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Research and development of manufacturing
protocols and new binders for the industrial
production of bio-composites materials

Experimental degree thesis

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1 Introduction

One of the challenges that our society is facing nowadays is the transition towards a more sustainable production system, the so called “**Circular economy**”. This new paradigm is based on three principles: eliminate waste and pollution, keep products and material in use and regenerate natural systems («Circular economy introduction - Overview» s.d.).

To this end, the use of non-renewable resources for the manufacturing of materials and consumer products has to be reduced. Given that, the demand for “green” materials and productive processes has been extensively increased in the last decades. Among these, **bio-based materials** represent an excellent alternative for product design and manufacturing, both in terms of sustainable manufacturing process and circular lifespan.

Particularly, the so called “**bio-composites**” are promising composite materials, constituted by bio-polymers or bio-derived polymers that are reinforced by natural fibres. The major attractive factor of bio-composites is the possibility to valorise instead of discarding biological wastes and/or residue, such as husks, waste fibres and residual stems, in agreement with the principles of Circular economy.

According to the definition of bio-composites just mentioned, the intervention of a further biological activity by living organisms is not excluded in the transformation process of materials. Given that, from the 1990s some research has been done in relation to the use of fungi as biomaterials. In fact, these organisms are able to give cohesion to incoherent materials due to the production of a mass of microscopic filaments (called “hypae”) that forms the **mycelium**. Over time, the substrate is replaced partially by the fungal biomass and the resulting mycelium is able to strongly cement the substrate itself, resulting in a bio-composite material.

All the productive processes of fungal bio-composites are based on the colonization of a substrate that is shaped contemporarily or subsequently to the mycelial growth. The material so obtained is then pressed and dried following different protocols of pressure and temperature. The diagram reported here below highlights the deep interconnection between the life cycle of mycelium-based materials and the principles of circular thinking. In fact, these renewable mycelium-based materials are fully natural and compostable. Moreover, since they have shown interesting performances, they have been further investigated to produce new materials for packaging, thermal and acoustic insulation (Girometta et al. 2019).

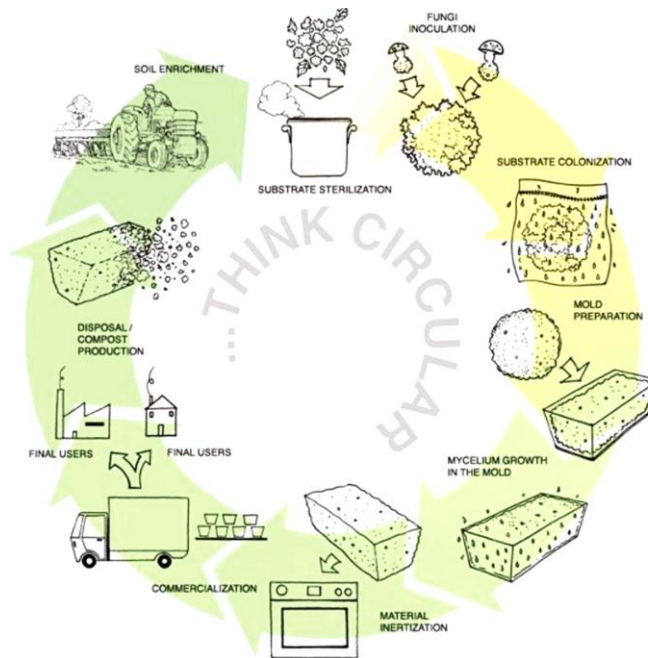


Figure 1.1 Production and life cycle of mycelium-based materials

Mogu S.r.l

Mogu S.r.l. is a small Italian Company that was founded four years ago “on the belief that it is possible to employ Nature’s intelligence to radically disrupt the design of everyday products” (muvobit s.d.). Thanks to complex and iterative R&D projects, different solutions based on mycelium have been explored, thus leading to the creation of responsible products for interior design.

In details, Mogu’s production protocols exploit agro-industrial residues such as cotton fibres or hemp-shives. Whereas the hemicellulose fraction of these plant-biomasses is easily attacked by fungi, the cellulose or lignin compounds are degraded in a preferential manner according to the fungal species. Thanks to minimal variations in the fabrication process, such as the substrate or fungal strains, a wide variety of technical and aesthetic properties can be achieved (Appels et al. 2019). This allows to tune interesting properties for mycelium-based composites, such as morphology, density, tensile and flexural strength, moisture- and water-uptake properties. Particularly, two different products were developed at Mogu: Mogu Acoustics and Mogu Floor.



Figure 1.2 Examples of a Mogu Acoustics set-up and a Mogu Floor tile

Mogu Acoustic modules are made from soft, foam-like mycelium materials and of upcycled textile residues. These mycelium-based foams are low density materials where the fungal biomass and the residual substrate coexist forming a highly anisotropic and fibrous structure. Given that, they have been tested for thermal and acoustic insulation. Particularly, Mogu Acoustic panels are characterised by 3D shapes, in order to maximise sound absorption in the range of frequencies from 130 to 5000 Hz.

Mogu Floor consists of a bio-composite core, coated by a poly-urethane layer 70% bio-based (**bio-PU**). Particularly, since resilient floor coverings may have a significant environmental impact during the entire life cycle of the product, the formulation of bio-PU was achieved strictly following principles of sustainability and **Green Chemistry**. In fact, this synthesis is solvent-free and involves materials and substances that are safe and have low environmental impacts, thus being in compliance with different principles of Green Chemistry: the development of “less hazardous synthesis” (principle number 3), the “design of benign chemicals” (number 4) and the use of “benign solvents and auxiliaries” (num. 5)-if needed. The different colours available for the bio-PU coverings derived from the addition of paints dispersed in castor oil, thus with low VOC emissions and heavy-metal free, as well as by the presence of inert natural materials that are provided as scraps from other productions, like powdered oyster shells from food industries or coffee grounds from local bars and restaurants. Given that, the utilization of these last materials targets also principles of Circular Economy. Thanks to the production principles just mentioned, Mogu’s bio-PU was awarded by the Blue Angel Certificate for resilient floor coverings (DE-UZ 120), the German environmental label that set stringent standards for environmentally friendly, healthy and durable products and services («Blue Angel_resilient floor coverings.pdf», s.d.).

The Internship project that will be discussed in the present Thesis was focused on the production process of bio-composites for flooring. Moreover, over the program’s duration, I had also the opportunity to collaborate with the Company’s technician for the preparation of samples of Mogu bio-PU. The experimental procedures followed for these last experimentations will be briefly discussed in *Section 3*.

1.1 Mogu Floor: BIOCOSCOMPOSITES

The production of Mogu Floor tiles started with the direct pressing of scraps from the production of acoustic panels, in order to valorise on site this waste. The processing conditions (160-200°C and 100 bar) were selected according to the main ones reported in literature that were suitable for the press still available at Mogu. However, the high humidity content of the material (rate of humidity-R.H.=65%) led to a consistent releasing of steam while pressing, and the obtained panels lacked cohesion.

The following tests were run using a mix of grinded colonized cotton fibres and raw hemp shives (total R.H.=35%), with kraft lignin as bio-adhesive.

As stated by Hubbe et al. 2017, there is a growing interest for this kind of hot-pressed biomass products that come from relatively low-valued lignocellulosic materials and natural binders. In fact, **formaldehyde** was traditionally used as adhesive in the production of lignocellulosic board but its emissions have been credited with an acute and chronic effect on human health (European regulation n° 2015/491). Given that, many technologies have been developed in order to manufacture high-performing wood-based products without using and generating any toxic substances. More precisely, from the 1920s the patented procedures followed by Mason and co-workers have demonstrated that with application of heat, pressure and time, the fragments of wood can be put back together, achieving a useful panel material. Particularly, the development of **bonding** by self-bonding or natural adhesives occurs through many different mechanisms:

- a. **Mechanical contact**=deformation of solid components/temporary conversion of at least some of the components into a liquid/viscoelastic state, capable of flowing.
- b. **Molecular contact**= wetting of reinforcing component (cellulose) by flowable matrices, such as plasticized lignin or hemicellulose.
- c. **Chemical bonding**=formation of covalent bonding or non-bonding interactions between the adhesive and the substrate or even between adjacent lignocellulosic components. (physical entanglements of polymeric chains)
- d. **Structural integrity**=suitable uniformity, connectivity and absence of large defects in particles of fibers, in order to allow a proper arrangement.

Many authors claim that **pretreatment** of cellulosic biomass can contribute to the mechanical performances of obtained panels. In relation to the bonding mechanisms described above, these treatments consist of:

- a. **Mechanical grinding** and various combinations of high temperature and added moisture, such as **thermo-hydrromechanical processes** and **steam explosion**.

Particularly, the last one has been widely applied right from the beginning (Mason process) and involves a first penetration of the material by an elastic fluid under high pressure, followed by its releasing that causes particles to rupture.

- b. Removal of substances blocking contact (e.g., a waxy layer coverings) by an **alkaline pre-treatment** with NaOH, that promotes the basic hydrolysis of inter-molecular ester bonds in order to remove hydrophobic compounds and make cellulosic surfaces more water-wettable for molecular contacts. Higher concentration of NaOH leads also to the degradation of hemicellulose and lignin.

Waxy materials can be also inhibited by **washings by solutions with ethanol/water or surfactants**.

- c. **Addition of natural organic compounds**, such as **lignin** that can carry additional free phenolic groups, especially if it has been firstly modified with oxidation/hydrolysis in alkaline conditions or reacted with hemicellulose in order to overcome limitations of its native form.
- d. A sufficient connectivity and continuity are ensured by **similar and small size particles** and **absence of any layering/steam-induced delamination** after both pre-treatment and production process.

Some **supplemental binding components** can also help in achieving the needed levels of bonding for a given application, like **cross-linkers** that behave as formaldehyde replacements. A wide variety of substances are listed in literature: diacids/diamines (e.g. dimethoxyethanal/ difunctional isocyanate with melamine, vanillin and dialdehydes), furan derivatives (furfural and 5-hydroxymethylfurfural), glyoxal and citric acid. Particularly, the latter is claimed to be a natural crosslinker with a catalytic effect on bonding also without adhesives.

There are also other factors that enhance the **activation of the chemical species** that participate in bonding reaction during pressing. They involve mainly **heat and steam-induced activations** to enable plasticization and pressure-induced flow of matrix materials, **acid hydrolysis** (e.g., with acetic acid), **chemical oxidative activation** with ozone/hydrogen peroxide, **enzymatic activation** with laccase enzyme (Gouveia et al. 2018), **plasma** for free radical activation and **curing conditions**.

All these concepts derive from the examination of both scientific articles and books that was carried out at the beginning of the present project of internship and thesis. However, only few of them were investigated with practical experimentations. This was so according to the company's target to produce tiles 100% biodegradable or bio-based, with low application of energy and without using any toxic or polluting compounds. In details, tests

were done using different particles' sizes to increase the structural integrity of panels and using substrates activated by a fungal enzymatic oxidation (see *Sections 2.3.1 and 2.3.2*). The addition of natural compounds to strengthen chemical bonding will be discussed more in details in the following paragraph.

1.1.1 Natural binders and biopolymers

With steadily increasing crude oil prices and increasing environmental awareness, natural binders from renewable resources (“**bio-based binders/adhesives**”, “**bio-binders/adhesives**”) regained attraction as alternative bonding agents (Kües 2007). The results of the state-of-art's analysis of these adhesives will be briefly discussed in this section but a complete list of all the documents investigated, both books and scientific articles, will be provided in the *Bibliography*.

Technical lignin

Lignin can be generally incorporated in phenolic adhesives thanks to the presence of chemical features that are generally involved in polymerization reactions, such as phenolic hydroxyl and aliphatic hydroxyl groups (Hemmilä et al. 2017).

Particularly, “**native lignin**” is wood component that consists of a large, complex hydrophobic polymer build from different monolignol units (e.g., coniferyl/synapyl/paracoumaryl alcohols) with source-dependent variations. Conversely, “**technical lignin**” refers to the derivatives of native-lignin that are obtained by delignification processes of lignocellulosic-biomass (Ekielski e Mishra 2020). These treatments modify the structure of lignin in a specific way, thus affecting also its potential application. In details, according to the applied treatments, the most common technical lignin can be classified in the following way

- a. **Kraft lignin** is major by-product of the kraft pulping process, where a mixture of hot water (155–175 °C), sodium hydroxide and sodium sulphide are utilized. Given that, this kind of lignin contains covalently bonded sulphur species which constitute the major impurities that limit and direct further applications and valorisation approaches, due to the fact that sulphur is a poisoning agent for many metal-containing catalysts.
- b. **Lignosulfonate lignin** is the sulphite salt that comes from the digestion of biomass over a wide range of pH, the so called “sulphite process”. This lignin is water-soluble (thanks to sulphonates groups) and can be considered as an anionic polyelectrolyte due to its large number of charged groups. The choice of cations for the precipitation of lignosulfonates also affects the chains' length and properties of the final lignosulfonates.

c. **Organosolv lignin** comes from the Organosolv pulping/Alcell process, a method that involves a mixture of solvents with/without catalyst and water as a processing medium. Delignification is carried out in the primary process by lignin solubilization using low-boiling-point solvents (mainly alcohols), high-boiling-point solvents (glycols and glycerol), organic acids, ketones, and others.

This sulphur-free extraction process allows to retain higher amounts of functional groups, thus making this type of technical lignin suitable for industrial applications such as biorefineries, nanocomposites, cosmetics.

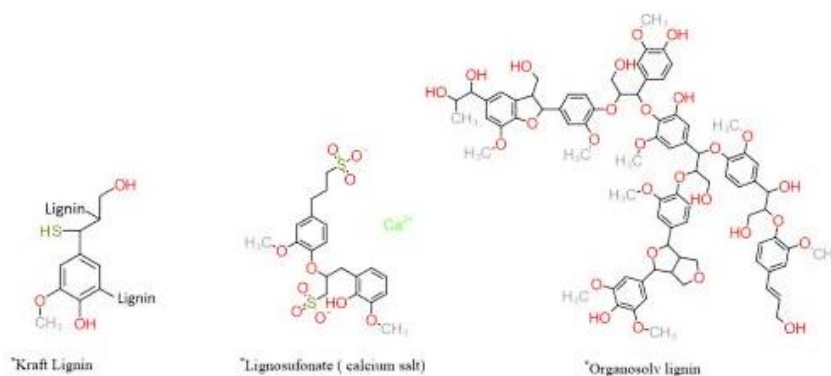


Figure 1.3 Chemical structures of different types of technical lignin

Generally speaking, the high hydrophobicity, low glass-transition temperatures (from 60–90°C for organosolv lignin to 100–170°C), low polydispersity and proportion of free phenolic -OH groups limit the bonding's formation between lignin and fibres. However, this issue can be slightly reduced by a chemical modification of the substrate (oxidation or alkaline hydrolysis), a careful choice of the type of technical lignin to be used, enhanced reactions between the lignin itself /with other wood components or by crosslinking with glyoxal (Navarrete et al. 2010).

On the basis of the results presented above, the most promising type of technical lignin to be used as bio-adhesive seems to be the organosolv one, but its high cost and limited availability did not allow to perform any tests at Mogu's. Conversely, kraft lignin has been mainly used for the tiles' production since the beginning. Lignosulphonate lignin, instead, has a higher cost than the kraft one and its hydrophilicity increases the possibility of steam releasing/bubble formation during pressing- operations. Anyway, it was also utilized for the production of some panels, as it will be discussed in *Section 2.4.2*.

Proteins (soy and whey)

Proteins are complex macromolecules consisting of amino acid monomers, which are chemically linked together to form polypeptide chains that coil into highly ordered global structures. They have been established as adhesives already from the earliest days of

production of wood-based panels but from the 1940s synthetic resins (urea- /phenol-formaldehyde resins) rapidly replaced them by superior durability, viscosity, and pot life. Nevertheless, in the last decades the interest in binders on protein basis has been revived, due to the fact that they come from renewable resources or even waste products, so their cost is low and are all-year around available. The four major groups of cheap and abundantly available proteins are animal albumins (e.g., blood albumins and egg white proteins), collagens from animal connective tissues, skins, and bones, caseins as main milk proteins and plant proteins from cereals, soybeans, etc. (Kües 2007). Particularly, for the present project soy protein isolates (**SPI**) and whey protein isolates (**WPI**) were utilized to produce bio-adhesives for bio-composites (see *Sections 2.4.3 and 2.4.4*).

In details, **soybean proteins** have been claimed to improve mechanical properties and water resistance of wood adhesives, in a different extent depending on the protein content. In fact, there are different soy protein products: **soy flour** (>50%/weight of protein) directly from processing of spent meal from oil production, **soy concentrate** (>70 %/w.) and **soy isolates** with a protein content >90%/weight.

As stated by Vnučec et al. 2017, a modification of soy proteins is necessary to break the strong internal bonds between molecules, thus making more functional groups available for reactions with wood components. Particularly, the main procedures reported in literature involve

- a. **Chemical modification** by acid/alkaline hydrolysis, anionic and cationic detergents (such as sodium dodecyl sulphate), salts and chaotropic agents/crosslinkers. Soy proteins could also be mixed with other natural binders (such as with sorghum lignin for a higher shear strength and water resistance), sucrose and fructose fractions (thus obtaining an improved water resistance and bonding strength thanks to Maillard reactions).
- b. **Thermal modification** that leads to a protein aggregation and decreased protein solubility, due to the combination of exposed -SH or hydrophobic side chains by interchange reactions or hydrophobic interactions. **Cross-linking** with synthetic resins (poly-isocyanate, water-borne epoxy latex and modified polyamide) can further improve the mechanical strengths and water resistances of the composites based on thermally acid-treated SPI.
- c. **Enzymatic modification** (e.g., with trypsin, chymotrypsin, papain and urease) that results in significant reductions in the molecular masses of soy proteins.

Among all these procedures, an alkaline hydrolysis with NaOH of soy protein isolates was selected as a treatment to perform at Mogu's because it could be easily carried out without using polluting or highly hazardous chemicals (see *Section 2.4.3*). Particularly, it was decided to use soy protein isolates in order to optimize the properties of the so obtained bio-adhesive, even though the cost of this material is higher than the one with a low protein concentration.

Whey proteins have a protein content around 50-53% w/w and are often called "waste protein" because they come from a by-product of cheese making and have a lower molecular weight than the soy ones or casein. Nevertheless, they are being gradually used in food and non-food applications e.g., as a food ingredient, for preparations of protective films, surfactants in hair creams and shampoos, pharmacology, biotechnological reagents and so on. It is believed that the use of whey proteins in adhesives might give them a higher added value than application in food, although no patent has been still registered for this application. Anyway, the globular structure of whey proteins must be firstly unfolded to release polar groups. This could be easily done by a **thermal denaturation** under gentle conditions, starting from 40°C and up to 65-70°C. Then, in order to offer additional cohesion strength and bond durability, whey proteins can be cross-linked. As stated by [Gao et al. 2011](#), a whey protein-based wood adhesive can be obtained by a **chemical crosslinking** with polymeric methylene diphenyl diisocyanate in a water-based glue (a solution of polyvinyl alcohol and polyvinyl acetate with emulsion of styrene-co-butadiene and ethylene-co-vinyl acetate). Given that this bio-adhesive requires the use of polluting chemicals, for the experimentation of tiles' production it was decided to further investigate the potentialities of thermally activated WPI without involving any other substances.

Starch

Starch is a polymeric carbohydrate produced by plants (such as corn, potato, cassava, wheat) with a hydrogen-bonding capability that comes from the polar nature of its large number of glycosidic and hydroxyl groups. The adhesive bonding strength of natural starch is not strong enough to glue wood but there are many starch-modification methods that can improve its cohesive properties -especially water resistance-, thus giving comparable performance as synthetic adhesives ([V. Gadhave et al. 2017](#)). These methods aim at creating ester groups and consist mainly in:

- a. **Cross-linking** with many different chemicals, such as isocyanates, melamine formaldehyde, glutaraldehyde and glyoxal

- b. **Acid hydrolysis** with citric acid, urea or polyvinyl alcohol in the presence of borate as crosslinker and catalyst to reduce its water sensitivity
- c. **Addition of emulsions** like epoxy resin or poly-vinyl acetate
- d. **Blending** with biopolymers like proteins and tannins with the addition of aldehyde hardeners like glyoxal, dioxal for an enhancement of properties.

None of these treatments meet the requirements for the production of Mogu's bio-composites because they all involve additional and polluting chemicals. However, some tests have been performed by using a thermoplastic starch, a bio-polymer obtained by the modification of natural starch. Particularly, the one selected (Amitroplast 8945) has been certified as bio-based, bio-degradable and can be disposed of in a home or industrial composting environment. Therefore, its plastic behaviour has been further investigated as it will discuss in *Section 2.4.2*.

Poly-lactic acid (PLA)

PLA is a bio-based and bio-degradable polymer produced by bacterial fermentation of sugar feedstocks, corn, wheat and other starch-rich products. It has slightly polar oxygen atoms which could form hydrogen bonds to the hydroxyl groups of the natural fibres. However, these interactions have a small influence on the overall adhesion so pre-treatments of the matrix are needed, such as the addition of **compatibilizers** or **chemical modifications** (esterification, alkali treatment and cyanoethylation). Particularly, they aim at reducing the hygroscopic nature of natural fibres because the presence of small amount of moisture can negatively affects the hydrolytic degradation of PLA under melt processing conditions (Mukherjee e Kao 2011).

The strength of bio-composites could be further improved by **functionalization/blending of PDLA and PLLA**. Moreover, by blending PLA with less expensive proteins, it is possible to make the blend cheaper, while also improving the performance of both (Misra, Pandey, e Mohanty, s.d., 210).

