CONTENT

INTRODUCTION[1]

The study of eggshells and the factors affecting their quality has been ongoing for the past 50 years [1-5]. Declining eggshell quality can be a serious problem, causing significant losses to the industrial food egg industry [1, 6, 7]. Often the seriousness of shell quality problems is underestimated, because the percentage of incrusted eggs and broken eggs increases significantly during transport and packaging [12–17]. At the same time, in the majority of enterprises engaged in the production of edible chicken eggs, from 2% and more belong to the category of technological breakage before reaching the egg sorter [17–21, 22–28]. The economic loss to the hatching egg industry is even more severe because the hatchability and safety of day-old chicks is reduced [22–29].

A significant decrease in eggshell quality is observed in laying hens above 45 weeks of age [1–3]. Due to age and some changes in reproductive organs, the formation of the protein matrix and the actual calcification of the eggshell is slower. In our book we offer practical steps to reduce the technological rejection of eggs.

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1. GENERAL CHARACTERISTICS OF MORPHOLOGICAL AND FUNCTIONAL PARAMETERS OF BIRD EGGSHELL AS A COMPLEX BIOMINERAL COMPOSITE BASED ON CALCIUM CARBONATES AND ORGANIC SUBSTANCES

Bird eggshells are a composite material with a calcite mineral phase and an organic phase whose interaction determines their ultrastructure and resulting mechanical properties. The egg is a widely used foodstuff throughout the world: more than 1300 billion eggs are produced per year [1–4]. It is therefore a staple food and an important agricultural product whose shell quality is critical for human food security. For this reason, the mechanism of shell formation is intensively studied [8–11, 13–16].

Natural biological materials are fundamentally different from conventional structural engineering materials, such as steel or concrete, in the sense that their internal embedded energy is low. This is due to the lack of high-temperature treatment in natural materials, which is facilitated by the use of large amount of information embedded in the genetic code.

Interaction between cellular organic constituents and the geological counterparts of the minerals that form on them is a fundamental component of biomineralisation research. Biomimicry is the imitation of structure and formation process of natural materials – has great potential for commercialization new structural materials without significant energy use associated with general engineering materials. The nanocomposite simulation structure of many natural materials also results in reproduce their relatively high stiffness, strength and impact strength per weight. Recent studies have studied the formation of biomimetics and the resulting material properties of natural materials such as bone, seashell nacre and eggshells. The developing biomimetic materials for widespread used in structural applications are the incomparable length scale between structural elements that are several meters long and nanometers dimensions in natural materials. Formation of eggshells in birds is one of the fastest known biomineralisation processes. The robust, rigid eggshell is formed in less than 24 hours at body temperature in chickens. Calcite ($CaCO_3$) shell (97 % calcite) is formed through heterogeneous germination on the outer most surface of the collagen double layer eggshell (Fig. 1.1, 1.2, 1.3).

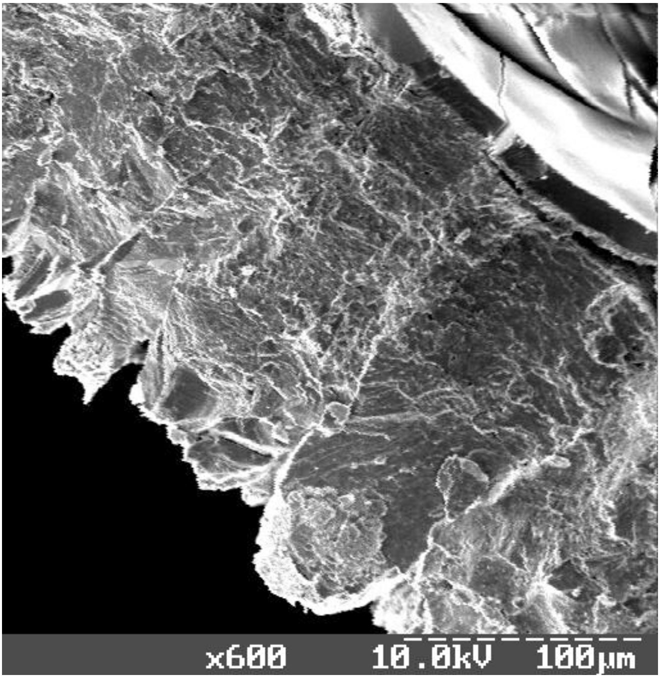

Fig. 1.1. Scanning electron image (SEM) of chicken eggshell in cross section (x 600)

