

**SEM APPLICATIONS FOR THE STUDY OF MODIFICATIONS
IN WOOD CELL MEMBRANE**

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Abstract

Scanning Electron-Microscopy (SEM) is a modern, non-invasive method for objective and specialized image analysis of anatomical material features at microscopic level. Referring to wood, it offers the possibility to view in 3D a bunch of neighboring cells, in all three grain directions. This allows the imaging of modifications that might appear in the structure of the wood cell membrane (e.g. micro-fissures) caused by different factors, including thermal treatments. This paper presents the results of the SEM analysis performed on European spruce wood (Picea abies) samples, subjected to different thermal treatments, including freezing, heating and drying. The main aim of this research was to reveal the conditions which determine the occurrence of micro-fissures and consequently strength losses in wood.

Key words: SEM, wood cell membrane, micro-fissures, imaging

1. INTRODUCTION

Wood is a heterogeneous anisotropic and hygroscopic material, characterized by a high variability of its properties, which vary not only from one species to another, but even within the same species from one tree to another, even within the same tree over its height and its section and even within the same piece of wood, depending on the grain direction (perpendicular or along the wood fibers).

Wood is made of vegetable cells (Fig. 1), which result from cambium cells, situated between the bark and the wooden tissue. Each cell is composed of the cell wall (also called cell membrane) and an empty space called cell lumen.

The cell membrane has a layered structure being composed of two walls:

- the primary wall (P) is made of microfibrils (3-6 lamellae), which are oriented more or less transverse to the cell axes. It is very thin and strongly lignified;
- the secondary wall (S) can be 10 times thicker than the primary wall and it comprises 3 layers:
 - the outer layer (S1) – a 0.076 – 0.35µm thin layer, consisting of few lamellae with the microfibrils wound helically around the main axes of the cell wall;
 - the middle layer (S2) – which can reach a thickness of 5µm or more and is a compact zone, as it contains more cellulose than lignin;

- the inner layer (S3) - with a thickness of 0.05 - 0.08 μm .

Two neighboring cells are bound by a strongly lignified connecting layer (L), with ca. 2mm thickness (Fig. 1).

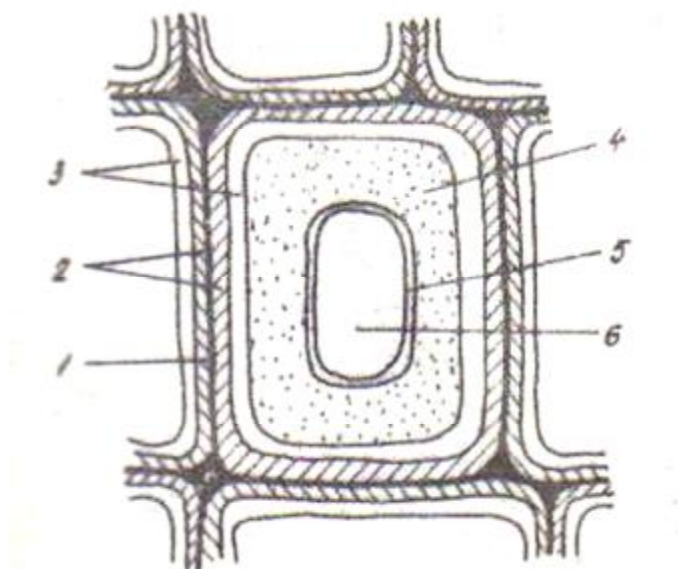


Fig. 1. Cross section of a wood cell:

- 1 - connecting layer (L)
- 2 - primary wall (P)
- (2+1+2 – middle lamella)
- 3 - outer layer (S1)
- 4 - middle layer (S2)
- 5 - inner layer (S3)
- (3+4+5 – secondary layer (S))
- 6 - lumen

During each growth period, a new concentric annual ring or growth ring is formed in the tree. Over its width, two separate zones can be distinguished even by the naked eye, due to the slight colour difference: the earlywood strap (formed in spring) and the latewood strap (formed in summer).

