INTRODUCTION

The eggshell, a thin mineral structure, protects the egg contents against mechanical impact, dehydration, and microorganism contamination. At the same time, the eggshell is permeable to gases and water necessary for the development of the chick embryo (Nys et al., 1999; Hincke et al., 2012). The permeability of the eggshell is in turn regulated by an organic-rich layer or cuticle that coats the outer eggshell surface and plugs the entry to the shell pores (Board and Halls, 1973). The cuticle is deposited by the epithelial cells lining the hen uterus during the last 1.5 h before oviposition (Nys et al., 1999). It has a variable thickness (up to 12 μm) and an uneven or patchy distribution on the eggshell surface (Board and Halls, 1973). The cuticle is composed of proteins and an inner part richer in sulfated polysaccharides and phosphates. It also shown that the cuticle composition, thickness, and degree of coverage are highly dependent on hen age and egg freshness. During the course of the first laying year, the thickness and degree of glycosylation of the cuticle decreases with hen age, and at the end of the laying cycle, the cuticle is significantly depleted in lipids. There are also well-defined compositional changes in the cuticle of freshly laid eggs as time passes and there is a notable increase in the permeability of the eggshell after 24 h due to cuticle drying. We discuss how these changes in the cuticle can affect the food safety of eggs in relation to the risk of trans-shell contamination by bacteria (i.e., Salmonellosis).

Additionally, the cuticle is rich in phosphorus (Wedral et al., 1974; Dennis et al., 1996; Cusack et al., 2003).

The cuticle limits movement of particles, water, and bacteria through the shell pores (Board and Halls, 1973; Board and Tranter, 1986), and thus, together with the mineralized shell and shell membranes, constitutes a physical barrier against microorganism invasion and contamination of the egg content (Board and Tranter, 1986; De Reu et al., 2006). These natural physical defenses of the egg are especially important considering that trans-shell contamination, in which microorganisms, by penetrating the shell, contaminate the egg after being laid, this being regarded as the prevalent route for bacterial infection of eggs (Bruce and Drysdale, 1994). In fact, eggs with an absent or partially removed cuticle are more susceptible to bacterial contamination (Board et al., 1974; Sparks and Board, 1984; Messens et al., 2005; De Reu et al., 2006; Bain et al., 2013). Moreover, the cuticle coverage has been shown to be the most important eggshell characteristic to resist bacterial shell penetration (De Reu et al., 2006). Also, its chemical composition plays an important role in limiting bacterial contamination. For instance, proteins extracted from the cuticle of different bird species possess antimicrobial activity against several bacterial species (Wellman-Labadie et al., 2008). Additionally, lipid components extracted from the cuticle

ABSTRACT For a fuller understanding of the functionality of the eggshell cuticle, we conducted a detailed study using a wide array of analytical techniques (scanning and transmission microscopy), energy dispersive x-rays, and attenuated total reflection-Fourier transform infrared spectroscopy to analyze the structure, morphology, and chemical composition of this organic coating. This study shows that the cuticle has a compositional gradation with an outer part richer in proteins and an inner part richer in sulfated polysaccharides and phosphates. It also shown that the cuticle composition, thickness, and degree of coverage are highly dependent on hen age and egg freshness. During the course of the first laying year, the thickness and degree of glycosylation of the cuticle decreases with hen age, and at the end of the laying cycle, the cuticle is significantly depleted in lipids. There are also well-defined compositional changes in the cuticle of freshly laid eggs as time passes and there is a notable increase in the permeability of the eggshell after 24 h due to cuticle drying. We discuss how these changes in the cuticle can affect the food safety of eggs in relation to the risk of trans-shell contamination by bacteria (i.e., Salmonellosis).

