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Micromechanical properties of hen's eggshell determined by nanoindentation technique

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Abstract: The paper describes applicability of nanoindentation as a novel experimental technique for the assessment of eggshell quality based on the evaluation of its micromechanical response. Traditionally, only overall mechanical properties are evaluated for the whole eggshell. Thus, any microstructural phenomena cannot be disclosed and averaged mechanical behavior is measured. Particularly, values of elastic modulus *E* were monitored in five different locations along the eggshell's meridian line. Detailed maps of elastic moduli at particular eggshell cross-sections revealed high variations in local values of *E*-moduli at individual points, but not significant differences of their means at distant parts of the eggshell. It shows on the relative homogeneity of the eggshell structure. Mean values of *E*-modulus in different meridian positions did not vary significantly and ranged from 48 to 53 GPa. The micro-scale values found during the research run correspond to the values reported in literature and obtained on macroscopic samples. The nanoindentation technique proved to be a suitable, easy-to-use, and powerful tool for assessing local variations of mechanical properties of a hen's eggshells.

Keywords: Nanoindentation, Hen's eggshell, Micromechanical properties, Modulus of elasticity

1. Introduction

The selection of hen's breeds with eggshells that are mechanically resistant to stresses imposed e.g. during transportation requires proper knowledge of the eggshell strength and stiffness under different stress states. Traditionally, the mechanical properties are assessed on macroscopic level, i.e. on the level of the whole eggshell. For example, elastic properties are often characterized by the static [1] or dynamic modulus of elasticity [2,3]. It was proven that the eggshell quality measured by strength or stiffness depends on many factors such as breeding conditions [4], the hen breed [5], diet [6], egg shape [7,8], and other parameters.

It is also obvious that the overall mechanical properties are influenced by the material microstructure. Eggshell microstructure influences critical phenomena,

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such as hatchability [9]. However, every microstructural phenomenon cannot be disclosed by means of macroscopic tests. Therefore, novel experimental techniques like nanoindentation may help to find out further material properties and dependencies.

Nowadays, the nanoindentation is widely used also for analyses of biological materials. The determination of hardness, elasticity, or fracture toughness through the direct measurements using sharp diamond indenters, such as Vickers, Knoop, Berkovich, or cube corner, can be an alternative to more traditional testing techniques [10]. Recently, these techniques are used with success in studying the mechanical characteristics of biomaterials and hard tissues [11,12]. Simplicity and expediency of the indentation technique make it attractive for the determination of micromechanical properties even of a hen's eggshell [13]. These approaches also potentially allow the characterisation of both local and bulk hardness, stiffness, and fracture properties.

The presented paper introduces new experimental results from nanoindentation using the Berkovich tip. The modulus of elasticity of a hen’s eggshell was determined as the main parameter, indicating the local microstructural quality. The experiments were performed on samples extracted from five different locations on the eggshell; maps of elastic moduli at individual positions (across the eggshell cross-section) were created along its meridian. Obtained values correlated well with macroscopic ones.

2. Materials and methods

2.1. Samples

Eggs (the *Hisex Brown* strain) were chosen for the experiment. Hens were kept in cage technology in a commercial breeding farm in the Czech Republic. Eggs were collected from hens that were 75 weeks old. Defective eggs were sorted and not included in the experiment.

2.2. Sample preparation

The eggshell fragments were extracted from five different locations: S1, S2, E, B1, and B2, see Fig. 1 (where "S" denotes "sharp end," "E" denotes "equator," and "B" denotes "blunt end"), and were embedded into metacrylate tablets. In order not to thermally affect the structure, the specimens were cold-prepared. Commercially available two-component resin was used for metacrylate mixture preparation and the specimens were left to dry and cure for 8 hours. The tablets were polished in order to achieve a flat surface with a maximum roughness of 10–20 nm.

2.3. Instrumentation and experimental methodology

All experiments were performed with nanoindenter Nanohardness tester by CSM Instruments equipped with the Berkovich tip (Fig. 2). The eggshell fragments were indented in directions perpendicular to the cross-sections. The load-controlled test was performed using the standard trapezoidal loading diagram as follows: linear loading (72 mN/min) up to the peak force (12 mN), then a 20 s holding period at the maximum force and linear unloading (72 mN/min) to zero force level. Selected locations were indented with rectangular grids of indents covering the whole eggshell thickness. Each grid contained 9×24=216 indents with mutual distances of 15 μm in both perpendicular directions. Elastic constants were extracted from unloading, assuming purely elastic behavior at this stage (Fig. 3) and using semi-analytical elastic solution [14]. Only indents lying on the eggshell were considered for the final results and statistics, based on high-magnification optical imaging (i.e. indents to metacrylate were excluded).

