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Behaviour of eggshell membranes at tensile loading

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The aim of this paper was to study the mechanical behaviour of the eggshell membrane using tensile tests at different loading rates. The eggshell membrane was obtained from commercial breeding lines of Japanese quails (*Coturnix japonica*). Samples were cut out of the membrane in latitudinal direction. TIRAtest 27025 tensile testing machine equipped with a 200 N load-cell was used. Tensile deformation exhibits both non-linear as well as linear region. The dependence of the stress on the strain in non-linear region can be described using of the Mooney-Rivlin equation. Linear region corresponds to the elastic strain. Parameters of the used equation are dependent on the strain rate. Generally, the strength of the eggshell membrane increases with the strain rate.

***Key words**:* Eggshell membrane, tensile loading, loading rate, stress, strain strength

INTRODUCTION

The eggshell membrane is a tissue found between the calcified eggshell and the albumen of eggs. This structure is a thin, highly collagenized fibrous membrane comprising inner (in contact with the albumen) and outer layers. It is mainly formed by types I, V and X collagen, making up 88 – 96 % of its dry weight. The presence of other proteins, such as osteopontin, sialoprotein and keratin, has also been reported [1]. The biologically active of the eggshell membrane is essential for the formation of the egg, retaining the albumen and preventing the penetration of bacteria [2]. The eggshell membrane also affects the eggshell strength [3]. Even if there are many reports on the use of the eggshell membrane, e.g. in the recovery of gold from waste water [4,5] not much information is known about its physical and structural properties, such as the pore and mechanical characteristics of the membrane. The only exception represents the paper of Torres et al. [6] which is focused on the study of hen`s egg membranes under tensile loading and nanoindentation.

The present paper deals with the mechanical behaviour of the eggshell membrane of quail's eggs using tensile tests at different loading rates. The knowledge of these properties is very useful namely at the study of the egg changes during its storage and at the numerical simulation of the egg loading [7].

EXPERIMENTAL

The eggshell membrane was obtained from commercial breeding lines of *Japanese quails (Coturnix japonica)*. The outer membranes were carefully removed using clamps and washed with distilled water. The membranes were then stored in physiologic saline solution in order to avoid dehydration. Samples were cut out of the membrane in latitudinal direction. TIRAtest 27025 tensile testing machine equipped with a 200 N load-cell was used. Rectangular samples (15 mm x 15 mm) were used for the measurements. It means the initial length of the specimen $l_0 = 15$ mm. The thicknesses of the membranes (around 50 μm) were obtained from digital micrometer. Specimens were glued to thin metallic plates – see Figure 1.

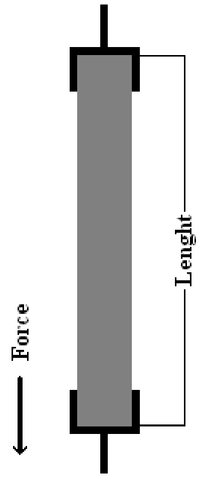

Fig. 1. Schematic of tensile test experiment and attached specimen.

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The deformation of the sample was assumed to be equal to the separation of the crossheads. The force *F* and the deformation $\Delta l = l - l_o$, where *l* is the instantaneous specimen length at the time *t*, are measured during tension and both quantities are recorded. The force-deformation data may easily be transformed into normalized quantities such as stress and strain. The Cauchy strain and Hencky's natural or true strain are of common use in representing compression curves. The Cauchy strain measure gives the relative deformation with respect to the initial sample length

$$\varepsilon_C = \frac{l - l_o}{l_o} = \frac{\Delta l}{l_o}.$$

Hencky's strain (often denoted as 'true' strain) derives from the integration of the infinitesimal strain and is given by

$$\varepsilon_H = \ln\left(\frac{l_o + \Delta l}{l_o}\right) = \ln(1 + \varepsilon_C).$$

The conversion of the force *F* into engineering stress is simple given by

$$\sigma_u = \frac{F}{A_0},$$

where A_o is the cross section of the undeformed specimen. In order to obtain some information on the true stress an assumption on the material incompressibility is often used. The true stress is than given by

$$\sigma_t = \sigma_u\left[(1 + \varepsilon_c)\right].$$

The specimen deformation is also described using the stretching parameter, λ, which is defined as

$$\lambda = \frac{l}{l_o} = 1 + \varepsilon_C.$$

The transformation force-deformation data into quantities given above have been performed using of MATLAB software. Four speeds, *v*, were used: 1, 10, 100 and 800 mm.min^{-1}. Loading rate can be converted to the strain rate:

$$\frac{d\varepsilon}{dt} \equiv \dot{\varepsilon} = \frac{v}{l_o}.$$

The corresponding values of strain rates are: 0.00111; 0.0111; 0.111 and 0.888 s^{-1}. Experiments were performed at the room temperature.

RESULTS AND DISCUSSION

It is shown in Figure 2 an example of the dependence of stress on the strain. The qualitative features of this dependence are the same for all used speeds. These curves are similar to those of other membranes as reported e.g. in Torres et al. [6], in which three different regions were found. In the first region (the toe region), little stress is needed to elongate the membrane. The second region is called the hill, and the stiffness of the membrane increases with elongation. Finally, a linear dependency is shown in the third region. The nonlinear dependence can be explained in terms of eggshell membrane microstructure [6].