Key words: eggshell, cuticle, glycoprotein, attenuated total reflection-Fourier transform infrared spectroscopy, Salmonellosis

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have potent antimicrobial activity (Wellman-Labadie et al., 2010). Moreover, recent studies have identified several proteins in the cuticle (e.g., lysozyme C, ovotransferrin, ovocalyxin-32, and ovolecidin-17) known to have antimicrobial activity (Rose-Martel et al., 2012). Thus, these studies confirm that specific chemical components of the cuticle are important chemical defenses of the eggshell against bacterial penetration and colonization of the eggshell surface.

On the other hand, eggs with a cracked shell or poor eggshell quality, besides posing a health risk to consumers (because eggs are the main source of food-related Salmonella enteritis poisoning), need to be downgraded, causing major economic losses to producers (Stadelman and Cotterill, 1995; Solomon, 1997). Thus, eggshell quality and integrity is of fundamental importance and great efforts have been devoted to study and improve it (Solomon, 1997; Dunn et al., 2009). An important aspect of eggshell quality, besides its thickness, is completeness of cuticle coverage, because this organic coating prevents water and bacteria moving across the shell (Musgrove et al., 2004; EFSA, 2005; Messens et al., 2005).

The eggshell characteristics and quality are greatly influenced by a wide array of factors including hen age, genetics, and diet as well as hen housing (Solomon, 1997; Jones et al., 2002; Rodriguez-Navarro et al., 2002; Travel et al., 2011). In the same way, cuticle properties are known to be affected by the same factors (Board and Halls, 1973; Board and Tranter, 1986; Bain et al., 2013). For instance, it is well known that there is a gradual decline in the quality of the cuticle with hen age (Board and Halls, 1973; Board and Tranter, 1986; Leleu et al., 2011). Eggs from end-of-lay hens generally have a very poor degree of cuticle coverage. However, there is scant information on how other important characteristics such as eggshell cuticle composition are affected by these factors (hen age, egg freshness; Haisiak et al., 1970; Rose-Martel et al., 2012). Thus, for a fuller understanding of the changes that occur in the cuticle as hens age and during egg storage, we have conducted a detailed study using up-to-date analytical techniques such as scanning and transmission electron microscopy (scanning EM and TEM, respectively), energy dispersive x-rays (EDX), and attenuated total reflection-Fourier transform infrared spectroscopy (ATR-FTIR) to analyze the structure, morphology, and chemical composition of the eggshell cuticle. This is the first comprehensive study available describing the variation of the cuticle composition with hen age and with egg freshness. The application of this methodology on a large scale (i.e., using ATR-FTIR to determine the cuticle chemical composition), for instance, during selection programs could be useful for breeders to improve the safety and quality of eggs, especially considering that cuticle composition determines the antimicrobial properties of the egg.

Materials and Methods

Samples

Hy-Line CV22 white hen eggs were collected within 2 h of being laid directly from a local farm (Avícola Garrido-García, Granada, Spain) and kept at room temperature. For the study of the influence of egg storage age, 160 eggs from hens of 2 age groups (16 to 70 wk) were collected. Groups of 10 eggs were analyzed at different times (2, 3, 4, 5, 6, 24, 26, and 72 h after laying). To study the effect of hen age, 20 eggs from each age group (16, 30, 36, and 70 wk) were analyzed after 24 h to allow for cuticle stabilization. Eggs were broken and analyzed immediately by ATR-FTIR. Analyses were made consistently around the egg waist.

Cuticle Staining

To assess the quality and degree of coverage of the eggshell cuticle, Cuticle Blue staining was used. A solution of 1% Cuticle Blue staining (MS Technologies Ltd., Northamptonshire, UK) was prepared and applied by immersing the samples in the solution for 5 min. This dye, having a strong affinity to proteins, stains the eggshell green if the cuticle coverage is good or does not stain at all where the cuticle is absent.

