Helsieni Oy 2741436-9
Uutiskatu 2, 00240 Helsinki, Finland
Stéphane Poirié: stephane@helsieni.fi
Chris Holtslag, chris@helsieni.fi

*Hanke on saanut tukea maa- ja metsätalousministeriön rahoittamasta **Ravinteiden kierrätyksen kokeiluohjelmasta**, jota hallinnoi Etelä-Pohjanmaan ELY-keskus*

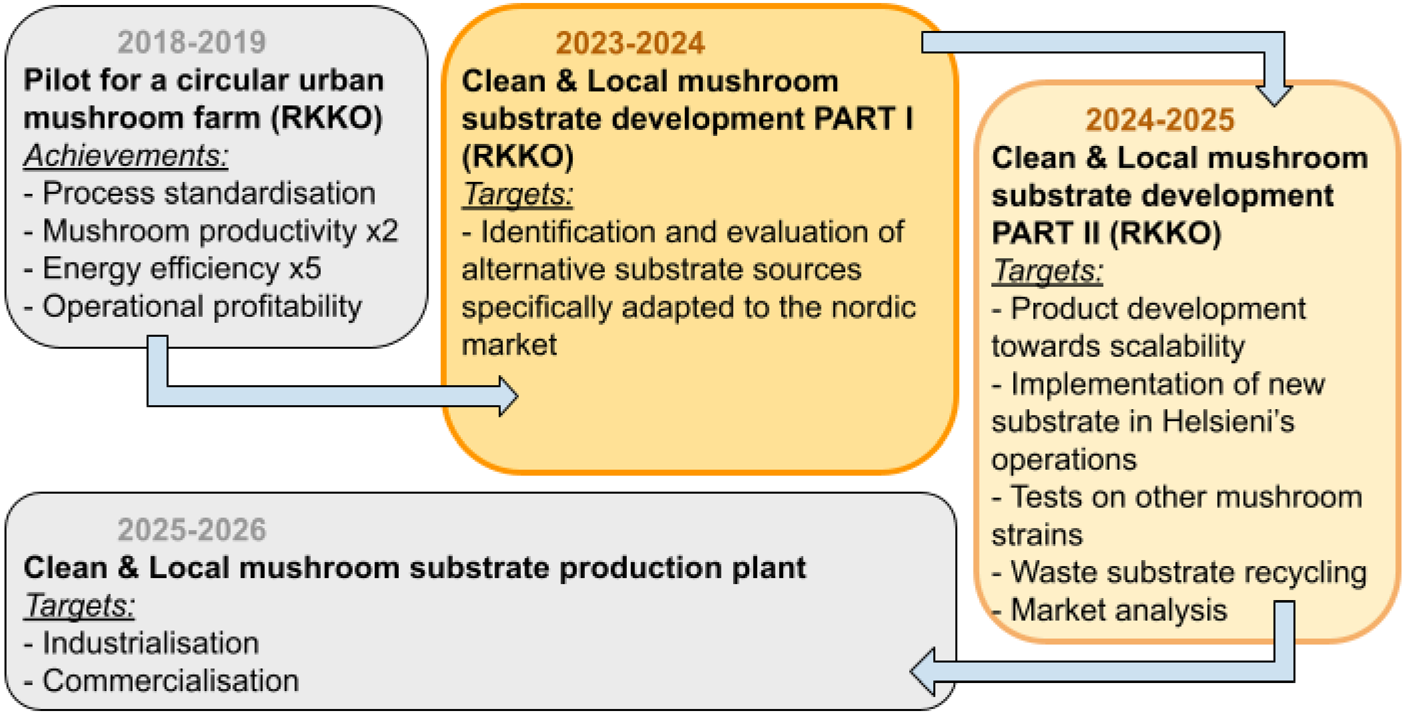
Clean & Local Mushroom Substrate Development
PART I

30.10.2023 - 30.11.2024

FINAL REPORT

INTRODUCTION

This project aims to identify, compare and develop new raw materials for commercial lignicolous mushroom substrates in European nordic countries. The focus will be set on locally abundant resources that are somewhat considered as waste or causing environmental issues. We will also compare different options for the use of spent substrate at the end of its life-cycle.
This project will be conducted in two parts: we will first test a wide range of available resources to select the few most promising, which will be developed in a second phase. This application only refers to the first phase of the project.
The goal is to be ready by the end of the project to start the commercial production of raw materials that give Finnish and nordic mushroom growers as good or better performance (mushroom yield) as the currently most used, while being more reliable, locally abundant, less energy intensive and less competing with other uses.



TEAM

Helsieni is currently run by its two of its founders, Chris Holtslag and Stéphane Poirié, whose educational and professional background are:

- Chris Holtslag: Industrial Ecology & Industrial Design (Master Of Science, Delft University of Technology & Leiden University).
- Stéphane Poirié: Electrical Engineering, spec. Integrated Production Systems (Master's Degree, INSA Lyon).

Helsieni' shareholders and advisers have competences in the fields of Sales, Mushroom Biology, IT and electronics, Circular Economy, Waste Management, Mushroom & Vegetable farming, Business Development, Design, Automation and Robotics, Communication and Marketing.

R&D BACKGROUND

Helsieni has grown oyster mushrooms, shiitake and lion's mane mushrooms in shipping containers for 7 years. Our current annual production is 3000 kg of fresh oyster mushrooms, 200 kg of fresh lion's mane and 200 kg of fresh shiitake. We also sell DIY mushroom products and we educate people about mushroom cultivation. The principles of circular economy apply to our whole process: all developments are made towards maximising added value while minimising energy inputs and waste outputs. For example, most of our spent substrate goes to home gardens where they produce more mushrooms and feed the soil. Everything that cannot be sold is composted. All crates in which we pack our fresh mushrooms are collected, washed and reused. Our DIY products are mostly produced in reused plastic buckets. Our production facilities are located nearby our customers (Uusimaa) and we educate other farmers elsewhere in Finland to start local mushroom farms to limit the transport of light, fragile and voluminous products. Our current substrate recipe consists of 25% of spent coffee grounds that we collect from restaurants.
However, another 20% of our substrate consists of pelletised straw, for which we cannot monitor sustainability, quality, price and availability.

Helsieni farm's performance and sustainability were improved after a project called "Pilot for a circular mushroom farm" conducted in 2019 and co-funded by ELY (RKKO). Thanks to its results, the use of spent coffee grounds has been systematised and optimised in our substrate. Our contamination issues have decreased by half after improving our pasteurisation process. As a result, our mushroom production capacity has doubled without extending facilities, and our energy consumption has been reduced by 80%.

By the end of the 2019 project, we were able to define our next needed developments. Amongst them:

- Developing easy-to-use cellulose-based materials from abundantly and locally available side-products or waste (reed stems, oat husks, cellulose-made plant substrates...)
- Research more specifically on three different mushroom species that we evaluate as worth cultivating: lion's mane, pheasant's back mushrooms, king oyster.

Amongst others, the current study will explore these points and thus continue the previous study.

Helsieni was also a founding member of the Metropolia Urban Farm Lab, where our team spent about a year in 2019 to explore circular economy opportunities with other urban food producers. This project gave birth to a modular farm prototype; a mushroom farm made of bricks that could be assembled to build the farm of the needed size in any context (for example, next to organic side streams).

INTERNATIONAL CONTEXT

Currently, lignicolous mushrooms are mainly cultivated on straw- and wood-based substrates, with the addition of supplements (soy husk, grain bran, coffee grounds, grains, etc.).

Straw, which used to be broadly available in Finland, has become a scarce resource as farmers tend to leave it on the fields to limit soil amendments. It is also in competition with other uses, such as animal bedding and in the case of pelletised straw, energy production. In our urban environment, we have preferred straw pellets over unprocessed straw for logistic, storage and production ease but their availability and quality have been fluctuating, especially since the start of the Ukrainian war. Most straw pellets available in Finland come from abroad and we have faced shortage periods and quality variations.
Hardwood-based substrates are necessary to cultivate certain mushroom species, but for non-forest-owners they have become hard to find and expensive, as the competition with other uses (construction, energy) is steadily growing. International sanctions towards Russia also had a big impact on the supply chain. Most available wood chips, sawdust and pellets on the Finnish market are softwood-based or mixed wood, making it improper for a large part of the cultivated mushroom species.

Most of the substrate research and development in the world was held in Asia, northern America and central Europe, sometimes using resources that are hardly available in Finland and other nordic European countries. However, a substantial amount of papers have been published in Sweden that are to be used in this study. Our role here will be to select the resources that can match with our context (a galaxy of small mushroom farmers in Finland) and push them towards industry standards and scalability.

OBJECTIVES

The project will be considered successful if it leads to the identification of at least one viable and sustainable alternative to the substrate material we currently use.
By viable we mean reliably available, scalable, biologically and financially competitive with the current most used materials.
By sustainable we mean local, circular (using side-streams that are not competing with other, more efficient uses) and if possible, organic.

By the end of the project (PART I) we will have identified if any of the tested substrates are worth further developments. If so, we will design further tests that need to be conducted to optimise recipes and production processes. These tests, together with a market analysis, will take place in PART II of the project, for which we will seek new fundings.