In general, PLA is used as a binder to obtain chipboard, fibreboard and OSB products with improved performance properties that make them suitable as auxiliary materials (formwork panels, box walls, pallets, etc.) (Kaddo 2020). Therefore, some tests were performed by using fine grains of PLA in order to investigate the potential mechanical performance of this binder without any blending or pre-treatments steps, that are time and energy consuming process that involve the use of additional chemicals (see *Section 2.4.2*).

Poly-vinyl alcohol (PVA)

PVA is a linear amorphous bio-degradable polymer that comes from petrochemical sources and has weak polar interaction among the molecular chains. Therefore, PVA has three main

drawbacks: low resistance towards water and humidity, poor elevated temperature performance and high susceptibility to creep. Anyway, its mechanical properties can be improved by **copolymerizing** with more hydrophobic monomers or functional monomers and **blending** with other adhesives or hardeners (Misra, Pandey, e Mohanty, s.d., 347). The risk of embrittlement can be especially reduced by adding **nanofillers** (montmorillonite and nano-clays), because they create an interaction zone which substantially alters the physical properties relative to the polymer matrix, such as higher or lower polymer mobility, entanglement density, and altered modulus (Kaboarani e Riedl 2011).

According to the studies just presented, the use of PVA as bio-adhesive for wood-based products surely requires additional and costly compounds. Therefore, only a few tests of tiles' production were done by using a mix of thermoplastic starch and PVA as binder (see *Section 2.4.2*). In fact, mixing these polymers decrease the cost of materials and the acidic properties of PVA are expected to enhance the interactions between the starch-based polymer and fibres, as previously discussed.

Natural rubber latex

Latex is a natural biopolymer obtained from *Hevea brasiliensis* (the "rubber tree") and constituted by isoprene units with alcohols or esters of fatty acids as terminal groups. It has excellent engineering properties, such as: high static tensile strength (22 MPa), high elongation (900%), degradation stability and flexibility.

Latex is considered as a promising component in green adhesive formulations for medium density fibreboards especially when mixed with other natural compounds such as lignin (Thuraisingam et al. 2019) or a starch solutions from the acid hydrolysis of rice starch (Akbari et al. 2014). As stated by [Nakanishi et al. 2018](#), it can be directly used as binder in particleboards, leading to an increase of thermal insulation and mechanical properties like modulus of elasticity and rupture, as well as a decrease in water absorption and swelling in thickness. In fact, the latex polymerization process with a natural fibre -such as sugar cane bagasse- is quite long (24 h to be completed) but it leads to the formation of an effective binder protection against water absorption.

In the light of the research presented above, natural latex rubber could enhance the physical and mechanical performances of wood-based panels. However, nowadays its production is limited and located in remote areas (south-east of Asia and the tropical part of Africa) so its utilization cannot be considered as fully sustainable. Moreover, the processing conditions reported in literature involve the application of a pressure much

higher than the one that can be reached using Mogu’s facilities. Therefore, no practical tests were performed to further investigate the use of natural latex rubber.

1.1.2 Mechanical properties and dimensional stability

Wood-based panels are classified by usage for structural or non-structural panels, to the exterior or interior grade panels and by the type of wood and materials used ranging from fibreboards to laminated beams (Hemmilä et al. 2017).

According to the UNI EN 13986 (“Wood-based panels for use in construction: Characteristics, evaluation of conformity and marking”), Mogu floor bio-composites belong to the category of **particleboard**, which are defined as “wood-particles manufactured under pressure and heat with the addition of an adhesive”. The related characteristics were further investigated in refer to the norm ISO 16893-2016 “Wood-based panels – Particleboard”.

Dimensional stability: warping effect

There are many studies in literature related to the dimensional stability of wood-based panels. Particularly, the warping effect is still a long-standing problem in the wood-based panels industries, although many mathematical models have been elaborated for its prevention and reduction. In details, warping is defined as the out-of-plane deformation of a panel from an initially flat condition. This effect is caused by both external stresses linked to panels’ geometry and internal strains, due to thermal changes and moisture movements. In details, there are many variables that influence warping and are related to panels’ composition and properties as well as to the processing conditions, as it is summarized in the following chart (Cai e Dickens 2004).

Table 1.1 Main variable that affect panels’ warping

variable	effect on warping
moisture content	↑ if high value
density and thickness	↓ if high values
modulus of elasticity	
temperature and pressing time	
amount of adhesive	
waterproofing agent	↓ if present
sanding thickness	↑ if smaller particles

Many tests have been performed in order to investigate the correlation of the applied experimental conditions and the factors listed above, changing one variable at each time. They will be fully described in *Section 2.3.7-Warping effect*.

2 Mogu floor: BIOCOSCOMPOSITES

2.1 Objectives

The current production protocol of Mogu Floor tiles consists in a continuous pressing at 180°C and 100 bar for 45-50 minutes of a mix of grinded colonized cotton fibres (40%/total dry weight), raw hemp shives (40%/dry w.) and kraft lignin (20%/dry w.). The obtained panels have a size of 55x55cm, a density as high as 0,95 g/cm³ and a thickness of 0,65 cm after calibration.

The purpose of the present project was the implementation of this protocol for the production of 100% biodegradable/biobased tiles with the mechanical performance of high-/medium-density fibreboards (e.g., dimensional stability, modulus of elasticity, modulus of rupture). The state of art and the final objectives of the project are resumed in *Table 2.1.1*.

Table 2.1.1 Comparison between the state-of-art and the project's objectives

feature	state-of-art	objectives
RH of material	35% in total (RH=65% for colonized cotton fibres)	0% (use only dry fibres)
Cooking time	45-50 minutes	15 minutes
Dimension	55x55 cm	>50x50 cm
Density	0,95 g/cm ³	0,8-0,9 g/cm ³
Thickness after calibration	0,65 cm	0,8-0,9 cm
Homogeneity	bigger particles (cotton) still visible	complete
Planarity	still variable	complete

The main properties and requirements that were targeted are summarized in *Table 2.1.2*. Particularly, they are related to different properties: Modulus of elasticity (MoE), bending strength, Internal bond strength and 24 h Thickness Swelling. Except for the threshold limits of Thickness Swelling, the reported values are the ones stated by the norm ISO 16893-2016 for “**load bearing particleboard for use in dry conditions**”. In fact, this classification well suits the manufactured panels, given their service conditions. Conversely, it was agreed to refer to the requirements of “**heavy-duty load-bearing particleboard for use in dry conditions**” for the thickness swelling tests because this could ensure better performances than the load bearing one. A detailed description of the setting of the above-mentioned tests will be presented in *Section 2.2.3*, whereas the recorded results will be discussed later on (see *Sections 2.3.6 and 2.4.5*).

Table 2.1.2 Tested property requirements of load bearing particleboards for use in dry conditions

Property	Units	Requirements
Residual indentation	mm	< 0,1
Modulus of elasticity (MoE)	Mpa	2200
Bending strength/Resistance to flexion	Mpa	15
Internal bond strength	Mpa	0,40
Thickness swelling 24 hours	%	16

In order to target the objectives presented above, the project was structured as

- study of **macro-variables**: particles' size and distribution, alternative fibres, substrates mixed with fibres activated by fungal enzymatic oxidation, different %/dry weight of kraft lignin as binder, temperature and pressure, density and thickness, warping effect
- study of **micro-variables**: alternative binders to be used both in a pure form and after pre-treatments

The results of these studies will be discussed thoroughly in *Sections 2.3. and 2.4.*

2.2 Material and methods

2.2.1 Tested substrates and bio-adhesives

A list of the different types of fibres and bio-binders utilized for panels' manufacturing in the present project is provided here below. In details, the separation of raw hemp shives fibres into different particles' size was carried out using a metal sieve with a meshes of 2- and 3-mm diameter. This allowed to obtain both a smaller fraction (<2 mm) and a bigger one (>3 mm). Moreover, also mycelium-based substrates were employed: grinded scraps of HS fibres from Mogu Acoustics' production and spent substrates of sawdust from the fungal cultivation for food purposes performed by a Dutch Company.

Among the tested bio-adhesives, both the kraft and lignosulfonate lignin are sold as scraps from other industrial processes. Conversely, the listed bio-polymers are produced by dedicated industries, whereas the protein isolates are sold online for food purposes.

The specifics related to experimental conditions of utilizations will be discussed in following sections. Particularly, panels' production with the same binder (kraft lignin) but different substrates will be discussed in *Section 2.3*, whereas the characterization and utilization of different bio-adhesives for the same fibres will be presented in *Section 2.4*.

Table 2.2.1 Summary of materials utilized in the present project

	material	specifics	appearance
Fibres	Hemp shives (HS)	<ul style="list-style-type: none"> • Normal fibres • Smaller fraction (diameter <2 mm) • Bigger fraction (diameter >3 mm) 	distribution of particles with different size and thickness
	Colonized HS	scraps from fungal cultivation on HS fibres for Mogu Acoustics	fibres covered by fungal residues in different extent
	Sawdust	scraps from fungal cultivation for food production	soft and uniform brownish agglomerates
	Miscanthus	scraps from agricultural production	flat short fibres
	Flax	scraps from agricultural production	distribution of particles with residues of tufted portions
Bio-adhesives	kraft lignin	kraft softwood lignin, sold as "lignin UPM"	fine brownish powder
	lignosulfonate lignin	commercial powder	fine ochraceous powder
	poly-lactic acid (PLA)	commercial powder	coarse whitish powder
	poly-vinyl alcohol (PVA)	viscosity=17 MPa*s, hydrolysis degree=88	fine whitish grains
	soy protein isolates	unflavoured commercial powder with 90% of protein content	yellowish powder
	thermoplastic starch	Amitroplast 8945 both bio-based and bio-degradable (by AGRANA Beteiligungs-AG)	yellowish granules
	whey protein isolates	unflavoured commercial powder with 97% of protein content	white powder

2.2.2 Pressing conditions

a. Pre-treatments of fibres

All the fibres were dried before for at least 24 hours at 80°C in an industrial drier. This was done in order to better approach the set objective of zero humidity rate in the material. Particularly, since sawdust and colonized HS fibres were provided in the form of blocks, they were grinded before drying by using an agricultural shredder. Moreover, flax fibres were firstly mixed in a concrete mixer, in order to manually separate their tufted portions.

b. Pre-activation of binders (if needed)

As it be presented in detail in *Section 2.4.3*, both Soy and Whey Protein Isolates must be treated before being used as bio-adhesive. The procedures followed for the basic hydrolysis of SPI and the thermal denaturation of WPI are described in the Mogu's short experiment canvas reported in the present Thesis as *Annexes III and IV*. Particularly, *Figure 2.2.1* shows the experimental setting for the activation of soy protein isolates.



Figure 2.2.2 Experimental setting for basic hydrolysis of SPI

c. Weighing of material

The calculation of the amount of material for panels' manufacturing was done according to the theoretical density to target and the dimensions of metal frame utilized while pressing. Then, the binder's content was derived as a percentage of the dry weight of substrates. In order to automatize these calculations, an Excel file was finally set up.

In relation to the requested amount, fibres were weighted in buckets or bigger plastic boxes. If the selected binder was a powder, it was directly added to the substrate and then the whole plastic container was mixed by a manual shaking and by using a trowel. Conversely, liquid binders such as activated proteins required a different and careful

mixing procedure. In details, after having added the estimated amount, the material was mixed using a drill at slow speed, thus minimizing any loss and heating of it.

d. Preparation of moulds

Metal frames were placed on a metal plate and a wood mould with slightly smaller dimensions was put inside them. Then the mixed material was carefully inserted in the mould and its surface was levelled as evenly as possible by using a wood support and a flat trowel. The whole apparatus was then transferred on the press' trolley and the wood frame was removed right before pressing.

e. Pressing process

The press utilized for almost all the experiments was a 400 tonnes Vi-Mach, a kind of machinery commonly used for plastic products' manufacturing. In details, it is composed by two trolleys that are inserted in between pistons by which the selected pressure and temperature are applied. During pressing, it is possible to observe a releasing of steam and even little explosions with spreading of fibres, in a different extent in relation to the humidity rate and experimental conditions. In order to limit this phenomenon, the substrates must have the lowest liquid content as possible, for instance by using dried fibres and powdered binders. Otherwise, some degassing steps could be done by a slightly opening of pistons, thus being able to deal also with liquid binders such as activated proteins. Particularly, in order to limit the formation of steam while pressing, these last bio-adhesives were prepared by using the minimum amount of water.

A summary of the different processing methodologies applied is provided in the present Thesis as *Annex I*. Each of them will be discussed in detail in *Sections 2.3 and 2.4.2-4*.

f. Curing and storage

At the end of the pressing process, panels were taken out of the press and the metal frame was removed, if they seemed already stiff and cohesive. Then, they were placed on a metal grid and left to cool down with a weight on top, thus limiting any swelling effect linked to environmental conditions. After having done some observations in relation to the warping effect (see *Section 2.3.7*), it was decided to cut the external edges of tiles using a table circular saw before they had fully cooled down. Finally, panels were put on a pallet, thus forming a pile that was tighten with belts and left under room conditions.

2.2.3 Experimental conditions of tests

At first, some research was done among the main worldwide manufacturers of medium- and high-density fibreboards, in order to understand which are the most important products' specifics requested from market. Therefore, it was decided to further investigate flexural properties, internal bond strength, residual indentation and swelling/expansion in thickness (%). In details, the mechanical performances of panels made with kraft lignin as binder were tested at first by a private laboratory, thus being able to check whether the process' development was approaching the set objectives or not. Afterward, other promising methodologies were characterized by doing additional tests both at the University of Bologna (Scanning Electron and optical Microscopy) and at Mogu's (swelling in thickness). An overall list of the studies performed will be provided in the Table here below, whereas the results will be presented in the following *Sections 2.3.6-7* and *2.4.5*.

In addition, the thermal degradation of all the tested binders was characterized by doing Thermo Gravimetric Analysis (TGA), as it will be discussed in *Section 2.4.1*.

Finally, the role of the enzymatic oxidation performed by fungi was further investigated by doing some analysis of TGA and Infrared Spectroscopy (IR), both for normal and colonized HS fibres (see *Section 2.4.6*).

The experimental conditions of all the tests mentioned in the present paragraph will be described in detail here below.

Table 2.2.2 Summary of tests performed for different methodologies

methodology	fibre composition	binder	mechanical tests				swelling in thickness		SEM and optical microscope**
			Residual Indentation	Bending strenght	Modulus of Elasticity (MoE)*	Internal bond strength (IB)*	Exposure to 99,9% humid environment for 8-10 days	Immersion in water for 24 hrs	
Current protocol	<ul style="list-style-type: none"> grinded colonized cotton fibres (40%/dry w.) normal HS (40%/dry w.) 	kraft lignin (20 %/dry w.)	x	x	x	x	x	x	-
Methodology E	normal HS	kraft lignin (10%/dry w.)	x	x	x	x	x	x	x
	smaller HS (<2 mm)	kraft lignin (10%/dry w.)	-	x	x	x	x	x	x
	<ul style="list-style-type: none"> normal HS (75%/dry w.) grinded colonized HS fibres (25%/dry w.) 	kraft lignin (10%/dry w.)	-	x	x	x	x	x	-
	normal HS	PLA (15%/dry w.)	-	-	-	-	-	x	x
Activated proteins	normal HS	activated SPI (10%/dry w.)	-	x	x	-	-	x	x
	normal HS	<ul style="list-style-type: none"> kraft lignin (5%/dry w.) activated SPI (5%/binder content) 	-	x	x	-	-	x	-
	normal HS	activated WPI (10%/dry w.)	-	x	x	-	-	x	x

* Tests performed also at University of Bologna, **Tests performed only at University of Bologna

Mechanical tests

The mechanical performances of these panels were analysed both by a private laboratory and at the University of Bologna, always in partial agreement with the related European and International Standards. In details, at university there were performed tests of Resistance to Flexion and Modulus of Elasticity, using a Dual Column Table Models testing machine (Instron 5966). The recorded data were then processed using the software Excel. The majority of tested tiles were manufacturing following “Methodology E” (see *Annex I*), since it allowed to reduce the warping effect, as it will be discussed in the following *Sections 2.3.4 and 2.3.7*. In order to check whether this Methodology was approaching the set objectives, a panel produced with the current Mogu’s protocol was examined as well. At last, the tiles with an activated protein as a binder were manufactured following a different process, as it will be explained in *Section 2.4.4*.

All the panels were firstly calibrated and then manually cut with a table circular saw in order to obtain samples with the dimensions and shape requested by these norms

- a. **Determination of Resistance to Indentation:** num. 3 specimens/methodology with dimensions of 6 cmx6cm (UNI EN 1534:2020)
- b. **Determination of Modulus of elasticity (MoE) in bending and of bending strength:** num. 8 transversal and num. 8 longitudinal specimens/methodology with dimensions of 20 x thickness + 50 mm) x 50 mm x thickness (mm) (UNI EN 310:1996 and ISO 16978:2003)
- c. **Determination of tensile strength perpendicular to the plane of the board (Internal bond - IB):** num. 10 specimens/methodology with dimension of 50 mm x 50 mm x thickness (mm), (UNI EN 319:1994)

Particularly, before cutting the samples to be tested for their **Resistance to Indentation**, a layer of bio-polyurethane was glued on their surface. Therefore, these specimens could be considered as representative samples of Mogu Floor and the results were compared with the values stated by ISO 16776 (Resilient floor coverings - Heterogeneous polyurethane floor coverings – Specification). It was decided to test three methodologies with different densities: 0,81 g/cm³ for the current protocol, 0,86 g/cm³ and 0,77 g/cm³ for the tiles made by normal HS as substrate. This range of values allowed to better set the optimal theoretical density to target for following experimentations, as it will discussed in *Sections 2.3.6 and 2.3.8*.

Swelling in thickness

At Mogu's some tests of swelling in thickness were done following two different approaches: the samples' exposure to a 99,9% humid environment for 8/10 days and the immersion in water for 24 hours (UNI EN 317:1993). Particularly, the last one does not represent actual conditions of utilization but it is commercially considered among the methods to determine panels' dimensional stability.

All the tiles were calibrated before being cut in specimens with dimension of 5cm x 5cm (3 specimens/methodology): This was done in order to remove any effect of protection from external layers and reproduce real use conditions as much as possible.

The experimental set-up of the two tests will be described in details here below.

a. Exposure to 99,9% humid environment for 8/10 days

Samples were firstly dried in a laboratory oven for 24 hours at 100°C. After having measured their weight and dimensions, they were immediately placed on the top of a plastic support (a perforated plastic cup) inside a box containing about 2 cm of water (see *Figure 2.2.3*). The box was carefully sealed, thus allowing to reach a stable rate of 99,9% of humidity in 24 hours. Width and thickness were measured after 1, 2, 3, 4, 8 hours and them almost every day, until constant values for both water absorption and swelling in thickness were obtained. After that, samples were taken out and left at environmental conditions until they were fully dried (about 50% R.H. and 20°C). At last, they were tested again for dimensions as well as for any textural change in respect to the starting conditions.



Figure 2.2.3 Experimental set-up for samples' exposure to 99,9% humid environment

b. Immersion in water for 24 hours

Samples were firstly placed for 24 hours inside a drawer with a stable humidity rate of 55%. Then, the first measures of weight and dimensions were taken and samples were placed inside a plastic cup. Particularly, a circular plastic piece, hold on the top of samples and kept in position by an elastic, allowed to maintain samples with 3 cm of water on top but not in contact with the bottom part of the container, as it is shown in *Figure 2.2.4*. This

allowed them to freely move while swelling during for the following 24 hours of immersion and in a sealed plastic box. After that time, they were taken out and left drying at environmental conditions, before being tested for dimensions and textural changes.



Figure 2.2.4 Experimental set-up for 24 hours of immersion in water

c. Immersion in water for 24 hours with a superficial thermal pre-treatment

As stated by Del Menezzi e Tomaselli 2005, a thermal treatment can decrease the wood hygroscopicity, which means reduction in dimensional instability. Therefore, it was decided to apply a thermal pre-treatment to some samples of normal HS with the 10%/dry w. of kraft lignin. At first, the selected specimens were heated at 250°C for 5 minutes by using the manufacturing press. Since with these experimental conditions some bubbles randomly formed, it was decided to apply a lower temperature of 200°C for 10 minutes. By doing so, it was possible to thermally treat samples in a replicable way, before performing the tests of immersion in water for 24 hours as described in the previous paragraph.

Scanning Electron and optical Microscope

Although different types of wood-based panels are currently manufactured at an industrial level, there is a general lack of microscopical characterization in literature. As a matter of fact, this kind of analysis allow to better correlate macroscopic properties with the panels' composition itself. Particularly, the article of Li et al. 2009 puts in comparison SEM images of fractured surfaces of wood fibreboards pressed at 170°C for 10 min with different initial moisture content (10% and 35%). Since the fibres that look highly flat and straight at microscopical level showed also better mechanical properties, it was possible to associate the entanglements of fibres to a good adhesion behaviour of the utilized soybean binder.

Given that, it was decided to further investigate some methodologies by performing analysis at SEM and optical microscope. In details, small calibrated specimens were manually fractured using a hammer and placed on a metal plate using a small amount of modelling clay. Differently from the article cited above, the surface of samples was not

coated with metals before the SEM analysis or dried. However, by setting a pressure as high as 100 Pa, it was possible to remove almost any interferences between the electron gun and electrons naturally present in air. The electron gun of the utilized instrument (Zeiss EVO 50 EP) was set up to 15 kV and samples' surfaces were investigated with different enlargements (20 and 100 μm).

In addition, some images of the same samples were acquired by using an optical microscope (Hirox KH 7700), thus being able to apply an enlargement as large as 2000 μm .

Thermo-Gravimetric Analysis (TGA)

These analyses were carried out with a PerkinElmer TGA 4000 with the following temperature programme: from 30°C to 600°C with a ramp of 10°C/min in inert atmosphere (nitrogen), 5 minutes at 600°C and room conditions.