Infrared Spectrometry

For the infrared (IR) analyses, the outer surface of intact eggshell samples was pressed against the ATR diamond crystal window and the IR spectra recorded at a 2 cm⁻¹ resolution over 100 scans using a FTIR spectrometer (model 6200, JASCO Analytical Instruments, Japan). The amount of water, proteins, sulfate, phosphate, carbonate, polysaccharides, and lipids were determined from the peak area of absorption peaks associated to a characteristic molecular group of each component (e.g., O-H: water; C-H: lipids or fatty acids; amide: proteins; C-O: carbonates; S-O: sulfates; P-O: phosphates; COC: sugars/polysaccharides; Rodriguez-Navarro et al., 2006). Overlapping peaks were resolved and their integrated areas measured using a specially designed curve-fitting software. Calculated peak areas of the main bands were normalized to the total area of the spectrum.

Microscopy Analyses

For scanning EM and scanning EM-EDX analyses, intact pieces of eggshells were carbon coated (Hitachi UHS evaporator, Hitachi, Tokyo, Japan) and analyzed with a high-resolution scanning EM (Leo Gemini 1530, Zeiss, Oberkochen, Germany) operating at 15 kV. For TEM analyses, small pieces of eggshell (1 mm²) were fixed for 24 h at 4°C in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer.
(0.01 M, pH 7.4). Each sample was then decalcified using a solution of EDTA (Sigma Aldrich, St. Louis, MO; 40 g/L, pH 8) for 15 d. Afterward, they were postfixed in 1% osmium tetroxide for 1 h and then dehydrated using an ethanol gradient (50, 70, 90, and 100%), embedded in EMbed 812 resin and sectioned to 70 nm (Ultracut R Leica, Leica, Wetzlar, Germany). Finally, sections were contrasted with uranyl acetate and lead citrate. These sections were examined in a Libra 120 Plus transmission electron microscope (Zeiss).

**Statistical Analyses**

Basic descriptive statistics were used to characterize cuticle properties. A 1-way ANOVA was used to compare cuticle properties between the different groups of samples analyzed. Pearson correlation analysis and linear regression models were used to study relationships among the different properties measured. The significance level chosen for all analyses was \( P < 0.05 \). All statistical analyses were performed using the software package SPSS 17.0 (IBM, New York, NY).

**RESULTS**

**Scanning EM and TEM**

Transmission electron microscopy examination of the cross-section of the outer surface of a decalcified eggshell revealed that the cuticle is a well-defined layer of a few microns in thickness constituted by densely packed spherical vesicles in close contact with the vertical crystal layer (Figure 1). The vesicular layer of the cuticle showed a gradual increase in contrast toward the outer surface. The higher contrast was due to the staining of vesicle membranes with osmium oxide, which gives them high electron density. Osmium oxide has a strong affinity to phospholipids of the vesicle membranes. On the other hand, the size of vesicles varied markedly, ranging from approximately 50 to 500 nm. The larger vesicles had an inner structure of radiating fibers, the remainder of spherical aggregates of needle-like apatite nanocrystals dissolved during the demineralization process (Dennis et al., 1996). Also, the cuticle thickness along the eggshell surface was quite variable, even being completely absent in some regions. The variability in the cuticle thickness was more evident in the case of eggs from old hens, which had a thinner and more irregular cuticle than eggs laid by young hens, which had a thicker and more regular cuticle. For example, Figure 1A and B shows TEM images of cuticles from eggs laid by old and young hens, the cuticle thickness measuring 2 and 5 microns, respectively.

Figure 1C, displaying an scanning EM image of an eggshell in which the organic cuticle was removed, shows an irregular eggshell surface with prominent pore openings. On the other hand, Figure 1D displays an scanning EM image of an eggshell with complete cuticle coverage. The organic cuticle coating the eggshell gives the eggshell surface a very smooth appearance. In this case, the pore openings are no longer visible, as they are completely covered by the organic matter of the cuticle. In contrast, a network of fissures appears in the cuticle surface as a result of drying.

