

PlastLIFE D6.6. Report on the technical and mechanical properties of new plastic-replacing materials

Raportti uusien muovia korvaavien materiaalien teknisistä ja mekaanisista ominaisuuksista

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PLASTLIFE

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Abstract

Report on the technical and mechanical properties of new plastic-replacing materials

We have developed at least 5 new bio-based materials, and this report comprises their technical and mechanical properties. We also show the summary of ecotoxicity of the materials against *Daphnia magna* tested at University of Jyväskylä (JYU).

T6.3.1 Fiber-based packaging material containing side streams from horticulture

Tomato fibers significantly improved mechanical performance, showing the highest tensile strength, burst strength, and fracture toughness among all boards, whereas cucumber fibers weakened most mechanical properties, performing below both tomato-fiber and reference kraft boards. Tomato fiber is a promising reinforcement material, while cucumber fiber requires further optimization for strength-critical applications.

T6.3.2 Thermomechanical processing and extrusion molding technology for packaging

Fibers, mostly consisting of sawdust, were processed at different temperatures above 60 °C. The fibers were mechanically treated to form wood-based isotropic structures. A water-based suspension was successfully incorporated after the thermo-mechanical treatment and final technical and mechanical properties will be presented in the final report.

T6.3.3 Mycelia-based composites

Fungal mycelium-based materials were developed to replace plastic foams. For that purpose, we utilized side streams from the agricultural and forest industry as feedstock for fungal growth. We tested several fungal species and combination of lignocellulosic fibers to achieve a homogeneous and mechanical resistant bio-based composite with fungal-based adhesive. A fungal strain was significantly increasing the compression strength of the composite in comparison with other strains. The highest maximum force (kN) at 20 mm deformation was observed in Strain 2 (3.79 kN)

T6.3.4 Translucent film material from fish and plant side streams

The developed film material has been tested in combination with the fiber-based materials - adding fish gelatin to fiber-based packaging material as well as stand-alone material. Gelatin addition enhanced the tensile strength and stiffness of the pure cellulose sheets. The resulting materials demonstrate foldability, with the package structure maintaining integrity during bending and creasing. These properties suggest that the materials have potential to perform well in food-packaging applications. As stand-alone materials, films have demonstrated antibacterial properties and different active ingredient additions affect the mechanical properties (tensile strength and elongation at break) of the films.

Ecotoxicity: Most tested prototype materials caused no significant acute or chronic toxicity to *Daphnia magna*.

Keywords: Bio-based materials; Plastic replacement; Sustainable packaging; Fiber-based composites; Mycelium-based materials; Side stream utilization; Mechanical properties; Ecotoxicity

Tiivistelmä

Raportti uusien muovia korvaavien materiaalien teknisistä ja mekaanisista ominaisuuksista

Olemme kehittäneet vähintään viisi uutta biopohjaista materiaalia, ja tämä raportti sisältää niiden tekniset ja mekaaniset ominaisuudet. Lisäksi esittelemme materiaalien ekotoksisuusyhteenvedon *Daphnia magna* -vesikirpuille, testattuna Jyväskylän yliopistossa (JYU).

T6.3.1 Puukuitupohjainen pakkausmateriaali, joka sisältää puutarhatalouden sivuvirtoja

Tomaattikuidut paransivat selvästi materiaalien mekaanista suorituskykyä. Ne osoittivat kaikista tutkituista arkeista korkeimman vetolujuuden, murtolujuuden ja murtositkeyden. Kurkkukuidut puolestaan heikensivät useimpia mekaanisia ominaisuuksia ja suoriutuivat huonommin sekä tomaattikuituarkkeihin että vertailun kraft-kuituarkkeihin nähden. Tomaattikuitu on lupaava vahvistusmateriaali, kun taas kurkkukuitu vaatii lisäoptimointia lujuuskriittisiin käyttökohteisiin.

T6.3.2 Lämpömekaaninen prosessointi ja ekstruusiomuovausteknologia pakkauksiin

Pääosin sahanpurusta koostuvia kuituja prosessoitiin eri lämpötiloissa yli 60 °C:n. Kuituja käsiteltiin mekaanisesti puupohjaisten isotrooppisten rakenteiden muodostamiseksi. Lämpömekaanisen käsittelyn jälkeen vesipohjainen suspensio pystyttiin onnistuneesti lisäämään materiaaliin. Lopulliset tekniset ja mekaaniset ominaisuudet esitellään loppuraportissa.

T6.3.3 Myseelipohjaiset komposiitit

Sienirihmaston perustuvia materiaaleja kehitettiin muovivaahdon korvaajiksi. Tätä varten hyödynnettiin maa- ja metsätalouden sivuvirtoja sieniviljelyn kasvualustana. Testasimme useita sienilajeja ja lignoselluloosakuitujen yhdistelmiä tavoitteena saada aikaan homogeeninen ja mekaanisesti kestävä biokomposiitti sienipohjaisella sidosaineella. Yksi sienikanta paransi komposiitin puristuslujuutta merkittävästi muihin kantoihin verrattuna. Suurin maksimaalinen voima 20 mm:n muodonmuutoksella havaittiin Kanta 2:ssa (3,79 kN).

T6.3.4 Läpinäkyvä kalvomateriaali kala- ja kasvipöeräisistä sivuvirroista

Kehitettyä kalvomateriaalia testattiin sekä yhdessä kuitupohjaisten materiaalien kanssa lisäämällä kalagelatiinia kuitupohjaiseen pakkausmateriaaliin että itsenäisenä materiaalina. Gelatiinin lisääminen paransi puhtaiden selluloosalevyjen vetolujuutta ja jäykkyyttä. Tuloksena syntyneet materiaalit ovat taivuteltavissa ja pakkausrakenteen säilyttää eheyttä taivutuksessa ja taittamisessa. Nämä ominaisuudet viittaavat hyvään soveltuvuuteen elintarvikepakkausten käyttöön. Itsenäisinä materiaaleina kalvot ovat osoittaneet antibakteerisia ominaisuuksia, ja eri aktiiviaineiden lisäykset vaikuttavat kalvojen mekaniisiin ominaisuuksiin (vetolujuus ja murtovenymä).

Ekotoksisuus

Useimmat testatut prototyyppimateriaalit eivät aiheuttaneet merkittävää akuuttia tai kroonista toksisuutta *Daphnia magna* -vesikirpuille.

Asiasanat: Biopohjaiset materiaalit; Muovin korvaaminen; Kestävä pakkaaminen; Kuitupohjaiset komposiitit; Myseelipohjaiset materiaalit; Sivujakeiden hyödyntäminen; Mekaaniset ominaisuudet; Ekotoksisuus

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1 Plastic-replacing novel packaging materials

The increasing global population and the growth of prosperity create a huge strain on our planet. The declining non-renewable resources must be replaced by renewable ones and resource scarcity must be addressed with comprehensive use of side streams. Additionally, there is a need to reduce food waste by prolonging the shelf-life of goods with smart packaging materials. The aim of the task is to develop novel alternative materials to replace plastic packaging materials. With the diverse prototype selection, we can substitute many kinds of fossil-based plastic materials and simultaneously increase the use of 2nd generation feedstocks and thus promote circular bioeconomy. T.6.3 consists of four parallel sub-tasks. Sub-tasks T.6.3.1-T.6.3.4. focus on material development and include testing and choosing of recipes and manufacturing techniques.