The selected range of temperature allowed to register the maximum weight loss (%) for each sample. Particularly, for the analysis of kraft lignin it was decided to raise the temperature up to 800°C in order to reproduce exactly the experimental conditions applied for previous analysis of other lignin samples. Moreover, by operating in presence of oxygen for the last 5 minutes, it was possible to fully burn any residues.

No pre-treatments were done on the tested samples, thus being able to investigate the actual conditions of both the fibres and binders utilized for panels' manufacturing. The registered data were converted into .txt files and then processed using the software Excel.

Infrared-Spectroscopy (IR)

A Spectrum Two FT-IR Spectrometer (PerkinElmer) was used to register the IR spectra of some fibres of normal HS and grided colonized HS. In details, a small random fraction and a thicker one of HS were chosen, thus taking into account the particles' size distribution of this substrates. Conversely, three different samples of the colonized substrate were analysed: a fibrous portion, a part fully covered by mycelium and a small fungal residue. This allowed to detect any differences between the normal and colonized substrate in relation to different content of mycelium.

The recorded results were processed in the same way of the ones from TGA studies and will be presented in details in *Section 2.4.6*

2.3 Results and discussion: studies on macro-variables

Many experiments were performed to identify the most impacting and favourable macro-variables towards the process' implementation. In details, it was changed a variable at a time to better determine its effects and only kraft lignin was used as binder for dried fibres. An overview of the features investigated is reported here below and a detailed summary of all the methodologies will be provided as well as *Annex I*.

2.3.1 Size and particles' distribution: normal, smaller and bigger HS

As it was discussed in *Section 1.1*, smaller particles are expected to enhance the structural integrity of bio-composites. Therefore, after having sieved and dried HS fibres, some panels were manufactured adding the 10%/dry weight of kraft lignin to the quantity of substrate calculated for 0,8 cm thickness. The process followed was 10 minutes of cooking with a degassing step after 5 min. and 5 min. of repressing at 180°C and 150 bar (see Methodology C of *Annex I*).

In the following chart, the recorded results are put in comparison with the ones obtained using a particle sizes' distribution ("normal HS") with analogous pressing conditions.

Table 2.3.1 Specifics of panels manufactured using HS particles with different sizes and distribution

fibres' dimension	av. Thickness (cm)	av. Density (g/cm ³)	defects
normal HS	8,21	0,85	warping as usual
smaller HS (<2mm)	8,69	0,80	reduced warping
bigger HS (>3 mm)	7,87	0,88	incohesive and with bubbles

In details, the **bigger fraction** of HS didn't allow a proper mixing with the binder, due to the fact that the available surface area is less, thus resulting in a poor packing of materials and uneven distribution of lignin. Given that, panels with many bubbles were obtained so it was agreed on not to proceed with further tests with this particles' size.

Conversely, dealing with **smaller particles** of HS made the process smoother. In fact, the increased surface area leads to a better spreading of lignin into the fibres, thus limiting also the bubbles' formation. Moreover, these panels showed a decreased warping just after the pressing than the ones made with normal HS. In fact, a particle sizes' distribution results in uneven and higher internal attraction between particles in the final panel, that causes an increased warping.

Anyway, also for the panels made by **normal HS** a good cohesion was observed with no bubbles' formation. Particularly, they have a slightly higher density in comparison with panels with sieved HS. This could be due to the fact that a particle sizes' distribution allows the fibres to better withstand the applied pressure during the manufacturing process thus being able to reach a lower thickness and so a higher density.

The mechanical performances and dimensional stability of panels made with different particles' size and distribution will be provided in *Sections 2.3.6 and 2.3.7*, respectively. Conversely, their internal structure will be deeply investigated in the following paragraph.

Analysis at Scanning Electron and optical microscope

The experimental observations mentioned above were further investigated at a microscopic level by performing some analysis both with the SEM and optical microscope at the University of Bologna. Particularly, it was decided to study panels processed with Methodology E and different substrates: the normal HS and its smaller fraction (diameter <2mm). By scanning at the SEM the surface of specimens with **normal HS**, it was possible to clearly distinguish bigger particles that prevent fibres to pack closely one on the others. Moreover, as it is highlighted by the red circle in the picture here below, some visible spots of melted lignin were detected. Their presence was recorded also while analysing the same substrate using the optical microscope (see *Figure 2.3.2*). Due to this microscopical accumulations of binder that did not reach with substrate, it was decided to test a lower %/dry weight of lignin, as it will be discussed in *Section 2.3.3*.

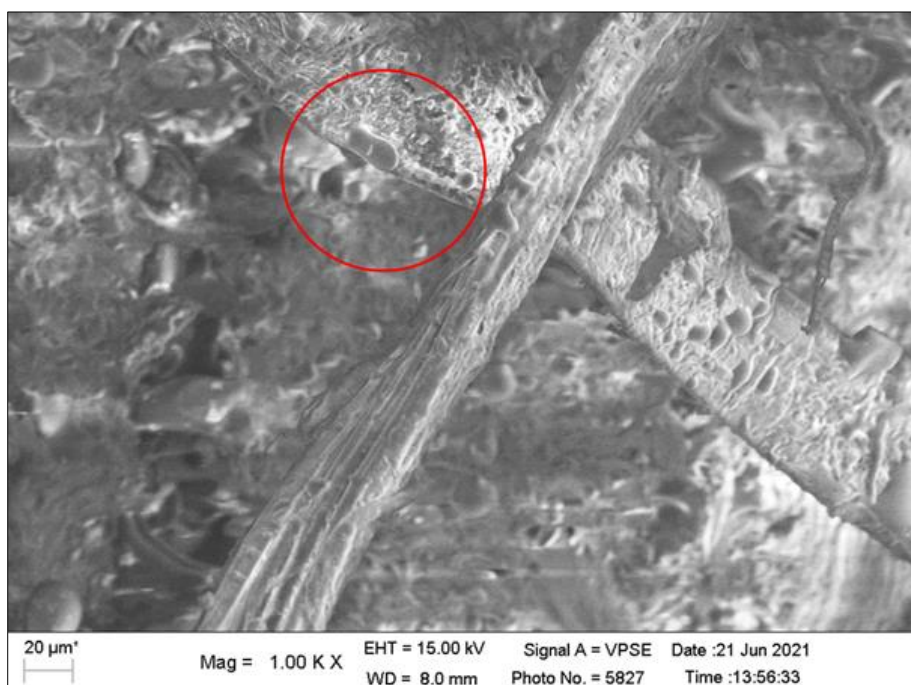


Figure 2.3.1 SEM image of sample made with normal HS and lignin UPM (10%/dry w.)



Figure 2.3.2 Image at optical microscope of sample made with normal HS and lignin UPM (10%/dry w.)

Conversely, the presence of melted spots of lignin was not detected for the sample of **smaller HS**, as represented in *Figure 2.3.3*. This confirmed what already noticed during the experimental manufacturing process, the fact that a uniform particles' size distribution allows a better spreading of lignin inside them.

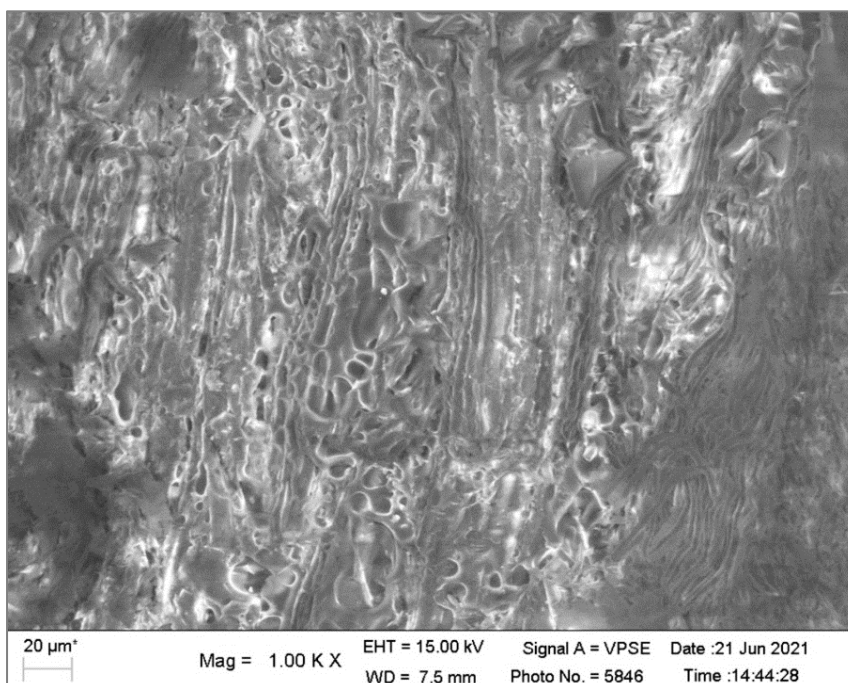


Figure 2.3.3 SEM image of sample made with smaller HS and lignin UPM (10%/dry w.)

2.3.2 Fibres' composition

Tests with mixed substrates: hemp shives and colonized substrates

As previously discussed in *Sections 1 and 1.1*, the fungal growth is an enzymatic oxidation that is claimed to add peculiar properties to its plant-based substrate. Given that, some experiments have been performed to further investigate this potentiality.

At first, tiles with **only mushroom-colonized substrates** were produced, following the **Methodology A**: 20 minutes of pressing at 100 bar and 180°C with a degassing after 10 minutes and 5 minutes of repressing. No bubbles formed for both grinded colonized HS and sawdust. In fact, these materials have a spongy texture and a very small particles' size because of the grinding process. Therefore, the spreading of lignin and packing of fibres is easier, as well as the releasing of steam during the manufacturing process, thus confirming what was already discussed for smaller HS. Moreover, as it is shown in the following Table, these panels have values of densities similar to the ones obtained with different particles' sizes and normal HS (see *Table 2.3.1*). Conversely, the tiles made of colonized substrates were highly fragile, especially the one with sawdust that also presents also the highest warping. However, cohesion could be slightly improved by an increase of the binder's content, as it was detected for panels made with 100% colonized HS. On the other hand, for panels made with sawdust, a higher %/w of lignin was directly used because of the highly granular texture of this substrate.

Finally, colonized HS and sawdust were **mixed in a 1:1 proportion** using both the 10%/w and 20%/w of lignin. These panels were extremely fragile and with a consistent warping. An enhanced binder's content seemed to provide a higher internal cohesion, but it also increased the warping effect.

Table 2.3.2 Results from panels made by 100% mushroom-colonized substrates

fibres' composition	%/w of lignin	thickness (mm)	density (g/cm³)	notes
100% colonized HS	10 %/w	8,65	0,80	very fragile
	20 %/w	8,70	0,75	less fragile
100 % sawdust	20 %/w	8,47	0,82	extremely fragile
50% colonized HS + 50% sawdust	10 %/w	9,09	0,75	less warping
	20 %/w	8,40	0,81	heavy warping

In order to understand if the use of a mushroom-colonised fibres (hemp shives or sawdust) could have any influence on the final properties, it was decided to mix both smaller and normal HS fibres with these substrates in different proportions.

At first, the **same mushroom-colonized substrate** was **mixed in a 1:1 proportion with HS fibres**. Particularly, it was decided to use sawdust in order to better distinguish the role of the fungal enzymatic oxidation when utilized with raw hemp shives fibres. It seemed that a different particles' size and distribution has no influence on the thicknesses and densities' values. However, the homogeneous particles' size of smaller HS could slightly decrease the warping effect, due to the fact that internal forces are weaker and well distributed along the tiles' profile.

Table 2.3.3 Results from panels made by mixes of 50% normal/smaller HS and 50% sawdust at 100 bar

HS fibres	% of sawdust	%/w of lignin	thickness (mm)	density (g/cm ³)	notes
50% normal HS	50%	10 %/w	8,93	0,78	huge warping
50% smaller HS	50%	10 %/w	8,98	0,78	almost negligible warping

Then **sawdust** was mixed in a **1:3 proportion with both normal and smaller HS**. Since comparable results were obtained, it seemed that the addition of sawdust does not have any influence of density within the applied processing conditions. In fact, all the densities obtained with these experiments are between 0,78 and 0,80 g/cm³.

Conversely, it was noticed that, when mixed with smaller HS, an increase in sawdust's content leads to a coherent and degressive evolution of the warping effect. Particularly, the flattest panel was obtained with the 50% of sawdust and 50% of smaller HS.

Table 2.3.4 Results from panels made by mixes of 75% normal/smaller HS and 25% sawdust at 100 bar

HS fibres	% of sawdust	%/w of lignin	thickness (mm)	density (g/cm ³)	notes
75% normal HS	25%	10 %/w	8,96	0,78	light warping
75% smaller HS	25%	10 %/w	8,70	0,80	light warping and 3 small bubbles

Anyway, these results did not allow to end up with the identification of a proper trend for the evolution of density and other mechanical properties in respect to the presence of mushroom-colonized substrates. Therefore, further investigations were done by changing the processing conditions.

In details, given the results of some other tests (see *Section 2.3.4-Pressure*), it was decided to repeat at higher pressure (**150 bar** instead of 100 bar) the combinations of both **normal and smaller HS with sawdust in a 1:1 proportion**. If compared to previous results, higher densities were reached for both normal and smaller HS (see *Table 2.3.5*). Since these panels were highly fragile, it was decided to increase the binder's content up to the 20%/w. However, this higher %/w of lignin enhanced the attraction forces within the panels thus worsening the warping effect and bubble formed in the panels made with smaller HS.

Table 2.3.5 Results from panels made by mixes of 50% normal/smaller HS and 50% sawdust at 150 bar

HS fibres	% of sawdust	%/w of lignin	thickness (mm)	density (g/cm ³)	notes
50% normal HS	50%	10 %/w	8,22	0,86	fragile panel with light warping in central part
		20 %/w	7,82	0,89	higher warping
50% smaller HS	50%	10 %/w	8,36	0,83	huge warping, one flat bubble, fragile panels
		20 %/w	7,48	0,92	huge warping, one big bubble, highly fragile panels

Given these last results, it could be concluded that a mushroom-colonized substrate like sawdust gives fragility to panels, regardless the %/weight of binder and particles' size and distribution.

Tests with different fibres: miscanthus, flax

After having implemented the process by using normal hemp shives as substrate (see *Section 2.3.8- Methodology F*), other fibres were tested for this methodology of panels' production. Particularly, Miscanthus and Flax were selected since they were already available at Mogu. As reported by Misra, Pandey, e Mohanty, s.d., both hemp and flax are classified as bast fibres, whereas miscanthus is considered among the perennial grasses. Although the basic chemical nature is similar in terms of constituents, there could be some varieties in their compositions depending on the type and origin of fibres. Therefore, the mechanical properties of resulting composite materials are highly dependent on the source of natural substrates. As shown by the Table here below, bast fibres are usually preferred for applications requiring high tensile strength, stiffness, and modulus.

Table 2.3.6 Specific from literature of the different fibres tested for panels' manufacturing

fibre	density (g/cm ³)	specific tensile strength (MPa)	specific tensile modulus (Mpa)	elongation at break (%)	cellulose content (wt. %)	moisture (wt. %)
flax	1.5	535-1000	18.4-53	1.2-3.2	64-71	8-12
hemp	1.47	372-608	47.3	2-4	70-74	6.2-12
miscanthus	1.41	-	6.7	-	38	-

In agreement with these data, the experimental manufacturing of tiles using **miscanthus** and the 8%/dry w. of kraft lignin as binder gave bad results. In fact, the obtained panel was highly fragile and had a lower density.

Conversely, the use of **flax** gave interesting results that could be compared to the ones of normal HS, obtained with an amount of material calculated for a theoretical density of 0,9 g/cm³ and 0,9 cm thickness and using a 0,8cm metal frame. In details, 12/13 panels made with normal HS were good and 13/18 for flax. According to the measurements of these last

panels (not calibrated), it was obtained an average density of $0,83 \pm 0,02 \text{ g/cm}^3$ and a thickness of $0,96 \pm 0,02 \text{ cm}$.

However, while dealing with flax, it was noticed a remarkable presence of tufted portions that were prone to aggregate with lignin, thus increasing the possibility of a local bubbles' formation. Therefore, it was necessary to separate these portions by spinning fibres in a concrete mixer, before adding the binder. Since this additional step made the whole manufacturing process with flax more time-consuming than the one with normal HS, it was agreed to proceed the experimentations by using only hemp.

2.3.3 Tests with different %/dry weight of kraft lignin

The utilized kraft lignin is industrially sold as a fine brownish powder, that can be more easily absorb by fluffy substrates. This is reason why the presence of grinded colonized cotton fibres in the current production protocol of Mogu's Biocomposites requires a lignin content as high as **20%/dry weight**. Given that this substrate was no longer utilized in the present project, it was decided to lower the amount of binder as much as possible. In fact, this could not only decrease the cost of materials but also the characteristic smell of lignin in the final products.

At first, the content of lignin was reduced down to the **10%/dry w.**, regardless the type and composition of fibres. However, this amount turned out to be still too high for the normal HS and its bigger fraction (diameter $>3\text{mm}$), whereas it was insufficient for substrates like sawdust and colonized HS (see *Section 2.3.2*).

Since the use of normal HS gave interesting results both regarding the mechanical performances and dimensional stability of panels (see *Sections 2.3.6. and 2.3.7*), it was decided to further investigate the best amount of lignin to be used with this substrate. Therefore, additional experiments were done by using the **8%/dry w.** of lignin. This content seemed to be the threshold value to get panels stiff and cohesive, although some bubbles formed where lignin was more concentrated. In fact, as previously described, the pressing conditions did not allow to have a fully homogeneous mixing between fibres and binders, as well as a standardized filling of moulds before pressing. This led to the formation of spots where lignin was more concentrated, thus increasing the possibility of local bubbles' formation. Given that, for further tests it is highly recommended to at least implement the mixing of fibres and binder, such as by always using a concrete mixer.

2.3.4 Tests with different pressing conditions

Pressure

The first tests were performed by applying a pressure of **100 bar**. However, it was observed that the top part of the press was not fully in contact with the metal plane below during the manufacturing process. Therefore, it was set an experiment to better investigate at which applied force the whole substrate could have been homogeneously pressed. Particularly, the same amount of material of previous experiments was put without the metal frame directly on the press' plane and then the applied pressure was increased until the top cylinder reached the plane below. The minimum thickness was reached with a pressure as high as **150 bar**, without doing any repressing step at the end (see *Annex I-Methodology B*). The obtained panel had fragile edges due to the lack of the metal frame and also presented a flat bubble on its surface.

Given that, in order to obtain cohesive panels and homogeneously pressed, it was agreed to enhance the applied pressure up to 150 bar and do a final repressing step without the metal frame, thus reducing also the final thickness. Particularly, dealing with such higher forces allowed also to reduce the manufacturing time, from 20 min. of cooking with a degassing after 10 min. + 5 min. of repressing to 10 minutes with a degassing after 5 min. + 5 min. of repressing, without the metal frame (see **Methodologies A and C** of *Annex I*).

For instance, the table reported here below puts in comparison the specifics of panels made by smaller HS and the same content of lignin at both 100 and 150 bar. By applying a higher pressure, it was possible to reach a lower thickness and so a higher density.

Table 2.3.7 Influence of pressure on panels with the same composition

pressure	composition	thickness (mm)	density (g/cm ³)
100 bar	100% HS<2 mm + 10%/dry w. kraft lignin	8,97	0,78
150 bar	100% HS<2 mm + 10 %/dry w. kraft lignin	8,41	0,82

While pressing at 150 bar, a high %/weight of adhesive caused more problems of bubbles' formation. Therefore, it was necessary to lower the %/w of lignin but the increased pressure was able to compensate this. In fact, similar results were obtained for panels made by normal HS, as it is reported in *Table 2.3.8*. Moreover, the softer was the material, the easier was reaching very low thicknesses and high densities. Given that, the best results were recorded for panels made with colonized HS as substrate. However, the high density of this panel did not correspond to an enhanced cohesion as well, thus confirming that activated substrates bring fragility to the tiles, as it was discussed previously in *Section 2.3.2*.

Table 2.3.8 Influence of pressure on panels with different binder content

pressure	composition	thickness (mm)	density (g/cm ³)
100 bar	100% normal HS+20%/w of lignin UPM	8,42	0,83
150 bar	100% normal HS + 10%/w of lignin UPM	8,21	0,85
100 bar	100% colonized HS + 20%/w of lignin UPM	8,7	0,75
150 bar	100% colonized HS + 10%/w of lignin UPM	7,98	0,86

The relation between pressure and thickness of the above-mentioned panels was further investigated by measuring it in with a proper calibre in 9 different points, evenly distributed among the surface. For panels manufactured at 100 bar, generally, lower values were recorded for the corners in respect to the central part. Conversely, by operating at higher pressure, the value-difference in respect to the middle point was reduced.

Considering what was discussed up to this point in the present section, it was agreed to apply a pressure of 150 bar for all the following experimentations.

Temperature

As reported in literature, the glass transition temperature of kraft lignin is about 124-174°C. Therefore, some tests were done in order to better estimate the lowest pressing temperature at which this kind of lignin exhibits plastic behaviour and cohesive and stiff tiles could be produced, with a decrease of energy requirements and production costs as well. In fact, by slowing down the lignin's melting process, any possible thermal shock responsible for bubbles' formation or eventually the warping of panels could be limited. On the other hand, it was set up also an experiment with a higher value of temperature in order to better understand its influence on panels' warping. In details, three different temperatures were investigated by performing a 15-minute cycle and 5 minutes of repressing at 150 bar, using normal HS with the 10%/w of lignin. The recorded results are reported here below

- **T=120°C:** incohesive panel
- **T=150°C:** cohesive panel with no bubbles and a density=0,82 g/cm³
- **T=200°C:** higher density (0,94 g/cm³) in respect to analogous panels manufactured at 180°C (around 0,89 g/cm³) but a huge bubble formed in the middle part from side to side.