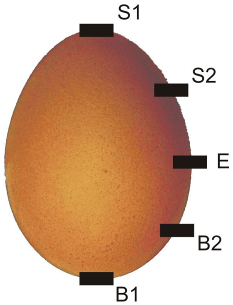

Fig. 1. Tested cross sections on the eggshell.

Fig. 2. Nanoindeter Nanohardness tester (CSM, Switzerland).

The estimate of Poisson's ratio is needed prior to the evaluation of *E*-modulus [14]. The value of Poisson's ratio ν=0.345 was adopted from measurements described in [8] and [13], where similar eggshells were used. Comparable values of ν, e.g. 0.307 in Lin et al. [15] were reported by other authors. Nevertheless, the sensitivity of the results of *E*-modulus on ν within the aforementioned range 0.3-0.35 is low.

Each indent was analyzed separately and a map of elastic moduli over the area of interest was constructed from these individual results. Although high variation in E-modulus appears in the eggshell cross-section, average values and standard deviations of E were computed for comparison purposes as shown later in this paper.

3. Results

Similar loading curves varying according to the local material hardness were obtained from grid nanoindentation. A typical example of a loading diagram is shown in Fig. 3. The chosen maximum load level (12 mN) revealed in the maximum penetration depths fluctuating around $h \approx 500$ nm. It is worth noted that evaluated properties are homogenized properties received from the influence zone under the indenter tip, which can be estimated as $3 \times h$ for the Berkovich indenter [16]. It yielded the affected volume from which local properties are extracted to be around 1.5 μm in depth.

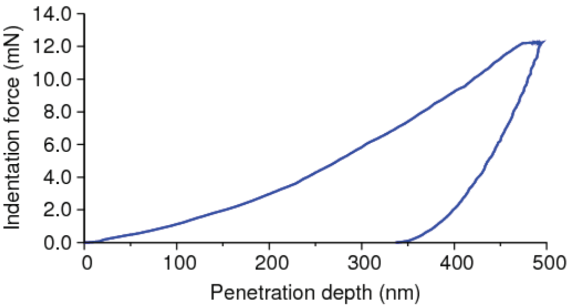

Fig. 3. Typical loading diagram received from the nanoindentation test (force vs. depth).

Elastic moduli averaged from all considered measurements in specific locations are summarized in Table 1. The values obtained from edges, membrane, inhomogeneities and pores were excluded from the statistical analysis. The overall view (≈200× magnification) on the eggshell cross-section with visible indents is shown in Fig. 4 (here, the equator is taken as an example). The circles surrounding the shell at the top and bottom of the picture represent undissolved particles of metacrylate matrix. The mammilary layer of the eggshell is visible in the upper part of the picture as an irregular dark zone. The black dots in the central part of the picture correspond to individual imprints in the grid.

The mean values of E ranged from 48 to 53 GPa. They are in accordance with values reported by Nedomová et al. [8], where macroscopic experiments were performed using similar eggshells. Nedomová et al. [8] found that elastic constants are independent on the egg shape as well as loading force orientation (egg loaded either on the equator and/or egg-tip). This conclusion was also supported by numerical simulations. Severa et al. [13] performed a series of nanoindentation tests in two different loading directions and found an isotropic nature of the eggshell structure. Although the mean values of E (detected by several approaches) were reported, the detailed distribution and differences in elastic moduli values at different locations over the cross-section were not established in these studies. In this context, it is clear that nanoindentation plays an indispensable role in the identification of the local variations in mechanical properties.

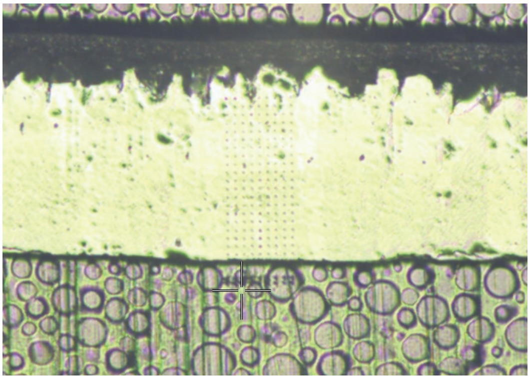

Fig. 4. Typical position of the indentation grid at the eggshell's cross-section (sample E).