**Phosphorus in the Cuticle**

The EDX analyses of eggshells showed that phosphorus and magnesium were concentrated mainly on the eggshell surface, in agreement with previous findings (Cusack et al., 2003), and that eggshells with a poor cuticle (i.e., from older hens) had significantly lower phosphorus and magnesium signals in the EDX spectra. However, the EDX analyses provided no information on the chemical form of cuticle phosphorus. The ATR-FTIR analyses of the eggshell surface were more informative, showing a well-defined phosphate band centered at about 557 cm\(^{-1}\) and a peak at 687 cm\(^{-1}\) associated with amide IV bands (Figure 2). The phosphate band was visible only in dry eggshells with a thin cuticle, suggesting that phosphates were concentrated mostly in the inner cuticle. Also, there is another phosphate band centered around 1,000 cm\(^{-1}\), but this band was not useful in this case because it was strongly overlapped by polysaccharide bands. It bears mentioning that, even though apatite crystals are found in the inner cuticle (Dennis et al., 1996), the cuticle phosphate band did not show an additional peak at about 600 cm\(^{-1}\) characteristic of crystalline apatitic phosphate. Moreover, the phosphate band of the cuticle was much broader than that of bone mineral (constituted by nanocrystalline apatite). All these facts indicate that most of the phosphorus in the cuticle was in a noncrystalline phosphate form, presumably as part of phosphoproteins (Gautron et al., 1997).

**FTIR Spectra of Eggshells with Different Cuticle Quality**

The analysis of the eggshell surface by ATR-FTIR provided detailed information about the cuticle chemical composition of the surface as well as its quality: defined as the thickness or degree of coverage over the eggshell mineral substrate. Figure 3 displays the ATR-FTIR spectra of the eggshell surface of eggs showing a good or a poor cuticle. The most prominent features of the FTIR spectra of the cuticle were: a broad band from 3,700 to 2,500 cm\(^{-1}\), associated with OH and amide A groups from water and proteins; 2 peaks centered at about 1,633 cm\(^{-1}\) and 1,540 cm\(^{-1}\), associated with protein amide I and amide II groups; a peak centered at about 1,390 cm\(^{-1}\) associated with carbonate groups from eggshell calcite crystals; and a broad band from 1,100 to 990 cm\(^{-1}\), associated with polysaccharides. On the other hand, the occurrence of a weaker band at about 1,240 cm\(^{-1}\), associated with sulfate bonds, implies that the polysaccharide moieties had some degree
of sulfation. There were other minor bands in the spectra at about 2,918 cm$^{-1}$, associated with C-H groups from lipids, and peaks at 1,447 and 1,410 cm$^{-1}$, associated with carboxylate groups from amino acid residues. In the case of an eggshell with a very good cuticle (Figure 3a), the amide and polysaccharides bands had high intensity, comparable with or higher than that of the main carbonate band (centered at about 1,397 cm$^{-1}$). In this case, the intensity of polysaccharide band was very high, indicating that the cuticle was strongly glycosylated. In contrast, for an eggshell with a very thin cuticle, the IR spectra displayed weak amide or polysaccharide bands and very strong carbonate absorption peaks (Figure 3b). Thus, as the cuticle coverage or thickness decreased, the carbonate peaks intensified as the eggshell mineral was more exposed to the surface. Finally, for an eggshell without any cuticle, the spectrum will be that of pure calcite (which is the mineral constituent of the eggshell). The calcite spectrum showed 3 main absorption bands of carbonate groups at 1,395, 817, and 713 cm$^{-1}$ (Figure 3c).