1.1. Fiber material with tomato and cucumber side streams (T6.3.1)

In Finland, approximately 9,130 tons of tomato and cucumber stems are generated annually as agricultural residues (see D6.7 TEA report). These materials are typically disposed of in landfills, contributing to environmental burdens such as methane emissions and resource inefficiency. Utilizing these stems as partial replacements for cellulosic pulp in board making offers several environmental benefits. Using these agricultural residues in board production significantly reduces organic waste sent to landfills. This diversion helps mitigate methane emissions, a potent greenhouse gas produced during anaerobic decomposition of organic matter. Traditional cellulosic pulp production is energy-intensive and often involves chemical processing. Replacing a portion of pulp with mechanically processed plant stems can reduce energy consumption and associated carbon emissions, contributing to climate change mitigation. Agricultural residues are currently underutilized. Their integration into industrial processes such as board making promotes circular economy principles by transforming waste into valuable raw materials, enhancing overall resource efficiency. Cellulosic pulp is primarily derived from wood, placing pressure on forest ecosystems. Substituting part of the pulp with non-wood fibers like tomato and cucumber stems can help reduce deforestation and preserve biodiversity. Avoiding landfill disposal of organic agricultural waste reduces the risk of leachate formation, which can contaminate soil and groundwater. This contributes to improved environmental quality and ecosystem health. Sourcing raw materials from domestic agricultural residues supports local bioeconomy initiatives, reduces transportation-related emissions, and strengthens regional sustainability efforts

This part of the report presents the characterization of three paperboard materials produced for Luke, each having a target basis weight (grammage) of approximately 185 g/m². The materials include:

- A reference board made solely from bleached kraft fibers (50% softwood, 50% hardwood).
- A board containing tomato stem fibers blended with kraft fibers.
- A board containing cucumber stem fibers blended with kraft fibers.

In commercial paperboard production, quality criteria are highly case-specific and depend strongly on the manufacturing process. For this reason, research in the field always includes reference material, such as the conventional kraft pulp used in this study. It is well established that kraft pulp performs reliably in many types of paperboards. Furthermore, the 50–50 softwood–hardwood fiber ratio used here closely resembles the typical top-layer composition of folding boxboard.

All three boards were subjected to a series of physical and mechanical tests both before and after calendering. The study aims to evaluate how agricultural fibers, specifically tomato and cucumber stems, affect paperboard performance and how calendering influences key material properties.

The tomato and cucumber fibers used in the paperboard were processed from greenhouse biomass. The biomass underwent mechanical pressing, alkaline digestion, washing and neutralization and subsequent refining before blending with cellulose fibers. Cucumber fibers lost approximately 20% of dry mass during washing, while tomato fibers lost about 15%. Tomato fibers required longer refining time, indicating structural differences between the two materials. The final fiber furnish for the agricultural fiber boards consisted of one part agricultural fiber and five parts kraft pulp, corresponding to approximately 16.7% agricultural fiber. All boards were produced with a target basis weight of 185 g/m² on a pilot paper machine. The produced rolls can be seen in **Fig. 1**.



Fig 1. Tomato fiber containing board (left), cucumber fiber containing board (right). Photographs by Risto Korpinen.

Calendering was performed to modify the surface and structural characteristics of the sheets. Each sample underwent five calendering passes with 120 kN/m nip pressure. Calendering is known to reduce thickness and bulk while improving surface smoothness. The evaluation therefore includes comparisons of these properties before and after the process. Prior to testing, all sheets were conditioned according to ISO 187 at 48% relative humidity and +24 °C.

The following standards were applied for material testing:

- ISO 536 – Basis weight
- ISO 534 – Thickness and bulk
- ISO 1924-3 – Tensile strength
- SCAN-P 77:95 – Fracture toughness
- ISO 1974:1990 – Tear strength

ISO 2758:2001 – Burst strength

All three materials reached the intended grammage of approximately 185 g/m². Tomato and cucumber fiber containing boards showed slightly higher grammage than the reference board, although deviations remained minor (**Fig. 2**).

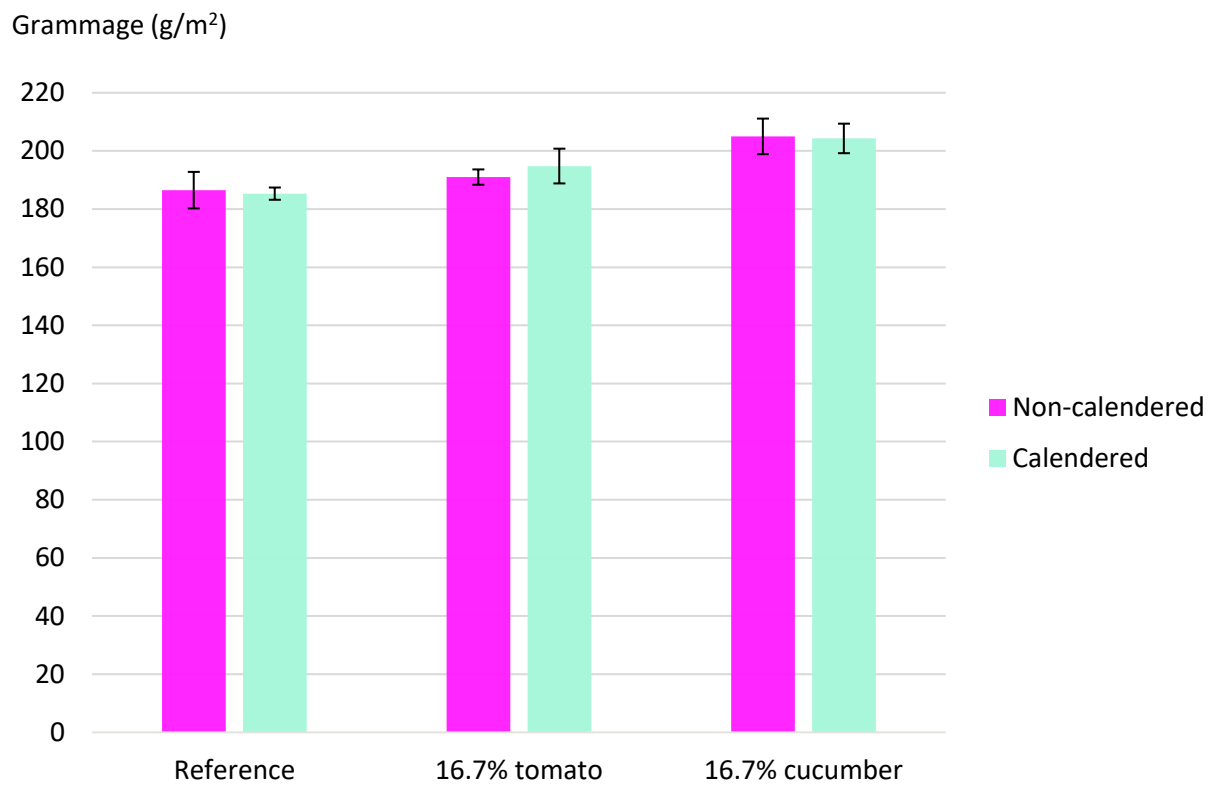


Fig 2. Grammage of the produced boards.