KEY PERFORMANCE INDICATORS

To evaluate what we stated as viable and sustainable substrates, we need to compare substrate performances through standardised key performance indicators. We have identified the following KPI to be well calibrated for this project:

KEY PERFORMANCE INDICATORS

BIOLOGICAL PERFORMANCE	PRODUCTIVITY	wet/wet productivity	biological efficiency	space productivity
	GROWING SPEED	# days from prod to end of first flush	% First flush / total	
	CONTAMINATION RATIO	% part. contaminated bags / total	% totally contaminated bags / total	
ECONOMICAL PERFORMANCE	MATERIAL COST	€ /kg of substrate	€ /produced bag	
	PROCESSING COST	€ /kg of substrate	€ /produced bag	
	COST OF GOODS SOLD	€ /kg of mushrooms produced		
SUPPLY	AVAILABILITY	# potential suppliers in Finland	periodicity & seasonality	
	SCALABILITY	Supply cost	Processing cost	potential yearly prod. at constant supply
ENVIRONMENTAL IMPACT	LOCALNESS	distance to the nearest supplier		
	CIRCULARITY	# other competing industrialised uses		
	ORGANICITY	Current availability of organic alternatives		

- BIOLOGICAL PERFORMANCE, for a given recipe (***abbreviation***[1])
    - Productivity:
        - Wet/wet productivity (***w/w prod***): kg of produced mushrooms per kg of wet substrate
        - Wet/wet productivity, normal cycle (***w/w 70d***): kg of produced mushrooms per kg of wet substrate within a normal production cycle (10 weeks = 70 days)
        - Biological efficiency (***BE***): kg of produced mushrooms per kg of dry matter in the substrate
        - Bag productivity (***bag prod***): kg of produced mushrooms per growing unit (20L bags)
        - Space productivity (***space prod***): kg of produced mushrooms per growing unit (20L bags) within a normal production cycle (10 weeks = 70 days)[2]. If multiplied by 1000, this number would give the maximum yearly production capacity of our farm for a given recipe, with our current setup[3].
    - Growing speed (First flush[4]):
        - Amount of days from substrate production to end of first flush (***#day to F1 end***)
        - kg harvested in the first flush compared to the total harvest (***%F1***)
    - Contamination ratio:
        - Amount of partially contaminated bags per total amount of bags produced (***% unhealthy***)
        - Amount of totally[5] contaminated bags per total amount of bags produced (***% OUT***)

- ECONOMICAL PERFORMANCE (for a given recipe)
    - Raw material cost[6]:
        - € per kg of substrate (***supply €/kg***)
        - € per growing unit produced (***supply €/bag***)
    - Processing cost: € spent to process[7] all needed raw materials for a given recipe in our current facilities
        - € per kg of substrate (***process €/kg***)
        - € per growing unit produced (***process €/bag***)
    - Total substrate cost[8]:
        - € per kg of substrate (***TT cost €/kg*** = supply €/kg + process €/kg)

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[1] Used in the entire report
[2] Bag productivity and other KPI depending on the amount of bags produced cannot be calculated for all samples in WP1 because the amount of bags produced is not a piece of data that we systematically record in our standard production process.
[3] We have space for 200 bags and one year is about 5 normal cycles.
[4] Mushroom substrates produce fruitbodies in waves that are harvested within a few days, with usually 10-20 days of break between each wave. Such waves are named “flushes” in the fungicultural jargon. The larger the first flush, the less economical it is to wait for consecutive flushes.
[5] A growing unit (substrate bag) is considered fully contaminated if it needs to be removed from the production system before it can produce any mushrooms. It is considered partially contaminated if it shows visual signs of contamination or if they need to be removed from the production system before the end of their normal production cycle.
[6] Also includes material logistics and transport to our farm. Costs are calculated with raw material prices as of 01.01.2024.
[7] Includes work to make the materials fit for production and to mix them into a substrate. It also includes pasteurisation costs (work + energy).
[8] Used only in some cases instead of Raw material and processing costs

    - € per growing unit produced (***TT cost €/bag*** = supply €/bag + process €/bag)
- Cost of goods sold: € Total substrate cost per kg of mushrooms produced (***COGS$_€$***)[9]

■ SUPPLY (for a given ingredient)

- Availability:
    - Amount of potential suppliers in Finland
    - Periodicity: amounts of months available per year
- Scalability:
    - Cost (supply + process) for a specific ingredient.
    - Amount of substrate bags that can be produced per year with the current supply

■ ENVIRONMENTAL IMPACT (for a given ingredient)

- Localness: Distance in km to the nearest identified supplier.
- Circularity: Amount of other (competing) industrialised uses for the raw material
- Organicity: Current availability of organic raw materials (Y/N)

[9] Excluding energy costs after substrate production (heating or cooling the substrate while incubating or fruiting).

WORK PACKAGE 1: PRELIMINARY WORK

Task 1. Data Analysis

WP1 T1.1: Analysed substrate batches

All analysed samples were selected amongst the Helsieni former commercial substrate production batches for their particularities (irregular recipe, unexpected outcome, remarkable health…) or on the contrary for their “average” characteristics. These substrates were not produced in a R&D context and therefore do not respond to the standards of laboratory tests. However, our consistent and routined substrate production process already enables a preliminary analysis. The goal is to give us references to define a standard process for the coming tests.

List of analysed samples and their recipes[10]:

ID #	Prod date; Weight	Carbon source (Brand / Provenance)	Carbon source pasteurisation (Y/N)	Coffee grounds	Chalk	Spawn variety[11]	Water content
2	24.01.23 30 kg	Straw pellets (Halmeko): 20.1%	Y	6.7%	1.0%	Allerpo: 2.4%	69.8%
3	24.01.23 105 kg	Crushed straw pellets (Strohfelder): 20.1%	Y	6.7%	1.0%	Allerpo: 2.4%	69.8%
5	01.02.23 90 kg	Straw pellets (Halmeko): 20.1%	N	6.7%	1.0%	Allerpo: 2.4%	69.8%
6	01.02.23 90 kg	Straw pellets (Halmeko): 20.1%	Y	6.7%	1.0%	Allerpo: 2.4%	69.8%
7	01.02.23 269 kg	Crushed straw pellets (Strohfelder): 20.1%	Y	6.7%	1.0%	Allerpo: 2.4%	69.8%
8	09.02.23 224 kg	Straw pellets (Baltic): 20.1%	Y	6.7%	1.0%	Allerpo: 2.4%	69.8%

[10] Unless specified elsewise, given ingredients in this study are measured in % of the total weight. Ingredients are considered dehydrated, that is their wet mass is added to the recipe water content while their dry mass is expressed in % of the total mass.
[11] See WP1 T1.4

9	21.02.23 90 kg	Straw pellets (Halmeko): 20.1%	Y	6.7%	1.0%	Allerpo: 2.4%	69.8%
10	21.02.23 90 kg	Straw pellets (Baltic): 20.1%	Y	6.7%	1.0%	Allerpo: 2.4%	69.8%
11	21.02.23 85 kg	Straw pellets (Baltic): 21.3%	N	7.1%	1.1%	Allerpo: 2.6%	68.0%
12	21.02.23 85 kg	Straw pellets (Halmeko): 21.3%	N	7.1%	1.1%	Allerpo: 2.6%	68.0%
13	09.03.23 237 kg	Straw pellets (Halmeko): 19.0%	Y	6.3%	1.0%	Allerpo: 2.3%	71.4%
14	09.03.23 237 kg	Straw pellets (Halmeko): 19.0%	N	6.3%	1.0%	Allerpo: 2.3%	71.4%
19	10.07.23 196 kg	Straw pellets (Baltic): 21.5%	Y	-	0.9%	Spoppo: 2.2%	75.4%
21	10.07.23 56 kg	Hemp shives (Hemparade): 18.9%	Y	-	1.4%	Spoppo: 3.2%	76.5%
23	28.09.23 428 kg	Straw pellets (Baltic): 12.7% Hemp shives (Hemparade): 3.0%	Y	8.2%	0.9%	Spoppo: 2.3%	72.9%
24	27.10.23 51 kg	Straw pellets (Baltic): 21.1% Hemp shives (Hemparade): 1.8%	Y	-	0.9%	Spoppo: 3.2%	73.1%
25	27.10.23	Straw pellets (Baltic): 13.7% Hemp shives (Hemparade): 2.9%	Y	7.9%	0.9%	Spoppo: 2.2%	72.4%

WP1 T1.2: Carbon source pasteurisation

Oyster mushroom substrates mainly consist of high-carbon ingredients containing cellulose, hemi-cellulose and/or lignin. This main component(s) can be completed with nutrient-rich ingredients and minerals to increase yield[12].

At Helsieni we have used coffee grounds as our nitrogen additive since we started. The results of our first subsidised development project in 2019 lead us to always pasteurise this ingredient, as it turned out to be a clear source of contamination. The chosen pasteurisation method has been to boil the coffee grounds and cool them overnight before using them. This added process solved half of our contamination problems, but occasional contamination waves still occurred later on.

We identified our carbon source (mainly straw pellets) as an occasional contamination factor, due to its quality variation throughout the year and depending on the batches we received. These problems might have been worsened by a change in our process: we decided to hydrate pellets with warm water (about 50°C) instead of cold (about 15°C) to increase water retention in our substrates and thus their productivity, water being an important limiting factor in mushroom substrates.

As an attempt to reduce contamination, we decided to try light pasteurisation: pouring 80°C hot water to the straw pellets and letting them cool down overnight. It showed satisfactory results in terms of contamination reduction (lost substrate amount reduced from 4% to 2% of the total produced) but because pasteurisation has an effect on biology, we need to control if this pasteurisation had any side effect on the substrate productivity. For this purpose we will analyse some of the data we collected.