In addition, Pearson correlation analyses for all IR peak areas provide information on the relationship between the main molecular components of the cuticle. Specifically, peaks associated with proteins (amides), polysaccharides, and sulfates proved to be highly and positively correlated. These results indicate that all these molecular components were structurally associated as glycoproteins, which are the main organic constituents of the cuticle. On the other hand, the intensity of amide peaks were strongly and negatively correlated with the intensity of main carbonate peaks. Thus, as the cuticle coverage decreases or becomes thinner, the amount of proteins coating the eggshell surface decreases and the carbonate mineral substrate becomes more exposed to the eggshell surface and contributes more to the IR spectra. Therefore, it is possible to define the intensity ratio of amide I to main carbonate peak as a measure or index of the cuticle quality. Also, another useful parameter could be the intensity ratio between the polysaccharide and the amide peaks, which is indicative of the degree of glycosylation of the cuticle proteins.

**Influence of Egg Freshness**

The ATR-FTIR analysis of the eggshell surface of freshly laid eggs showed well-defined changes in the
composition and structure of the cuticle over time. The eggshell surface is moist shortly after an egg is laid and the ATR-FTIR spectra of a freshly laid egg showed a very large OH band (Figure 4). With the passage of time, a gradual reduction appeared in the intensity of the OH band, due to moisture loss as the cuticle dries (Figure 4 and 5). Also the carbonate peaks became better defined and their relative intensity increased with time. As the cuticle dried and became thinner, the eggshell carbonate mineral became more exposed to the

Figure 2. Attenuated total reflection-Fourier transform infrared spectra in the 800 to 400 cm\(^{-1}\) range of the eggshell surface (a) and that of a powdered cortical hen bone used as a reference material (b). Abs = absorbance.

Figure 3. Attenuated total reflection-Fourier transform infrared spectroscopy analyses of the surface of eggshells: a) with a good cuticle, b) with a poor cuticle, and c) calcite powder. Abs = absorbance.
surface, contributing more to the IR spectra (note that the ATR signal had very low penetration of about 2 microns). Simultaneously, there was a gradual decrease in the intensity of amide I peak and an increase in the area of peaks associated to polysaccharides and sulfates. The decrease in amide peaks and the increase in polysaccharide and sulfate peaks could have resulted from the diffusion of sulfated polysaccharide components from the inner cuticle. Alternatively, this could be explained by a gradient in the chemical composition of the cuticle in which the outer part is richer in proteins and the inner part is richer in polysaccharides. As the cuticle dries and becomes thinner, the inner layer of polysaccharides that are associated with sulfates contributes more to the spectra. On the other hand, the increase in the amount of polysaccharides in the surface suggests that proteins become more glycosylated as the cuticle matures. A higher degree of glycosylation should improve the mechanical properties of the cuticle during the first hours after the eggs are laid. At longer times (72 h), amide I (proteins) and OH (water) peaks were found to sharply increase, whereas the carbonate peaks fell. As the cuticle dried, many fissures formed in this coating, leaving pore openings exposed. These changes in the cuticle facilitated the diffusion of the water from the egg white through the eggshell pores. Thus, the observed increase in the amount of protein at the eggshell surface was due to increased eggshell permeability to water, which could mobilize and transport soluble proteins from the egg white or from the eggshell itself.

Parallel to the compositional changes occurring at the eggshell cuticle during its maturation and drying, there were concomitant structural changes in its constituting proteins. In particular, there was a notable shift of the amide I peak to higher wave numbers from 1,636 to 1,645 cm\(^{-1}\). This shift implies that a change in the conformation of cuticle proteins from having a disordered structure (typical of proteins in solution) to a more ordered structure. At longer times (after 72 h), the protein peak in the eggshell surface changed back to 1,636 cm\(^{-1}\) due to hydration.

The changes described in the eggshell surface of freshly laid eggs were similar regardless of the hen age, though these changes occurred more rapidly and were more evident in the case of eggs laid by older hens. This could be due to the poorer cuticle quality of eggs from older hens but also to the fact that the albumen (egg white) in older hens is thinner and has a higher water content (Travel et al., 2011), making it easier for water to permeate to the eggshell surface.