The result is a complex multilayered calcite deposition process, which occurs in three distinct phases: beginning in the cone (or mammillary) stratum, linear mineral deposition in a pockmarked layer, and calcification stops at the surface mineral germs of the conical layer on organic nodules are scattered in a semi regular pattern over the surface of the fibrous membrane of the outer shell, and the mineral then fuses into a single shell structure as it grows outwards from the shell (Fig. 1, 2). As the mineral is deposited proteins are incorporated into the calcite matrix. Thus, the composite eggshell has two distinct non-mineral matrix components: a fibrous eggshell membrane with nodules of organic mineralization, and incorporated organic material in crystals. Because egg formation is one of the most fastest known forms of biogenic mineralization as organic elements have attracted attention as key elements of biomineralisation and biomimetics studies.

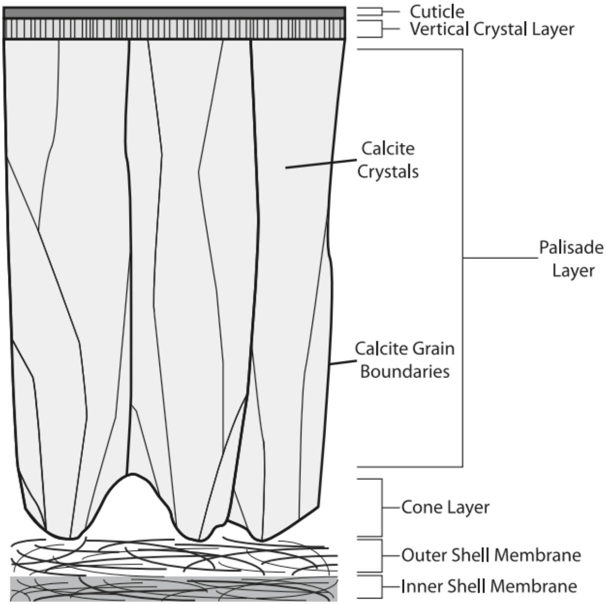

Fig. 1.2. Schematic cross-section of eggshell and bilayer eggshell membrane. Adapted from Ref [31]

Damage to the eggshell disrupts its function as an incubator for developing chicks or as packaging for an important human foodstuff. Thus, there are many stakeholders involved when considering the formation and destruction of eggs. Some physical properties of an intact egg have been investigated across a wide range of species, demonstrating that the force to break an entire egg is approximately linearly related to the weight of the projectile and is proportional to the square of the shell thickness. The shape of the egg varies greatly between species, and can be roughly spherical, ellipsoidal, conical origin-between, depending on the bird and its evolutionary adaptation. More recent studies have investigated the processes of egg shell fracture and destruction from a material science and engineering approach aimed at quantifying properties such as elasticity modulus of elasticity and fracture toughness, taking into account understanding of the underlying structure and properties relationships in biomineralized composites. However, insufficient attention has been paid to mechanical importance of the shell structure which holds the shell parts in place, even when cracked.

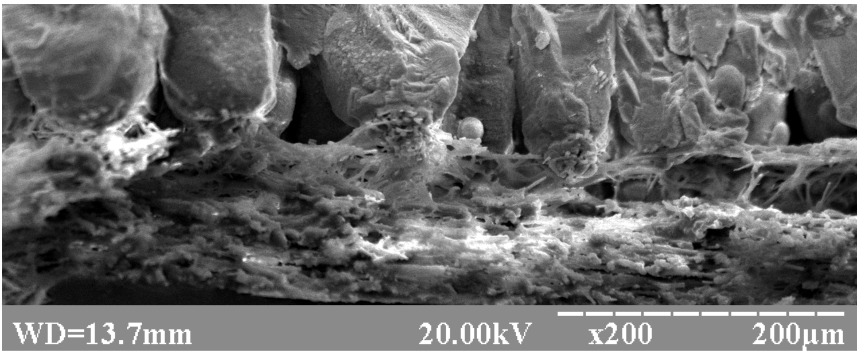

Fig. 1.3. Scanning electron image of chicken eggshell in cross section (x 200). The lower part of the image shows the double-layered under-shell membrane