The best range of temperatures for this panels' manufacturing process seems to go from 150°C to 180°C. In fact, within these values, cohesive panels are obtained with comparable warping. However, since the majority of other production protocols' that involve the use

of lignin as bio-adhesive operate at temperatures around 180°C, it was decided to keep this value for the following experimentations.

2.3.5 Panels' characteristics: density and thickness

At first, the amount of fibre to be used for panels' manufacturing was calculated according to the metal frame's dimensions (55x55 cm with a thickness of **0,9 cm**) and a theoretical density of 0.9 g/cm³. In details, it was decided to target this value of density since it is the one of high- and medium-density fibreboards, the wood-based panels that are claimed to have the highest mechanical performances.

As mentioned in the previous section, increasing the pressure could slightly enhance panels' actual density in a replicable way, whereas higher temperatures make the manufacturing process less reliable. Nevertheless, the real density of the so obtained tiles was always lower than the theoretical one, regardless the type of substrate that was used. In fact, comparable issues occurred during the manufacturing processes: a small amount of material was lost while preparing the mould before pressing, some fibres remained stucked to the metal frame, steam was released during the pressing and so on. Given that, the mass of panels was always lower than the weighted material and, to be more precise, this difference was about 80 grams on average. In order to overcome these losses and better approach a density of 0,9 g/cm³, it was agreed to use an amount of substrate estimated for a higher panels' thickness, while keeping the same metal frame for the manufacturing process. This was so because the metal frame showed to play an essential role for the cohesion and homogeneity of panels.

Firstly, some experimentations were done using the amount of fibre calculated for a thickness as high as **1,2 cm**. Due to this higher amount of material, the process' time was slightly increased in respect to the one of Methodology C. In detail, this new methodology (see **Methodology D** in *Annex I*) consists in 15 minutes of pressing with degassing each 5 minutes and 5 minutes of repressing at 180°C, after having flipped the panels to increase the homogeneity between the two sides. The same recipe was tested by applying both a lower and higher pressure (100 and 150 bar). This confirmed that a pressure of 150 bar allows to achieve lower thickness and higher density values, as it is shown in the following Table. Both these panels were not sufficiently cohesive and showed a comparable warping effect, with edges more pressed. Given that, it was decided to use an amount of material estimated for a lower thickness.

Table 2.3.9 Results of panels with material estimated for 1,2 cm of thickness

Type of fibre	%/w of lignin UPM	pressure (bar)	thickness (mm)	density (g/cm ³)	notes
100% HS for 1,2 cm thickness	10%/w	100	13,24	0,79	warping as usual
		150	11,96	0,88	warping as usual, 3 bubbles after the repressing step

Moreover, taking into account all the tests made before, it was noticed that repressing steps worsen the warping effect, both with flipped panels or not. Therefore, it was decided to test a quantity of material for 0,9 and 1 cm thickness with a new methodology: 16/20 minutes of pressing with degassing steps each 4/5 minutes at 150 bar and 180°C (see **Methodology E-Annex I**). No bubbles formed and the obtained panels were very cohesive with a slightly lower warping effect. Since the panel made with an amount of material estimated for **1 cm** thickness showed the highest density, this recipe was further investigated, as it will be discussed in the following *Section 2.3.8*.

Table 2.3.10 Results of panels with material estimated for 1 cm and 0,9 cm of thickness

Type of fibre	%/w of lignin UPM	thickness (mm)	density (g/cm ³)	notes
100% HS for 1 cm thickness	10%/w	10,5	0,87	lower warping effect
100% HS for 0,9 cm thickness		9,54	0,83	lower warping effect

2.3.6 Panels' mechanical performances

The requirements of the different mechanical tests performed have been already listed in *Section 2.1*, whereas the experimental conditions were reported in *Section 2.2.3*. Therefore, the following paragraphs will discuss more in detail the results of each test.

Residual indentation

The resistance to indentation is determined by applying a loaded indenter to the face of the test specimen. After the loading, the unloaded specimen is left to recover and then the diameter of the residual indentation is used to evaluate the material's resistance.

In the present project, this test was performed on three different specimens for each selected methodology: the current production protocol ("CONV") and panels made with normal HS following Methodology E and different values of density (0,77 and 0,86 g/cm³ after calibration). The following graph presents the average results of indentation after the samples had fully recovered and the decreasing in thickness after only 150 minutes from the releasing of the applied load (50 kg).

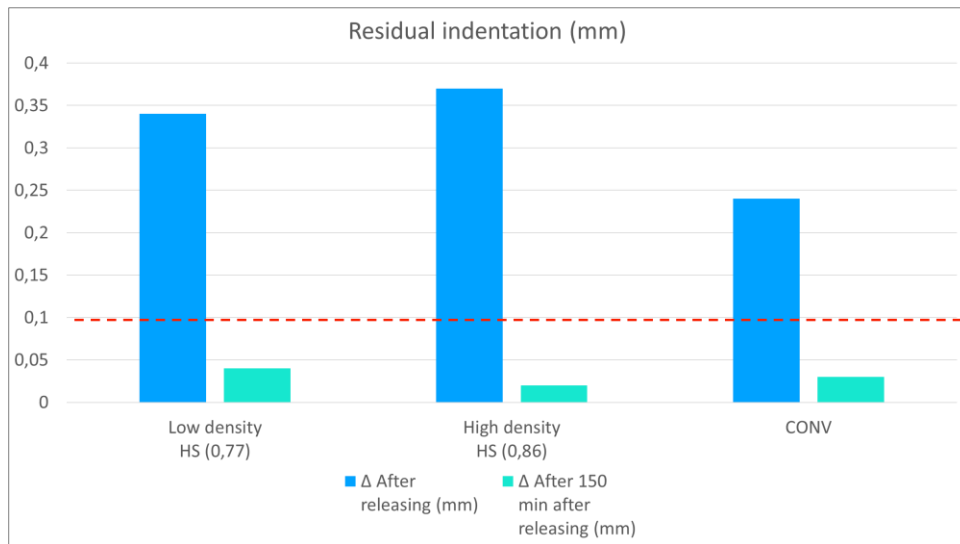


Figure 2.3.4 Average results of Residual Indentation of analysed methodologies

All the results of measurements taken after 150 minutes from the load's releasing are in compliance with the values stated by ISO 16776 (Resilient floor coverings - Heterogeneous polyurethane floor coverings – Specification). Although higher values of density showed a higher resistance, the lowest one still fitted the above-mentioned requirements.

These results allowed to better select the theoretical density to target for panels manufacturing. Particularly, the real value of $0,86 \text{ g/cm}^3$ was obtained targeting a theoretical density of $0,95 \text{ g/cm}^3$, whereas the samples with $0,77 \text{ g/cm}^3$ derived from calculation made considering a theoretical density of $0,82 \text{ g/cm}^3$. In order to obtain feasible results but with lower amount of substrate, it was agreed to target a theoretical density of $0,9 \text{ g/cm}^3$ for the following tests, as it will be discussed in *Section 2.3.8*.

Modulus of elasticity (MoE) in bending and bending strength

The modulus of elasticity in bending and the bending strength are determined by applying a load to the centre of a test piece supported at two points, as it is shown in *Figure 2.3.5*. The MoE is calculated by using the slope of the linear region of the load-deflection curve, whereas the bending strength corresponds to the ratio of bending moment at the maximum load to the moment of its full cross-section. Anyway, the calculation of these values is automatized by the instrument' software.



Figure 2.3.5 Experimental apparatus utilized at the University of Bologna

The first analyses were performed at a private laboratory. In details, the tested methodologies were manufactured following the current production protocol and the Methodology E with different substrates (normal and smaller HS, mix with 25% grinded colonized HS+25% normal HS). For each of these methodologies, 6 longitudinal and 6 transversal specimens were studied. The following graphs present the average value derived from the average of the longitudinal and transversal ones.

The best performances were reached by the samples made with normal HS as substrate. Particularly, these specimens were the only ones that exceed the threshold values of MoE set by the norm ISO 16893-2016, although also the current protocol was really close to them. This could be due to the fact that bigger/longer particles generally provide a good ability to flex to wood-based panels.

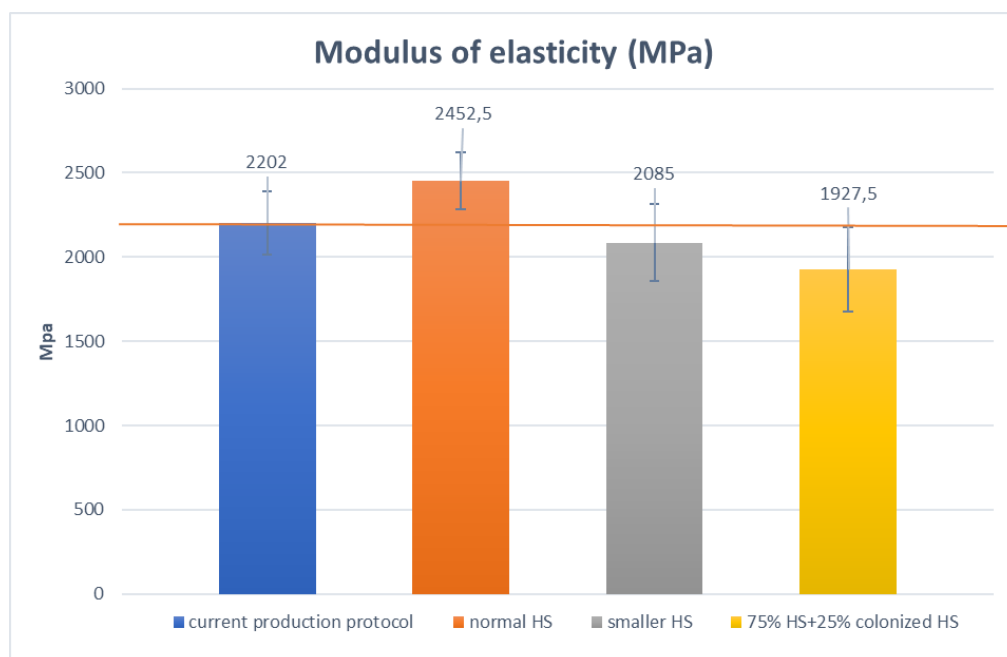


Figure 2.3.6 Average values of MoE recorded for methodologies tested by a private laboratory

For the bending strength, the second highest result recorded is attributed to tiles made with smaller HS (diameter <2 mm). In fact, little particles are able to form a highly packed structure that provide a good resistance to flexion. However, all the tested methodologies did not exceed the threshold value of bending strength.

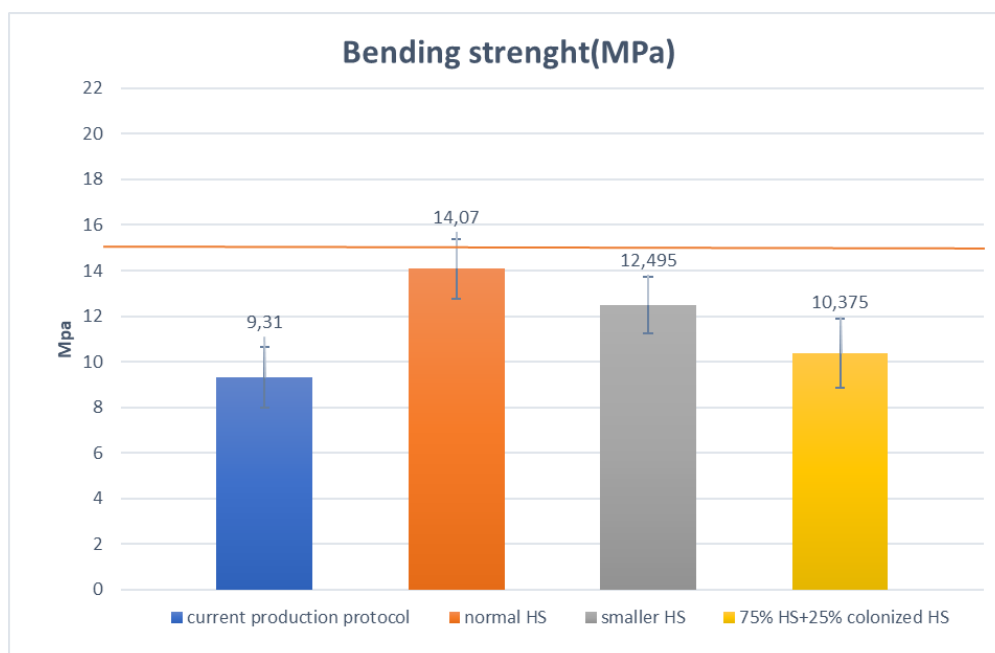


Figure 2.3.7 Average values of bending strength recorded for methodologies tested by a private laboratory

Since the experimental conditions of tests performed at the University of Bologna were different from the ones of the private laboratory, it was decided to repeat the analysis done on specimens with smaller HS as substrate. In details, the actual recipe followed for the two tested tiles were slightly different: a mass calculated for 1 cm thickness for used for specimens studied by the private laboratory, whereas it was estimated for 0,8 cm for the ones analysed at the University of Bologna. Moreover, for this last panel also a repressing step was done, as set by the Methodology C. Nevertheless, as it is shown in the following graph, the recorded values of bot MoE and bending strength were comparable. In fact, if the standard deviations of average results are taken into account, the related results recorded by the two instruments fit the same range. Given that, it could be said that repressing tests do not influence these mechanical performances for panels made with the same composition. In addition, the results of other methodologies tested at the University of Bologna could be compared to above-mentioned ones, as it will be discussed in *Section 2.4.5*.

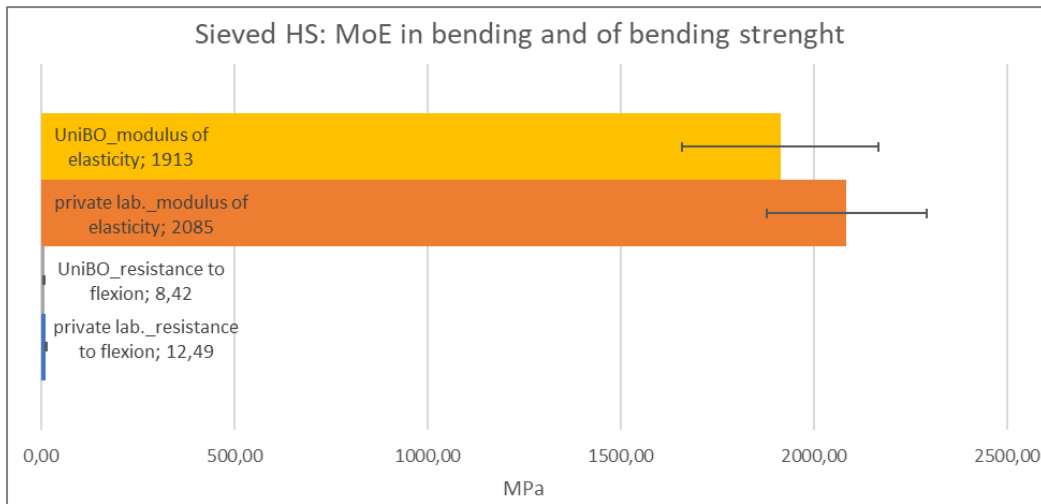


Figure 2.3.8 Comparison of average values of MoE and bending strength obtain by the private laboratory and University

Internal bond strength (IB)

The resistance to tension perpendicular to the plane of the board (“Internal Bond”) is tested by submitting specimens to a uniformly distributed tensile force until rupture occurs. In details, IB results from the ratio of maximum load to the surface area of the test piece. For each methodology tested by the private laboratory, 8 specimens were firstly bonded to test blocks made with hardwood, compatible with the fixing device. The results of tensile strength perpendicular to the plane of the board were directly provided by the private laboratory.

The graph here below shows the average values for each methodology (current protocol and methodology E with different substrates). Both the panels made with normal HS and smaller HS were able to exceed the threshold values set for particleboards. Particularly, the best results were recorded for specimens with HS<2mm of diameter, due to the fact that littler particles are able to distribute the applied force in a highly homogeneous way along their closely packed structures.

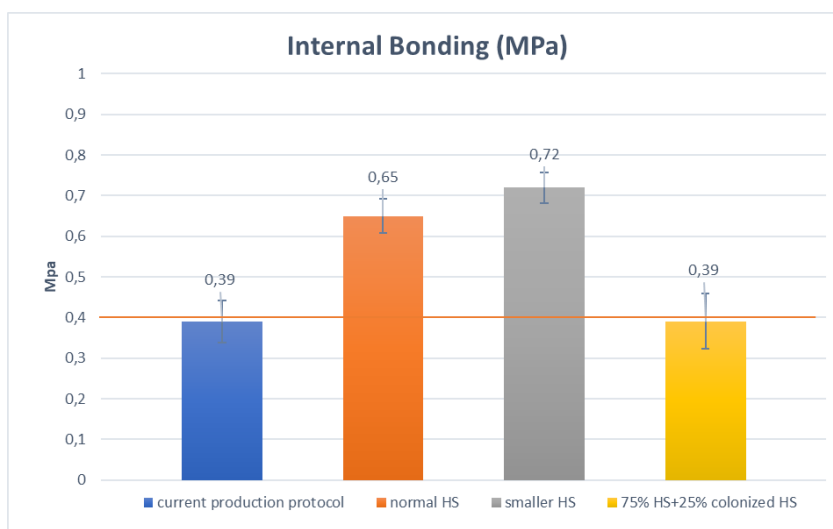


Figure 2.3.9 Average values of Internal Bond strength recorded for methodologies tested by a private laboratory

2.3.7 Panels' dimensional stability

Warping effect

After having produced a consistent number of panels, it was possible to recognise a similar pattern of warping and to distinguish between the variables that could have an effect on it, or not. In details, the thickness profile, drawn by measuring panels with a calibre in nine different positions, showed the highest value in the centre whereas a corner was sensibly thinner, with a difference around 0,18 cm in respect to the middle point. This led to a higher swelling of the central part of panels that prevented the corner to stay in contact with the ground. Therefore, the results from previous and following tests have been further investigated in order to understand the correlation of the applied experimental conditions and the main factors that affect warping, as listed in literature (see *Section 1.1.2*). At first, panels were put in contact with a regular surface on both sides and the maximum deviation of sides from the ground was measured using a meter. Firstly, it was noticed that the warping effect was not dependent on the **applied temperature and pressure**, as well as on the **panels' density**. These observations are in disagreement with literature, where higher values of temperature, pressure and density are claimed to decrease warping. This could be due to the fact that slightly differences were applied in the manufacturing conditions (see *Section 2.3.4*) and comparable values of density were reached.

On the other hand, many other variables seemed to have an impact on warping, as listed here below.

- a. **Type of substrate:** a uniform particle size's distribution -like the smaller fraction of sieved HS- improved panels' dimensional stability, since residual internal forces are lowered. Nevertheless, for tiles made with smaller particles it was recorded an increased warping over time, especially if storage conditions were not properly set.
- b. **Thickness:** the panels made with an increased amount of material (see *Section 2.3.5*) showed a lower warping effect in respect to the others. However, even if they were stored underweight and tighten with belts, their flatness did not improve over time. Conversely, after a month, it was recorded a reduction in warping for thinner panels.
- c. **Content of kraft lignin:** a higher %/weight of binder leads to the formation of stronger internal forces, that worsen the panels' dimensional stability. These experimental observations are contrary to what is reported in literature, where a high amount of adhesive is claimed to lower the warping effect.
- d. **Repressing steps** generally badly affect warping, especially if they are done without using the metal frame as panels' protection. In fact, this causes an uneven distribution

of forces during pressing that is responsible for the peculiar thickness profile previously described.

- e. **Cooling phase** plays an important role in determining the final shape of panels. In fact, as soon as they are taken out from the press, tiles could be highly affected by environmental conditions, like the humidity rate. Therefore, to prevent any additional swelling effect, it is fundamental to let panels gradually cool down under a weight and on a metal grid.
- f. **Storage conditions** are fundamental for panels' preservation. In fact, as reported also in literature, a dry environment and a system of belt or weights to keep tiles as flat as possible not only maintains the dimensional stability but could also improve it, as previously described.
- g. **Cut edges** have also a role in improving flatness over time. A pre-formatting is the first step that is usually done in the wood-based panels' manufacturing process, regardless the types of press and tiles. Indeed, the edges of raw panels are initially rough and unclean so they could worsen the distribution of residual internal forces during the cooling phase. It was experimentally verified that by cutting panels from 55x55 cm to 50x50 cm of dimensions right after pressing, this so called "**edge effect**" was sensibly reduced, thus leading to a decreased warping.

The actual setting of manufacturing press at Mogu's represented an obstacle in eliminating the warping effect. However, thanks to the observation presented here above, panels' dimensional stability was improved, with a reduction of the value-difference in respect to the highest middle point down to values around 0,13 cm. The whole manufacturing process was then implemented, as it will be discussed in detail in *Section 2.3.8*.

Tests of swelling in thickness

As already explained in *Section 2.2.3*, two different procedures of swelling in thickness were followed at Mogu's for testing the dimensional stability of some methodologies: the exposure of specimens to a 99,9% humid environment for 8/10 days and their immersion in water for 24 hours, also with thermal pre-treatments. Particularly, four different materials were tested following both the procedures: the conventional production protocol and panels manufactured with Methodology E but different fibres (normal HS, smaller HS, mix with HS and grinded colonized HS). This allowed to both evaluate the effects of pressing process on panels' dimensional stability but also make a comparison between the different substrates' behaviours.

a. Exposure to 99,9% humid environment for 8/10 days

Three samples were analysed for each of the four selected methodology. In details, the graphs here below show their evolution of the swelling in thickness' percentage over time.