Sample choice

Amongst the data samples we collected and listed above, 4 pairs can deliver results concerning the impact of straw pasteurisation:

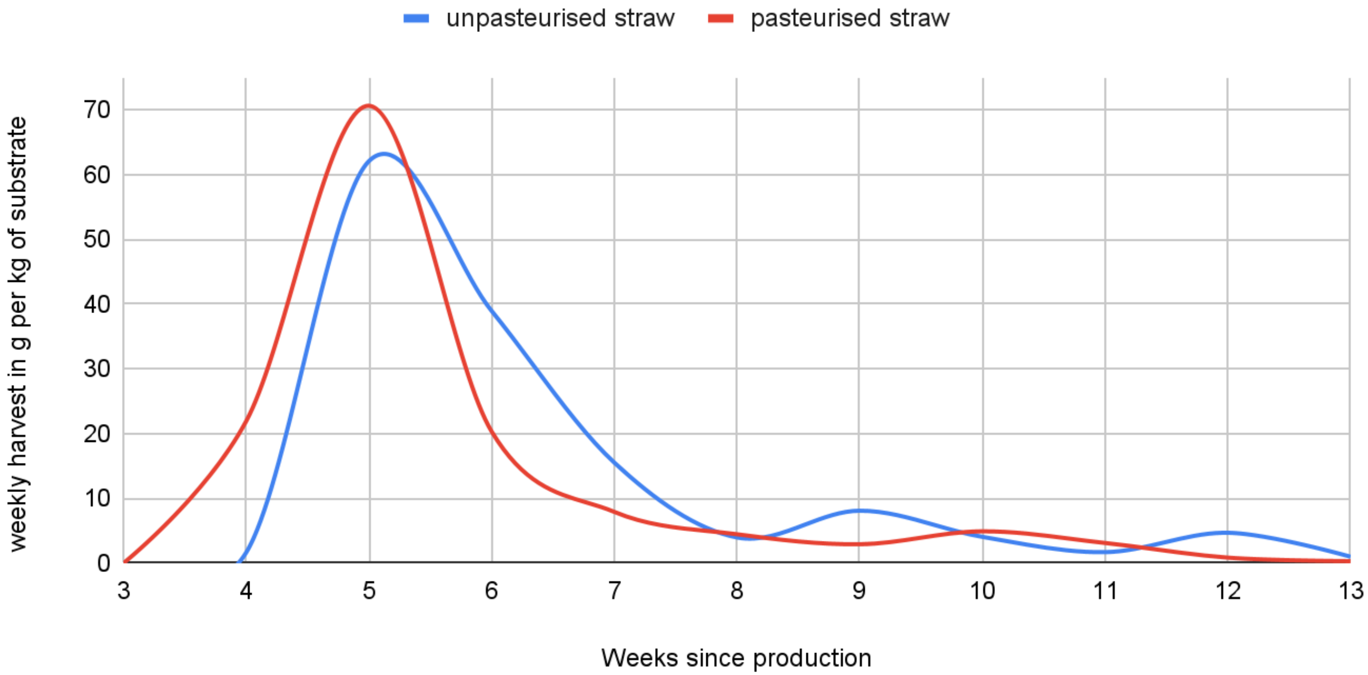
- **Samples 5** (unpasteurised) **and 6** (pasteurised): Produced on 1.2.2023 with an otherwise identical recipe based on Halmeko pellets.
- **Samples 10** (pasteurised) **and 11** (unpasteurised): Produced on 21.2.2023 with an otherwise closely related recipe based on Baltic Straw pellets. The recipe differences result from observations made on previous samples, where we learned that the pellets could not absorb as much lukewarm water as what they could absorb in hot water. We thus reduced the amount of water in sample 11 to avoid excess drainage.
- **Samples 9** (pasteurised) **and 12** (unpasteurised): Same as above with Halmeko pellets.
- **Samples 13** (pasteurised) **and 14** (unpasteurised): Produced on 9.3.2023 with the same recipe as samples 10 and 11, but with Halmeko pellets. These 2 samples are of much greater size than the previous 4.

[12] See WP1 T3.2: Nitrogen-rich supplementations

Aggregated results

Here is the timeline of the harvest we made on the aggregated samples (5+11+12+14 vs 6+9+10+13) to compare performance of unpasteurised and pasteurised straw:

Graph 1: Timelime of harvested oyster mushrooms on unpasteurised and pasteurised straw pellets



Unpasteurised and pasteurised straw pellets gave very similar productivity. However, substrates made with pasteurised straw pellets gave their first flush quicker (on average half a week).

KPI analysis[13]

		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	$COGS_€$
Sample #		w/w prod	BE	#days to F1 end	%F1			
5		0.144	0.478	40	64%	0.24	0.41	4.47
6		0.134	0.443	34	91%	0.24	0.42	4.92
9		0.154	0.508	31	90%	0.24	0.42	4.29
10		0.118	0.390	42	72%	0.24	0.42	5.60
11		0.157	0.490	43	84%	0.25	0.42	4.27
12		0.158	0.495	34	56%	0.25	0.42	4.23
13		0.140	0.488	41	79%	0.23	0.41	4.55
14		0.129	0.451	43	80%	0.23	0.40	4.83
Unpast average[14]		0.142	0.470	41	74%	0.24	0.41	4.56
Past average[14]		0.137	0.466	38	82%	0.23	0.42	4.76

Samples made with unpasteurised straw pellets gave on average a slightly better productivity (biological efficiency +0.4%) and economical performance (COGS -4.2%) than those made with pasteurised straw pellets. The difference is too small to be considered significant[15]

Samples made with pasteurised straw pellets produce quicker (First flush comes 7.1% faster and its absolute weight is bigger than with unpasteurised pellets).

On a wider timeframe and looking at all substrates produced in 2023, we calculated that pasteurisation of straw decreased our contamination by half (2% of lost substrates versus 4% without pasteurisation). With our current production capacity and pricing, 2% of contamination cost us 1200€. The measured output cost difference between unpasteurized and pasteurised substrates (0.20€ per kg of mushroom produced) costs us less than 600€ per year, making it worth pasteurising. As a result, all pellets used in the following tests are pasteurised.

[13] In WP1 we use a partial selection of KPI.
[14] Averages are weighted with sample weights.
[15] Much smaller than standard deviation within each group.

WP1 T1.3: Straw pellet brand

We have been testing various brands of straw pellets since we decided to use them as the main carbon source in our recipes. We noticed that their products have different aspects (color, pellet size…), density, particle size and water absorption capacity, resulting in different performance.
We collected data about 3 of them: Baltic straw, Halmeko and Strohfelder.
All 3 suppliers' production plants are located in Lithuania. The Strohfelder product we used (Strohfelder Platinum) is a slightly different product as the others, as it consists of "granulate" (crushed pellets).

Sample choice

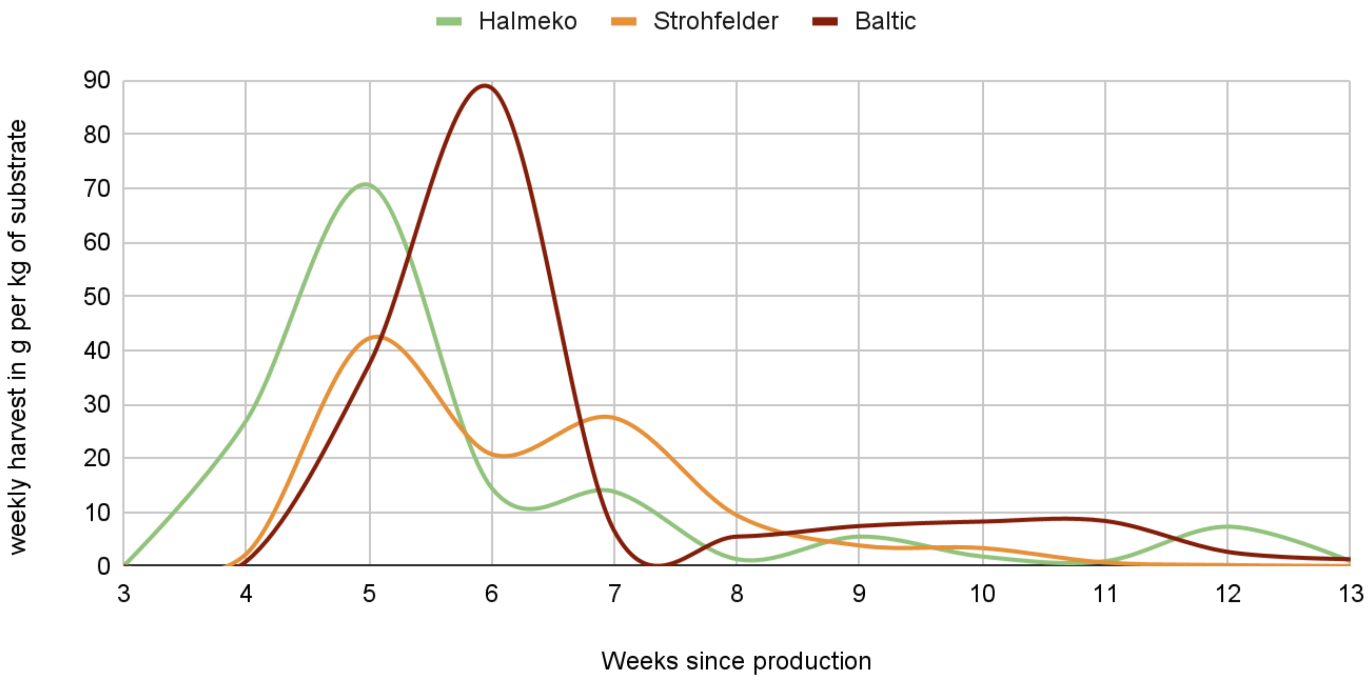
Amongst the data samples we collected and listed above, 4 pairs can deliver results concerning the impact of straw pasteurisation:

- **Samples 2** (Halmeko) **and 3** (Strohfelder): Produced on 24.1.2023 with an otherwise identical recipe.
- **Samples 7** (Strohfelder) **and 8** (Baltic): Produced on 1.2 and 9.2.2023 with an otherwise identical recipe.
- **Samples 9** (Halmeko) **and 10** (Baltic): Produced on 21.2.2023 with an otherwise identical recipe.
- **Samples 11** (Baltic) **and 12** (Halmeko): Produced on 21.2.2023 with an otherwise identical recipe.

