PROPERTIES OF FRESH AND FROZEN FISH SKIN AT CYCLIC LOAD

Josef ZEMAN[1], Jan SEDLÁČEK[1]

[1]Department of Physics, Faculty of Engineering, Czech University of Life Sciences Prague, Kamýcká 129, Praha 6, 165 21 Prague

Abstract
Our paper deals with the methodology for determining of changes in rheological properties during various technological adjustments of biological structures. An example of the methodology is performed on the deformation curves during the cyclic tensile load in the elastic region. With the number of cycles, the absorption capacity of mechanical energy decreases exponentially during increasing and decreasing of the load. We observed the final value of the percentage of mechanical energy that the skin is able to absorb and the number of cycles that lead to this value. The measurements were made on a sample of freshly decapitated fish and the fish after quick freezing and thawing. We found the effect of this technological process on both the absorption capacity of mechanical energy and the number of stabilization cycles. We also found a strong positive correlation between the number of relaxation stabilization cycles and the thickness of fish skin.

Key words: *absorption energy; relaxation time; rheological properties.*

INTRODUCTION

The deformation of biological samples often results in the absorption of deformation mechanical energy. The value of this energy is determined as the area of the hysteresis loop, after measuring the curve of loading and unloading. It turns out that when repeating a load cycle, this area (and thus the absorbed energy) decreases, up to the finite non-zero value. This relaxation process can be described as a decreasing exponential function. When we fit the exponentials to the gradually decreasing surfaces of the hysteresis loops, we determine the number of relaxation cycles $\tilde{n}$. (Then we relax only 1/e changing parts of the hysteresis loop.) In practice, this relaxation process is often underestimated. This transition state is omitted in many works (see e.g. *Hadraba, Janacek, Filova, Lopot, Paesen, Fanta, Jarman, Necas, Ameloot & Jelen, 2017*) and the properties of the already relaxed material are evaluated. Experimenters tends to determine the number of load cycles, which are used for evaluation, completely arbitrarily. There are two primary reasons for absorbing mechanical energy when deforming biological materials. The first of these mechanisms is wringing (see e.g. *Fransson & Stading, 2003*). This mechanism consists in the fact that the formation of mechanical stress releases the water molecules that are present in the structure. These free molecules pass through the tissue that forms the structure of the biological material. In this process, hydrogen bonding arise and disappear continually. After each release of the bond, a part of the mechanical energy changes to a disordered movement of water molecules. Thus, as the entropy increases, the temperature of the material is increased. The second mechanism of mechanical energy absorption is the release of tertiary bonds of proteins and polymers that are present in the structure. Superfast freezing (*Dinu, Ozmen, Dragan & Okay, 2007*) is a process that is gentlest of all biomaterial storage processes, but it disrupts both types of bonds. It can therefore be expected, that its consequences will be identifiable by this method. The aim of this study is to demonstrate the usefulness of the proposed methodology on the example of determining rheological changes occurring in fish skin under rapid technological freezing.

MATERIALS AND METHODS

A sample of skin 3 cm length (beginning at 2 cm behind the gill arch), 1 cm width and *d* thickness, was attached in the jaws of the DEFORM02 deformation device from the firm of Pemar. The measuring cycles were defined as follows for the material used: The maximum force of 2 N (generated by the sample) was achieved at a constant rate of 0.1 mm / min. Immediately thereafter, the force in the sample was reduced to 0.1 N at the same speed. Again we increased the force to 2 N and decreased the force to 0.1 N at the same speed. We used adult individuals to measure. There were four species: Erpetoichtys calabricus (3 tested fish) - further species A, Gnathonemus petersii (3 tested fish) -

further species B, Oncorhynchus mykiss (8 tested fish) - further species C and Squalius cephalus (3 tested fish) - further species D. The fish sample of skin was always taken from its right flank immediately after being killed. The preparation of the frozen samples was as follows. The fish was shock-frozen and left for 14 days at -40 °C. The fish was taken from the freezer and left at 25 °C for 8 hours. Equally large sample of skin was taken from left side of fish as in the case of fresh samples. Both types of samples were mechanically strained in an isotonic bath of Ringer's solution. The stress was carried out with the above-described 8 cycles at 35-38 °C. The resulting hysteresis loops of the individual cycles were analyzed as follows. For the measured absorbed energy E_i in the *i*-th cycle:

$$E_i = E_i^{UP} - E_i^{DOWN} = \int_{l\,(F=0.1\,N)}^{l\,(F=2\,N)} F(l)\mathrm{d}l - \int_{l\,(F=2\,N)}^{l\,(F=0.1\,N)} F(l)\mathrm{d}l, \quad i \in \{1,\dots,8\}, \qquad (1)$$

E_i^{UP} – the energy supplied to the sample as it is stretched,
E_i^{DOWN} – the energy taken from the sample as it is shortened,
$l(F)$ – the length of the sample corresponding to the force F,
$F(l)$ – the force induced by the sample with length l in the given direction of deformation.