The behaviour of the toe and hill regions of the eggshell membrane was modelled by using the Mooney-Rivlin equation [8]. According to this theory the strain-dependent behaviour can be represented by the Mooney-Rivlin hyperelastic potential, U_{MR},

$$U_{MR} = C_1(I_1 - 3) + C_2(I_2 - 3),$$

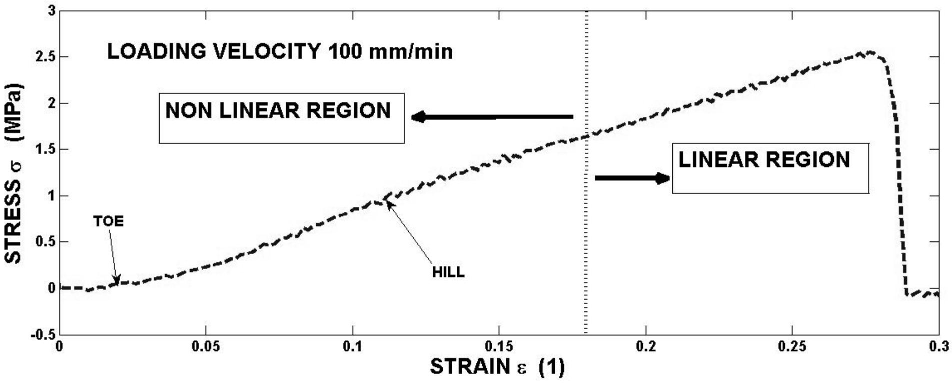

Fig. 2. Example of stress-strain dependence.

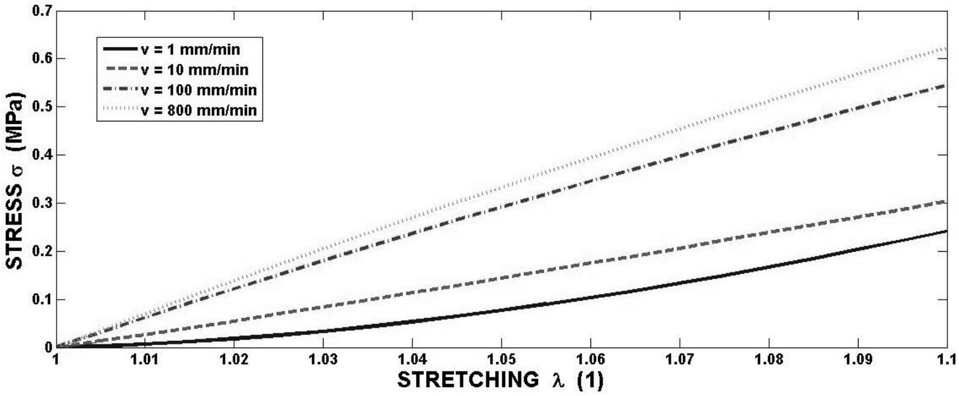

Fig. 3. Mooney-Rivlin model of the stress-strain dependence.

where C_1 and C_2 are material constants with dimensions of stress and:

$I_1 = \lambda_1^2 + \lambda_2^2 + \lambda_3^2 \qquad I_2 = \lambda_1^2\lambda_2^2 + \lambda_2^2\lambda_3^2 + \lambda_1^2\lambda_3^2$ λ_1, λ_2, λ_3 are the stretch ratios in the three principal axes. For uniaxial loading the stress is expressed as:

$$\sigma = \left(\lambda - \frac{1}{\lambda^2}\right)\left(2C_1 + \frac{2C_2}{\lambda}\right), \quad (1)$$

where λ is the stretch ratio in the direction of load. Values of the constants C_1 and C_2 are given in the Table 1. The dependence of stress on the stretching is displayed in the Figure 3. It is evident that these parameters are dependent on the loading velocity. At low strain levels the behaviour is modelled with Equation (1) until the strain is about 10 %. Beyond that point, the sample behaves as a Hookean material. Linear part of the stress – strain dependence enables to evaluate of the Young modulus, *E*. Values of this material parameter are given in the Table 2. The values of this parameter which describes the elastic properties of membranes are independent on the loading rate. This is in agreement with results reported in Bing et al. [9] but in disagreement with the conclusions found by Torres et al. [6] for the eggshell membrane also affects the eggshell strength of the hen`s eggs. The Mooney-Rivlin relation is typically used for the study of rubber elasticity. Rubber is an elastomer formed by a network of cross-linked polymer chains and the deformation of its chains has an entropic origin. The evidence shown here might indicate that, as in the case of collagen molecules and fibrils, the initial deformation of the eggshell membrane has an entropic origin.

Table 1. Parameters of Mooney-Rivlin model.