Aggregated results

Here is the timeline of the harvest we made on the aggregated samples (2+9+12 vs 3+7 vs 8+10+11) to compare performance of different straw pellets:

Graph 2: Timelime of harvested oyster mushrooms on different straw pellet brands



Halmeko and Baltic straw pellets clearly give better results than Strohfelder. Baltic straw seems to be more productive on first flush but slower than Halmeko (one week). This speed difference might be partially explained by the position of analysed samples on the carts: most Baltic straw samples were positioned at the bottom of the cart whereas most Halmeko samples were placed on top. We noticed later a difference of about 2°C between top and bottom shelves. Temperature has a determinant impact on growing speed.

KPI analysis

		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	$COGS_€$
Sample #		w/w prod	BE	#days to F1 end	%F1			
2		0.071	0.236	40	77%	0.24	0.42	9.24
3		0.090	0.299	38	54%	0.30	0.42	7.92
7		0.118	0.389	40	48%	0.30	0.42	6.09
8		0.190	0.629	44	77%	0.24	0.42	3.47
9		0.154	0.508	31	90%	0.24	0.42	4.29
10		0.118	0.390	42	72%	0.25	0.42	5.60
11		0.157	0.490	43	84%	0.25	0.42	4.27
12		0.158	0.495	34	56%	0.25	0.42	4.23
Halmeko average[16]		0.143	0.463	34	74%	0.24	0.42	4.99
Strohfelder average		0.110	0.364	39	50%	0.30	0.42	6.61
Baltic average		0.167	0.546	43	77%	0.24	0.42	4.12

A closer number analysis translates what was visible on the graphs: Halmeko is a performant brand of pellets if looking for a fast growth while Baltic gives a bigger harvest. We note that the results of batch number 2 looks like a statistical outlier but its relatively small size makes it of little influence on the results. .
Due to its better performance in our context and easier availability in Finland, Baltic straw will be our reference straw pellet source for further tests.

Note that this study only shows results for specific pellet batches. Each batch of product from each brand can be different from the previous depending on many factors, many of which being a consequence of the changing quality of their raw material (straw) throughout seasons and years. Still, some quality factors result from the used machinery, production process and storage facilities, which we consider constant over time. A batch of pellets from each brand shall be tested regularly to update this data.

[16] Averages are weighted with the sample sizes in kg

WP1 T1.4: Spawn supplier

We decided long ago to work with sporeless oyster mushroom varieties, even if they are not the most productive, to avoid the health hazard of hypersensitivity pneumonitis (“spore allergy”). At the time of this study, there are two varieties of sporeless oyster mushroom, which are genetically close but produced by different suppliers:

- Spoppo produced by Sylvan
- Allerpo produced by Amycel.

For the European market, both strains are produced in France.
We have always used Spoppo but last year we decided to test Allerpo.

We did not find any suitable sample in the collection we made to compare them quantitatively.
But observations and production numbers in the period we used Allerpo showed poorer performance than with Spoppo, especially in colder environments. Allerpo only produces well above 16°C when Spoppo already gives good results with 13°C. In the period we used Allerpo (autumn-winter-spring 2022-2023) our fruiting room temperature was below 15°C and the productivity was significantly lower than what we have been used to. The last batches produced in spring gave satisfactory results, making it maybe worth trying again Allerpo in summer conditions. However, we will use Spoppo as the only strain for this study.

WP1 T1.5: Hemp shives

We found out that our main straw pellet supplier also proposed hemp shives chopped into 5mm - 2cm long pieces and pressed into bales. We decided to try it in various proportions in some of our production batches. We identified immediately a key characteristic: Hemp shives can absorb and hold 5 to 6 times their weight of water, exactly twice as much as what straw pellets can do. Water being a determinant limiting factor in mushroom production, this characteristic made it worth a data analysis.

Sample choice:

Amongst the data samples we collected and listed above, the following are of interest to consider the impact of Hemp shives on oyster mushroom substrate performance:

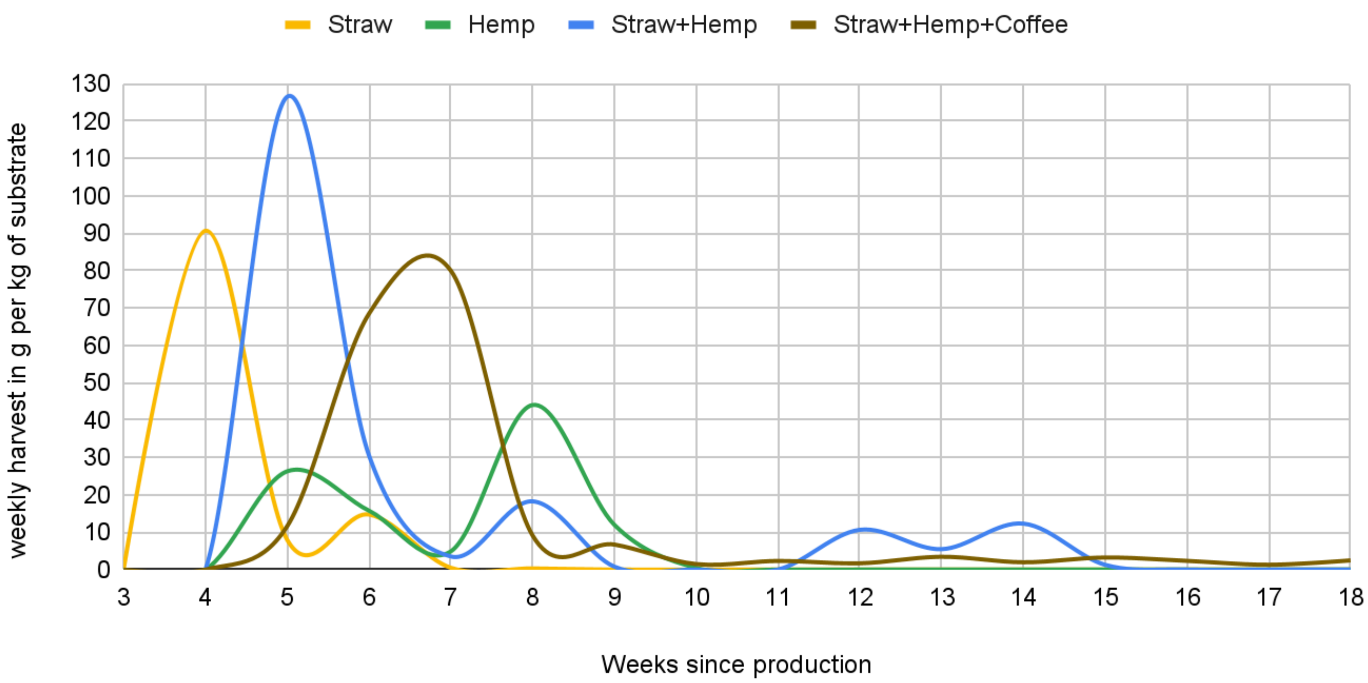
ID #	Prod date; Weight	Straw pellets Brand: Baltic	Hemp shives Brand: Hemparade	Coffee grounds	Chalk	Spawn Spoppo	Water content
19	10.07.23 196 kg	21.5%	-	-	0.9%	2.2%	75.4%
21	10.07.23 56 kg	-	18.9%	-	1.4%	3.2%	76.5%
23	28.09.23 428 kg	12.7%	3.0%	8.2%	0.9%	2.3%	72.9%
24	27.10.23 51 kg	21.1%	1.8%	-	0.9%	3.2%	73.1%
25	27.10.23 445 kg	13.7%	2.9%	7.9%	0.9%	2.2%	72.4%

We will group them in 4 groups:

- **Unsupplemented straw**: sample #19
- **Unsupplemented hemp**: sample #21
- **Unsupplemented straw+hemp**: sample #24
- **Coffee-supplemented straw+hemp**: samples #23+#25

Aggregated results:

Graph 3: Timelime of harvested oyster mushrooms with different hemp concentrations



- Because all samples were not produced in the same season and therefore their fruiting temperatures were different, we cannot strictly compare growing speeds here. However, unsupplemented straw and hemp substrates were produced on the same day. We can conclude that straw-based samples are very much faster to produce than hemp-based substrates
- Hemp alone seems to give a poor productivity, especially on the first flush. The second flush is bigger than the first, confirming its slow digestion by oyster mushrooms.
    - Hypothesis: this might be due to the coarseness of hemp shives. Big pieces are always longer to digest for mushrooms. We could consider trying ground hemp shives instead but the product requires a mill and does not exist as a standard product.
- The combination of straw and hemp in small amounts, supplemented or not, seem to give much better results than straw or hemp alone.
    - Hypothesis: this clear productivity increase can be explained with improved structure/texture: better mixing, better airflow in the substrate thanks to the bigger hemp particles.

KPI analysis

		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	$COGS_€$
Sample #		w/w prod	BE	#days to F1 end	%F1			
19		0.114	0.464	31	86%	0.27	0.41	5.87
21		0.103	0.439	39	33%	0.45	0.44	8.63
23		0.225	0.831	47	85%	0.28	0.40	3.01
24		0.209	0.778	42	75%	0.36	0.44	3.79
25		0.168	0.609	49	78%	0.27	0.40	4.03
Unsupplemented straw		0.114	0.464	31	86%	0.27	0.41	5.87
Unsupplemented hemp		0.103	0.439	39	33%	0.45	0.44	8.63
Unsupplemented straw + hemp		0.209	0.778	42	75%	0.36	0.44	3.79
Coffee-supplemented straw + hemp		0.196	0.718	49	81%	0.28	0.40	3.53

A close number analysis of these numbers show an increase in productivity of about 80% and a biological efficiency increase of about 70% when including a small amount of hemp shives in a straw-pellet-based substrate. This result is to be confirmed with further studies but it is remarkable enough to be considered a potential game changer.