**Influence of Hen Age**

All eggs treated with Cuticle Blue dye showed some degree of staining and turned green. However, eggs laid by older hens (70 wk old) showed a lesser degree
of staining and lighter green hues compared with eggs laid by younger hens. This result confirms previous observations that the quality of the cuticle decreases as hens age (Board and Halls, 1973). On the other hand, ATR-FTIR data indicated that the composition of the eggshell surface (the cuticle) was highly dependent on hen age (Figure 6). Furthermore, a detailed study of eggs laid by hens of different age groups showed that during the course of the first laying year, there were well-defined compositional changes in the cuticle—specifically, a gradual reduction in the amount of polysaccharide in the cuticle as the hen aged. By contrast, the amount of sulfate increased with hen age. Meanwhile, the amount of lipid gradually increased toward the middle of the laying season and decreased, reaching its lowest value at the end of the laying year. However, the intensity of the amide peaks remained almost constant over the course of the first laying year, indicating that the amount of protein also was independent of hen age. It bears mentioning that the relative intensity of the main carbonate peak remained almost constant during the course of the first laying year. These findings indicate that the thickness of the cuticle remained almost constant. On the other hand, the decrease in the intensity of the polysaccharide peak implies that the degree of glycosylation of the cuticle proteins decreases as hens age. Lower glycosylation could reduce the mechanical properties of the cuticle and could diminish the resistance of the cuticle to bacterial penetration.

Figure 5. Time course of the intensity of attenuated total reflection-Fourier transform infrared spectroscopy peaks associated with main chemical components of the eggshell cuticle over time since the eggs were laid. a) Intensity of OH band. b) Intensity of main infrared peaks associated to polysaccharides, sulfates, carbonates, protein-amide, and lipids. Data shown correspond to eggs from 70-wk-old hens. Color version available in the online PDF.
Notable differences were also found in the water content in eggs laid by older and younger hens. Fresh eggs from older hens had a larger amount of water on the eggshell surface than eggs laid by younger hens. These differences can be attributed to such factors as a different cuticle coverage, leaving more eggshell pores exposed, and also to the fact that the albumen (egg white) in older hens is thinner and has a higher water content (Stadelman and Cotterill, 1995; Van Den Brand et al., 2004), making it easier for water to permeate to the eggshell surface.

DISCUSSION

Because a solid eggshell with an intact cuticle is an effective physical barrier against bacterial shell penetration (Board and Tranter, 1986; Messens et al., 2005; De Reu et al., 2006), a good-quality cuticle is essential to ensure the safety of eggs. Previously, cuticle quality (i.e., its thickness and degree of coverage) have been assessed qualitatively by the degree of staining of the eggshell with specific dyes (i.e., Edicol Pea Green; Board and Halls, 1973; De Reu et al., 2006). More recently, a quantitative methodology has been developed based on spectrometric color analyses of dyed eggs (De Reu et al., 2006; Leleu et al., 2011; Bain et al., 2013). Complementary information concerning the degree of coverage of the cuticle is assessed by scanning electron microscopy (Solomon, 1997; Leleu et al., 2011). In the present study, we describe an alternative methodology based on ATR-FTIR spectrometry to measure the quality of the cuticle, gaining not only measurements of cuticle thickness but also detailed information on its chemical composition. Additionally, due to the low penetration of the ATR signal (about 2 microns), this analytical technique provides information exclusively on the cuticle on the intact eggshell without the need to use tedious extraction processes that may also alter its composition (Pereira-Mouries et al., 2002). Moreover, from the IR analyses, it is possible to define compositional parameters to quantitatively measure the cuticle quality.

Previous studies have shown that in the cuticle, 2 structurally distinct layers can be differentiated: an organic rich outer layer and a mineralized inner layer (Dennis et al., 1996; Fraser et al., 1999; Chien et al., 2008). The present study reflects a compositional gradation of the cuticle in which the outer part is richer in proteins and the inner part is richer in sulfated polysaccharides and phosphates. Most of the phosphate in the cuticle is in a noncrystalline form, presumably as part of phosphoproteins (i.e., Ovocalyxin-32, osteopontin), as previously hypothesized (Gautron et al., 1997; Chien et al., 2008). The presence of crystalline phosphate (hydroxyapatite) described by other authors (Dennis et al., 1996) must be restricted to a minor fraction not detectable by our analyses.