The dimensions of the anatomical constituents are slightly different in the two zones. Thus, the cell lumen diameter in tracheids of resinous wood is: 12.1...61.6 μm in earlywood and 6.4...28.8 μm in latewood, depending of wood species. Cells produced at the beginning of the growing season are commonly larger with thinner cell walls, and so this earlywood appears less dense than the latewood produced towards the end of the season.

Two main categories of wood species are distinguished according to their anatomical features: resinous wood species – mainly composed of tracheids – and hardwood species – with a more complex structure, composed of several types of cells (fibers, vessels, rays etc.).

Considering that the topic of the present paper refers to resinous wood, Fig. 2 presents a 3D image of a representative species of this regnum, namely eastern spruce (Perre 2007).

The European spruce wood (*Picea abies* L.) comprises 93 – 95% tracheids, 5 -7% medullary rays and 0,2 - 0,3% resin canals. At microscopic level, these components have the following dimensions (Wagenfuhr 2000):

- axial tracheids:
 - thick cell walls (two walls) of 1.9...3.5...4.9 μm , in earlywood and 9.3 ... 10.7 ... 11.6 μm in latewood;
 - lumen thickness of 16.0...32.0...45.0 μm in earlywood and 6.4...17.4...22.0 μm in latewood;

- lumen length of 1300...2800...4800 μm
- proportion of lumen 94.5...95.3...96.5%;
- radial tracheids:
 - height: 100...150...190 μm (3...20 cells);
 - width: 8-14 μm (4...7 cells);
 - proportion: 4.4...4.7...5.5%
- resin canals: with diameter of 30...150 μm .

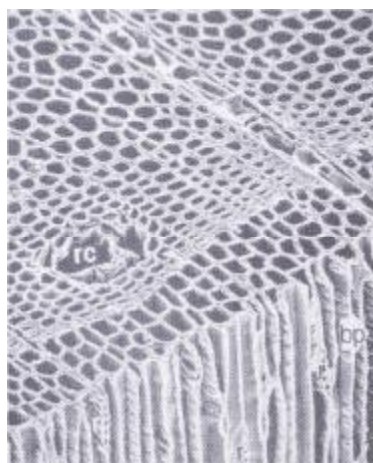


Fig. 2. SEM 3-D picture of eastern spruce wood.

Wood also contains a considerable amount of water. In green wood, moisture is located both in the cell walls and in the cell cavities (lumena), in liquid and gaseous (vapour) form and it is bound to the wooden substance by four different types of bonding forces. The water located inside the cell lumen is called “free water”, as it is retained by relatively weak mechanical forces, which can be destroyed even without heat supply. The water located inside the cell membrane is called “bound water”, as it is retained by stronger forces, of chemical and physical nature, which can be destroyed only when heating wood

2. THEORETICAL BACKGROUND CONCERNING INTERNAL STRESSES GENERATED IN WOOD BY FREEZING

Internal stresses are generated in wood by various factors: they may occur due to growth conditions, drying, but also due to climate changes which modify the state of water in wood. When the stresses exceed the rupture limit, wood cracks (Fig. 3).

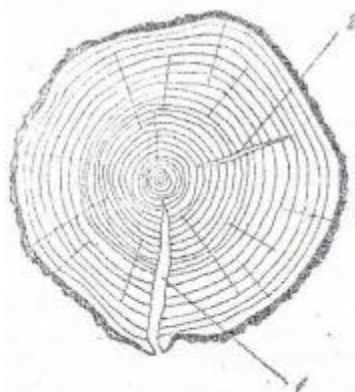


Fig. 3. Frost crack (Pescarus and Cismaru 1979).

1- opened frost crack, 2- closed frost crack

During winter, in temperate climate zones, when outside temperatures drop frequently below 0°C, logs and timber stored in an open yard freeze.