Coffee supplementation does improve productivity here.

Task 2. Desktop Study

The questions we address are:
- What is a good chemical composition to produce oyster mushrooms (Cellulose / Hemicellulose / Lignin) and the most needed minerals?
- What are the most promising carbon sources for oyster mushroom prod in Finland (performance, availability, sustainability)?
- Are there available tips on how to design a working substrate / mixing process?

WP1 T2.1: Carbon sources literature review

Here is a literature study of different carbon sources available in Finland and neighbouring countries.

Hardwood

Wasser (2005) finds that Shiitake in its natural habitat mainly feeds on trees in the Fagaceae family, such as Castanea, Castanopsis, Fagus, Lithocarpus and Quercus. But also the genera Acer, Alnus, Carpinus, Liquidambar, Morus and Populus occur as a substrate.

Hannus (2017)[17] writes that wood species that break down quickly are considered in most cases to be the best to use as a substrate, as its poor resistance to rot means faster colonisation by the mycelium of the fungus and, as a rule, greater yield (Stamets, 2000). Stamets particularly recommends alder (Alnus) for this purpose.

Crowe 2021[18] finds a wet/wet productivity of almost 50% of oyster mushrooms, (2,2 kg of fresh mushrooms per 4,5 kg of fresh substrate) on a substrate of 50% oak pellets and 50% of sterilised soybean hulls.

Straw

Straw from wheat, oats, barley and rye are relevant in the nordic context. The four species have similar fibre content of 16-21, 24-38 and 29-37 percent of lignin, hemicellulose and cellulose respectively. Of the four, wheat straw is the most proven, even though, judging by the fibre content, they should perform similarly as mushroom substrate (Hansson & Hansson, 2014; Sánchez, 2009). Rapeseed straw is also a possible material. Analysis of rapeseed straw has shown a content of 48.5% and 20% on cellulose and lignin respectively (Housseinpour et al., 2010[19]).

[17] https://stud.epsilon.slu.se/10035/
[18] https://www.youtube.com/watch?v=SmzNdVxpO5o
[19] https://brill.com/view/journals/iawa/31/4/article-p457_6.xml

Oat husk

Our first RKKO subsidised study in 2019[20] gave slightly better performance for straw pellets than oat husk. However, the oat husk used for the tests then were unprocessed and therefore did not absorb enough water. It would be worth investigating the possibility of breaking the husk into smaller pieces and measuring its influence on their water retention capacity.

Reeds

Hultberg & Persson (2017)[21] used a substrate of common reed (*Phragmites australis)* and broadleaf cattail *(Typha latifolia)* harvested in summer from constructed wetlands. It was chopped and hydrated to a moisture level of around 70%, pasteurised and inoculated at a rate of 5% (inoculum weight / dry substrate weight). A biological efficiency (BE or fresh mushrooms / dry substrate) of 1.40 was measured. The mushrooms were also tested for the heavy metals cadmium and lead and found that the produced mushrooms were 100 times below the limit value for cultivated mushroom which for cadmium is 0.2 mg/kg fresh weight and for lead 0.3 mg/kg fresh weight (EU, 2006).

Hultberg (2018)[22] found that 'the harvested wetland biomass consisted exclusively of common reed, with an average biomass yield of 13 ton dry matter per hectare (DM/ha) (Table 1). Moisture content at harvest was 41 ± 4% and 44 ± 4% in 2016 and 2017, respectively. The concentrations of nitrogen, phosphorus and potassium (Table 1) were similar to those reported previously for common reed (Granéli et al., 1982). The resulting removal of plant nutrients from the wetland in 2016 was 234 kg/ha nitrogen, 22.8 kg/ha phosphorus.

The author also found that no amendment of the biomass was necessary. The harvested mushrooms had low levels of heavy metals. The biological efficiency sounds extremely high. Reed is an abundant and underused resource in Finland, making it interesting to study further in this research.

Coffee silverskin

Our first RKKO subsidised study in 2019[23] taught us that silverskin is hard to use as a primary carbon source due to its lightweight and compactness when humidified. No data was found regarding its performance as a supplement.

Hemp shives

Reiss 2022[24] found a BE of approximately 66% for a substrate composed of 100% Hemp. Siwulski et al. 2010[25] found a BE of 82%.

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[20] Ravinteiden Kierrätys Pilot for a circular urban mushroom farm Final Report | Helsieni Oy p.50
[21] https://partnerskapalnarp.slu.se/uploads/faktablad/914.pdf
[22] https://www.sciencedirect.com/science/article/abs/pii/S0048969718317418
[23] Ravinteiden Kierrätys Pilot for a circular urban mushroom farm Final Report | Helsieni Oy p.50
[24] https://vtechworks.lib.vt.edu/server/api/core/bitstreams/ce7cb1ec-2611-42e9-bb5f-11b017339ac5/content
[25] https://czasopisma.up.lublin.pl/index.php/asphc/article/view/3360/2329

Flax by-products

Vilppunen (1995)[26] did a study on growing oyster and shiitake mushrooms on a substrate of flax by-products and found that as a material it yields as good as any other commercial substrate. For oyster mushrooms the best performing substrate was made from the by-products of the enzymatic soaking of flax: shives (8 parts), flax seeds and hulls (4 parts) and water (12 parts). The substrate material was neutralised with chalk (5%), and was pasteurised for 7 hours at 85 ºC. The resulting wet/wet productivity was 18-25%. For shiitake mushrooms the same substrate yielded 16,6 % (w/w prod) in 81 days and was slightly superior to a control substrate containing sawdust and wheat bran.

Sunflower seed hulls

Curvetto 2002[27] show sunflower seed hulls can be used as a nitrogen supplement but that different strains presented significantly different rates for whole substrate colonisation, so not all oyster mushroom strains work equally well. Curvetto 2004[28] shows that sunflower seed hulls contain 4% protein, 5% lipids and 50% carbohydrates.

Faba bean hulls *(also called: Broad bean, Fava bean)*

Ivarsson et al. (2021)[29] found that a substrate of 100% faba bean (*Vicia faba L.*) hulls proved very suitable as substrate for production of oyster mushrooms, with biological efficiency of 109 ± 28% (mushroom (fresh weight) / substrate (dwt)) based on the first flush only[30]. The dried hulls were rewetted with distilled water to a moisture content of 70%. The substrate was pasteurised at 65 ◦C for 8 h. These results warrant further exploration of the hulls as a substrate or as a supplement for this study.

Cardboard

Jonsson (2019)[31] found that cultivation of oyster mushrooms with cardboard as a substrate is possible, but yields of a 100% cardboard substrate were around 50% of the control in the study. Better results comparable to the control of sawdust were achieved by adding an ammonium based biofertilizer. Further studies are also required to ensure that the fruit bodies grown on cardboard are suitable for consumption, considering any harmful substances that the fungus may have absorbed from the carton. For this reason this study will not pursue cardboard as a potential candidate to be tested.

Cellulose Fibre Rejects

Grimm et al. (2019)[32] found that oyster mushroom mycelium growth was faster on substrates based on fibre rejects than a control batch made of birch sawdust. The average biological efficiency (BE) of the first flush of fruit bodies grown on fibre rejects substrates was slightly lower than the BE of the control batch. The faster mycelium colonisation could, somehow, compensate the lower BE. The fruit bodies grown on fibre-rejected substrates have good

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[26] https://jukuri.luke.fi/bitstream/handle/10024/447295/asarja73.pdf?sequence=1&isAllowed=y#page=47
[27] https://www.sciencedirect.com/science/article/abs/pii/S0960852402000135
[28] https://www.fungifun.org/docs/mushworld/Oyster-Mushroom-Cultivation/mushroom-growers-handbook-1-mushworld-com-chapter-5-3.pdf
[29] https://www.sciencedirect.com/science/article/pii/S0959652621021879
[30] *The equivalent in fresh/wet productivity here is 109 x 0,3 = 33%*
[31] https://stud.epsilon.slu.se/14468/
[32] https://link.springer.com/article/10.1007/s12649-020-01311-y

nutritional values and low contents of heavy metals. There is room for improving the substrate formulation by removing ash from the raw fibre rejects or by the addition of supplements and additives. Grimm et al. (2023)[33] attempted to remove the contaminants such as microplastics and ash, that is, calcium carbonate, kaolin, and sand. It required processing with a cyclone dryer followed by a sieving process. Due to the cost of these treatments, this material is not deemed interesting for the current study.

Spruce/Softwood

Sundelin (2018)[34] researched the suitability of using sawdust from spruce (Picea abies) as substrate for production of oyster mushroom (Pleurotus pulmonarius) and found a biological efficiency (BE) of 51% (fresh mushrooms/dry substrate) for the first flush of an autoclaved substrate based on spruce sawdust supplemented with wheat bran[35]. The substrate contained 64.6% sawdust, 33.0% wheat bran, 2.4% CaSO4 and the moisture content was 65%. The amount of inoculum was 12%.

Chen et al. (2020)[36] compared substrates of softwood spruce (Picea abies) versus hardwood alder (Alnus glutinosa) and found on softwood a BE (fresh mushrooms/dry substrate) ranging from 14.0 to 33.8%.

The results of these studies do not give better productivity than our current recipes, and softwood needs to be stored a long time before use, to release most of its fungicide resins. Thus we will decide not to conduct any further study or test on softwood.