Before calendering, the bulk values of all materials were similar regardless of fiber composition. After calendering, bulk decreased consistently for all samples as expected, and the values converged, indicating that the calendering process had a stronger effect than fiber type, see **Fig. 3**.

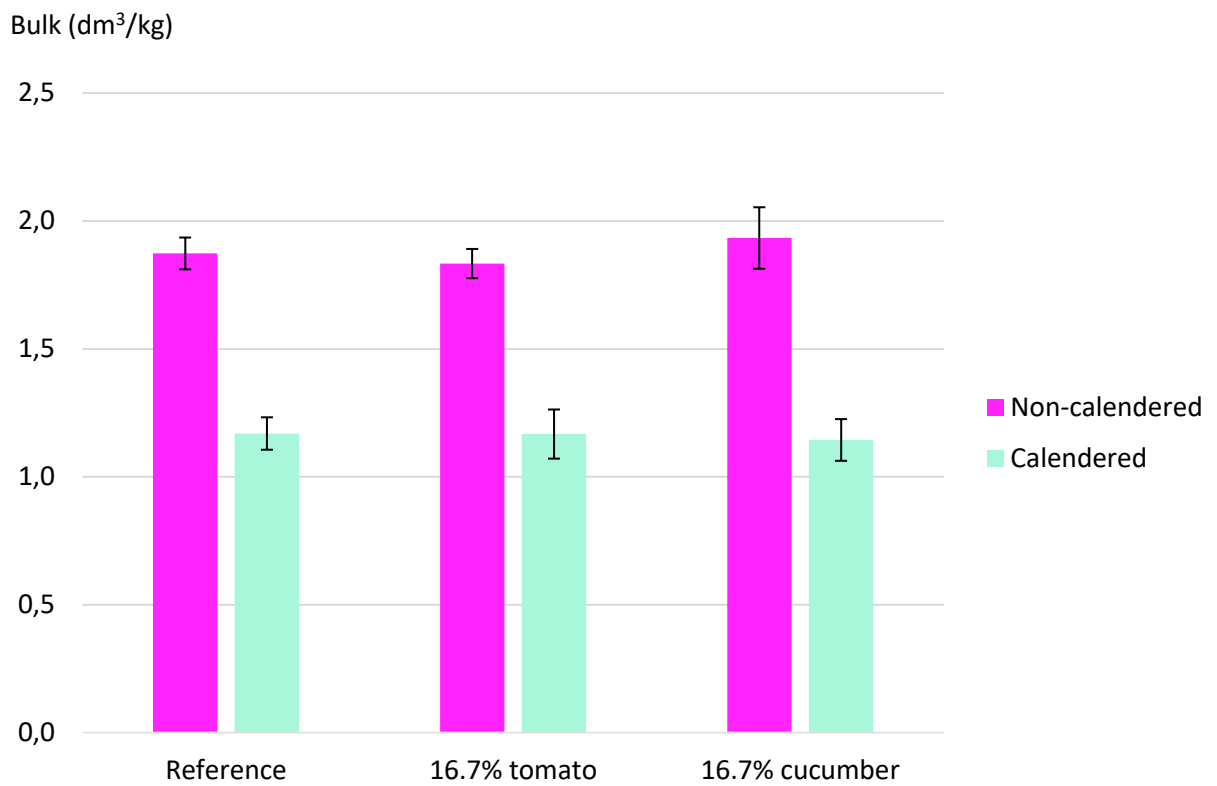


Fig 3. Bulk of the produced boards.

The tomato fiber containing board demonstrated the highest tensile index among the fiber containing materials and performed better than the reference. In contrast, the cucumber fiber containing board showed lower tensile strength than both the tomato fiber containing board and the reference. Calendering caused a slight decrease in tensile strength across all materials, as seen in **Fig. 4**.

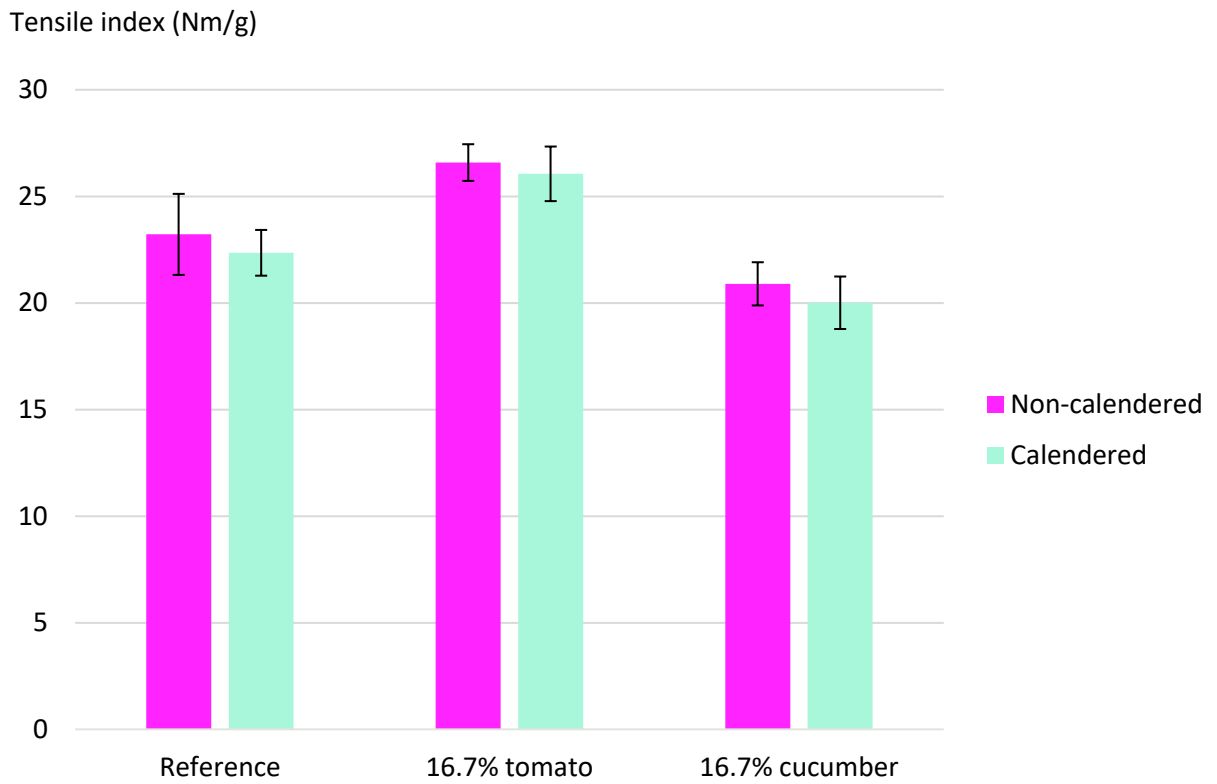


Fig 4. Tensile index of the produced boards.

Both tomato and cucumber fiber containing boards exhibited lower tear strength than the reference board. Calendering further reduced tear strength in all samples except the reference board, which remained stable (Fig. 5).