In the numeral current works, both classes of organic constituents have been studied while considering the overall behavior of the eggshell. Moreover, functional information must now be linked to genomic data. Several collaborative programmes with industrial poultry farmers are currently underway that combine the latest advances in our knowledge of egg shell mineralization mechanisms with advances in genomic selection to ensure the accuracy of selecting animals capable of laying eggshells with improved mechanical properties under various physiological and production conditions [30]. It is also of interest that eggshells represent the most studied model of calcium carbonate biomineralisation. It is therefore also an excellent model for the development of new bioinspired materials. The shell of bird eggs is a natural protective nanobiocomposite whose formation is based on the phenomenon of biomineralisation. The eggshell consists of calcium carbonate ($CaCO_3$) in the form of calcite, which contains hundreds of proteins that interact with the mineral phase, controlling its formation and structural organization, and thus determine the mechanical properties of the mature biomaterial. Its mineralogy, structural features and the regulatory interactions that unite the mineral and organic components are sufficiently well described. An outstanding discovery is the recently shown presence of vesicular transport of amorphous calcium carbonate ($CaCO_3$ACC) as a new pathway providing an active and continuous supply of ions Ca^{2+} necessary for shell mineralization [30]. Over 900 proteins and thousands of regulatory transcripts have now been identified during the formation of the chicken eggshell [30]. Bioinformatics predictions relate to their functionality during the biomineralisation process. Matrix proteins have been quantified to understand their role during key spatially and temporally regulated shell mineralization events. Finally, J. Gautron et al. proposed the latest version of an updated scheme with a global scenario covering the mechanisms of mineralization of bird eggshells [30]. With this large dataset, it is now possible to identify specific motifs, domains or proteins and peptide sequences that perform critical functions during avian eggshell mineralization. Combining this understanding with genomic data (non-synonymous single nucleotide polymorphisms) and accurate phenotyping (eggshell biomechanical parameters) on "pure" lines of bird breeds will result in consistently better eggshell characteristics to improve food safety. This information will also answer the question of how evolutionarily optimized chicken eggshell matrix proteins affect and regulate calcium carbonate mineralization as a good example of biomimetic and bioinspired materials.

Oviparous bird species are characterized by extra-uterine development of the embryo in a closed chamber, the egg. An avian egg has a protective, mineralized shell that limits microbial contamination of its contents and, due to its porosity, allows gaseous exchange between the external environment and the embryo. Distinctive features of the shell of bird eggs, compared with bone or teeth, are the nature of the mineral substance of the deposit – calcium carbonate in the form of calcite, as well as the absence of cell-directed assembly during its manufacture. Poultry egg shells are distinguished by their mechanical properties. In chickens, this thin mineral layer, about 0.3 mm thick, can withstand static pressure of more than 3 kg; its formation within 20 hours is one of the fastest known biomineralisation processes. Our knowledge of eggshell mineralization has advanced considerably over the past 10 years, thanks to the identification of the components of the organic matrix and the demonstration that they interact with calcium carbonate to determine its mineral crystallographic texture and hence the mechanical properties of this biomaterial.

Structure and composition of eggshells

Shells have a highly ordered and mineralized structure and form rapidly (< 20 hours for laying hens) at physiological temperatures (< 40 °C) [1–4]. Shell thickness, shape and size of the whole shell and its structural elements as well as features of the porous system vary among different species, but the general structure of eggshells is essentially the same in all birds [5, 6]. The shells of all avian eggs consist of the trigonal phase of calcium carbonate, calcite, which is its most stable polymorph at room temperature.

In the vast majority of bird species, eggshell weight is proportional to egg weight [7] and amounts to 10–11 % of egg weight. Chicken egg shells have been the most studied to date. It contains 1.6 % water, 3.3 to 3.5 % organic matrix if eggshell membranes are included and 95 % inorganic minerals. It consists mainly of calcium carbonate (98.4 % of its mineral content), which is permeated by the organic matrix, accounting for 2.3 % of the shell weight. In addition to calcium (37.5 %) and carbonate (58 %) (2, 3), phosphorus is also present in the outer part and in the cuticle (8). Moreover, numerous trace elements (e.g. magnesium, manganese, copper and zinc) are present throughout the shell. The shell of bird eggs consists of six layers, as shown in Fig. 1.1, 1.2, 1.4.