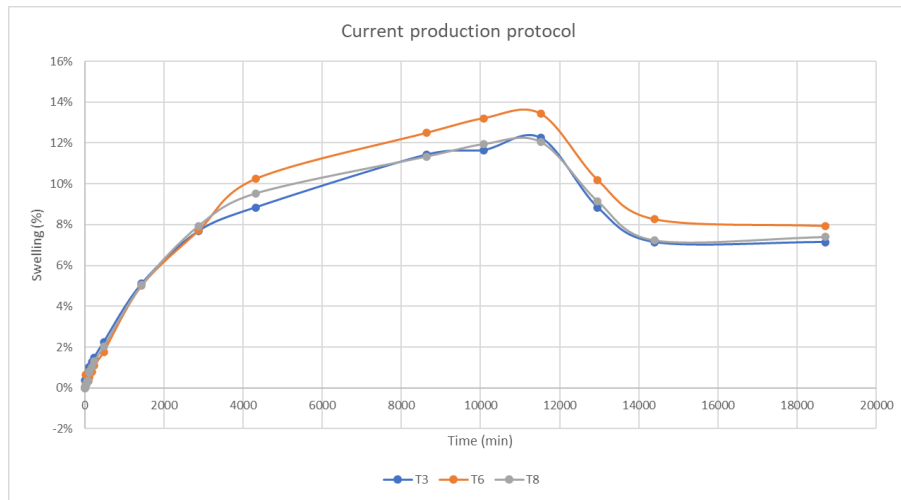


Figure 2.3.10 Swelling in thickness (%) of Current production protocol after exposure to 99,99% humid environment

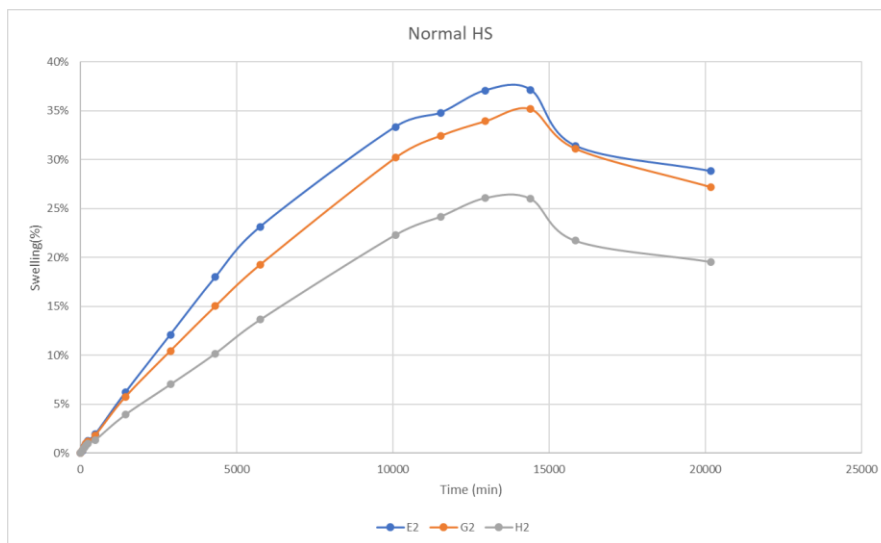


Figure 2.3.11 Swelling in thickness (%) for normal HS after exposure to 99,99% humid environment

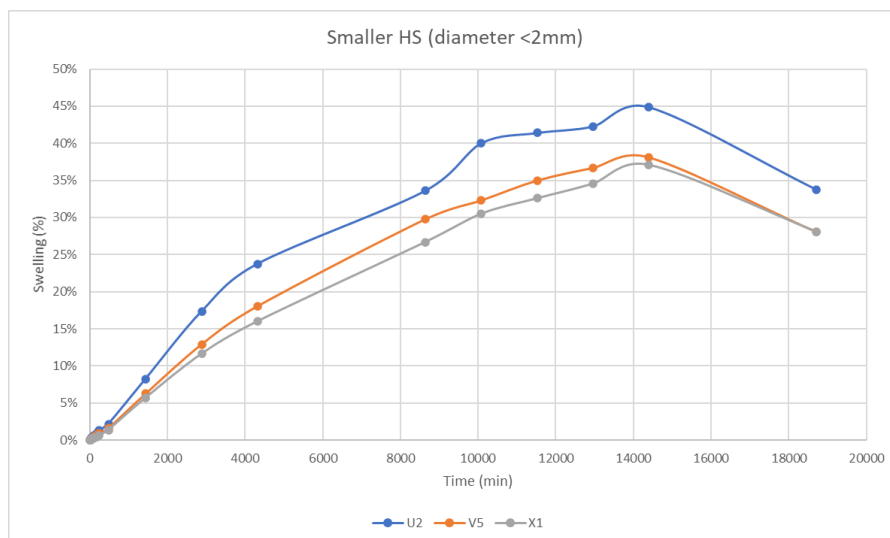


Figure 2.3.12 Swelling in thickness (%) for smaller HS after exposure to 99,99% humid environment

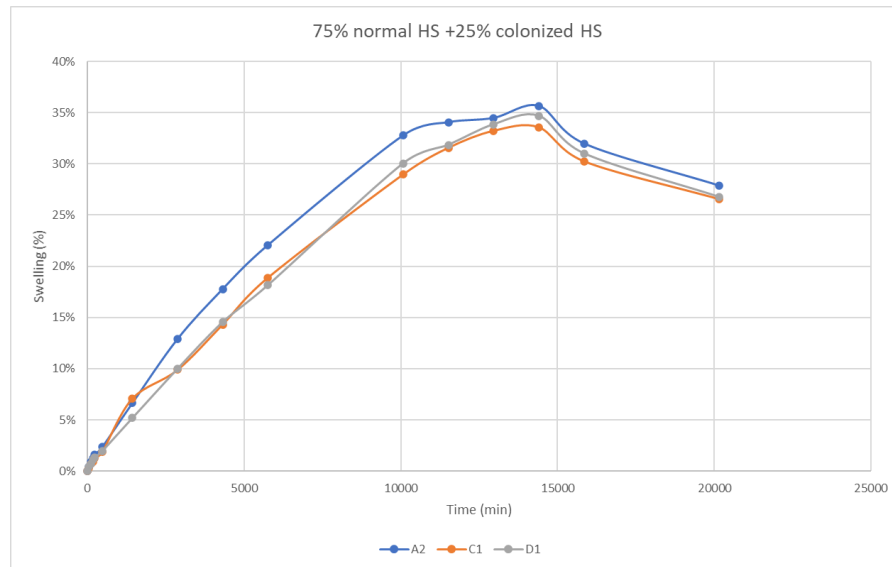


Figure 2.3.13 Swelling in thickness (%) for mixed substrate after exposure to 99,99% humid environment

At a first sight, it can be noticed that tiles manufactured with the current production protocol swelled about 3 times less than the other ones (maximum 13% after 10 days in respect to the minimum value of 26% after 10 days recorded for Methodology E). This could be easily explained by considering the total time of pressing of the two manufacturing methodologies, being of 45 minutes for the current protocol and 15 minutes for Methodology E. In fact, with longer processes such as the current production protocol, the surface of panels become almost burnt, thus forming a sort of protective layer against humidity's absorption. Because of this observed trend, it was agreed to perform an extra thermal pre-treatment on specimens manufactured by Methodology E, as it will be discussed in following paragraphs (see subsection c.).

The specimens of conventional production protocol were the only ones that got mouldy after 8 days, in correspondence to the grinded cotton fibres. This strengthens the decision of not using cotton fibres anymore, as already mentioned in *Section 2.1*.

By taking into account the results of panels made with same manufacturing methodology but different substrates, it can be noticed that specimens with normal HS swelled on the less, around the 33% on average (in comparison to 35% for mixed substrates and 40% for smaller HS particles). Anyway, in order to correlate the performances of the tested methodology to the threshold values set by norms, the tests of immersion in water for 24 hours must be considered (see subsection b.).

Another notable thing is the fact that panels' density seems to have an influence on the resulting swelling. In fact, by making a comparison between the % of swellings and the specifics of related samples listed in *Table 2.3.11*, it can be concluded that specimens with high densities show a higher dimensional stability than the ones with low values.

Table 2.3.11 Classification of samples tested with the exposure to 99,9% humid environment

	current production protocol			normal HS			smaller HS (<2mm)			75% normal HS +25% colonized HS		
sample	T3	T6	T8	E2	G2	H2	U2	V2	X1	A2	C1	D1
Density after 24 h of drying (g/cm ³)	0,71	0,78	0,73	0,73	0,80	0,88	0,75	0,77	0,84	0,72	0,80	0,85

b. Immersion in water for 24 hours

At first, the same methodologies mentioned in the previous subsection were tested for their dimensional stability following this procedure. This allowed to compare the recorded results with the threshold value of 16% of swelling in thickness after immersion for 24 hours, set by ISO 16893-2016 for “heavy-duty load-bearing particleboard for use in dry conditions”. As it is showed by the graph here below, the current production protocol performed at the best, in agreement also with the results recorded with the other testing methodology. In fact, it was the only process that almost reached the threshold limit, with the average value of 17% of swelling in thickness. Conversely, the data related to panels manufactured following Methodology E are at least a hundred times higher than it. Anyway, in order to better reproduce the actual condition of utilization of Mogu Floor, it was decided to perform further studies on tiles made with normal HS, which seemed to be the most promising substrate among the ones tested for Methodology E. In details, all the samples’ free surfaces were sealed using the same elastic filler applied for grout lines while setting the floor. No improvements for the swelling in thickness were noticed, maybe also because the manual application of the filler.

Finally, in order to compare these last results with the ones of other products already present on the market, it was decided to test also some commercial specimens of Medium Density Fiberboards (MDF) and a thick particleboard. Since also these samples performed much better than the panels manufactured following methodology E, it is clear that further adjustments of this process are needed.

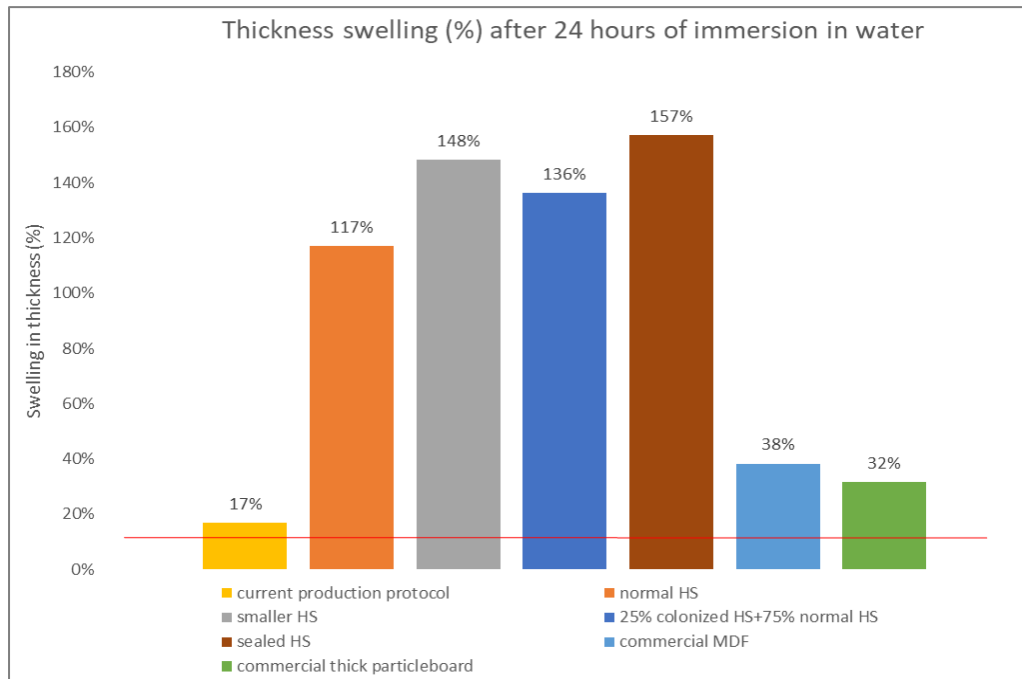


Figure 2.3.14 Swelling in thickness (%) after 24 hrs. of immersion for samples with kraft lignin as binder

c. Immersion in water for 24 hours with a superficial thermal pre-treatment

In order to decrease the swelling in thickness of panels made with Methodology E and normal HS, it was decided to apply a thermal pre-treatment on specimens before testing them, as explained in detail in *Section 2.2.3-Swelling in thickness*, subsection c. At first, samples were heated up inside the press at **250°C** and for different amounts of time. Given this extreme conditions, **5 minutes** of pre-treatment were sufficient. Anyway, in order to operate in a lower range of temperature, thus limiting bubbles' formation, it was also decided to heat up sample at **200°C for 10 minutes**. The graph below summarises the average results of the recorded data. In details, thanks to the milder treatments, the swelling in thickness was reduced from 117% to 70%. However, treating specimens at 250°C allowed to improve panels' dimensional stability even more, leading to a swelling in thickness as low as the 25%. These last results are not in compliance with the thresholds value of 15% set by the reference norm, but they are highly promising for further process' implementation.

Since performing additional treatments on panels is surely time-consuming at an industrial level, other bio-adhesives were tested and compared with the performances of the ones containing only kraft lignin, as it will be discussed more in details in the following *Sections 2.4.2. and 2.4.5.*

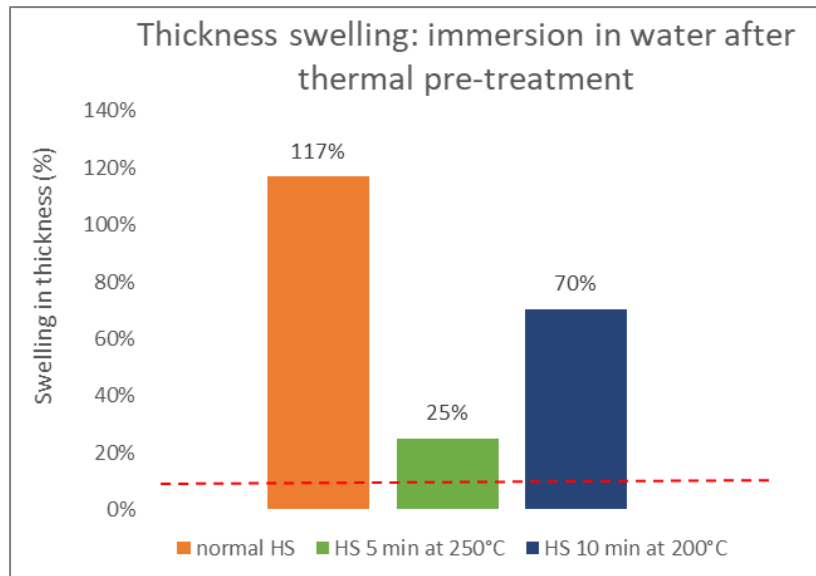


Figure 2.3.15 Swelling in thickness (%) after 24 hours of immersion for samples thermally pre-treated

2.3.8 Process' implementation and repeatability

As discussed in the previous *Sections 2.3.6 and 2.3.7*, **Methodology E** gave promising results, both for the mechanical performances and dimensional stability of the so obtained panels. It was thus decided to further test this manufacturing process for its repeatability and reproducibility.

At first, the recorded data related to panels with normal HS were studied more in details to draw additional observations. This was so because obtaining the smaller HS was no longer feasible. In details, according to the results of Residual Indentation tests, an intermediate value of (real) density could not only be in compliance with the related norm but also limit the problem of bubbles' formation during the manufacturing process. By considering the data of Swelling in thickness, it was noticed that higher density values gave the best results. Therefore, it was agreed to target a theoretical density of 0,9 g/cm³ for the following experimentations.

In order to better approach the industrial requirements of a manufacturing process (e.g., total time, comparison with competitors' products, costs of materials and so on), other targets were set before proceeding with repetitions:

- Decreasing thickness down to 0,8 cm with a range of $\pm 0,25$ mm (before calibration)
- Further reduction of manufacturing time, ideally down to 5 minutes
- Use the lowest %/dry w of kraft lignin, thus limiting both costs and residual smell in the final product.

Given that, after having performed other tests, the "**Methodology F**" was developed. The amount of HS was calculated for a density of 0,9 g/cm³ and thickness of 0,9 cm, using a metal frame with dimensions of 55x55x0,8 cm while pressing. The lignin's content was

decreased down to 8%/dry weight. Degassing steps were implemented to shorten the process' time as much as possible. In details, the manufacturing process consisted of 12 minutes of pressing at 150 bar and 180°C with degassing each minute for the first 5 ones and then two more at the 8. and 10. minute. Before panels had fully cooled down, edges were cut so to reduce the “edge effect” and tiles were stored as organized pile tighten by belts.

This methodology gave excellent results for its reproducibility. In fact, all the 13 panels produced in a day were stiff and cohesive, no bubbles formed and warping was comparable to the best results previously obtained. The measurements before calibrations gave an average density of $0,85 \pm 0,02 \text{ g/cm}^3$ and a thickness of $0,93 \pm 0,02 \text{ cm}$.

Since both the mechanical performances and dimensional stability of the so obtained panels were not studied due to a lack of time, it will be surely interesting to further investigate this promising methodology for future developments.

2.4 Results and discussion: studies on micro-variables

After having implemented the procedure for panels' manufacturing, some experiments were performed in order to test other potential materials to be used as bio-adhesives. The aim of these tests was finding substitutes that could facilitate the pressing process, improve panels' performances and overcome the problem of smell in the final products manufactured with lignin. Firstly, the native form of all the utilized compounds was characterized by performing Thermo-Gravimetric Analysis with the experimental conditions described in *Section 2.2.1*. Then, given the potentialities reported in literature (see *Section 1.1.1*), the utilization of both Soy and Whey proteins was deeply investigated, as it will be discussed in the following paragraphs.

Finally, the role of fungal enzymatic oxidation for fibres activation was also studied more in details by performing both IR and TGA studies.

2.4.1 TGA of all the tested bio-adhesives

Kraft lignin

The kraft lignin utilized as bio-binder for the majority of manufactured panels was further characterized by a Thermo-Gravimetric Analysis. The following graph represents the recorded variation of mass in respect to the temperature's increase. In details, the small peak in the % of weight variation over time around 100°C highlighted the presence of some tracks of humidity in the analysed sample. However, since it could correspond to a humidity rate of 5%, this is in agreement with the storage of material at environmental conditions. The maximum thermal degradation occurred around 400°C and the final weight loss was equal to the 45%. Even though the experimental settings do not represent actual conditions of utilization, this kind of lignin could surely have a role in extending the time needed for panels' thermal degradation.

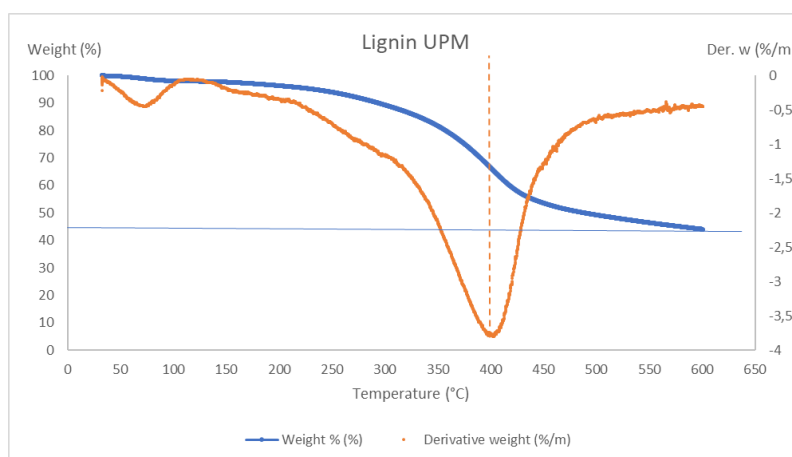


Figure 2.4.1 TGA of kraft lignin

Biopolymers: PLA, PVA, Thermoplastic starch

Differently from lignin, the final % of weight recorded for the analysed biopolymers was at maximum as high as 15% (see TGA of Thermoplastic starch). To be more precise, PLA fully degraded in the set experimental conditions and the maximum variation % of weight occurred slightly above 350°C. Conversely, for PVA and Thermoplastic starch, the peak of degradation occurred some degree below 350°C. Both these last biopolymers seemed to contain tracks of humidity, due to the small loss of weight recorded around 100°C. Moreover, three different peaks could be distinguished in the graph of PVA (see *Figure 2.4.3*). This could be attributed to the presence of hydroxyl groups both esterified and hydrolysed, in different proportions according to the declared hydrolysis degree of 88. In the light of these results, it could be said that the utilization of these compounds as bio-adhesives could be surely helpful in improving the times of panels' thermal degradation.