According to the present state of knowledge (Cividini 2001; Marinescu 1980), only free water freezes in wood if the temperature does not drop below -30°C. The transformation of liquid water into ice inside the cell lumen develops a certain pressure upon the cell walls. It can be assumed that the generated mechanical energy is capable of breaking some bonds between the bound water molecules and the wooden substance, “squeezing” out a certain amount of water from the cell walls and re-locating it into the cell lumena. Due to this compressive stress exerted by the expansion of liquid water into the lumen, it is most likely that certain micro-fissures occur within the cell wall structure, diminishing thus the mechanical properties of wood (Ilic 1995).

Mishiro (1990) showed that the freezing of water in different Japanese species, like balsa, sugi, katsura, karamatsu and keyaki, treated at -196...-20°C for 15...45 minutes, is accompanied by cracks, which occur in the cell membrane and that their severity is influenced by the initial moisture content of wood, its density, by the negative temperature and the duration of exposure.

The thawing process plays also an important role in generating internal stresses in wood, especially when wood is subjected to a sudden temperature change, like when frozen wood is introduced in a drying kiln at temperatures above +30°C. Heating of frozen wood differs essentially from wood that contains only water in liquid form. Experience shows that during thawing, the temperature of an arbitrary point changes according to different waved curves depending on its position within the cross section. Thus, in the first stage of the process, an arbitrary point of the body increases its temperature rapidly from baseline (negative) to 0°C. In the second stage, it remains constant (close to 0°C), when water in the outer area (relative to the considered point) goes from solid to liquid. In the third stage of the temperature begins to rise again, tending to ambient temperature (for heating water and saturated steam) or cooling the temperature limit (Marinescu 1980)(Fig. 4).

The question is: how severe conditions induce such strong stresses that wood cracks?

In order to answer this question, a research was initiated concerning the structural and property modifications which occur in spruce wood when being subject to various climate changes.

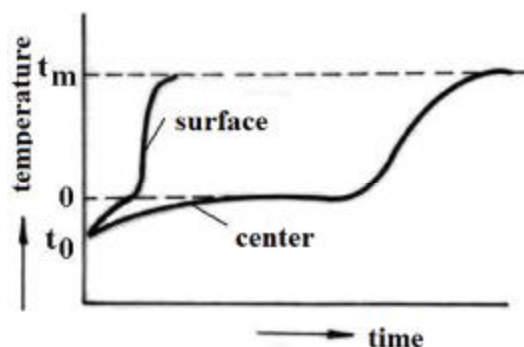


Fig. 4. Temperature variation curves during the thawing process.

3. OBJECTIVES

The present paper concerns the appearance of internal stresses and microfissures in wood after being subjected to freezing and thawing. Three evaluation methods were employed in order to compare the results, namely: the imaging method by SEM microscopy, meant to visualize eventual microfissures that occur in the cell membrane and two classical methods, usually applied in order to assess casehardening (internal stresses) for solid wood, namely the mid-cut test and the prong test.

4. MATERIALS, METHOD AND EQUIPMENT

4.1. Material

The material used within this research consisted of sample boards from European spruce (*Picea abies*) wood with an initial moisture content of 65%. The dimensions of the board samples were: 35 x 80 x 500mm. In order to manufacture the microscopic samples, small 10 x 10 x 40mm pieces were cut out of one board, which were then subjected to the same treatment as the large boards.

4.2. Method and Equipment

The experimental procedure involved freezing, thawing, kiln drying, planing and cutting samples, both for the SEM analysis (microscopic samples) and for the classical evaluation through the mid-cut test and prong test (macroscopic samples).

Both the small pieces and the boards were first frozen at - 35°C for 24 hours in a KPK 200 type climatic chamber from FEUTRON (Fig. 5). A control sample was left unfrozen.

After freezing, half of the small pieces were thawed in cold water and half of them were thawed in hot water (30°C). Then, they were planed with a sledge microtome (Fig. 6) and were sliced into microscopic samples with dimensions 3x3x3 mm. The microscopic samples were dehydrated by immersion in different baths (50% ethyl alcohol, ethyl alcohol 70%, ethyl alcohol 80%, absolute alcohol and acetone) for 7 minutes each.