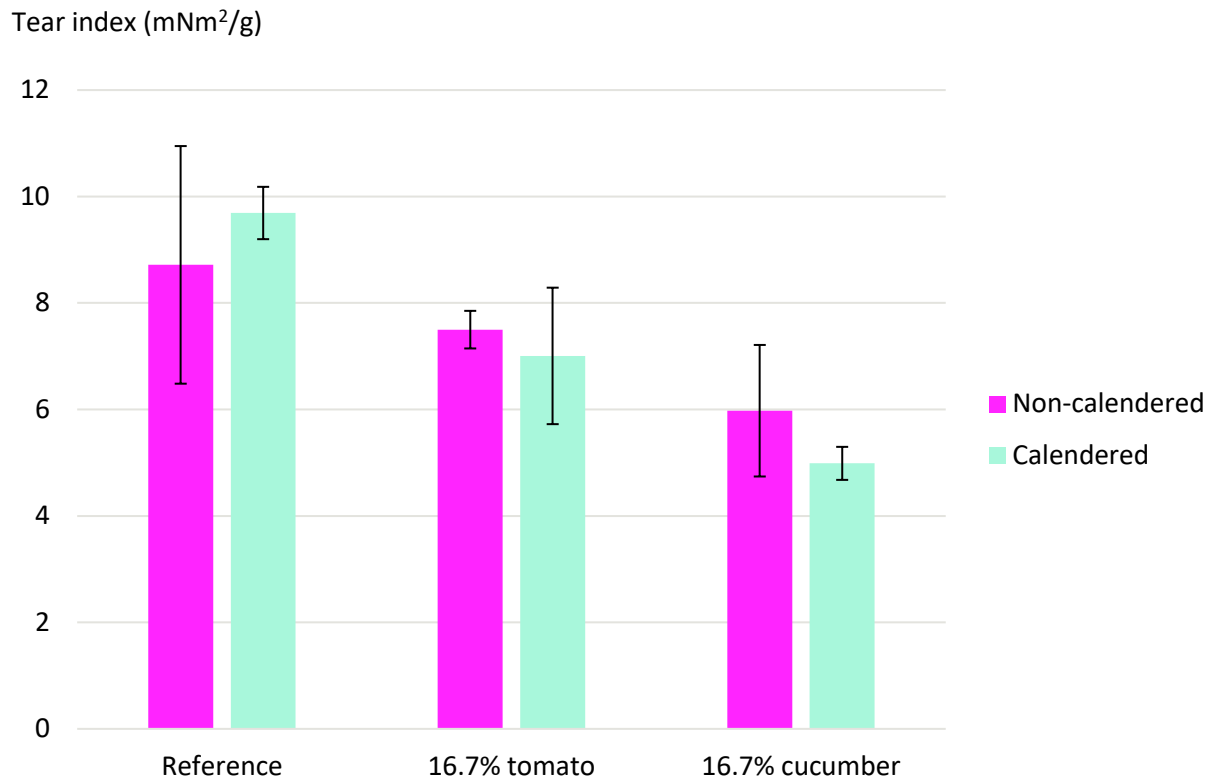


Fig 5. Tear index of the produced boards.

The tomato fiber containing board showed the highest burst strength in both uncalendered and calendered states. The cucumber fiber containing board had the lowest values, while the reference material performed at an intermediate level. Calendering produced a slight increase in burst strength across all boards, see Fig. 6.

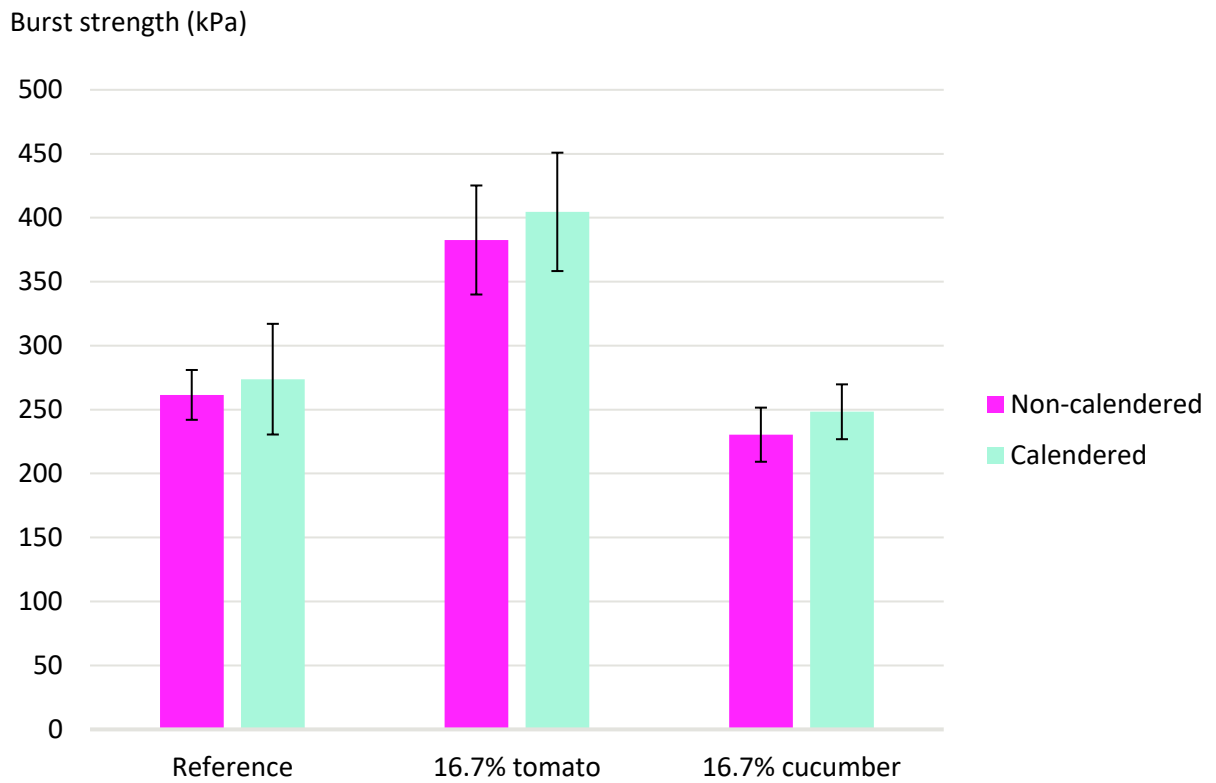


Fig 6. Burst strength of the produced boards.

Tomato fiber containing board exhibited the best fracture toughness among the three materials. Reference and cucumber fiber containing boards showed similar but lower values. Calendering caused a minor increase in fracture toughness, but the ranking between materials remained the same, as seen in Fig. 7.