In addition, this study shows that cuticle composition is variable, being highly dependent on hen age and egg freshness. Particularly, during the course of the first laying year, well-defined compositional chang-
es were found in the cuticle. Specifically, eggs laid by old hens were found to have a cuticle composition that was significantly depleted in polysaccharides and lipid components compared with eggs laid by younger hens. Haisiak et al. (1970) reported a gradual decrease in the lipid content in the eggshell cuticle with increasing hen age. However, we observed this decline only at the end of laying season. It is worth mentioning that in the study by Haisiak et al., the analyses were made in cuticle extracts, which could include lipid components from other eggshell structures. Regarding the content of polysaccharides, a decrease in their amount indicates a lower degree of glycosylation of the cuticle proteins (Khajehpour et al., 2006). Glycosylation define the structural stability of glycoproteins and their adhesive properties to substrates (Nicholson et al., 1998; Sola et al., 2007). A lower glycosylation could decrease the mechanical properties of the cuticle, reducing the resistance of the cuticle to bacterial penetration and thus magnifying the risk of bacterial contamination of the egg content. Also, a reduced lipid content could have a negative impact on the cuticle antimicrobial properties (Wellman-Labadie et al., 2010) as well as the ability of the cuticle to resist water. These changes added to a cuticle with a reduced degree of coverage could partly explain the higher incidence of bacterial contamination of eggs laid by older hens (Wells, 1968; Nascimento et al., 1992; Jones et al., 2002). On the other hand, rapid changes in the cuticle properties (degree of moisture, composition, and permeability) in fresh eggs could strongly influence the effect of bacterial contamination, depending on the timing of the penetration. For instance, an immature and moist cuticle, such that of freshly laid eggs (less than 6 h), could be more vulnerable to bacterial penetration. Also, increased eggshell permeability detected after 24 h would also increase the risk of bacterial penetration. Additionally, cuticle compositional changes could influence the ability of cuticle-digesting bacteria (i.e., Pseudomonas) to colonize and spoil eggs (Board and Halls, 1973) because cuticle polysaccharides and proteins are sources of the carbon and nitrogen necessary for microbial growth. In this respect, eggs from older hens might have a lower risk of colonization by these bacteria.

To compensate for a poor cuticle coverage or cuticle loss, for example in sanitized/washed eggs (Sparks and Burgess, 1993; Hutchison et al., 2003; Rose-Martel et al., 2012), specific strategies have been developed such as coating the eggs with mineral oil or with other edible coating materials (Stadelman and Cotterill, 1995; Bhale et al., 2003; Caner and Cansiz, 2008). These restorative treatments offer beneficial effects in eggs such as a reduced moisture and CO₂ loss, which slows down the natural decline of egg internal quality, thereby extending the shelf life of eggs. Additionally, these coatings can also provide additional protection against bacterial penetration. However, these coatings do not have the antimicrobial properties of the cuticle chemical components and might allow the bacterial colonization of the eggshell surface.

Finally, the functionality and effectiveness of the cuticle as a barrier against bacterial penetration depends heavily on the degree of coverage and thickness of this layer, but also, as this study shows, protection could depend on cuticle composition, as specific components have antimicrobial properties. We have shown that cuticle composition is in turn highly dependent on hen age and egg freshness. This information could be useful to design strategies to evaluate and reduce the contamination risks during egg processing. Additionally, a rapid analytical tool such as ATR-FTIR spectrometry could be helpful in breeding hens genetically selected for eggs with the best cuticle characteristics (i.e., high degree of glycosylation, high lipid content) to improve egg quality and safety.

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