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[33] https://pubs.acs.org/doi/full/10.1021/acsomega.2c06453
[34] https://stud.epsilon.slu.se/13617/
[35] *The equivalent in fresh/wet productivity here is 51% x 0,35 = 18%*
[36] https://www.sciencedirect.com/science/article/abs/pii/S0959652620320588

Note: Source quality for this type of information is uneven and greatly dependent on soil types, raw material properties (for example wood can be trunk, bark, branches,...) and research protocols. However we tried to keep only the most relevant information and for some of the data, we averaged several results.

	Cellulose[37]	Hemicellulose[37]	Lignin[37]	N	P	K	Na	Fe	Cu	Mn	Mg	Ca	Se	Zn
	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Birch[38] [39] [40]	40	36	20		130-430	500-900	20-30	<10-1100	1.1-2.2	26-408	200-380	500-1100		18-165
Alder[41] [42] [43]	48	31	23											
Wheat straw[44] [45] [46] [47]	36	23	20		700	11200-12000	100-170	167-184	4	32-46	1100-1200	4400-4800	0.2	14-17
Oat husk[48]	40	25	26		1800-1900	5900	100-180	146	3	66	600-1500	1900-2200		24
Common reed, winter[49]	44	20	22	3700-6700	200-400									
Common reed, summer[50]				13200-20300	1100-1800	10899		58.8	8.5	97	2649	2660		27

[37] Averaged values
[38] www.mdpi.com/1996-1073/12/15/2952
[39] https://www.soilsa.com/Nutrient-distribution-in-silver-birch-Betula-pendula-Roth-biomass-growing-on-post,195922,0,2.html
[40] https://pubmed.ncbi.nlm.nih.gov/31642944/
[41] www.ncbi.nlm.nih.gov/pmc/articles/PMC9460749/
[42] https://www.silvafennica.fi/article/1260
[43] https://www.researchgate.net/publication/52001729_Nutrient_allocation_accumulation_and_above-ground_biomass_in_grey_alder_and_hybrid_alder_plantations
[44] www.sciencedirect.com/topics/agricultural-and-biological-sciences/wheat-straw
[45] https://www.feedtables.com/content/wheat-straw
[46] https://www.feedipedia.org/node/12758
[47] https://pubs.acs.org/doi/10.1021/ef901181h?utm_source=chatgpt.com#
[48] www.sciencedirect.com/science/article/pii/S030881461631665X
[49] https://www.google.com/url?q=https://www.doria.fi/handle/10024/97313&sa=D&source=docs&ust=1743755315397803&usg=AOvVaw1cBq61_dGEujiob5iCdbF6
[50] https://www.google.com/url?q=https://drive.google.com/file/d/12VNc_0VevgsGfxgzAyCrNDbr3Ke-P1Bu/view?usp%3Ddrive_link&sa=D&source=docs&ust=1743755315396956&usg=AOvVaw0S_8JRSrzXiDn2clrQ5A_k

Coffee silverskin[51 52 53 54 55 56]	18	13	N.A.[57]			10000	200	1000	98	145	>2000	>10000		25
Hemp shives[58 59 60 61]	47	20	18											
Sunflower seed hulls[62 63 64]	41	20	20		900-1300	10800-11900	90-100	137	10	16	2400-2600	4100-4400	0.6	23
Flax shives[65 66 67 68]	50	20	25											
Faba bean hulls[69 70 71 72 73]	31	21	16		2559	6118.3	148.4	39.1	10	4.9	1805.2	2173.5	978.3	11.4

[51] www.tandfonline.com/doi/full/10.1080/10942912.2016.1253097
[52] www.researchgate.net/figure/Chemical-composition-g-100-g-of-coffee-silverskin-CS-and-spent-coffee-grounds-SCG_tbl2_225402675
[53] https://pmc.ncbi.nlm.nih.gov/articles/PMC8392354/
[54] https://www.sciencedirect.com/science/article/abs/pii/S0308814621021944
[55] https://pubmed.ncbi.nlm.nih.gov/34624779/
[56] https://www.researchgate.net/publication/332063958_COFFEE_SILVERSKIN_AND_EXPIRED_COFFEE_POWDER_USED_AS_ORGANIC_FERTILIZERS
[57] www.ncbi.nlm.nih.gov/pmc/articles/PMC8392354/
[58] www.ncbi.nlm.nih.gov/pmc/articles/PMC8587414/
[59] https://pmc.ncbi.nlm.nih.gov/articles/PMC9875026/
[60] https://www.researchgate.net/publication/353970128_Restoration_of_minesoil_organic_matter_by_cultivation_of_fiber_hemp_Cannabis_sativa_L_on_lignite_post-mining_areas
[61] https://www.sciencedirect.com/topics/engineering/hemp-shives
[62] www.researchgate.net/publication/333312616_Microwave-assisted_extraction_of_antioxidant_compounds_from_sunflower_hulls
[63] https://www.feedtables.com/content/sunflower-hulls
[64] https://www.feedipedia.org/node/733
[65] www.sciencedirect.com/science/article/abs/pii/S0926669020302405
[66] https://www.sciencedirect.com/science/article/pii/S0926669025001311
[67] https://www.researchgate.net/publication/281369210_Nitrogen_Phosphorus_Potassium_Calcium_Magnesium_and_Zinc_in_Southeastern_USA_Harvested_Flax
[68] https://cjes.guilan.ac.ir/article_7334_8fbf2a13a956b3251cc2e01e6ba9d1a7.pdf
[69] https://www.sciencedirect.com/science/article/pii/S0142941823001277
[70] https://www.jafs.com.pl/pdf-69602-7547?filename=7547.pdf
[71] https://www.feedipedia.org/node/4926
[72] https://pulsecanada.com/uploads/resources/Pulse-Canada-Faba-Bean-Feed-Guide.pdf
[73] https://www.sciencedirect.com/science/article/pii/S0959652621021879

Task 3. Preparation of WP2

WP1 T3.1: Local availability of carbon sources

Carbon source & shape	**Competing uses**	**Supplier & product**	**Place of origin**	**Km to Helsieni farm**
Hardwood - Pellets - Chips - Sawdust	- Energy - Animal bedding	- Greenfull alder pellets / apple tree pellets - Karjaan puupörssi Birch coarse sawdust	Sipa, Estonia Karjaa, Finland	235 4
Straw - Chopped - Pellets	- Animal bedding - Soil amendment	- Nina Långstedt Chopped straw on the field, Baled straw - Baltic straw: Straw pellets - Biodela: Straw pellets - Biohansa: Straw pellets	Mustio, Finland Vilnius, Lithuania Vilnius, Lithuania Peetri, Estonia	17 765 770 170
Oat husks - Whole - Ground	- Animal feeding - Soil amendment - Energy	- Anna Alm, Morby mill Whole oat husks - Anne Lukana, Kranni farm Whole / ground oat husks	Mustio, Finland Nurmijärvi, Finland	19 78
Reeds - Chopped - Pellets	- Animal bedding	- Kaislanleikkuu Chopped reed - Nine voices / Reed pellets	Siuntio, Finland Dreverna, Lithuania	41 785
Coffee silverskin	- Energy	- Helsingin kahvipaahtimo - Cafetoria roastery	Helsinki, Finland Lohja, Finland	81 42
Hemp shives - Chopped	- Animal bedding	- Korkeaojan luomutila Chopped hemp shives - Biohansa Chopped hemp shives - HempFlax (Hemparade) Chopped hemp shives	Kokemäki, Finland Peetri, Estonia Oude Pekela, NL	220 170 1700
Linseed & Flax - Flax shives - Linseed by-products	- Animal bedding - Energy	- Linenstories Chopped flax shives - Vihervakka Refused linseeds	Narpiö, Finland Pöytyä, Finland	370 135
Faba bean hulls	- Soil amendment	- Makelan luomutila - Nojosniemen tila - Vihreän Härän vihannestila	Mäntsälä, Finland Laukaa, Finland Uusikaupunki, Finland	125 335 180

WP1 T3.2: Preliminary tests

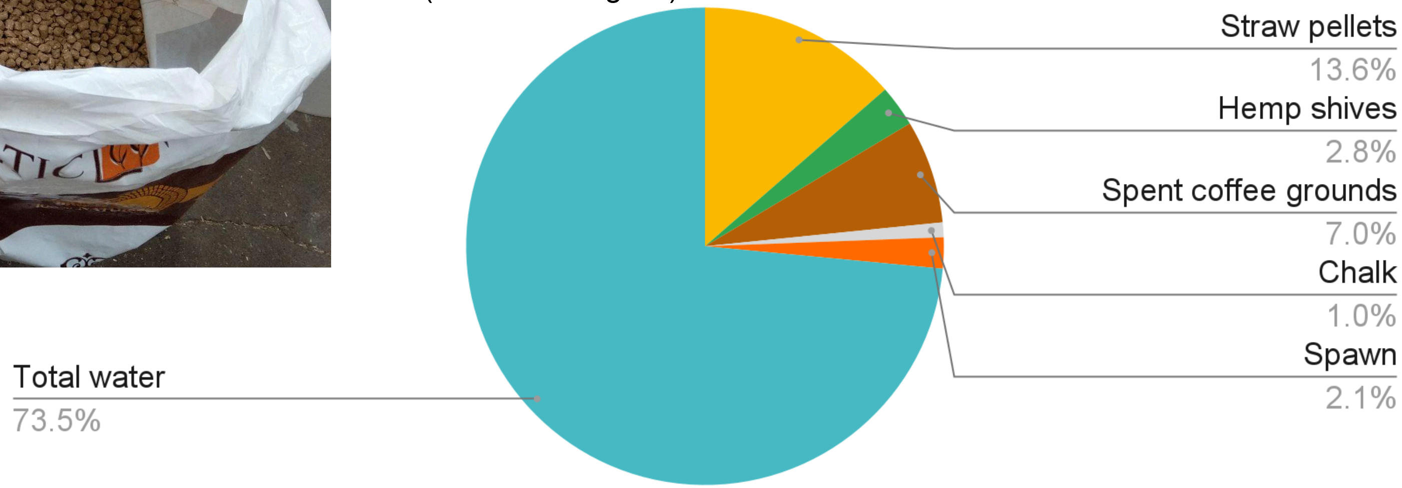
We decided to use the resource easily available on the market (not necessarily local or organic) to conduct a few preliminary tests that will help us refine our further test protocols.