Each dehydrated sample was then glued on a metal base with an adhesive composed of graphite and epoxy glue.

The prepared samples were metallized with 14 karat gold. The metallization of the samples was performed with a vacuum evaporation aggregate (Fig. 7).



Fig. 5. FEUTRON climatic chamber.



Fig. 6. Sledge microtome.



Fig. 7. Vacuum evaporation aggregate.

The microscopic imaging of the wooden structure was performed with a scanning electron microscope (SEM), model VEGA II LSH (Fig. 8), produced by Czech TESCAN coupled with an EDX detector QX2 QUANTAX type, manufactured by Bruker / ROENTEC Germany.



Fig. 8. SEM microscope, model VEGA II LSH.

In parallel, the large boards for confectioning the macroscopic samples were thawed under laboratory conditions and hereinafter, two types of samples for evaluation of internal stresses were cut out, according to the two applied methods:

- for the **mid-cut test** (ENV 14464), a 15mm wide test piece was cut at 500mm distance from the board end, which was then sliced in two at half thickness; the two halves were conditioned at 20°C and 55% RH for 24h, and then the maximum gap between them was measured (Fig. 9); the gap size is a measure of the casehardening degree and the allowable values are given in Table 1;

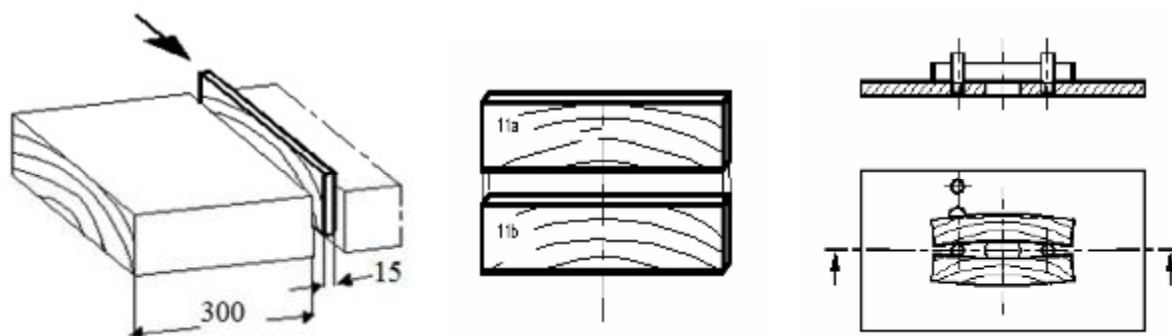


Fig . 9. Mid-cut test for evaluating the casehardening degree in wood.

- for the **prong test** (TRADA), a fork sample with two teeth was cut according to the dimensions shown in Fig. 10a, then conditioned at 20°C and 55% RH for 24h and then aligned to the left corner of a jig, as shown in Fig. 10b; the casehardening degree is read on a scale at the upper part of the jig, as

function of the deformation of the exterior margin of the left tooth (Fig. 10c); after this, the sample is rotated, and the reading is repeated for the other tooth; the allowable values are given in Table 1.