In this way, we obtain a series of eight absorption energies. The sequence of this series, as mentioned above, can be approximated by the following function:

$$E(i) = E_0 - (E_0 - E_\infty)e^{-i/\tilde{n}}, \qquad (2)$$

$E(i)$ – the approximated absorption energy in *i*-cycle,
E_0 – absorption energy of the initial deformation,
E_∞ – the residual absorption energy required to deform the sample, in the order of the endless cycle of deformation,
$\tilde{n}$ – the number of relaxation stabilization cycles (see Introduction).

The fitting function $E(i)$ to values of E_i was realized by Solver of Excel 2010. Subsequent statistical processing was performed in the R application (ver. 4.3.0).

RESULTS AND DISCUSSION

First, we present a typical example of a measured hysteresis loop - see Fig. 1, from which the value of energy absorbed in one particular cycle was calculated according to (1).

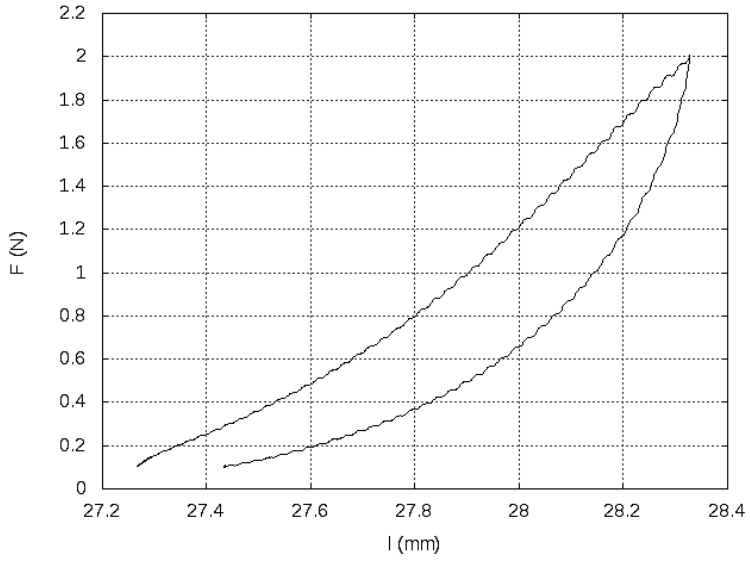

Fig. 1 Third loading and unloading cycle of the fifth sample of species C

If we plot the energies absorbed in eight consecutive cycles, we get the result shown in Fig. 2. The found exponential dependence, that approximates the measured data with equation (2), is shown in this figure too.

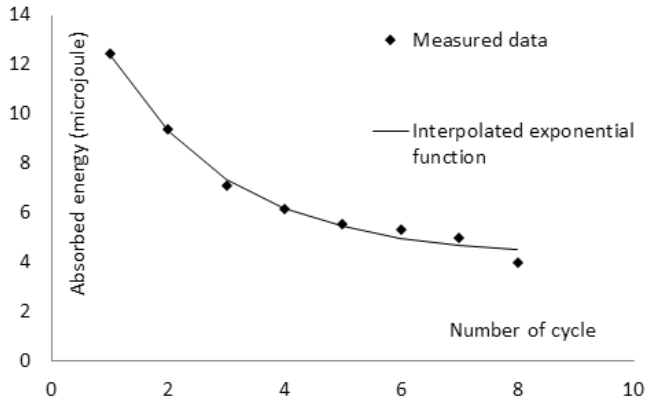

Fig. 2 The energies absorbed in each cycle of the fifth sample of species C

Based on the Kolmogorov-Smirnov test (= 0.65) we were forced to reject the hypothesis of the normality of all the examined quantities. Therefore, no average values are in Tab. 1, but medians of measured values.