Straw pellets (new suppliers)

We concluded our data analysis with the fact that Baltic straw was our preferred supplier compared to Strohfelder and Halmeko. Here we compare it with 2 more brands of straw pellets (Biodela and Biohansa) to decide the one we finally use as a comparison base for our final tests. All pellets are quickly pasteurised and hydrated by pouring 80°C water onto them.

Picture 1: Biohansa and Baltic straw pellets

Straw Pellet Supplier - Test Recipe
(% of wet weight[74]):



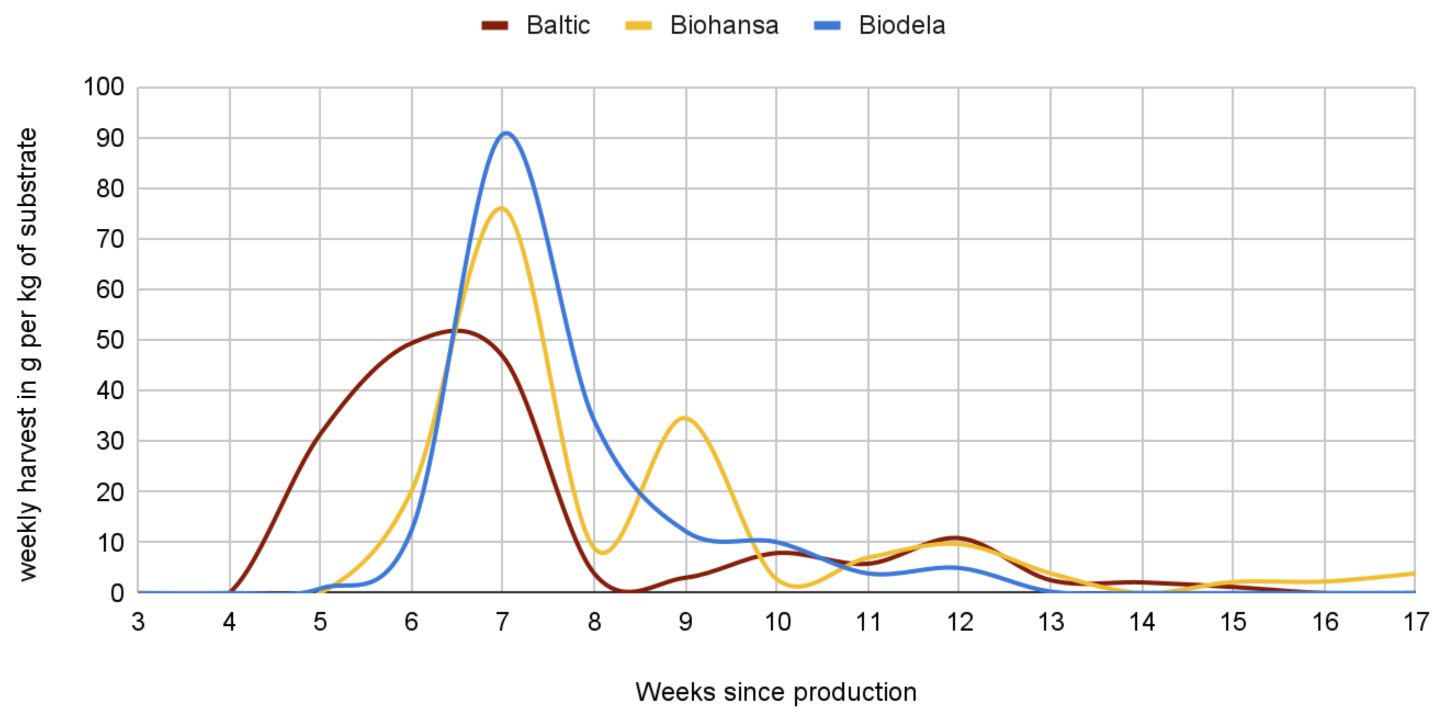
[74] All ingredients but water are measured dry (own humidity deduced); precision ±10%.
Total water includes added water and the sum of all other ingredients' humidity contents; precision ±2%.
Spawn supplier: Sylvan; Variety: Spoppo
Hemp shives brand: Hemparade, pasteurised together with straw pellets

Straw Pellet Supplier - Production batches

ID #	Prod date	Weight (kg)	Straw Pellet brand
37	12.01.24	216	Biohansa
38	12.01.24	233	Baltic
39	16.01.24	231	Baltic
40	16.01.24	222	Biohansa
43	01.02.24	218	Biohansa
44	01.02.24	188	Baltic
54	28.02.24	229	Baltic
55	28.02.24	227	Biodela
56	29.02.24	179	Biohansa
57	29.02.24	90	Biodela
58	29.02.24	225	Biodela
	TOTAL WEIGHT:		
	Baltic	**881 kg**	
	Biohansa	**836 kg**	
	Biodela	**542 kg**	

Straw Pellet Supplier - Results and KPI analysis:

Graph 4: Timelime of harvested oyster mushrooms on different straw pellet brands



The production graph above shows different production patterns for the 3 studied brands. We can describe them as such:

- Baltic pellets seem to give a first flush earlier than other brands.
  - Hypothesis 1: This property can be the sign of a less dense or smaller-particle product, making it quicker available for the mushroom to digest.
  - Hypothesis 2: When mixing this product with other ingredients, we notice a more homogeneous result than with other brands. Pellets are slightly less compact and break easily once hydrated, making a smooth mixture without clumps. Clumps are always a slowing factor for mycelium growth, as they do not contain any spawn and are of hard consistency.

- Production with Baltic is more spread in time than with other brands.
    - Hypothesis 1: This can be due to a less homogeneous product, for example if several batches of pellets were mixed on the same pallets. It is possible, as the Baltic pellets we used were conditioned in bags.
    - Hypothesis 2: Incubation time and fruiting temperature were not precisely consistent during these tests. It has surely influenced growing speeds of different batches.
- Biohansa and Biodela pellets almost have the same production timeline but Biodela gives most if it (88% on avg) in the first flush while Biohansa gives a decent second flush.
    - Hypothesis: for a reason that can be physical (particle size, production process…) or biological (nutritive content, sterility,..) Biodela pellets are quicker available for mycelium to feed on.

		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	COGS$_{€}$
Sample #		w/w prod	BE	#days to F1 end	%F1			
37		0.178	0.682	53	84%	0.27	0.40	3.76
38		0.145	0.544	47	80%	0.26	0.40	4.58
39		0.164	0.630	49	73%	0.26	0.39	4.00
40		0.190	0.716	54	77%	0.27	0.39	3.49
43		0.179	0.681	52	72%	0.29	0.40	3.89
44		0.187	0.695	49	78%	0.28	0.40	3.68
54		0.169	0.636	51	81%	0.25	0.39	3.88
55		0.168	0.633	54	87%	0.26	0.39	3.88
56		0.141	0.513	65	82%	0.27	0.40	4.80
57		0.121	0.464	65	87 %	0.26	0.39	5.34
58		0.193	0.733	58	90 %	0.26	0.39	3.38
Baltic average		0.165	0.623	47	78%	0.26	0.40	4.06
Biohansa average		0.173	0.655	53	79%	0.28	0.40	3.95
Biodela average		0.170	0.643	56	88%	0.26	0.39	3.94

The KPI table (left) shows a high proximity of results within these 3 pellet brands. We can consider them equivalent, as their standard deviation between each group is far below the standard deviation within each brand[75].

[75] σ(Baltic) = 0.030; σ(Biohansa) = 0.037; σ(Biodela) = 0.037; **σ(brand groups) = 0.006**

However, finer comparison in the 70 first days from production day (our standard growth cycle) give additional information:

BRAND		w/w 70d	% of full potential (comparison with above)
Baltic average		0.142	86 %
Biohansa average		0.141	82 %
Biodela average		0.161	95 %

The standard deviation within each brand makes it difficult to conclude for sure, but we decide that this difference in first flush ratio is a good reason to choose Biodela as a straw pellet brand for our following tests.

According to these preliminary tests, Biodela would be a good substrate base for one-flush production (only one harvest). However, results with more nutritive recipes might be different. We will use Biodela in our systematic testing (WP2).

Hemp shives

We already stated in our preliminary data analysis (WP1 T1.5) that hemp shives are a potential game changer for our substrate. Questions remained about our supply, as Hemparade-branded shives are of changing provenance and quality. They are also not the most local resources, as they come from central Europe.
We identified one source of Finnish organic hemp shives (Korkeaoja farm) of which we got samples of different natures that we will test here as well as Hemparade in different recipes.
We also received hemp fibre samples from the Korkeaoja farm. We identified when mixing that these long fibers would be unadapted for mushroom cultivation, as they create big clumps while turning in the mixer. They are also a valuable material that is suitable for higher-value uses. However, we managed with efforts to make one test with this product, to understand its suitability if included (finely chopped) in small amounts together with shives. Small amounts of fibers are indeed often found in shives, as a result of imperfect machinery.