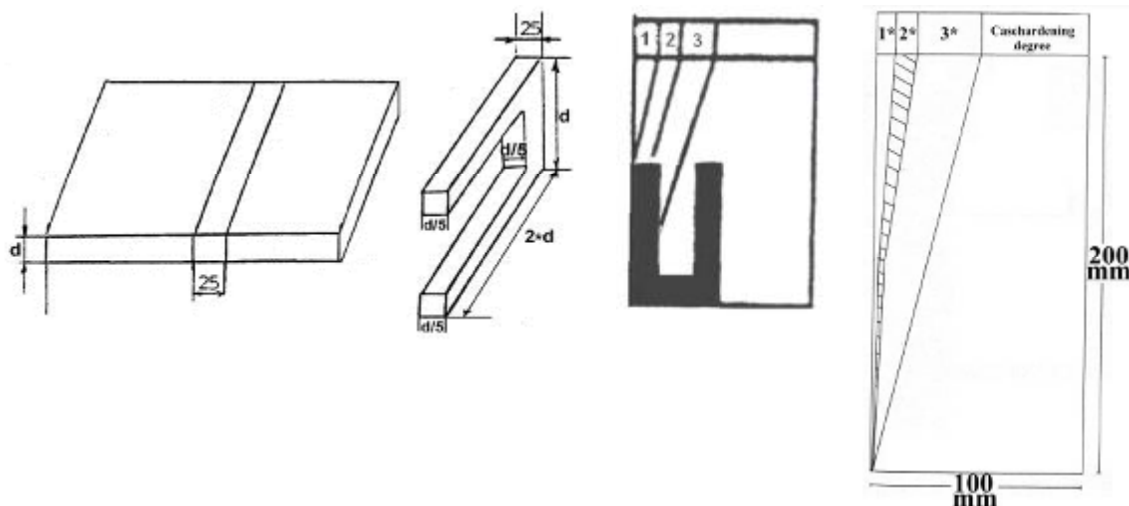


Fig. 10. Prong test for evaluating the casehardening degree in wood.

Table 1. Assessment of casehardening severity according to results obtained by the two methods.

<i>Casehardening degree]</i>	<i>Maximum gap measured [mm] (MID-CUT test)</i>	<i>Average deformation degree of both teeth (PRONG test)</i>
<i>Low</i>	1	1*
<i>Moderate</i>	2	2*
<i>Severe</i>	3	3*

5. RESULTS AND DISCUSSIONS

5.1. Microstructure Observations of Frozen Spruce Wood by SEM

Figures 11, 12, 13 show the results of the SEM observations of the tracheid walls and pits in all three grain directions, for the control (non-frozen) sample (Fig. 11), for a frozen sample thawed in cold water (Fig. 12) and for a frozen sample thawed in hot water (Fig. 13).

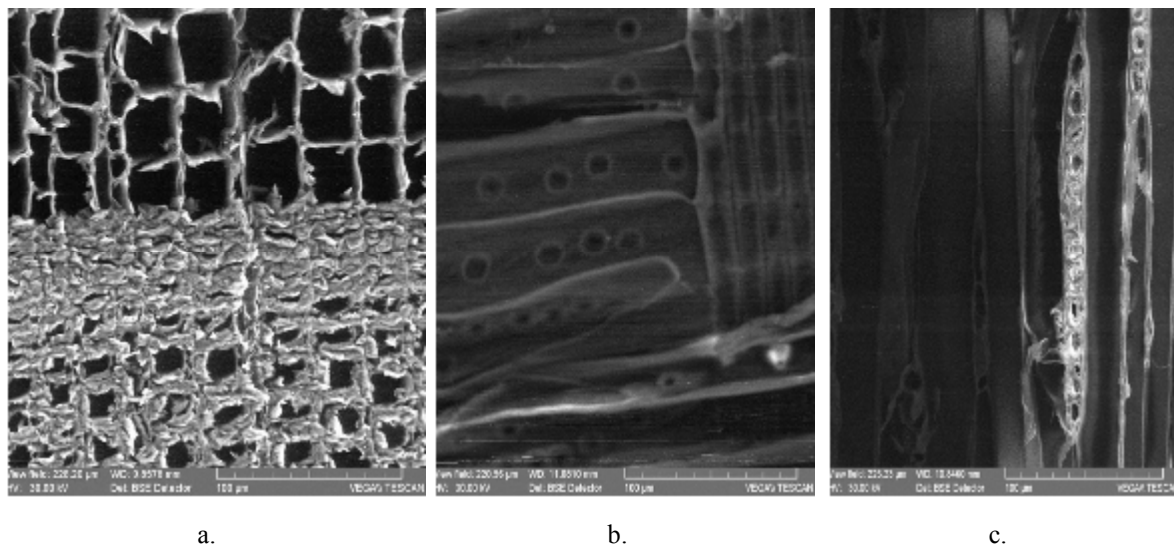


Fig. 11. SEM micrographs of control spruce sample in three grain directions, magnification 1000X:

a-cross section; b-radial section; c-tangential section.

