distilled-deionized water until all visible egg yolk was removed. The membrane was spread on a glass plate, and samples the size of a penny were cut and placed in small glass vials containing fixing solution (6 mL glutaraldehyde solution + 100 mL of 3%, 0.1 M potassium phosphate buffer). The vials were refrigerated overnight before SEM analysis. Samples were then post-fixed by 1% osmium tetroxide in phosphate buffers for 1 h at room temperature. Samples were washed in distilled water and dehydrated in a series of ethanol washes (25, 50, 75, and 95% water and dehydrated in a series of ethanol washes (25, 50, 75, and 95% ethanol, and were observed with a Cambridge S: 90 scanning electron microscope operated at 15 kV. Images were recorded with a Semi-Caps digital imaging device.

Table 1. Effect of aging on egg characteristics

<table>
<thead>
<tr>
<th>Egg type/age</th>
<th>Yolk Wt (g) pH</th>
<th>Albumen Wt (g) pH</th>
<th>Whole eggs Wt (g) pH</th>
<th>Yolk index</th>
<th>Haugh units</th>
<th>Vitelline membrane strength</th>
<th>TA force (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W/Fresh</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>47.37a</td>
<td>7.31a</td>
<td>0.41a</td>
<td>80.47a</td>
</tr>
<tr>
<td>W/Aged</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>49.89a</td>
<td>7.41a</td>
<td>0.31b</td>
<td>55.12b</td>
</tr>
<tr>
<td>Y/Fresh</td>
<td>16.41a</td>
<td>5.93a</td>
<td>31.88a</td>
<td>8.95a</td>
<td>—</td>
<td>0.39a</td>
<td>463.40b</td>
</tr>
<tr>
<td>Y/Aged</td>
<td>17.80b</td>
<td>6.21b</td>
<td>31.85b</td>
<td>9.44b</td>
<td>—</td>
<td>0.28b</td>
<td>162.80b</td>
</tr>
</tbody>
</table>

4-5 = Means within a column with no common superscript differ significantly (P ≤ 0.05).
6Stored 1 wk at room temperature (25°C).
7W = whole eggs, Y = yolk-only eggs, and TA = texture analyzer. Mean = 24 eggs.}

RESULTS AND DISCUSSION

Texture Analyzer

The TA was effective in determining differences in VMS between fresh and aged whole eggs and fresh and aged yolk-only eggs (Table 1). Specifically, the TA method indicated aged eggs in both groups (whole and yolk-only eggs) had decreased yolk membrane strengths, as expected. Aging has been reported to influence the vitelline membrane and to cause it to lose weight. Specifically, degradation of one of the major structural glycoprotein (glycoprotein II) and the disulfide bonds of the ovomucin causes a loss in membrane integrity over time (Kido et al., 1976; Kato et al., 1979). In the process of aging, water is also displaced from the egg albumen to the yolk (Trziszka and Smolinska, 1980). The excess water in the egg yolk causes the vitelline membrane to stretch and lose elasticity. Moran (1936) observed a similar decrease in elasticity of the vitelline membrane with age. Because water is transferred to the yolk, changes in viscosity and egg yolk weight may be observed and may influence measurements of VMS. In the current study, no differences were observed among egg weights (Table 1); however, viscosity of aged eggs was lower when compared to fresh eggs (111 vs. 81 s, respectively). Therefore, egg weight did not impact VMS, but it is likely that egg viscosity did influence VMS.

The TA data (Table 1) also indicated that removal of the egg albumen and the process thereof further reduced VMS in fresh and aged yolk-only eggs compared to fresh and aged whole eggs. Previous research has suggested that VMS appears to be related to the density or quantity of the chalaziferous layer that encircles the yolk (Fromm, 1966). Therefore, it would be expected that removal of this layer would affect VMS.

Froning et al. (1983) utilized an Instron Universal Testing Machine equipped with a back-extrusion food cell and a 2-kg tension load cell to determine the force (g) required to rupture the vitelline membrane of eggs obtained from hens fed different levels of vitamin E. With the Instron, these researchers found differences in VMS (g) based on the level of vitamin E supplied to the hens. Froning et al. (1983) have suggested that the compression head on the Instron method makes contact with a larger surface area of the yolk membrane than other methods
previously used to determined VMS. The TA also measures force using a load cell and compression head. We used a TA equipped with a 5-kg load cell in our study instead of a 2-kg load cell used in the previous study with the Instron (Froning et al., 1983). The TA equipped with a 5-kg load cell, a compression head, and the modified food cell was effective in determining VMS. In addition, the TA method, like the Instron procedure, makes contact with a larger surface area of the yolk compared to traditional methods of determining VMS.