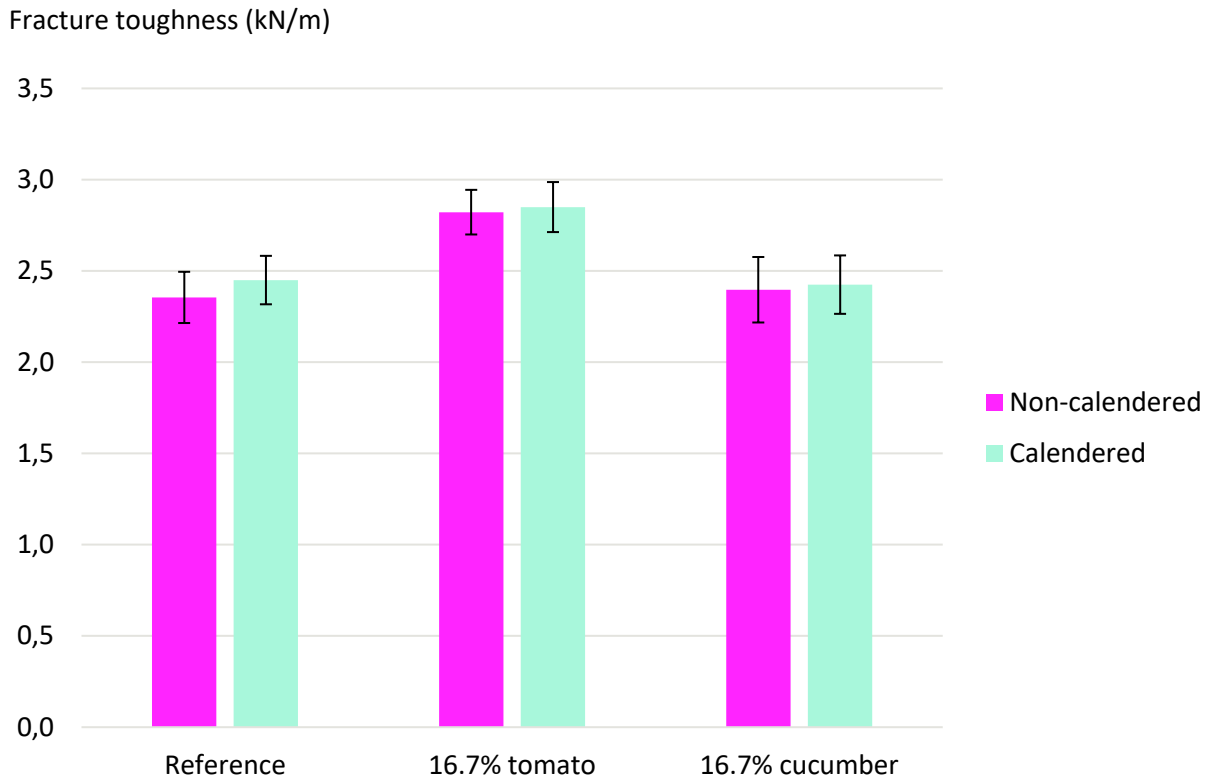


Fig. 7. Fracture toughness of the produced boards.

The results indicate significant differences in performance between tomato and cucumber derived fibers. Tomato fibers contributed positively to tensile strength, burst strength, and fracture toughness, suggesting good bonding characteristics and reinforcing capability. Cucumber fibers, on the other hand, consistently reduced mechanical performance. Possible reasons include weaker fiber structure, lower bonding ability, and higher material losses during processing.

Calendering had predictable effects: reduced bulk and minor decreases in most strength properties. However, the relative performance between the materials was not altered by calendering. Tomato fiber containing board remained the strongest across most metrics, while cucumber fiber containing board remained the weakest. Overall, tomato fiber is a promising reinforcement material, while cucumber fiber requires further optimization for strength-critical applications.

1.2. Thermomechanical processing and extrusion molding technology for packaging (T6.3.2)

In Task 6.3.2, thermo-mechanical processing of fibers was evaluated as a method to produce fiber-based packaging materials. The work focused on the combined effects of thermal treatment and mechanical modification of biomass on fiber structure and processability.

Wood sawdust-based fibers were processed at several defined temperature levels to assess the influence of thermal conditions on fiber behavior. Following the thermal treatment, the fibers were subjected

to thermo-mechanical processing using a planetary roller extruder (PRE). This processing step enabled intensive mechanical treatment, resulting in the formation of wood-based isotropic fiber structures (**Fig. 8**).

After thermo-mechanical processing, a water-based suspension was incorporated into the treated fiber matrix. The incorporation was successful and produced a homogeneous mixture suitable for further material development. Based on the applied processing conditions, a prototype packaging material was produced. Mechanical strength testing of the prototype is ongoing, and the results will be reported during final reporting.

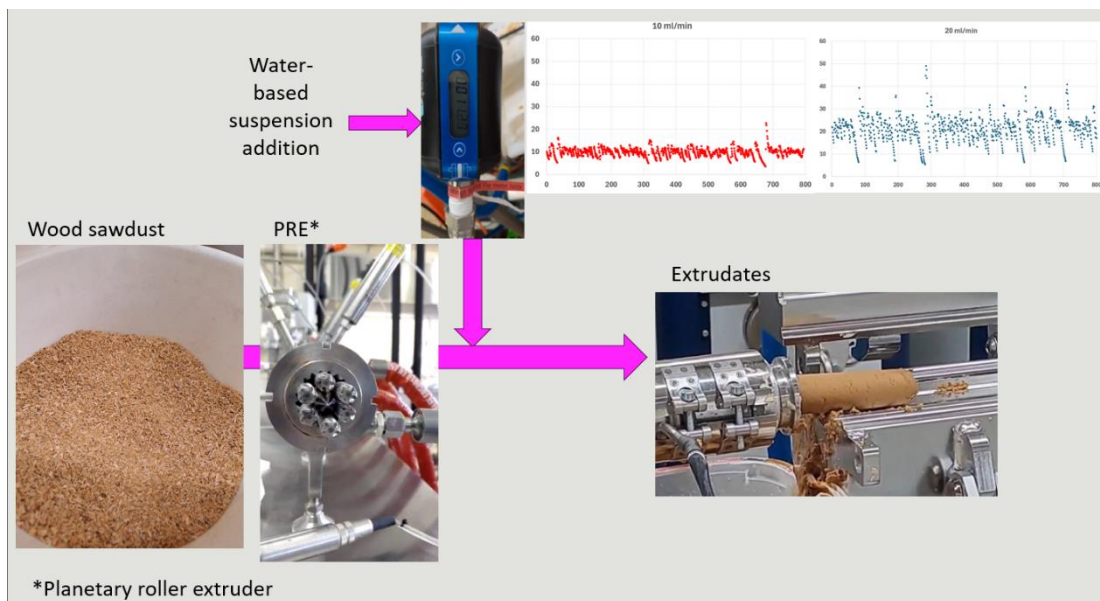


Fig. 8. Schematic representation of the thermomechanical processing protocol for plastic replacing fiber materials. PRE= planetary roller extruder. Photographs by Martin Ramos Diaz.

1.3. Mycelial composite materials (T6.3.3)

The mycelial composite prototype developed in this project utilizes agro-industrial and food industry side streams as feedstocks. The material is intended for applications such as packaging for beverages or fragile fruits, where fungal mycelium from LUKE’s culture collections is grown within prefabricated casting molds. These molds are filled with nutrient-rich side streams derived from breweries, mechanical wood processing, and vegetable processing, providing an eco-friendly alternative to conventional packaging materials.

The environmental benefits of this approach are significant. Valorization of industrial side streams directly reduces waste by diverting organic materials from landfills and incineration. Feedstocks such as brewery spent grains, wood dust, and vegetable peels are instead repurposed as substrates for fungal growth. For larger prototypes produced within the project, including glass bottle packaging and insulation panels, up to 5 kg of organic side-stream material is incorporated per product. During growth, mycelium sequesters carbon, contributing to biogenic carbon storage. In contrast to plastic- or polystyrene-based solutions, mycelium composites require minimal energy during production, resulting in a lower carbon footprint. Furthermore, the developed materials are designed to be fully compostable under natural conditions, thereby reducing microplastic pollution. Mycelium cultivation also requires substantially less water than the production of paper- or plastic-based packaging materials.