Loading rate	$2C_1$, MPa	$2C_2$, MPa	R^{2*}
1 mm/min	13.320	-13.1200	0.9933
10 mm/min	5.966	-4.6370	0.9840
100 mm/min	3.787	-0.2228	0.9896
800 mm/min	3.632	-0.1364	0.9929

*R^2 is the correlation.

Table 2. Young modulus of elasticity E.

Loading rate	Min E, MPa	Mean E, MPa	Max E, MPa	Standard deviation
1 mm/min	9.23	9.618	9.89	0.27105
10 mm/min	9.67	9.804	9.93	0.10164
100 mm/min	9.35	9.728	9.91	0.23499
800 mm/min	9.63	9.718	9.82	0.09039

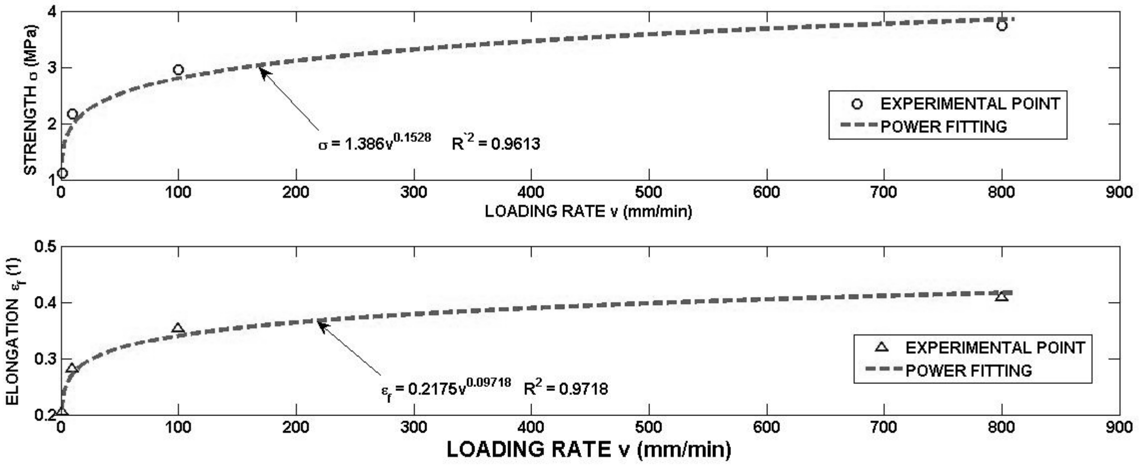

Fig. 4. Effect of loading rate on the fracture parameters of the eggshell membranes.

Table 3. Fracture parameters – ultimate tensile strength, maximum of elongation, strain energy density.

Loading rate	Ultimate tensile strength, MPa			
	Min	Mean	Max	Standard deviation
1 mm/min	1.05	1.11	1.15	0.040
10 mm/min	2.09	2.16	2.24	0.055
100 mm/min	2.80	2.95	3.08	0.106
800 mm/min	3.57	3.74	3.91	0.140
Loading rate	**Elongation [1]**			
	Min	**Mean**	**Max**	**Standard deviation**
1 mm/min	0.184	0.204	0.247	0.0248
10 mm/min	0.242	0.281	0.311	0.0255
100 mm/min	0.339	0.352	0.373	0.0139
800 mm/min	0.387	0.408	0.421	0.0130
Loading rate	**Strain energy density, J/m^3**			
	Min	**Mean**	**Max**	**Standard deviation**
1 mm/min	76874.22	91869.40	128544.590	20844.65
10 mm/min	340874.07	362883.64	376454.469	13293.23
100 mm/min	389065.19	462359.97	486152.422	41308.33
800 mm/min	664204.87	686757.33	704406.124	15044.17

In the next step the values of ultimate tensile strength has been evaluated together with maximum elongation ε_f. The volume density of work, W, up to the membrane fracture has been also determined using of the relation:

$$W = \int_0^{\varepsilon_{ff}} \sigma d\varepsilon.$$

The values of these properties are given in the Table 3. The ultimate tensile strength increases with the loading rate as well as the value of the maximum of the elongation – see Figure 4. The dependence of these parameters on the loading rate can be fitted by a power function. Parameters of this function are presented in the Table 1 together with the correlation coefficient R^2.

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ОТНАСЯНИЯ НА МЕМБРАНАТА В ЯЙЧЕНАТА ЧЕРУПКА ПРИ ЕДНООСНА ДЕФОРМАЦИЯ НА ОПЪН

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(Резюме)

Целта на настоящата работа беше да се изучи механичното поведение на мембраната в яйчена черупка при деформация на опън с различни скорости. Мембраната беше получена от търговски породи на японски пъдпъдъци (Coturnix japonica). Образците бяха нарязани по ширината на мембраната. За експериментите беше използван динамометър TIRAtest 27025, снабден с клетка за натоварване 200 N. Деформацията на опън се характеризира с линеен и нелинеен участък. Зависимостта на напрежението от относителната деформация в нелинейния участък може да се опиши посредством уравнението на Мууни-Ривлин. Линейният участък съответства на еластична деформация. Параметрите на използваното уравнение зависят от скоростта на деформация. Като правило силата на разрушаване се увеличава при увеличаване на скоростта на деформация.