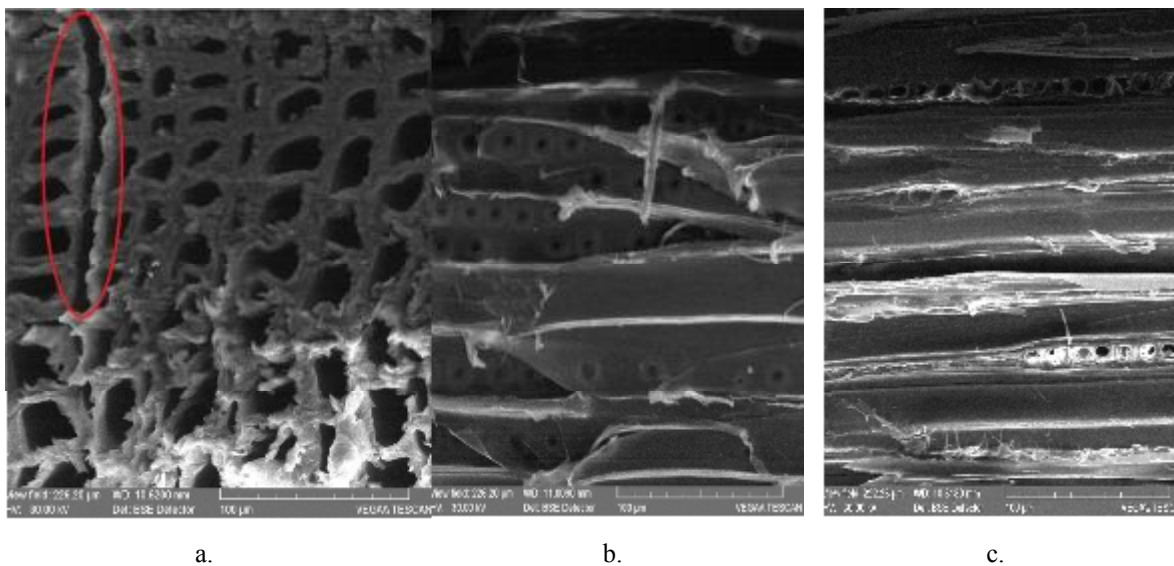


Fig. 12. SEM micrographs of spruce sample thawed in cold water in three grain directions, magnification 1000X:

a-cross section; b-radial section; c-tangential section.