Table 1. Overall results of elastic moduli *E* (GPa) on specific eggshell locations

Location/grid	S1	S2	E	B1	B2
Mean *E*	52.981	50.565	50.484	48.275	50.195
Std. dev.	6.360	5.891	6.115	5.268	6.054
Min. *E*	30.170	27.664	23.695	33.036	23.210
Max. *E*	81.702	64.153	72.526	63.812	65.460
Number of indents	162	168	180	162	162

The maps of elastic moduli for all monitored locations are given in Fig. 5, at which the bottom left corner (point 0,0) of the map starts at the exterior eggshell surface (cuticle) and the top end is located close to the mammilary layer. The macroscopic measurements performed by Lin et al. [17] indicate that mechanical properties show a certain degree of variability when measured at different locations on the same egg. This conclusion was generally confirmed by the presented research (see Fig. 5 and Table 1), giving much more precise information on the local variations in *E*. Nevertheless, the detailed maps resulting from aforementioned indentation procedure did not reveal any general trend of *E* increasing or decreasing across eggshell cross-sections and the variation seems to be randomly distributed (Fig. 5). It is evident that all the maps in Fig. 5 are characterized by more or less random heterogeneity and the values of elastic moduli are dependent on other parameters, but not the location. It is supposed that chemical composition, especially calcium content on the local level, may affect the micromechanical properties and thus play a significant role. Comparative data, however, is not available at this moment and confirmation or disproof of this hypothesis will be a subject of future research.

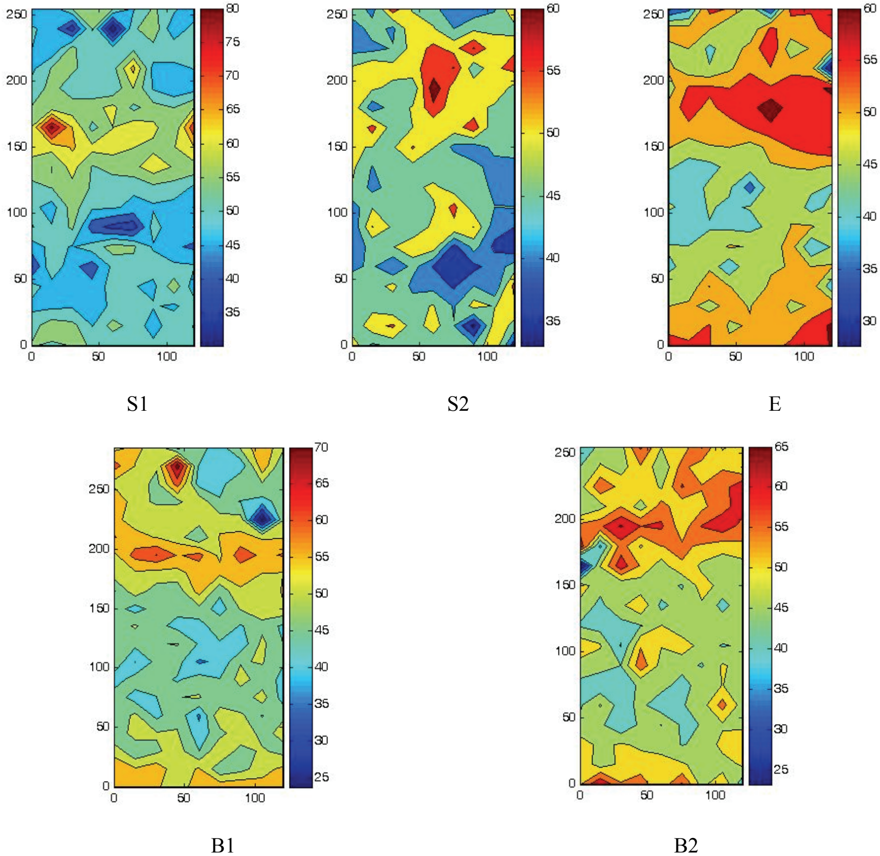

Fig. 5. Maps of elastic moduli on particular eggshell sections (axial coordinates are shown in μm, color scale of *E*-moduli is in GPa).

4. Conclusions

It was shown in the paper that nanoindentation offers a precise tool for determining micromechanical properties in the scale of nano/micrometres and can directly access individual variations in these properties on samples with biological nature. So far, no other current technique is able to give such a detailed and accurate description of the properties. The *E*-modulus maps created for five different locations positioned along the eggshell meridian line indicate that there is no clear correlation between the position on the eggshell and *E*-modulus value. Moreover, the local variations in *E* modulus seem to be randomly distributed over the eggshell cross section. On the other hand, mean values of elastic moduli assessed by nanoindentation correspond to values detected by traditional methods. Nevertheless, the presented approach offers a much closer view into the micro-level and properties of individual structural layers and components. This knowledge can serve both animal breeders and technologists. Further research on the correlations of the local stiffness with the microstructural composition and/or dependency on the food/breeding factors are planned for the future.

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