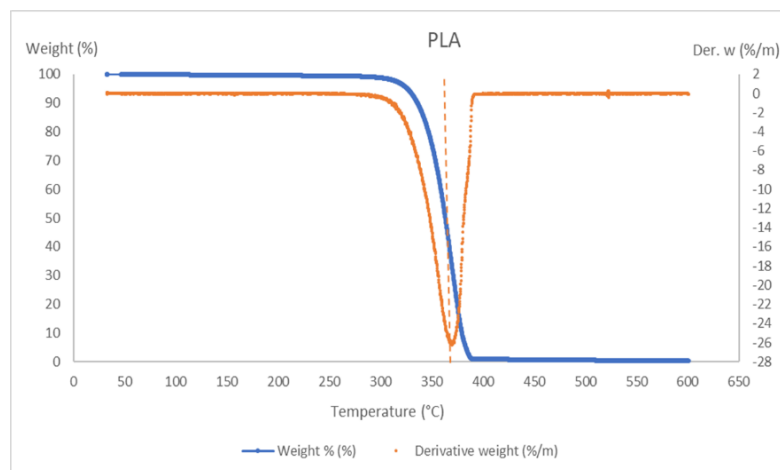


Figure 2.4.2 TGA of PLA utilized as a binder

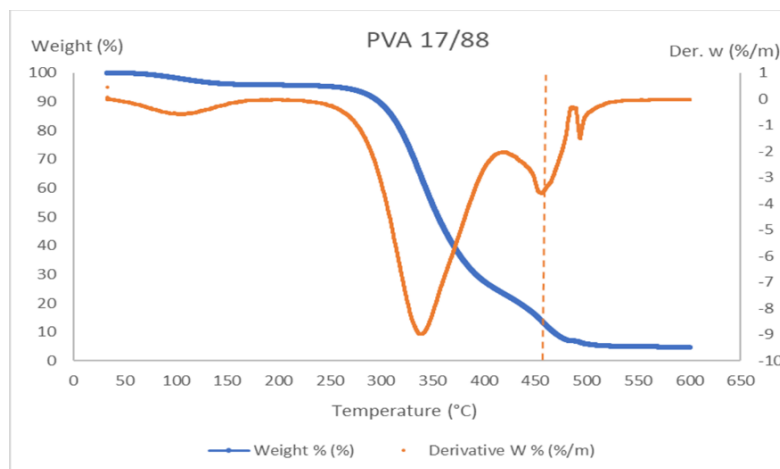


Figure 2.4.3 TGA of PVA utilized as a binder

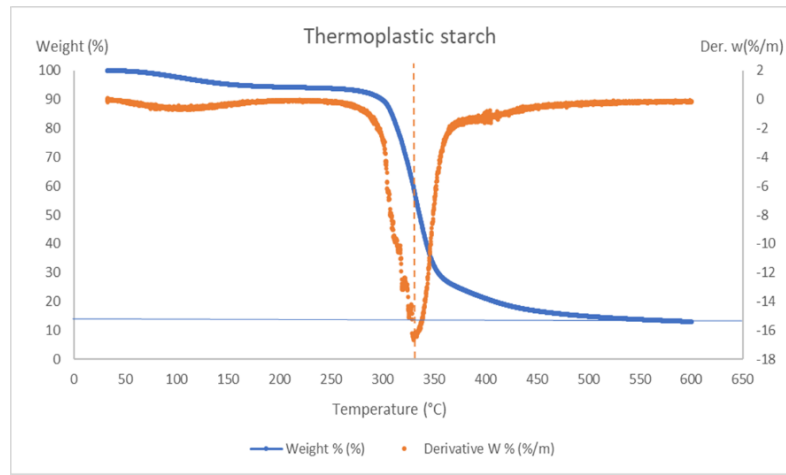


Figure 2.4.4 TGA of thermoplastic starch utilized as a binder

Protein Isolates: soy and whey proteins

The graphs related to the TGA of both SPI and WPI can be almost overlapped. In details, for both these compounds, it can be noticed a first peak in the derivative weight (%/w) around 100°C. This is due to the humidity content of the samples that was compatible to the standard room R.H. of 5%, anyway. The degradation peaks are sharp and around 315°C, whereas the maximum percentage of weight remained was equal to the 30%.

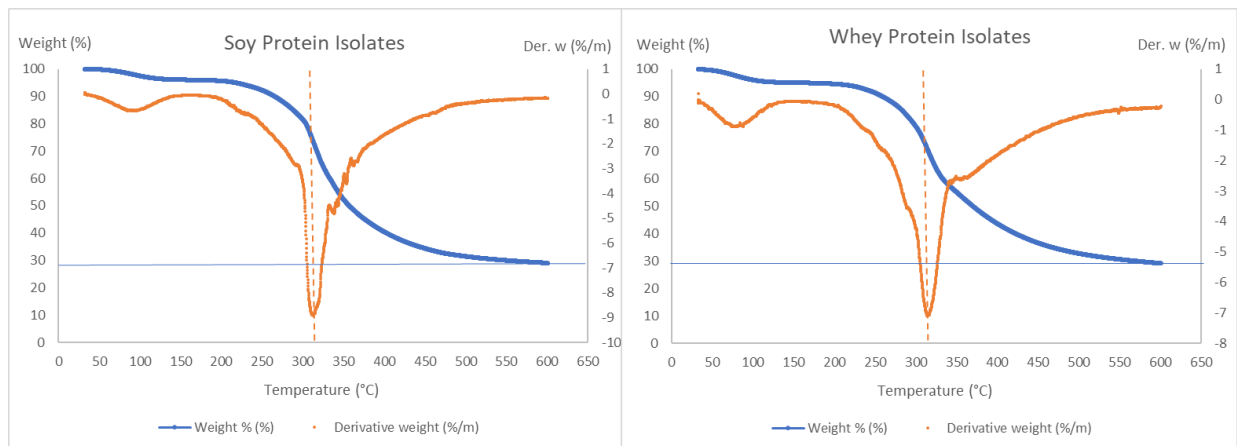


Figure 2.4.5 TGA curve of soy and whey protein isolates

2.4.2 Tests with different bio-adhesives

Lignosulfonate lignin

As stated by literature, the reactivity of lignosulfonate lignin is expected to be higher than the kraft's one. Therefore, it was decided to test the compound already available at Mogu's for panels' manufacturing. In details, an amount of binder equal to the 20%/dry weight was mixed with dried HS fibres and then pressed continuously for 20 minutes at 180°C and 100 bar. At the end of the process, not only a consistent amount of steam was released while opening the press but also a huge bubble formed on almost the whole surface of the obtained panel. Since this could be attributed to the humidity content gained by the binder

over storage, it was decided to dry it in a chemical oven before further tests. After having left the sample for one night at 105°C, it was registered an average weight loss around the 10%. However, not all the dried material was feasible for panels' manufacturing since a thick dried layer formed on surface. The replication of previous process allowed to manufacture a panel with no bubbles but almost bended.

These results could not be compared to the ones obtained by using kraft lignin, maybe because the storing conditions of the lignosulfonate one badly affect its reactivity. Given that, it was decided not to proceed with other studies related to this kind of lignin.

PLA

The poly-lactic acid already available at Mogu's was firstly grinded to obtain a fine powder. Then an amount of compound of 15%/dry w. was added to HS fibres and panels were manufactured following Methodology E. It was noticed that, immediately after the pressing process, panels were not fully formed and their surface was sticky so they were left cooling without removing the metal frame. The final tiles seemed to be stiff and cohesive, without any bubbles on their surfaces and with an average value of density equal to 0,74 g/cm³. Given these promising results, it was agreed to perform additional studies on the obtained panels. However, while cutting the tiles, a strong internal delamination was detected. This could be due to the absorption of humidity during the cooling phase because the solidification of PLA takes a long time, thus allowing air to penetrate inside panels a create voids in between layers that have already polymerized.

Although it was not possible to perform mechanical tests on these tiles, it was decided to both perform a test of swelling in thickness and analyse the internal fractured surface at the SEM and optical microscope. In fact, as a biopolymer, PLA could behave as a plasticizer agent that could reduce the absorption of humidity, as it is reported in literature.

In details, the sample's surface presents many tracks of delamination while analysed using at the SEM. It was also noticed the formation of peculiar patterns among internal layers that could have been created by the melt PLA during the manufacturing process (see *Figure 2.4.6*). This suggests that the applied pressing conditions were not adequate to this compound, so further adjustments will be needed for future experimentations.

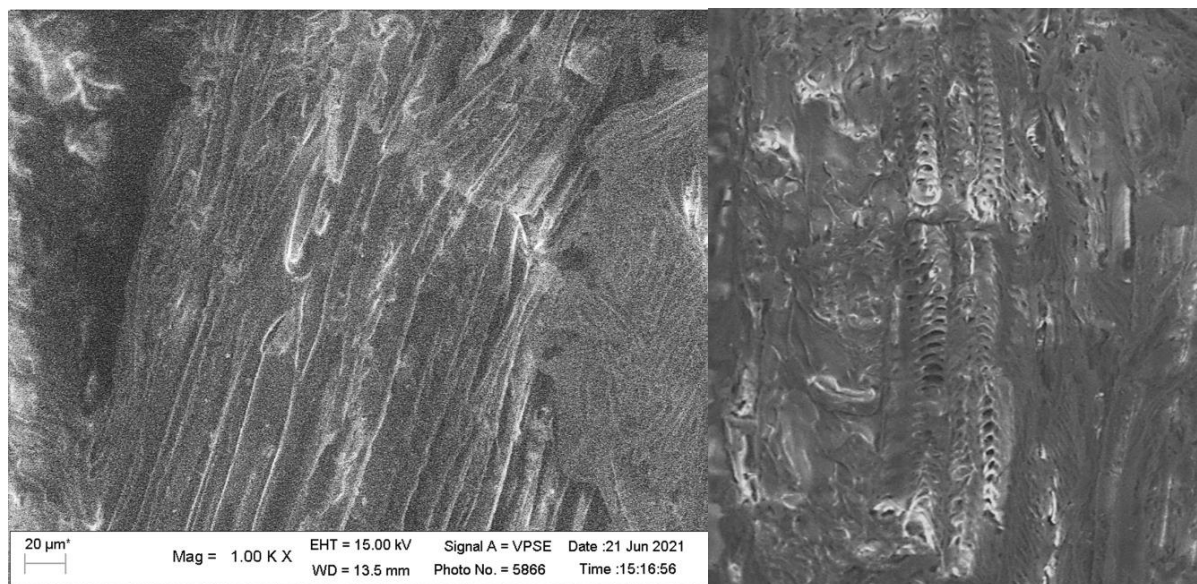


Figure 2.4.6 SEM analysis of fractured surface of panels with PLA

The images acquired with the optical microscope confirmed the tracks of delamination mentioned above, as it is highlighted by the red boxes in the picture here below. Moreover, this analysis allowed to clearly distinguish the plasticisation effect performed by PLA after it had fully polymerized. In fact, the portions in the blue boxes have a shiny appearance thanks to the plastic covering made by the biopolymer.

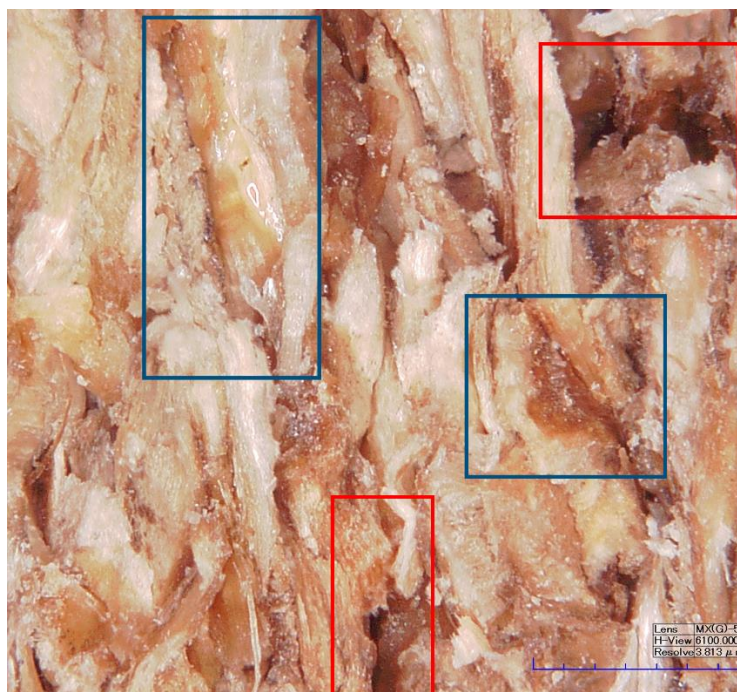


Figure 2.4.7 Image of panels made with PLA taken with the optical microscope

A plastic covering of fibres could surely help in increasing panels' dimensional stability. In fact, after the immersion in water for 24 hours, it was recorded a swelling in thickness of 71% for panels made with PLA as binder. Although this value was lower than the one recorded for samples made with normal HS and kraft lignin (T.S.=117%), it still exceeds the threshold limit of 15% set by the norm. However, it could be interested to further

investigate the potential role of PLA in reducing the swelling in thickness of the manufactured panels, after having better set the pressing process.

PVA + Thermoplastic starch

As mentioned in *Section 1.1.1*, the combination of starch with poly-vinyl alcohol could improve its bonding capacity. Therefore, it was decided to test as bio-adhesive a mix of thermoplastic starch and PVA 17/88 in a 1:1 proportion (20%/dry weight in total). In details, the last biopolymer mentioned was chosen because a high degree of hydrolysis - equal to 88 in this case- is expected to increase both the crystallinity and the melting temperature (around 160-240°C), thus making the compound more suitable for the applied pressing conditions.

Both these compounds were manually grinded using a small grinder before being added to HS fibres. Following the same methodology of lignosulfonate lignin, it was manufactured a panel almost flat but with a very low density. Therefore, it was decided to replicate these experimental conditions but using a higher amount of fibres in order to increase panels' density. However, even though a density of 0,78 g/cm³ was reached, the tile was extremely fragile. Given that, it was concluded that this combination of binders did not allow to improve their bonding capacity and was not feasible for panels manufacturing.

2.4.3 Protein Isolates: experimental pre-treatments

In order to assess the need of activation treatments for SPI and WPI, some tests were run using directly these compounds as powders. The obtained panels did not present any bubbles but were extremely fragile. Moreover, their surface was fully covered by the dust of unreacted protein isolates, thus meaning that adhesive properties were not strongly enough. Given that, different procedures were set for the activation of both SPI and WPI, as it will be discussed in the following paragraphs.

The internal structures and mechanical properties of the panels obtained with the activated protein isolates were further investigated by performing some tests at the University of Bologna: the modulus of elasticity in bending and bending strength, analysis at SEM and optical microscopes. All these results will be discussed in detail in *Section 2.4.5*.

Basic hydrolysis of SPI: experimental procedure

The setting of this experiment derives from a combination of two procedures from literature. Particularly, the moderate **pH/temperature combination of 10.0/50°C** was targeted, since it is claimed to be the optimum treatment condition for producing alkali-modified soy protein adhesives with the highest bonding strengths (Hettiarachchy et al., 1995). However, in order to limit the liquid content of the activated binder, it was decided

to apply experimental proportions between the NaOH solution and dry protein content more similar to those described by Zhang et al. 2013. Given that, firstly theoretical calculations were done in order to estimate the amount of NaOH needed to get a solution with a pH around 10. Then, the highest amount of SPI as possible was solubilized in this solution under stirring and heating (**T=50-70 °C**). On the basis of the recorded results, other tests were conducted in order to better approach the targeted pH value and a highly viscous solution as well. Particularly, it was selected a dry protein content of **12% dry mass/V** because this solution's texture allowed a proper mixing both during the basic hydrolysis and the preparation of materials for tiles' production.

The procedure that allowed to achieve the targets just mentioned with a consistent repeatability is fully described in the Mogu's short experiment canvas reported as *Annex III* in the present Thesis.

Some investigation about the **pot-life** of the activated SPI were done as well. For a solution stored in a glass jar at room conditions, it was registered a change in the appearance and smelling after 9 days. Conversely, by storing the product in a fridge (temperature around 5°C), its adhesive properties were still suitable for tiles' production after two weeks, even though the pH was slightly lower (from 10,62 to 9,78).

Thermal activation of WPI: experimental procedure

There are not many articles in literature regarding the use of whey proteins as bio-adhesive for wood-based product. As reported by Gao et al. 2011, however, whey protein isolates can be utilized for the production of an aqueous polymer solution-isocyanate adhesive, that is environmental friendly and suitable for bonding wood in both structural and non-structural applications. However, this application requires some chemicals, so it was decided to further investigate the potentialities of using just activated WPI as bio-adhesive. In fact, the polar groups of whey proteins can be easily unfolded by a thermal denaturation under gentle conditions, starting from 40°C and up to 65-70°C. Particularly, the viscosity of denatured WPI sharply reduces with increasing time because of gels' formation before or soon after the denaturing temperature is reached, which occurs at $T < 65^{\circ}\text{C}$ for solution with a concentration $> 15\% \text{ p/V}$. With the heat-induced gelation, pot-time decreases as well, especially when the treatment lasts for more than 35 minutes. Given that, the first attempts for activating WPI were performed with a careful monitoring of experimental conditions. It was observed a change in the solution -from a liquid creamy-like to a uniform and highly viscous texture- after **20-22 minutes of heating at 60-62°C**. After 2 hours ca. from the heating treatment, some lumps formed and aggregated over time, leading to a

complete separation of the liquid and solid phases after a couple of days. Therefore, in order to ensure the product's stability, it was decided to activate WPI within just few hours before tiles' production. The final setting of the experimental procedure for this thermal denaturation will be fully described in *Annex IV*.

2.4.4 Activated Protein Isolates: pressing process

Use of activated SPI as binder: setting of the process

The setting of the procedure for tiles' production using activated SPI as a bio-adhesive took lots of effort since there is no literature related or suitable for Mogu's processing facilities. A brief summary of the followed path is reported here below.

a. Identification of best %/dry weight of activated SPI

Experimentations started with the production of tiles with dimensions of 35x35cm by directly mixing the activated SPI with dried HS fibres. In details, the amount of HS was fixed and different %/w of binders were derived by doing proper proportions in order to target a theoretical density of 0,9 g/cm³ and a thickness of 0,9 cm. A **20 minutes cycle** of cooking at 180°C and 50 bar was firstly applied. With a binder content of **10 %/dry weight** it was possible to obtain cohesive panels without any bubbles and a **density of 0,72 g/cm³**.

b. Shortening the processing time

Some tests were done to shorten processing times, keeping constant the 10%/dry w of activated SPI. Anyway, a cooking cycle of 15 minutes seemed to be needed in order to limit bubbles' formation. Moreover, since all the panels so obtained with these methodologies were cohesive but with a very low density, it was agreed to increase the amount of HS for the following tests. Particularly, by doing some mathematical proportion on the basis of recorded results, it was decided to target a theoretical density of 1,1 g/cm³.

Aiming at shortening the pressing's time as much as possible, it was agreed to do degassing steps each 3 minutes to enhance the steam's release linked to the utilization of such a liquid binder. Moreover, in order to approach a real density of 0,9 g/cm³, repressing steps were done keeping a bigger metal frame as "protection", thus reducing the thickness even with a higher amount of HS fibres. A **density as high as 0,89 g/cm³** was achieved with the 10%/w of SPI applying a **9 minutes cooking cycle with degassing each 3 minutes and 3 minutes of repressing**.

c. Scale-up of the process: from 35x35 cm to 50x50 cm panels

Dealing with bigger panels' dimensions is expected to enhance the releasing of steam even more. Therefore, process' scalability was firstly tested using a new methodology: **7 minutes of cooking with degassing each single minute and one minute of repressing**. Before

proceeding further with repetitions, the so obtained panels were cut in half and this highlighted a strong internal decohesion for all of them. Conversely, this was not the case for the cut 35x35 panels obtained with 15/20 minutes of continuous cooking cycle. Therefore, it was assumed that the above-mentioned lack of internal cohesion was linked to the degassing steps. However, avoiding them in processing 50x50cm panels will lead to a consistent increase of cooking time, much higher than the 15 minutes cooking cycle applied for the 35x35 panels.

In order to continue with the degassing steps and shorter process' times, it was decided to add a small %/w of kraft lignin. In fact, differently from the activated SPI, lignin is a thermoplastic material thus being able to melt again after degassing/during repressing steps. Therefore, some experimentations were done in order to identify the best amounts of activated SPI and lignin to be used. Particularly, the tested proportions were obtained by considering only lignin as dried binder and then deriving the proper amount of activated SPI keeping a constant proportion (total binder %/dry weight equal to 10). All the samples were stiff and cohesive and, particularly, the panel with **5%/dry w of lignin UPM and 5%/binder content of activated SPI** showed almost no delamination once cut so some repetitions of this methodology were done. However, further repetitions present still some delamination in a random way, so it was necessary going back to a continuous and longer processing cycle: **20 minutes of cooking with degassing step each single minute just for first 2 ones and 5 minutes of repressing.**

From the perspective of an industrial production, further studies are needed to identify the best methodology to be used for the production of 50x50cm panels.

Use of activated WPI as binder: setting of the process

The experimentations of tiles' production with activated WPI followed more or less the same path of the SPI's ones. To be more precise, the last ones were always done firstly due to limited pot-life of activated WPI and other problems that rose during the tiles' production. In fact, any slightly change in the texture of activated WPI hindered the mixing process leading to the formation of aggregates and darker spot on the final panels, as it is showed in the following photo.



Figure 2.4.8 Darker spots on tile produced with 10%/dry weight on activated WPI

Given that, the tested methodologies are summarized here below.

a. Identification of best %/dry weight of activated WPI

The best results for the production of 35x35 cm panels were obtained by using the **10 %/dry w.** of activated WPI and performing a continuous cooking cycle of **20 minutes at 180°C and 150 bar**. In details, the amount of material was calculated for a theoretical density of 1 g/cm³ and 0,9 cm thickness. The **average density** of these panels was about **0,82 g/cm³**.

b. Scale-up of the process: from 35x35 cm to 50x50 cm panels

The first attempts to scale-up the process were done using this methodology: **9 minutes cooking cycle with degassing each 3 minutes and 3 minutes of repressing**. Particularly, by using the amount of material calculated for a theoretical density of 1,1 g/cm³ and thickness of 0,9 cm, it was possible to reach **densities around 0,9 g/cm³**.