Within Task T.6.3.3, fungal mycelium-based materials were developed as replacements for plastic foams. To achieve this, side streams from the agricultural and forest industries were used as substrates for fungal growth. Fungi bind lignocellulosic fibers through the formation of an interconnected hyphal network, known as mycelium, resulting in a lightweight composite material. Several fungal species and combinations of lignocellulosic fibers were evaluated to produce a homogeneous, mechanically robust bio-based composite in which the mycelium functions as a natural adhesive.

1.3.1. Preparation of lignocellulosic fibers

The lignocellulosic materials used to produce mycelium-based packaging consisted of wood chips and straw. Prior to fermentation, the fibers were subjected to thermomechanical treatment. The general processing steps were as follows:

1. The raw lignocellulosic material was first size reduced using a grinder, yielding particles approximately 1.2–1.5 mm in size.
2. Moisture content was adjusted to 60–75% by adding water and homogenizing the material. Several moisture levels within this range were evaluated to determine optimal processing conditions.
3. When formulations included multiple lignocellulosic substrates, the materials were thoroughly mixed.
4. The substrates were sterilized in an autoclave at 121 °C for 90 minutes to ensure aseptic conditions and eliminate contaminating microorganisms.
5. After sterilization, the substrates were cooled and stored in a laminar flow hood until inoculation with the selected fungal species.

1.3.2. Composite formulation

Biobased composite materials were produced using different ratios of wood fibers and straw. Fungal species from the Basidiomycetes class were selected due to their strong ability to form extensive mycelial networks, making them suitable as biological adhesives. The selected fungal strains were inoculated into the sterile substrates, followed by solid-state fermentation for 1–2 months. During this period, the mycelium colonized the lignocellulosic matrix and bound the fibers into a cohesive composite structure (**Fig. 9**).

1.3.3. Evaluation of composite performance

The technical performance of the developed bio-based composites was evaluated using several analytical methods. Mechanical strength testing was carried out to assess structural integrity, while composite densities were measured to support interpretation of mechanical performance and material uniformity. Quantification of mycelial growth within the composites is currently ongoing. In addition, biodegradability tests are being conducted to evaluate the environmental performance of the materials.



Fig 9. General steps for the production of mycelial composite materials. Photographs by Marta Cortina-Escribano

1.3.4. Technical and mechanical properties of new bio-based materials based on fungi

The influence of different fungal strains on the density and compression strength of the bio-based composites was evaluated. The resulting composite densities ranged from 0.12 to 0.22 g/cm³, reflecting variation in substrate composition and the colonization efficiency of the fungal species. Notable differences were observed in mechanical performance between the strains. One fungal strain significantly increased the compression strength compared with the others. The highest maximum force at 20 mm deformation was recorded for Strain 2, which reached 3.79 kN, indicating superior reinforcement of the composite structure by this strain (Fig 10).

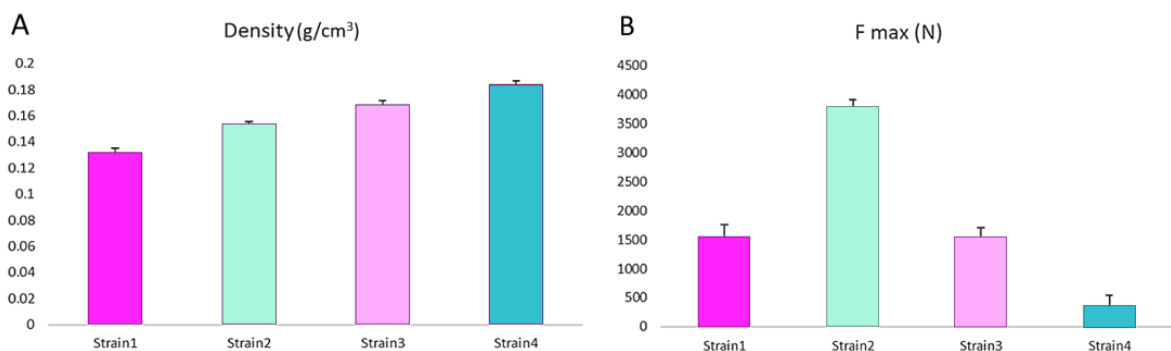


Fig 10. Technical performance of the new mycelium-based materials with fungal strains 1–4. On the left the density of the mycelial-based composites (A); on the right the compression strength of the mycelial-based composites (B).

1.4. Transparent films from fish gelatin and functional additions (T6.3.4)

Fish gelatin prototype (**Fig. 11**) aims to replace single-use plastics in sushi and fish products packaging. The prototype development utilizes fish processing residues (e.g., skins, scales) as well as side stream extracts, and waxes from agro/forestry/berry industries. The processing of salmon and rainbow trout generates about 23 percent of the side streams. In salmon, the skin accounts for about eight percent of the weight of the gutted fish. However, the majority of salmonids are sold as unskinned products, and it has been estimated that in Finland only about ten percent of processed salmonids are skinned. Thus, about 262 t of salmonids side stream are skins. Gelatin yield from fish skins is about 10–14 %, i.e., it is possible to get about 36.7 t of gelatin from 10,000 t of salmon side streams (**Fig. 12**). In addition, fish bones and fish backbones of salmonids contain collagen, thus being potential sources of gelatin. We are utilizing also berry processing side streams, such as berry press cakes, for active ingredients and total aim is that up to 80 % of the film is side stream originated. Our hypothesis (still to be confirmed by biodegradability tests) is that gelatin-based films are biodegradable and can degrade in soil or marine environments. Compared to petroleum-based plastics, gelatin and plant-based materials have lower embodied energy. Fish gelatin is naturally water-soluble, which further decreases the post-consumer waste.