Tab. 1 Medians of parameters of individual measured fish species (fresh samples)

Species of fish	$\hat{d}$	$\widehat{E_\infty}$	$\widehat{E_0}$	$\hat{\tilde{n}}$
	mm	%	%	
A	0.56	37.7	87.3	2.6
B	0.05	18.9	32.0	3.3
C	0.33	7.8	29.2	16.6
D	0.07	14.5	18.9	19.0

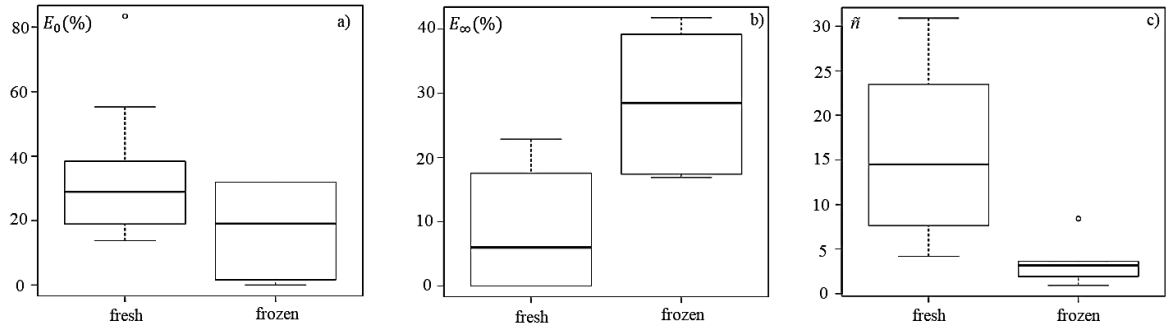

Fig. 3 Comparison of fresh and frozen samples values

A comparison of the fresh and frozen samples is shown in Fig. 3. This statistical comparison was conducted by Wilcoxon unpaired test. We found that the values of the examined parameters $\tilde{n}$, E_∞ a E_0 differ at the 10% level of significance for all species, even for our small number of samples (E_0 p-value = 0.1078, E_∞ p-value = 0.004891, $\tilde{n}$ p-value = 0.01198). The Kruskal-Wallis test found significant differences also among all four fish species. Subsequently, a moderately strong correlation was found between sample thickness d and the number of relaxation stabilization cycles $\tilde{n}$. Pearson correlation coefficient r = -0.3433 only and Spearman's rank correlation coefficient ρ = -0.3904. This indicates a moderately strong, slightly non-linear negative correlation. Further results (dependence of the number of relaxation stabilization cycles $\tilde{n}$ on the thickness d of the fish skin sample) can be seen in Fig. 4.

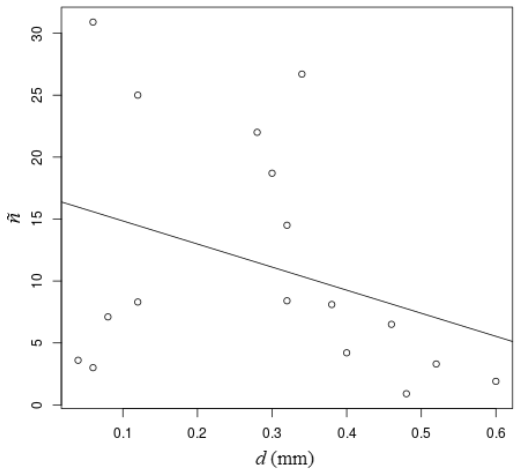

Fig. 4 Dependence of the number of relaxation stabilization cycles $\tilde{n}$ on the thickness d of the fish skin sample

DISCUSSION

On the example of the use of our methodology, we have shown the effect of freezing on the examined mechanical parameters for all examined species A, B, C, D Thus, there appears to be structural changes during the freezing of the fish skin, and the flow of tissue fluid through the microporous medium is increasing. Furthermore, it appears that significant differences among the values of the quantity $\tilde{n}$ exist for individual examined species. Mutual ratio between the energies E_∞ and E_0 is also significantly different for each species. This illustrates the structural differences of skin among individual fish species. The proven negative correlation between $\tilde{n}$ and d indicates that thick skin (probably due to higher collagen content) relaxes at a lower number of load cycles.

CONCLUSIONS

We managed to describe a method of how to effectively use the first load cycles to describe the rheological properties of a material. With this method, not only the ratio between elastic and plastic deformation can be obtained, but also the number of relaxation cycles can be determined. We applied the method to the case of fish skins distribution. It turned out that our method is applicable both to differentiation of individual fish species and to indicating of technological treatments (freezing fish meat).

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Corresponding author: Mgr. Josef Zeman, Ph.D., Department of Physics, Faculty of Engineering, Czech University of Life Sciences Prague, Kamýcká 129, Praha 6, Prague, 16521, Czech Republic, phone: +420 22438 3317, e-mail: zeman@dzeta.cz