In order to shorten the process' time while dealing with bigger panels, degassing steps were then implemented. Because of internal delaminations due to degassing steps, it was decided to add a 5%/dry weight of lignin, as previously discussed for activated SPI. However, differently from these last ones, the panels thus obtained in a process of **7 minutes of cooking with degassing each single minute and one minute of repressing** presented many bubbles. Therefore, no further investigations were done, and it was necessary going back to a continuous and longer processing cycle: **30 minutes of continuous cooking**. It goes without saying that this methodology is not suitable for an industrial process, so further studies are needed to identify the best methodology to be used for the production of 50x50cm panels.

2.4.5 Characterization of panels with activated proteins

The most promising methodologies mentioned in the previous section were further investigated by performing some tests both University of Bologna and at Mogu's, as it will be discussed here below.

Table 2.4.5 Methodologies analysed at University of Bologna and at Mogu's

binder	pressing conditions	average thickness (cm)	average density (g/cm ³)
activated SPI (10%/dry w)	25 min of cooking (35x35 cm panels)	0,93	0,81
kraft lignin (5 %/dry w) + activated SPI (5%/binder content)	20 min of cooking with degassing each one min for first 2 one+5 min repressing (50x50 cm)	1,04	0,82
activated WPI (10%/w)	20 min of cooking (35x35 cm panels)	0,94	0,82

Analysis at Scanning Electron and optical microscope

The internal structures of panels with the 10%/w of both activated SPI and WPI was examined by analysis at SEM and optical microscope.

In details, the images of panels with **activated SPI** present tracks of delamination along the fibres' planes, as it is showed in the enlargements reported here below (see *Figure 2.4.9*). This lack of internal cohesion could be caused by the highly thermosetting behaviour of this bio-binder or by an insufficient adhesive strength. However, this delamination was visible only at a microscopic scale and the analysis of the mechanical properties showed still good results, as it will be discussed in the following paragraph. Anyway, as stated before, further experimentations are needed to assess the best processing methodologies to be used with this bio-adhesive.

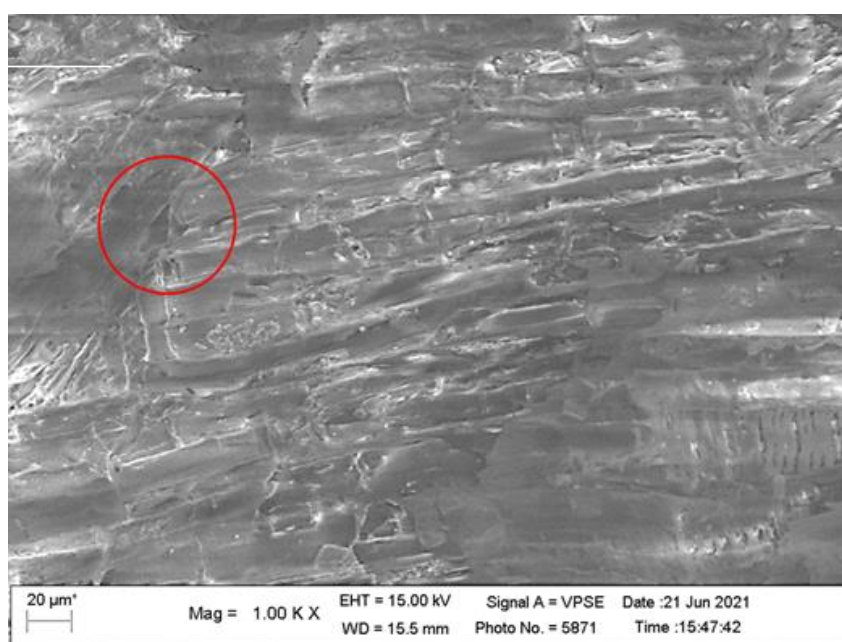


Figure 2.4.9 SEM image of panel with 10%/dry w of activated SPI



Figure 2.4.10 Optical microscope's image of panel with 10%/dry w of activated SPI

The SEM analysis of the panel with the 10%/dry w of **activated WPI** as bio-adhesive reveals a highly iso-orientation of fibres. Such a very effective packing and interactions led to promising results related to the panels' mechanical performance, as it will be discussed in the following section. Moreover, as it is highlighted in *Figure 2.4.11*, it was possible to detect some tracks of binder's aggregation due to the jellification that is likely to occur during the thermal denaturation of WPI.

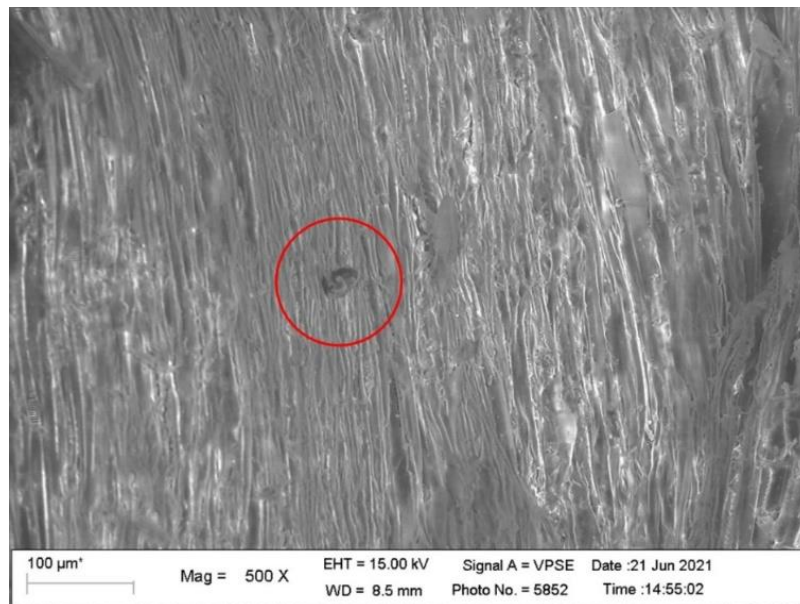


Figure 2.4.11 SEM image of panel with 10%/dry w of activated WPI

Modulus of elasticity (MoE) in bending and bending strength

In the following graphs, the average values of MoE and bending strength related to the panels with activated proteins are put in comparison with the ones recorded for the sample of sieved HS with kraft lignin as binder. In details, for this last methodology, 6 longitudinal and 6 transversal specimens were studied, whereas 10 samples were analysed for the

other ones. This was so because the manufacturing process of panels with activated proteins as binders did not allow to obtain panels with the same dimensions of the ones made with smaller HS, as already explained in the previous section.

For both the MoE and the bending strength, all the tested methodologies containing activated proteins as bio-adhesives exceed the threshold values set by the ISO 16893-2016 for load bearing particleboard for use in dry conditions. Particularly, the tiles with activated WPI as bio-adhesive show the best performances, reaching a value of MoE twice the one set as threshold limit. These promising results could be linked to the highly iso-orientation of fibres, that was highlighted by performing the microscopic analysis previously discussed.

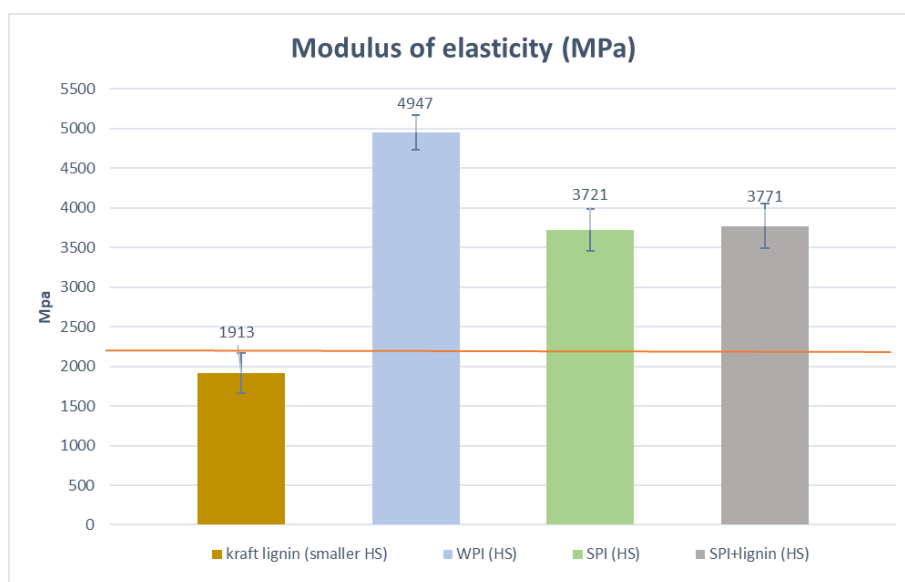


Figure 2.4.12 Average values of MoE of tiles with activated proteins and smaller HS with lignin

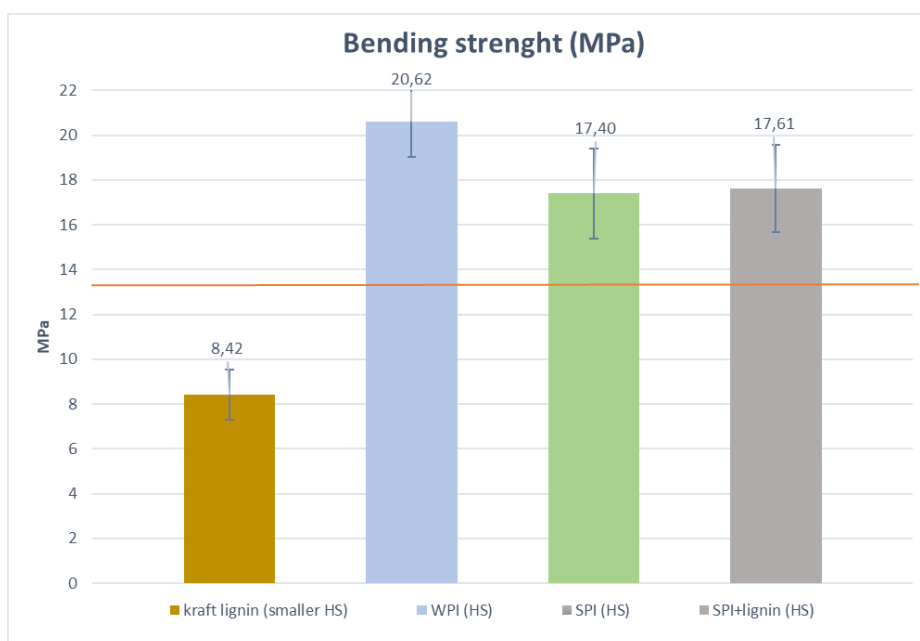


Figure 2.4.13 Average values of bending strength of tiles with activated proteins and smaller HS with lignin

Swelling in thickness

The dimensional stability was investigated by some tests of swelling in thickness after immersion in water, following the standard EN 317. The following graph shows an average of the recorded values in relation to the limits set by the norm for heavy-duty load bearing particleboard for use in dry conditions.

All the tiles with activated proteins as bio-binder swell less in thickness than the one with lignin and without a thermal pre-treatment. Particularly, the activated WPI performed better than SPI. It can be noticed also that adding a small % of lignin to activated SPI slightly increases the swelling in thickness, from to 76% to the 90%. Anyway, the dimensional stability of panels with activated proteins as bio-adhesive is not competitive to the one of HS with lignin thermally treated and the conventional protocol. Given that, further investigations of processing conditions are recommended in order to better approach the threshold values.

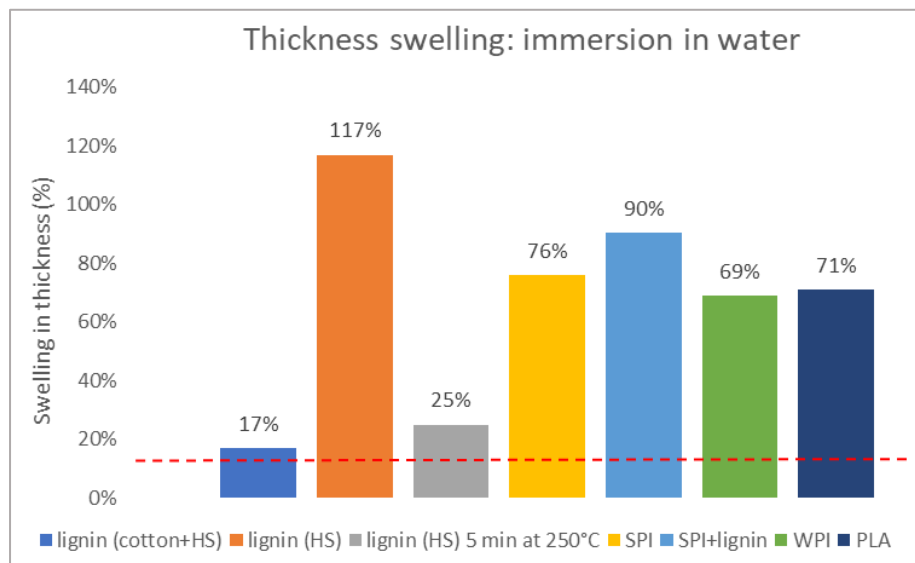


Figure 2.4.14 Thickness swelling in immersion of panels with different bio-adhesives

2.4.6 Characterization of normal and colonized HS

According to literature, any oxidation treatments of fibres is expected to generally increase their bonding capacity with bio-adhesives. However, as previously discussed in *Section 2.3.2*, all the panels produced with spent substrates from fungal cultivation were highly fragile, regardless the amount of lignin utilized. In order to further investigate the role of this enzymatic oxidation, it was decided to do some IR and TGA analysis of both normal and colonized HS fibres.

The acquisition of IR spectra was intended to highlight any tracks of oxidation at a chemical level. At first, three different samples of the colonized substrate were analysed, in order to see if any differences could have been detected in relation to different content of mycelium.

The graph here below reproduces the data related to a fibrous portion (“Colonized HS”), a part fully covered by mycelium (“Col HS-lots of mycelium”) and a small fungal residue (“Mycelium only”). These spectra could be almost overlapped, except for some peaks with different intensities among the three samples. Since the main differences were recorded in the fingerprint region (600-1400 cm^{-1}), other kind of analysis are needed.

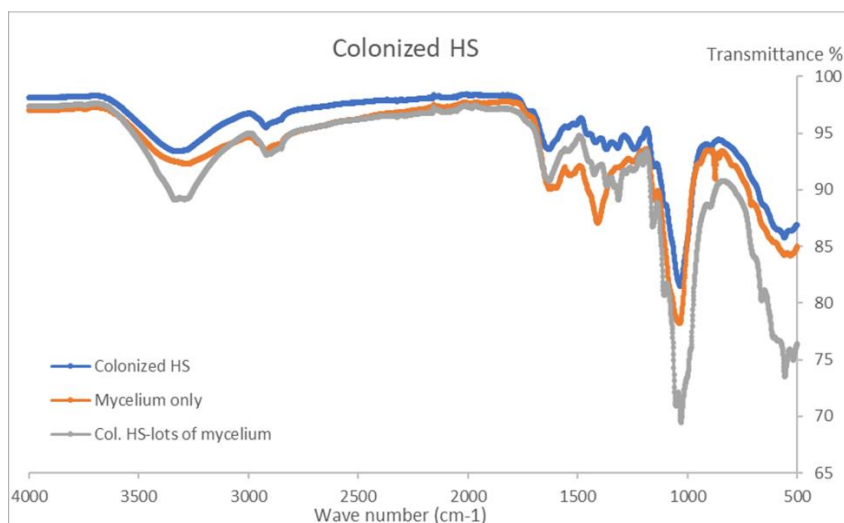


Figure 2.4.15 IR spectra of the three analysed samples of colonized HS

Even though all the analysed samples were not fully representative because randomly chosen, it was possible to detect some differences among fungal residues and normal substrates. In fact, the series “normal HS” in Figure 2.4.16. presents a sharp peak around 1635 cm^{-1} that, according to online libraries, characterises the stretching movement of a C=C group, an alkene. Conversely, this peak has a lower intensity for samples of colonized fibres, maybe because a reaction occurred on the double bond detected for the normal substrate. Moreover, the series “Mycelium only” shows a peak at 1411 cm^{-1} , that could be attributed to the bending of a hydroxyl group.

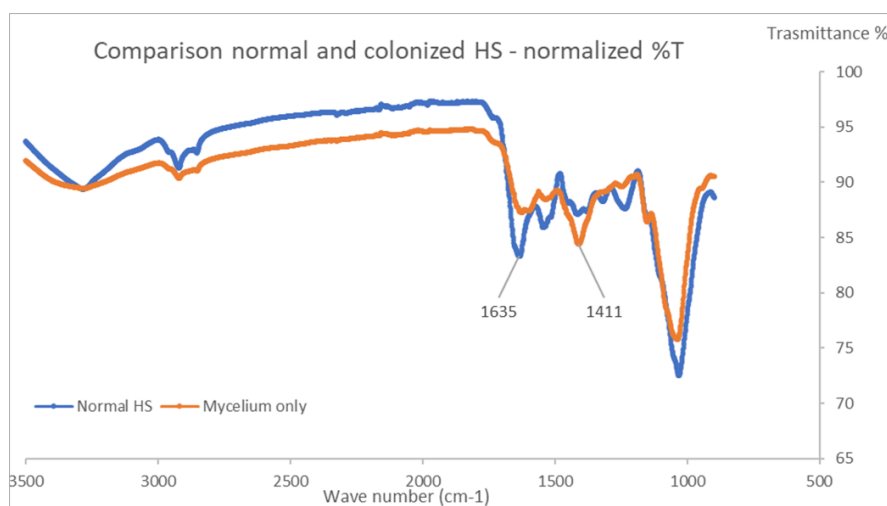


Figure 2.4.16 Comparison between the IR spectra of normal and colonized HS

The graphs here below put in comparison the thermal degradation of the two different substrates. At first, it can be noticed that the degradation peak of normal HS was not as sharp as the one of colonized substrate. This could be due to the fact that particles of normal HS burn in a different extent over time, thus causing some oscillations of sample's weight during the analysis. Conversely, a previous oxidation of fibres seemed to have a role in making fibres more compact. In fact, the left % of weight recorded for grinded colonized HS was slightly higher than the one of normal HS (30% for oxidized fibres and 20% for the normal one). Anyway, the peak of weight loss in relation to the starting amount occurred around 350°C for both the substrates. As specified in *Section 2.2.3*, these tests were performed in inert conditions and the analysed samples were randomly chosen, thus they cannot be considered as representative for their substrate. Therefore, other investigations are needed to access the actual thermal behaviour of these fibres.

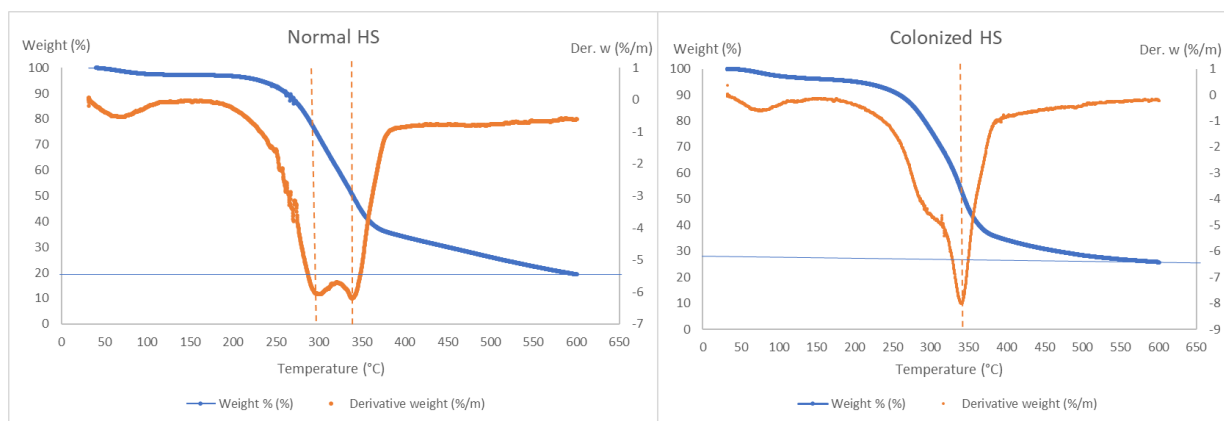


Figure 2.4.17 TGA analysis of normal and colonized HS

2.5 Conclusions

The Internship project presented in this Thesis confirmed that it is feasible to reach interesting properties with natural components coming from leftovers of several industries and even surpass some conventional products already on the market.

Particularly, thanks to the studies performed in relation to **macro-variables** (see *Section 2.3*), many of the set Objectives presented in *Section 2.1* were reached or at least improved, in respect to the current production protocol. In fact, by using only dried HS fibres with kraft lignin as binder, it was possible to develop the “Methodology F” with good repeatability results. The following Table puts in comparison this last manufacturing process with the current production protocol of Mogu Floor bio-composites and the target values set by the related norms: ISO 16893-2016 for “load and heavy-duty load bearing particleboard for use in dry conditions” (* and **, respectively) and EN 16776 (***).

The different colours highlight if the process’ implementation approached or reached the set targets (colour yellow and green, respectively) or further developments are needed (colour red). Precisely, the total manufacturing time reported for Methodology F takes into accounts also the minutes loss while opening the presses during the degassing steps.

Table 2.5.1 Comparison between the current production protocol and the implemented process “Methodology F”

features	Current production protocol	Methodology F	Targets
Manufacturing time (min)	45	17	10
Density (g/cm ³)	0,95	0,85	0,9
Thickness after calibration (cm)	0,65	0,95	0,8-0,9
Warping effect	++	+	+
*Bending strength (MPa)	9	14	15
*Modulus of Elasticity (MPa)	2152	2453	2200
*Internal bond (MPa)	0,39	0,65	0,40
**Swelling after immersion (%)	17	117%	16
***Residual indentation after 150 min of rest (mm)	0,03	0,02	<0,1

On the other hand, the studies performed in relation to **micro-variables** allowed to explore the use of alternative bio-adhesives. At first, the utilization of another kind of lignin and commercial biopolymers did not allow to obtain feasible results. Therefore, according to the researches done on literature, some experimental procedures have been developed to process powdered soy and whey protein isolates in order to obtain suitable binders. Although the manufacturing process will require further studies, it was possible to produce small panels that exhibit interesting mechanical performances. In details, the highest

values for both Modulus of elasticity and Bending strength were recorded for tiles containing activated whey proteins isolates (4947 MPa and 20,62 MPa, respectively).