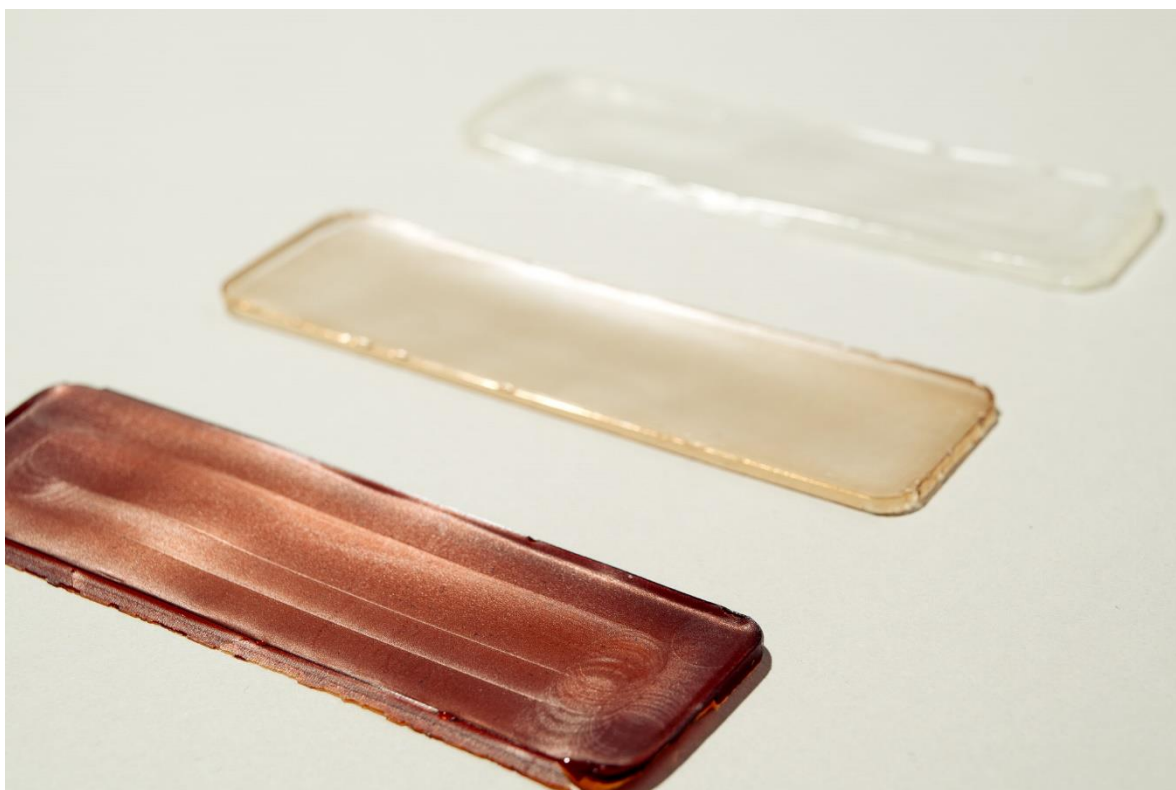


Fig 11. Gelatin films with different active ingredient additions produce varying coloration to the material. Photograph by Jarkko Mikkonen.

1.4.1. From fish side streams to active packaging

Gelatin films with plant side stream additions have been identified with antimicrobial barrier properties, making them interesting in the view of food packaging. Antimicrobial properties can be used to enhance the food safety and prolong shelf-life of beverages.



Fig. 12. Gelatin is extracted from salmonid skin. Photographs by Taru Kariniemi

Gelatin exhibits several attractive characteristics for use as packaging material, particularly due to its bio-based origin and simple extraction processes (**Table 1**). It is widely used in biomedical, food, and cosmetic applications, and its transparency, lack of strong odor or taste, and bioactive collagen-derived peptides further enhance its functional versatility. In addition, gelatin poses no risk of zoonotic disease transmission and is acceptable across religious dietary frameworks, including halal and kosher requirements.

Despite these advantages, gelatin also presents notable limitations in packaging applications. Its inherent water solubility and hygroscopic nature restrict performance in humid environments, while low melting and gelling temperatures constrain thermal stability. Furthermore, gelatin may cause allergic responses in sensitive individuals, and variability between production batches can affect the consistency of material properties, posing challenges for large-scale or standardized applications.

TABLE 1. Advantages and disadvantages of gelatin as packaging material

Advantages	Disadvantages
biomaterial (scaffolds, carriers, foods, cosmetics)	water soluble/hygroscopic
safety (no risk in transference of zoonotic disease)	potentially allergenic
no religious barriers (halal/kosher)	low melting and gelling temperatures
simple isolation methods	variation between batches (biomaterial)
bioactive properties; collagen peptides	
quite odorless and tasteless	
water soluble	
transparency (films and gels)	

In addition, we have identified three differing ways of manufacture for the films: casting, extrusion and heat pressing. Different techniques yield differing films, and they can all be upscaled.

1.4.2. As stand-alone films

Films produced from salmonid gelatin demonstrated tunable functional properties through the incorporation of different bioactive side stream additives, ranging from forest-based materials to berry processing residues. Antibacterial barrier performance was successfully optimized, and antimicrobial activity was observed against both Gram-positive and Gram-negative bacterial strains, indicating the potential of these films for active packaging applications. In addition, certain additives were found to reduce the inherent water solubility of gelatin films, thereby improving material durability under humid conditions.

Mechanical properties (**Fig. 13**) were notably influenced by the type of berry side stream incorporated. Films containing berry side stream A exhibited reduced tensile strength but increased elongation at break compared to the control, indicating a more ductile and flexible material behavior. Such films can undergo substantial deformation before fracture, making them suitable for applications where flexibility and conformability are required rather than high load-bearing capacity. In contrast, the addition of berry side stream B resulted in decreases in both tensile strength and elongation at break, suggesting a weaker and more brittle material with reduced toughness. These results demonstrate that side stream selection enables targeted tailoring of the mechanical and functional performance of salmon gelatin films.

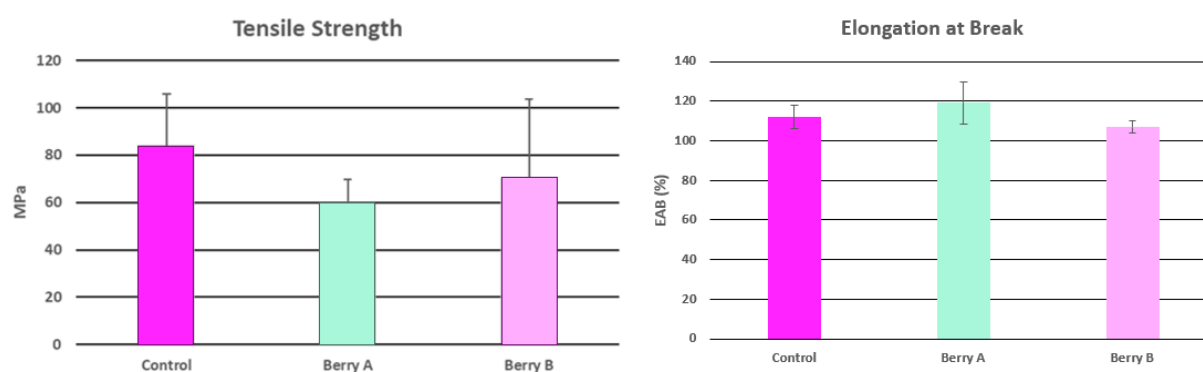


Fig. 13 Tensile strength and elongation at break measurements for stand-alone films show variation with different berry side stream additions.

1.4.3. Fish gelatin-coated cellulose-based fiber prototypes

The incorporation of fish gelatin into cellulose-based sheets was investigated as a means to enhance the functional performance of fiber-based packaging materials. Gelatin addition resulted in a clear improvement in tensile strength and stiffness of pure cellulose sheets, indicating effective reinforcement through the formation of a continuous coating layer. In contrast, puncture resistance was not significantly affected, suggesting that the gelatin layer primarily contributes to load-bearing and stiffness-related properties rather than resistance to localized penetration forces. As expected, gelatin incorporation also led to an increase in the overall weight of the paperboard materials.

When cellulose sheets were reinforced with lignocellulosic side streams such as tomato, cucumber, and hop stems, the effect of gelatin addition was less pronounced. In these formulations, the heterogeneous nature of the fibrous substrates likely limited the efficiency of gelatin as a reinforcing agent.

Nevertheless, preliminary studies indicate that gelatin still functions as a protective and cohesive coating layer on cellulose-based cartons reinforced with tomato and cucumber stems. The resulting materials exhibited measurable foldability, with the package structures maintaining integrity during bending and creasing. This behavior is a critical requirement for packaging applications involving forming, folding, and handling.

Overall, the results suggest that fish gelatin can act as a functional bio-based coating for cellulose fiber structures, improving mechanical performance and handling properties while maintaining structural integrity. These characteristics highlight the potential of gelatin-coated fiber materials as sustainable alternatives for food-packaging applications, particularly where flexibility and formability are essential.