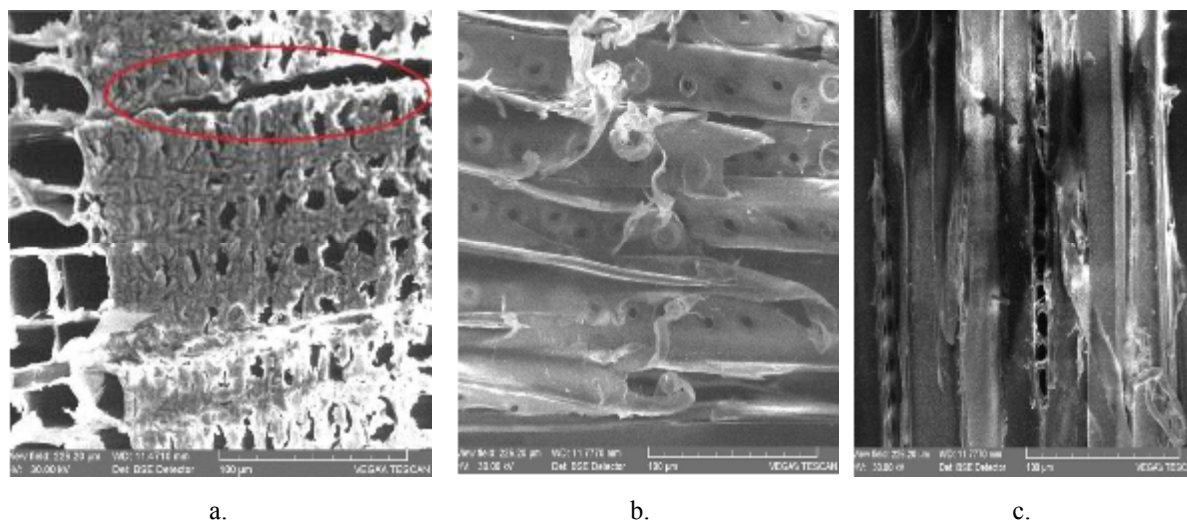


Fig. 13. SEM micrographs of spruce sample thawn in hot water in three grain directions, magnification 1000X:

a-cross section; b-radial section; c-tangential section.

The most eloquent results are obtained on the transversal sections (Fig. 12a, 13a, 14a). By comparing the images obtained from the frozen samples and from the control sample, one can clearly notice the fissures in the tracheid walls of the frozen samples, localized in the transition area from earlywood to latewood. Their length encounters 200 - 500 μ m. No significant differences could be observed according to the thawing temperature.

The results show that even with this low time of exposure (24h), microfissures are born in wood due to freezing.

5.2. Determination of Internal Stresses by Classical Methods

The results obtained by means of the mid-cut test and prong test are presented in Tables 2 and 3.

Table 2. Results concerning the casehardening degree recorded by applying the mid-cut test.

<i>Wood samples</i>	<i>Maximum gap [mm]</i>	<i>Appreciation on casehardening degree</i>
<i>Spruce, not-frozen</i>	0	Not existent
<i>Spruce, frozen and thawn in ambiental conditions</i>	2	Moderate

Table 3. Results concerning the casehardening degree recorded by applying the prong test.

<i>Wood species</i>	<i>Deformation of first tooth</i>	<i>Deformation of second tooth</i>	<i>Average deformation</i>	<i>Appreciation on casehardening degree</i>
<i>Spruce, not-frozen</i>	0	0	0	Not existent
<i>Spruce, frozen and thawed in ambient conditions</i>	2	1	1.5	Moderate

The results obtained through the two classical tests are in good accordance, as they both indicate a moderate casehardening degree of the frozen samples and no internal stresses in the control samples. This means that the internal stresses established in the frozen samples are due to the freezing process and it excludes the possibility that they may have been caused by growth conditions.

Connecting now to the results obtained by SEM microscopy imaging method, it seems that the revealed level of fissuring (moderate fissure length, low proportion) corresponds to a moderate casehardening degree, with good chances not to affect seriously the strength properties of wood (Campean *et al.* 2008).

However, the advantages of employing modern imaging tools in the study of the behaviour of heterogenous and complex materials like wood are obvious. With a very small amount of material, one gets able to “see” the causes, not only intuit them by measuring through indirect parameters the effects.

CONCLUSIONS

Knowing from reference literature that only free water freezes in wood when the temperature does not drop below -30°C , spruce wood samples with high initial moisture content (above the saturation point) were chosen, in order to evaluate the influence of freezing & thawing upon their mechanical integrity.

Modern imaging and classical evaluation methods were employed to assess the appearance of internal stresses and microfissures in the cell walls of the frozen samples, compared to non-frozen control samples.

3D images of the cross section clearly showed the occurrence of 200 – 500 μm long microfissures in the cell wall of the frozen samples, located in the transition area from earlywood to latewood.

The classical evaluation methods confirmed the result obtained through SEM imaging, revealing a moderate casehardening degree of the frozen samples, while the control samples showed no stresses.

The present paper gives only an example on how modern technology can provide better understanding of the phenomena that occur in organic materials such as wood, letting us “see” the causes, not only intuit them by measuring the effects. The research will be continued by subjecting wood also to different other processes, in order to clearly establish the influence of freezing temperature, time of exposure, level of initial moisture content upon the properties of ex-frozen wood in service, after thawing and drying.

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