For all the alternative bio-adhesives tested, there were recorded data of swelling in thickness lower than the ones related to panels manufactured with kraft lignin. In details, bio-composites containing activated whey protein isolates shown also the highest dimensional stability (69% of swelling in thickness). Conversely, the lowest value of swelling in thickness obtained for panels with kraft lignin was equal to the 117% (normal HS). However, further studies proved that thermal pre-treatments play an important role in improving panels' dimensional stability. In fact, after having heating sample at 250°C for 5 minutes, it was recorded a value of 25% of swelling in thickness.

Finally, thanks to the analysis performed at the SEM and optical microscope, it was possible to correlate observations done at a macroscopic level to the internal structures of panels.

In the light of these results, further studies are recommended to better identify a minimum viable product for a new production protocol. In fact, this will require additional data in relation to input and outputs of all productive processes, as well as the consideration of other important parameters such as economic and ecological issues. A draft of a procedure to follow in order to evaluate all the different methodologies was developed at last. In details, it is constituted by different steps:

1. Insert recorded data in a structured chart (see *Figure 2.5.1*)
2. Identification of the best and worst results for each category.
3. In another blank structured chart, give marks to the previous results, following a scale: 1=best, 5=worst, 3=middle, 2=towards best, 4=towards worst
4. Sum these marks in rows
5. Select the most relevant criteria for an industrial production and weight the related marks on the basis of the importance of their category.

INPUTS						
		kind (size, origin, ...)	quantity for 1 panel		pre-treatments (drying, sieving, ...)	
Materials	particles					
	binder(s)					
Processing parameters	set-up (metal&wood frame, ...)	Temperature (°C)	pressure (bar)	time of cooking (min)	time of process (min)	fluency of process (pre-/post-treatments, precautions, ...)
OUTPUTS						
Mechanical results	residual indentation (mm)	internal bonding (MPa)	modulus of elasticity (MPa)		resistance to flexion (Mpa)	
Dimensional stability	thickness (mm)		warping: no (-), normal (+), huge (++)		swelling in thickness (%)	

GENERAL FEATURES	ECONOMICAL ISSUES	ECOLOGICAL ISSUES		
Reproducibility (%)	Total costs (€) (production, energy, ..)	Biodegradability (days)	Bio-based content (%)	VOC emissions

Figure 2.5.1 Hypothetical criteria to follow for the identification of the minimum viable product

3. Mogu floor: BIO-POLYURETHANE COATING

Mogu's bio-PU production is made by external industries that have plants with four different coating heads. Conversely, the preparation of materials for the sample boxes is currently performed at Mogu's. As mentioned in the Introduction, some experiments have been made in relation to the manufacturing process of these last bio-PU layers.

Firstly, the preparation of bio-PU sheets was done by a **casting method**. In fact, after having mixed the different compounds, the resin was directly applied on a plastic support and manually levelled using a tube. Due to the high thickness (around 1,2 mm) and the low catalyst's content, these layers required a long time to dry. Moreover, while detaching it from the plastic support, the material was often damaged. Therefore, in order to limit these scraps and the waste of compounds, it was decided to adapt formulations to a production by coating, using a laboratory coating machine.

The first attempts in producing bio-PU sheets **by coating** were done following the industrial formulation already tested. However, since the different processing conditions required other adjustments, it was agreed to perform some tests using **different catalysts** before proceeding further. In details, the aim of these experiments was speeding the reactions but by maintaining a long pot-life for the utilized resins. In fact, this could allow not only to shorten the manufacturing time but also to limit the waste of compound over process that were caused by a limited pot-life. Different concentrations were tested for all the four catalysts selected (the 1%, 0,5% e 0,1%) and the resins that gave feasible results in the manufacturing of bio-PU by coating were left in a chemical oven at 25°C, in order to monitor any changes in texture over time. The catalyst that showed the longest pot-life at the lowest concentration as possible was then selected for further studies.

The production of different colours of bio-PU by coating took quite a long time. In fact, both the amount of catalyst and the processing conditions (e.g., the thickness by which resins were applied to a siliconized paper sheet over manufacturing, as well as temperature and time) had to be adapted for each specific formulation. Moreover, some recipes required the addition of natural pigments in order to match the colours of bio-PU sheets produced by casting. However, thanks to these experiments, all the formulations were properly adapted for the production by coating.

Finally, these bio-PU sheets were applied on Mogu Floor biocomposites using a glue, that was prepared following a formulation in agreement with the principles already listed in *Section 1*. These tiles were left to fully dry underweight and then were cut into pieces as required by the Mogu's sample boxes.

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Annexes

I. Complete overview of processing methodologies

methodology	A	B	C	D	D*	E	F
Degassing step	after 10 minutes	after 10 minutes	after 5 minutes	each 5 min	each 5 min	each 4/5 min	each min. for the first 5 minutes + at 8. and 11. minutes
Pressing time (minutes)	20	20	10	15	15	16/20	12
Re-pressing step	5 minutes	-	5 minutes without the metal frame	5 minutes	5 minutes	-	-
Temperature (°C)	180	180	180	180	120, 150, 200	180	180
Pressure (bar)	100	150	150	100 and 150	100 and 150	150	150
Theoretical density targeted (g/cm ³)	0,9	0,9	0,9	0,9	0,9	0,9	0,9
Theoretical panels thickness (cm)	0,8	0,8	0,8	1,2	0,9	1,0	0,9
others						edges cut	edges cut

II. Complete results of applied processing methodologies

Methodology A

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
11/02	1a	HS, 1950	lignosulfonate lignin, 220	10	1,01	0,67
	1b	HS, 1950	kraft lignin, 220	10	1,07	0,64
12/02	2	HS, 1950	SPI, 490	20	1,1	0,69
	3	HS, 1950	kraft lignin, 290 WPI, 190	20	1,09	0,69
	4	HS, 1950	PLA, 490	20	1,31	0,59
	5	HS, 1950	PVA, 240 thermoplastic starch, 240	20	1,14	0,71
	6	HS, 1950	lignosulfonate lignin, 490	20	1,06	0,71
2-03/03	1	HS, 1950	PVA, 240 thermoplastic starch, 240	20	9,6	0,78
	2	HS, 1950	lignosulfonate lignin, 490	20	9,7	0,805
	3	50% HS + 50% sawdust, 1960	kraft lignin, 220	10	8,93	0,78
	4	sawdust, 1740	kraft lignin, 440	20	8,47	0,82
	5	smaller HS, 1960	kraft lignin, 220	10	8,97	0,78

	6	colonized HS, 1960	kraft lignin, 220	10	8,65	0,80
	7	colonized HS, 1740	kraft lignin, 440	20	8,7	0,75
	8	50% sieved HS + 50% sawdust, 1960	kraft lignin, 220	10	8,98	0,78
	9	1960 (50% colonized HS + 50% sawdust)	kraft lignin, 220	10	9,09	0,75
	10	50% colonized HS + 50% sawdust, 1740	kraft lignin, 440	20	8,4	0,81
	11	75% HS + 25% sawdust, 1960	kraft lignin, 220	10	9,17	0,74
	11 bis	75% HS + 25% sawdust, 1960	kraft lignin, 220	10	8,96	0,78
	12	75% HS + 25% sawdust, 1960	kraft lignin, 220	10	8,7	0,8
	13	HS, 1740	kraft lignin, 440	20	8,42	0,83

Methodology B

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
2-03/03	14	colonized HS, 1740	kraft lignin, 440	20	5,127	0,99

Methodology C

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
2-03/03	15	1960 (50% HS + 50% sawdust)	kraft lignin, 220	10	8,22	0,86
	16	1740 (50% HS + 50% sawdust)	kraft lignin, 440	20	7,82	0,89
16/03	1 (cfr13)	HS, 1960	kraft lignin, 220	10	7,8	0,89
	1b (cfr13)	HS, 1960	kraft lignin, 220	10	8,21	0,85
	2 (cfr5)	smaller HS, 1960	kraft lignin, 220	10	8,41	0,82
	3	bigger HS, 1960	kraft lignin, 220	10	7,87	0,88
	4 (cfr7)	colonized HS, 1960	kraft lignin, 220	10	7,98	0,86
	5 (cfr8)	50% sieved HS + 50% sawdust, 1960	kraft lignin, 220	10	8,36	0,83
	6	50% sieved HS + 50% sawdust, 1740	kraft lignin, 440	20	7,48	0,92
	layered 7	50% smaller HS + 50% HS	kraft lignin, 220	10	8,1	0,86
	layered 9	50% sawdust + 50% HS	kraft lignin, 220	10	7,84	0,89
19/03	8	HS, 1740	PVA, 220 thermoplastic starch, 220	20	8,15	0,85
19/04	layered 4	HS, 1960	kraft lignin, 220	10	0,79	0,9

Methodology D

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
16/03	combined parameters tests 10	HS, 2160	kraft lignin, 330	10	12,32	0,77
19/03	3	HS, 2940	kraft lignin, 330	10	13,24	0,79
	4	HS, 2940	kraft lignin, 330	10	11,96	0,88

Methodology D*

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
19/03	1 (T=120°C)	HS, 1960	kraft lignin, 220	10	10,36	0,67
	2 (T=150°C)	HS, 1960	kraft lignin, 220	10	8,71	0,82
16/03	8 (T=200°C)	HS, 1960	kraft lignin, 220	10	7,5	0,94
02/04	10 (T=200°C)	HS, 2450	kraft lignin, 270	10	9,735	0,88

Methodology E

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
19/03	5	HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	10,5	0,87
	6	HS, 2205 (m for 0,9 cm thickness)	kraft lignin, 245	10	9,54	0,83
02/04	1 (th. 0,92 g/cm ³)	HS, 2500	kraft lignin, 280	10	10,49	0,84
	2 (th. 0,87 g/cm ³)	HS, 2370	kraft lignin, 260	10	10,35	0,82
	3 (th. 0,82 g/cm ³)	HS, 2230	kraft lignin, 248	10	10,3	0,74
	4 (th. 0,9 g/cm ³)	HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	10,18	0,86
	5	HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	10,32	0,86
	6	HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	10,5	0,87
	7	smaller HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	10,4	0,84
	8	smaller HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	10,14	0,86
	9	smaller HS, 2350 (m for ca. 1 cm thickness)	kraft lignin, 260	10	10,2	0,82
	12	HS, 2205 (m for 0,9 mm thickness)	kraft lignin, 245	10	9,73	0,79
09/04	1	HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	0,94	0,88
	2	25% colonized HS+75% HS, 2450	kraft lignin, 270	10	1,06	0,83
	3	25% colonized HS+75% HS, 2450	kraft lignin, 270	10	1,06	0,83
	5	25% colonized HS+75% HS, 2450	kraft lignin, 270	10	1,06	0,83
	6	25% colonized HS+75% HS, 2450	kraft lignin, 270	10	-	-
	7	25% colonized HS+75% HS, 2450	kraft lignin, 270	10	1,04	0,84
	8	HS, 2450 (m for 1 cm thickness)	kraft lignin, 270	10	1,02	0,87
	9	25% colonized HS+75% HS, 1960	kraft lignin, 220	10	0,96	0,74
	10 (0,95 g/cm ³)	HS, 2590 (m for 1 cm thickness)	kraft lignin, 290	10	1,06	0,88

19/04	1bis	HS, 1960 (for 0,8 cm thickness)	kraft lignin, 220	10	9,75	0,69
	1	HS, 1960 (for 0,8 cm thickness)	kraft lignin, 220	10	9,3	0,76
	2	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	10	0,804
	3	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	9,7	0,833
	4	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	9,6	0,825
10/05	7	HS, 2080 (calculated for th. thickness=0,9 cm)	PLA, 370	15	0,96775	0,78
	8	HS, 2080 (calculated for th. thickness=0,9 cm)	Thermoplastic starch, 370	15	1,00725	0,76
09/06	6 (rep. 7 of 10/05)	HS, 2080 (calculated for th. thickness=0,9 cm)	PLA, 370	15	1,105	0,69

Optimization of Methodology E

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)	process
28/05	1	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	-	-	8' cooking cycle
	2	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	-	-	8' cooking cycle
	3	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	0,97	0,78	10' cooking cycle
	4	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	0,98	0,79	10' cooking cycle
	5	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	0,98	0,78	12' cooking cycle
	6	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10			10' cooking cycle
	7	HS, 1960 (for 0,8 cm thickness)	kraft lignin, 220	10	0,95	0,72	10' cooking cycle
	8	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10			12' cooking cycle
	9	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	0,979	0,77	12' cooking cycle
	10	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10	1,02	0,76	12' cooking cycle
	11	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,985	0,77	10' cooking cycle
	12	HS, 2210 (for 0,9 cm thickness)	kraft lignin, 250	10			12' cooking cycle
31/05	1-8	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8			10' cooking cycle


Methodology F

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
31/05	1 top	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9432	0,84
	2 bottom	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9104	0,89
	3 top	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,952	0,84
	4 bottom	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9148	0,86
	5 top	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,927	0,87
	6 bottom	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,904	0,86
	7 bottom	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9166	0,86
	8 top	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9428	0,84
	9 top	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9332	0,87
	10 bottom	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9228	0,86
	11 top (alone)	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9764	0,78
	12 bottom	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9288	0,82
	13 top	HS, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9448	0,80

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
14/06	1	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	x	x
	2	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	1,024	0,79
	3	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,939	0,83
	4	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,963	0,83
	5	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9798	0,81
	6	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,955	0,83
	7	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9666	0,83
	8	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9724	0,81
	9	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,963	0,83
	10	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,962	0,83
	11	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,955	0,83
	12	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9498	0,83
	13	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9598	0,82
	14	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9214	0,83
	15	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9636	0,81
	16	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9692	0,84
	17	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,942	0,86
	18	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9722	0,84

data	sample	fibre: type and weight (g)	binder: type and weight (g)	binder: %/dry w. (%)	average thickness (mm)	density (g/cm ³)
21/06	1	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	-	-
	2	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	-	-
	3	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	-	-
	4	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,758	0,85
	5	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,71	0,9
	6	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,699	0,93
	7	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,7086	0,89
	8	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,717	0,9
	9	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,6968	0,92
	10	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,7244	0,88
	11	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,6872	0,93
	12	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,703	0,9
	13	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,6872	0,93
	14	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,7044	0,91
24/06	1	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	1,0312	0,74
	2	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9926	0,77
	3	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	1,012	0,77
	4	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9942	0,79
	5	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	1,0044	0,78
	6	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,986	0,79
	7	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9576	0,81
	8	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9656	0,78
	9	Flax, 2250 (for 0,9 cm thickness)	kraft lignin, 200	8	0,9452	0,73

III. Alkaline hydrolysis of SPI: experiment's canvas

EXP.ID #		Project:	Mogu Composite R&D	
Title:	Alkaline hydrolysis of Soy Protein Isolates			
Author/s:	Sara Gransinigh	Starting date:	01/02/2021	
Supervisor:	Marco Cartabia, Daniele Cielo	Finished on:	30/06/2021	

Keywords (max. 5):

Soy Protein Isolate, activated binders, compressed panels.

Background: As stated by *Vnučec et al. 2017*, a thermal/chemical/enzymatic modification of Soy Proteins is necessary to break the strong internal bonds between molecules, thus making more functional groups available for reaction with wood components. Among all the main procedures of modification cited in literature, an alkaline hydrolysis with NaOH of Soy Protein Isolates (SPI) was selected. In fact, this treatment can be easily carried out without using polluting or highly hazardous chemicals.

Goal: The setting of this experiment is a combination of two procedures from literature (*Hettiarachchy et al. 1995, Zhang et al. 2013*). This aims at taking advantage of the equipment still available at Mogu, as well as limiting the liquid content of the final solution.

Materials & Details:

- Heating plate with magnetic stirrer (stirring speed=0-1500 rpm, capacity=20 L, ceramic plate, dimensions=280x160x85 mm)
- Becker with V=2 and 3 L (borosilicate glass)
- Metal sieve
- Cowles mixer for varnishes
- Soy Protein Isolates (unflavoured commercial powder with 90% of protein content)
- Demineralized water
- NaOH (≥99% assay, micropearls low in metals)

PROPORTION	for 1L of V	for 2L of V
V NaOH sol (L)	0,5	1
m NaOH (g)	2,9	5,8
V H2O (L)	0,5	1
m SPI (g)	120	240
th. [NaOH]	0,07	0,14

Methodology:

1. Partial solubilization of SPI in demineralized water

The proper amount of water (0,5L for 1L of final solution and 1L for 2L of it) is firstly heated up to T=65°C in a 3L becker.

The maximum amount possible of SPI (almost half of the total-see the "Proportion" chart above) is then solubilized under magnetic stirring. To limit lumps' formation, the SPI powder is sieved using a metal sieve.

2. Preparation of NaOH solution

The estimated quantity of NaOH is dissolved in water under stirring: 2,9g in 0,5L of water for 1L of final solution and 5,8g in 1L of water for 2L of product. Since NaOH is corrosive and vapours could be released at this stage, gloves and fume extraction plant are needed.

3. Final solubilization of SPI in water and NaOH solution

Further aliquots of SPI are added gradually as well as the NaOH solution, under stirring and at 65°C. Before adding the whole NaOH solution, solution is mixed using a cowles mixer to limit lumps' formation.

Solution is maintained at 65°C under magnetic and manual stirring for 1,5 h. in total. Particularly, also a manual stirring is useful because of the high viscosity of the product. Therefore, this feature could be easily improved if mixing is enhanced, such as by using the cowles/other equipment during the whole process.

Results:

The final solution is ochraceous and highly viscous, with a pH in the range 10,25-10,80.

The pot-life is of 9 days at room temperature but it can be extended up to two weeks if the solution is stored in a fridge (at about 5°C). An experiment was also set to increase of the %p/V of SPI, in order to limit the aqueous content of the solution. However, already the 15% p/V of SPI leads to a highly jelly product that was difficult to prepare and then mix with fibres during the preparation of compressed panels. For this reason, it is recommended to use a %p/V of SPI as high as the 12%.

Conclusion & Next Steps:


The procedure was easily scaled-up from 1L to 2L of solution, keeping the 12%p/V of SPI constant.

For further scaling-up, mixing must be improved in order to be as homogeneous as possible.

References:

- Hettiarachchy, N. S.; Kalapathy, U.; Myers, D. J. Alkali-Modified Soy Protein with Improved Adhesive and Hydrophobic Properties. *J Am Oil Chem Soc* **1995**, *72* (12), 1461-1464. <https://doi.org/10.1007/BF02577838>.
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IV. Thermal denaturation of WPI: experiment's canvas

EXP.ID #		Project:	Mogu Composite R&D	
Title:	Thermal denaturation of Whey Protein Isolates			
Author/s:	Sara Gransinigh		Starting date:	01/02/2021
Supervisor:	Marco Cartabia, Daniele Cielo		Finished on:	30/06/2021

Keywords (max. 5):

Whey Protein Isolate, activated binders, compressed panels.

Background: As stated by Gao et al. 2011, the globular structure of whey proteins must be unfolded for releasing polar groups, in order to offer additional cohesion strength to a whey-protein based environmentally friendly wood adhesive. This could be easily done by a thermal denaturation under gentle conditions, starting from 40°C and up to 65-70°C. The experimental conditions must be carefully monitored during this treatment because the WPI solution tends to be gelled before or soon after it reaches the denaturing temperature.

However, the adhesive mentioned above is obtained through an additional cross-linking of the denatured WPI with an aqueous polymer-isocyanate, thus requiring the use of polluting chemicals.

Goal: The purpose of this experiment was to investigate the potentialities of thermally activated WPI without involving any other substances and limiting the liquid content of the final solution as well.

Materials & Details:

- Heating plate with magnetic stirrer (stirring speed=0-1500 rpm, capacity=20 L, ceramic plate, dimensions=280x160x85 mm)
- Becker with V=2 L (borosilicate glass)
- Whey Protein Isolates (unflavoured commercial powder with 97% of protein content)
- Demineralized water

Methodology:

1. Dissolution of WPI in water at 40-49°C

WPI is fully dissolved in water at 40-49°C under stirring to form a solution with 40 %w/V concentration (e.g., 200g for a V=0,5L, 400g for 1L etc.). The obtained solution is liquid and creamy-like and has a thick foam layer on top.

2. Thermal denaturation at 60-63°C

The solution is then heated up to T=60-63°C under magnetic and manual stirring. The temperature must be carefully kept as constant as possible for 20-25 minutes, until viscosity increases and the solution becomes white and uniform. The process must be immediately stopped, otherwise it could fully jellified thus becoming almost unsuitable for the compressed panels' production. In fact, having lots of lumps in the binder does not allow a proper mixing with fibres and leads to the formation of darker spots on the panels' surfaces.

Results:

The final solution is white, uniform and highly viscous.

The pot-life is of only few hours at room temperature. In fact, over time jellification is more likely to occur, followed by the separation of components.

Conclusion & Next Steps:

The procedure can be easily scaled-up, if the experimental conditions are kept carefully under control.

Since the change of consistency viscous-jellified solution occurs within a very narrow gap of temperature and time, further studies are recommended to make the whole procedure steadier.

References:

Gao, Z.; Yu, G.; Bao, Y.; Guo, M. Whey-protein Based Environmentally Friendly Wood Adhesives. *Pigment & Resin Technology* 2011, 40 (1), 42-48. <https://doi.org/10.1108/03699421111095937>.