2 Ecotoxicity of the materials

Ecotoxicity testing is essential for the developed bio-based materials despite their natural origin, as the use of diverse side streams and bioactive additives may introduce compounds with unknown environmental effects. During degradation or composting, these substances can be released into soil or water, where they may affect non-target organisms. In particular, the intended antibacterial properties of some materials raise concerns about potential impacts on beneficial microbial communities. Moreover, biodegradability alone does not guarantee environmental safety, as degradation products must also be proven non-toxic. Ecotoxicity assessments therefore help verify that the materials are environmentally benign throughout their life cycle. Such data also support regulatory approval and strengthen the credibility of sustainable material solutions.

Although the developed materials are based on natural and bio-derived components, ecotoxicity assessment remains a critical step in their evaluation. The use of side streams from agricultural, forest, and food processing, as well as bioactive additives such as berry residues and fungal mycelia, introduces chemical complexity that may result in the presence of compounds with unknown or unintended environmental effects. During material degradation, processing, or composting, these substances may be released into soil or aquatic environments, where they could affect microorganisms, plants, or invertebrates.

In addition, bioactive functionality, such as antibacterial properties intentionally incorporated into gelatin films, raises justified concerns regarding potential impacts on non-target organisms. Antimicrobial activity that is beneficial in packaging applications may adversely influence beneficial microbial communities if released into the environment, potentially disrupting ecosystem functions such as nutrient cycling and soil health. Therefore, assessing ecotoxicological effects is essential to ensure that antimicrobial performance does not come at the expense of environmental safety.

Furthermore, the biodegradability and compostability of the materials necessitate evaluation beyond material performance. While biodegradation is generally considered environmentally advantageous, the breakdown products themselves must be proven non-toxic under realistic environmental conditions. Ecotoxicity tests provide evidence that degradation residues do not accumulate or exert harmful effects throughout the material life cycle.

Finally, ecotoxicity testing supports regulatory acceptance and market uptake of novel bio-based materials. Demonstrating environmental safety through standardized tests strengthens the credibility of sustainable material solutions and ensures compliance with emerging regulations addressing circular bioeconomy products. Overall, ecotoxicity assessments are an essential component in validating that these innovative bio-based materials are not only functional and biodegradable but also environmentally benign across their full life cycle. Ecotoxicity of the materials was tested at University of Jyväskylä by Sami Taipale and Emma Kemppainen. This report only contains a short summary of the results and full report can be found in the master's thesis by E. Kemppainen.

2.1. Ecotoxicity

Daphnia magna was used as a model organism, since it is commonly used in toxicity testing. Acute toxicity test followed the OECD 202 "*Daphnia* Acute Immobilization Test" standard (OECD 2004). Test lasted for 48 hours and used a concentration of 320 mg/l. Chronic toxicity test followed the OECD 211 "*Daphnia magna* Reproduction Test" standard (OECD 2012). Test lasted for 21 days, and the tested concentrations were 6.4 mg/l and 64 mg/l.

In the acute toxicity test, none of the materials caused any toxic effects on mortality. The main findings of the chronic toxicity test are summarized in tables. **Table 2** summarizes the control treatments included in the experiments. **Table 3** shows the conventional plastics used for comparison, and **Table 4** lists the novel natural materials tested and their observed effects. In the chronic toxicity test mortality was significantly higher for GF 1 and GF 2 at 64 mg/l. No other treatments at either concentration caused significant changes in mortality.

TABLE 2. Summary of the control treatments and observed effects on *Daphnia magna* in both acute and chronic toxicity tests.

Material	Description	Main effects in the acute test	Main effects in the chronic test
Control I (C I)	Lake water	No effects	No effects
Control II (C II)	Artificial freshwater medium	No effects	Not tested
Kaolin (K)	Natural clay mineral	No effects	No effects
Potassium dichromate	Known to be toxic for aquatic organisms	High mortality	Not tested

TABLE 3. Conventional plastics, the novel natural materials they were compared to, and observed effects on *Daphnia magna* in the chronic toxicity test.

Material	Compared to	Main effects in the chronic test
Low-density polyethylene (LD-PE) plastic film	Recycled fibers	No effects
Translucent polypropylene (PP) plastic bag	Gelatine films	No effects
Foamed polystyrene (PS, Styrofoam)	Mycelium-based material sample REL 43	No effects

TABLE 4. Summary of the novel natural materials, their composition, and observed effects on *Daphnia magna* in the chronic toxicity test.

Material	Contains	Main effects in the chronic test
Recycled fibers 1 (RF 1)	80 % bleached pulp, 4 % gelatin, 20 % side stream pulp	No effects
Recycled fibers 2 (RF 2)	80 % bleached pulp, 4 % gelatin, 20 % tomato	No effects
Gelatine films 1 (GF 1)	Gelatin, glycerol, berry extract(i)	Higher mortality and reduced reproduction at 64 mg/l
Gelatine films 2 (GF 2)	Gelatin, glycerol	Higher mortality and reduced reproduction at 64 mg/l
Mycelium-based material sample REL 43 (MBM)	50 % straw, 50 % birch sawdust, <1 % barley (fermented) and <1 % mycelium	No effects

Unexpected effects were observed in the chronic ecotoxicity tests for gelatin-based films, particularly considering that the gelatin was extracted from salmonid skin, i.e. from an aquatic organism. Gelatin was also the only tested material that showed clear solubility in water, which may partly explain the observed biological responses. Water solubility may facilitate exposure of test organisms to the material itself, as well as to any residual impurities or potential microbial contaminants present in the gelatin films, such as enterobacteria or other unintended microbiota. In addition, the incorporation of berry extracts into the gelatin films was associated with increased mortality in *Daphnia magna*, despite the low additive concentration (0.4%). This effect may be related to indirect factors such as local pH changes or the release of bioactive compounds, although the exact cause remains unclear. At present, the specific mechanisms underlying the observed effects cannot be conclusively identified, and therefore further targeted testing has been agreed to elucidate the drivers of toxicity, representing a scientifically interesting avenue for continued investigation.

3 Conclusions

This report demonstrates that bio-based materials derived from industrial side streams, including fiber-based, mycelium-based, and gelatin-based solutions, have strong potential as sustainable alternatives to conventional packaging materials. The developed prototypes showed promising functional and mechanical performance, and the results highlight the flexibility of these material concepts to be tailored for different packaging applications. At the same time, the work identified key trade-offs related to material composition, performance variability, and processing conditions, underlining the importance of formulation-specific optimization.

Further validation of environmental performance is ongoing. Biodegradability testing (D6.8) of the developed materials is currently in progress, and comprehensive life cycle assessment (LCA) studies (D6.11) for both carton-based and mycelium-based materials will be completed to quantify their environmental impacts across the full life cycle. In parallel, the techno-economic feasibility of the material solutions is addressed in a separate report (D6.7), providing insight into scalability and cost-effectiveness. Together, these results will support informed decision-making regarding the most promising pathways for further development, commercialization, and integration into sustainable packaging systems.

References

- OECD. 2004. Test No. 202: *Daphnia* sp. Acute Immobilisation Test. OECD Guidelines for the Testing of Chemicals, Section. 2.
- OECD. 2012. Test No. 211: *Daphnia magna* Reproduction Test. OECD Guidelines for the Testing of Chemicals, Section. 2.