Traditional methods used to determine VMS include direct application of force to rupture the yolk membrane (Haugh, 1937), vacuum (in mm Hg) required to rupture the membrane (Munro and Robertson, 1935), and vacuum time required to rupture the vitelline membrane (Fromm and Matrone, 1962). In each of these methods, only one position can be measured on each yolk. Holder et al. (1968) found variability in the VMS from different loci on the same yolk; therefore, the TA might be more advantageous compared to the other methods because the TA method would apply force over a larger surface area.

Characterizing Aging of Eggs

Another aspect of this study was to analyze other egg characteristics and correlate those to VMS expressed as grams of force. Egg characteristics examined in whole eggs were egg weight, whole egg pH, yolk index, and Haugh units. No differences were detected between fresh and aged whole eggs for egg weight or pH (Table 1). However, decreases in yolk index and Haugh units were observed in aged whole and yolk-only eggs compared to fresh whole and yolk-only eggs (Table 1). Although whole egg pH was not different between aged and fresh eggs, albumen pH and yolk pH were different in aged eggs (Table 1). Specifically, aged yolk-only eggs had higher albumen pH and higher yolk pH compared to fresh yolk-only eggs. These types of changes are commonly observed during egg aging. Heath (1976) found an increase in albumen pH from 8.4 to 9.4 and a lower yolk index for eggs stored at room temperature for 1 wk. Others have also noted the characteristic decline in Haugh units as a result of egg white thinning due to aging (Donovan et al., 1970; Kato and Sato, 1972; Stadelman and Cotterill, 1995).

The TA results were used to establish the relationships between VMS and other egg characteristics. Correlation coefficients (r) indicated that membrane strength was significantly related to yolk index, Haugh units, albumen pH, and yolk pH (Table 2). Specifically, as yolk membrane strength decreased, yolk index (r = 0.59), and Haugh units (r = 0.56) decreased; however, yolk pH (r = −0.64) and albumen pH (r = −0.57) increased. Fromm and Lipstein (1964) postulated that the factors associated with egg white thinning were the same factors associated with a decline in the quality of the vitelline membrane. Aging affects many egg characteristics; therefore, it is not surprising that VMS was correlated with other egg quality characteristics. Moreover, the fact that these relationships exist suggests that the TA method was a viable and useful measurement of VMS.

Scanning Electron Microscopy

Because aging weakens the structure of the vitelline membrane as evidenced by the TA measurements, SEM

FIGURE 2. (a) Scanning electron micrography (SEM) images of hen’s egg yolk vitelline membrane (fresh/outer surface/2.40 kV × magnification). (b) SEM images of hen’s egg yolk vitelline membrane (aged for 1 wk/outer surface/2.50 kV × magnification).
was utilized in an attempt to visualize these changes. Yolk membrane images obtained by SEM indicated a loss of structural integrity in the vitelline membranes of aged eggs as compared to fresh eggs (Figure 2a,b). These findings are similar to those of Fromm (1966) who concluded that during aging the vitelline membrane became weaker and lost fibrous material from the outer surface. Specifically, he reported that the surface of the vitelline membrane has a fibrous network when fresh but which begins to disappear after 3 d at 35 C. Similarly, visual observations in our study indicate that a fine layer present on the surface of the vitelline membrane dissipates with age. In contrast, images obtained from SEM of the vitelline membrane of fresh eggs revealed a distinct feature of the surface. The distinct structural feature of the vitelline membrane obtained from fresh eggs was similar to that observed previously by Bellairs and Harkness (1963). Images of fresh eggs in our study indicated the outer layer of the membrane was a latticework of fine fibrils (Figure 2a). In addition, observations from our study suggest that the original structural integrity of the membrane was lost as a result of aging.

Based on the results obtained in this study, the TA (within the given parameters) combined with the modified extrusion cell may be an effective method for determining VMS. Furthermore, those factors influencing VMS of eggs appear to be related to those influencing Haugh units, yolk index, albumen pH, and yolk pH. Finally, SEM was a useful tool in visualizing the loss of structural integrity of the vitelline membrane as a result of aging.

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