Pictures 3, 4, 5: Preparation and mixing of Hemp-based substrates

Hemp shives - List of analysed samples and their recipes[76]:

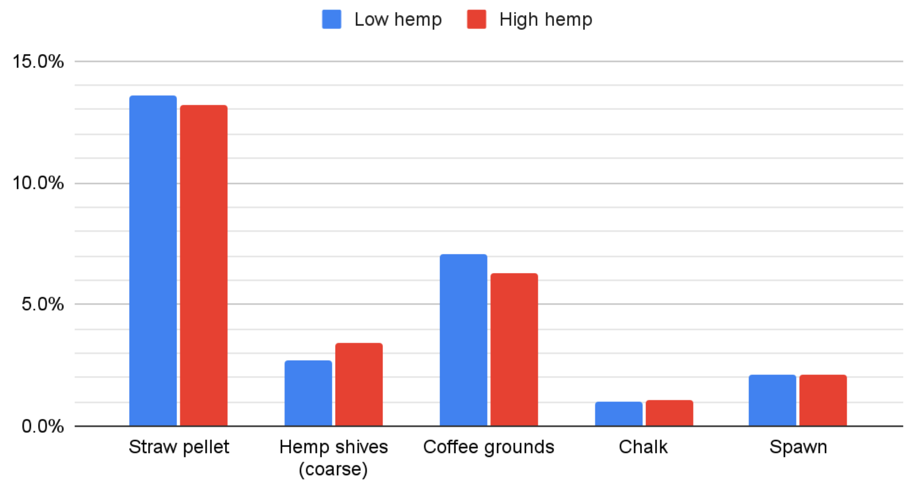
ID #		Prod date; Weight	Straw pellets (brand)	Hemp/Reed[77] (Product, brand/ provenance)	Coffee grounds	Chalk	Spawn Spoppo	Water content
35		05.01.24 233 kg	Straw pellets (Baltic): 13.6%	Hemp shives (Hemparade): 2.7%	7.1%	1.0%	2.1%	73.6%
36		05.01.24 215 kg	Straw pellets (Baltic): 13.2%	Hemp shives (Hemparade): 3.4%	6.3%	1.1%	2.1%	74.0%
64		22.03.24 145 kg	Straw pellets (Biodela): 9.1%	Hemp shives (Korkeaoja): 2.3%	12.3%	1.9%	2.2%	72.2%
65		22.03.24 55 kg	-	Finely chopped shives (Kork.): 9.7%	12.9%	1.3%	2.6%	73.6%
68		05.04.24 211 kg	Straw pellets (Baltic): 12.9%	Winter reed (Kaislanleikkuu[78]): 3.1%	6.6%	1.0%	2.4%	74.0%
69		05.04.24 234 kg	Straw pellets (Biodela): 12.5%	Hemp shives (Korkeaoja): 3.1%	7.7%	1.0%	2.1%	73.7%

[76] Spawn supplier: Sylvan; Variety: Spoppo
[77] Hemp shives and reeds are pasteurised at 80°C together with straw pellets.
[78] Pirkkalan Kaislanleikkuu is a company specialised in harvesting reeds from wetlands.

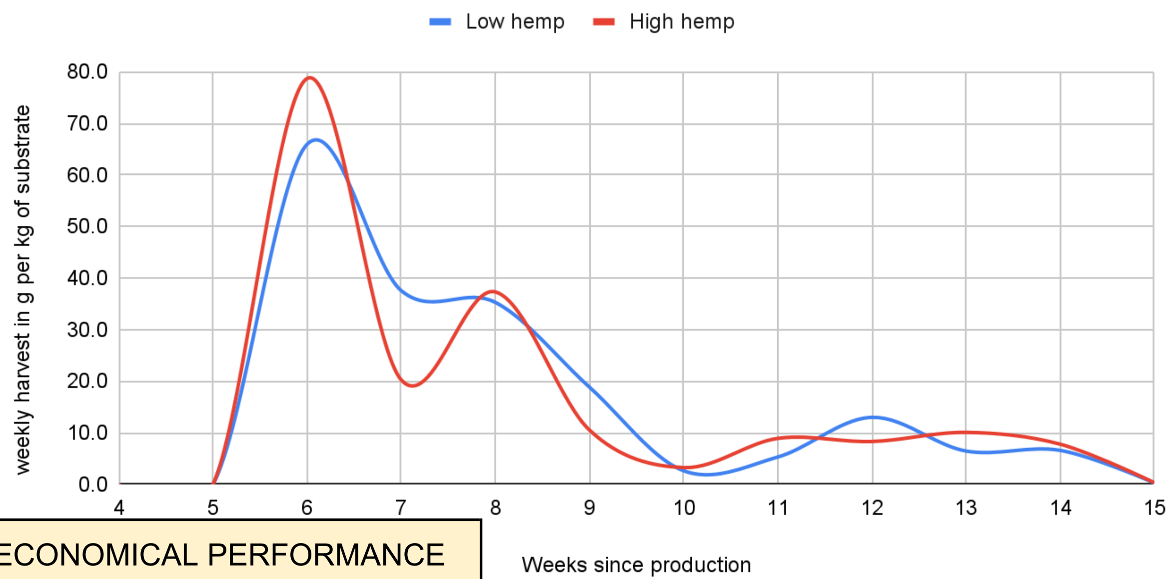
Hemp shives - Results and KPI analysis:

- HEMP CONCENTRATION

05.01.24 Hemp concentration tests; Recipes (ex-water):



Graph 5: Timelime of harvested oyster mushrooms on different hemp shive concentrations



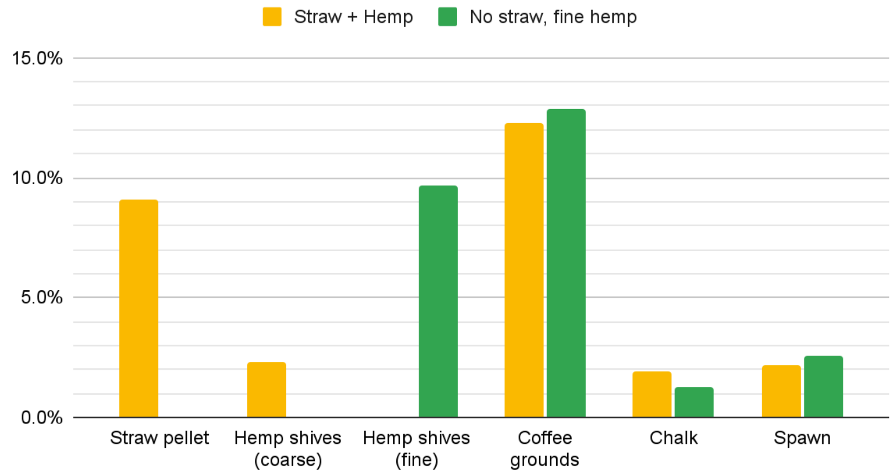
		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	$COGS_€$
Sample #		w/w prod	BE	#days to F1 end	%F1			
35		0.192	0.727	55	68%	0.26	0.39	3.52
36		0.185	0.712	57	74%	0.27	0.40	3.58

These 2 recipes give equivalent results. The studied range of hemp shives concentration might have been too narrow.

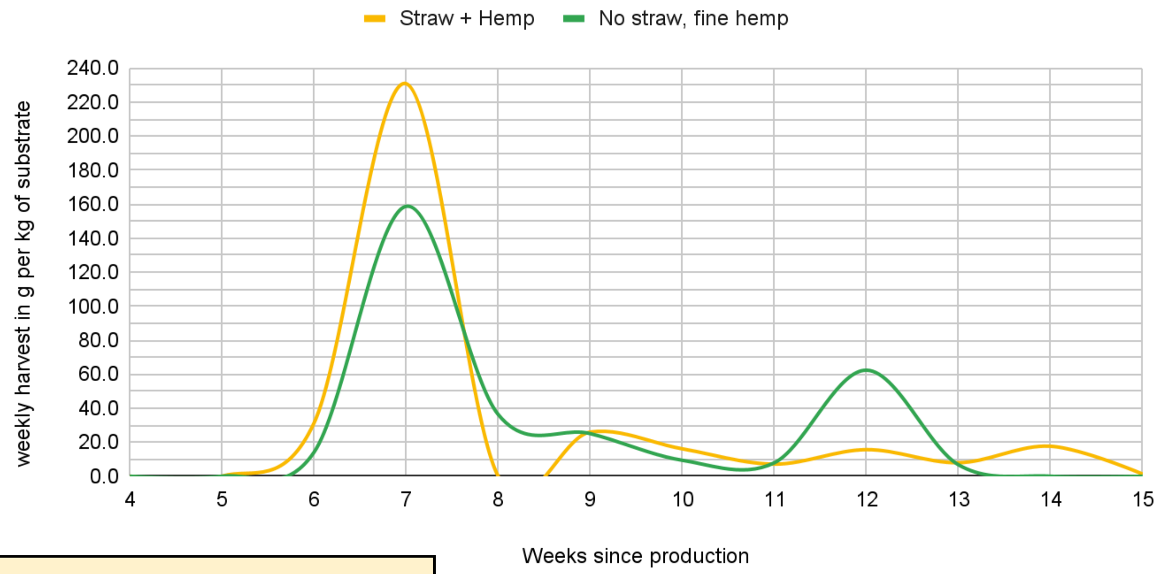
- STRAW+HEMP versus HEMP ONLY

Data analysis above informed us that coarse hemp shives were not suitable as the main substrate ingredient, mainly because they were too sparse and slow-access for the mushrooms to digest. We thus decided to test a finely chopped hemp shive product of which we obtained a sample from the Korkeaoja farm.

22.03.24 Straw+Hemp vs Fine Hemp; Recipes (ex-water):



Graph 6: Timelime of harvested oyster mushrooms on finely chopped hemp



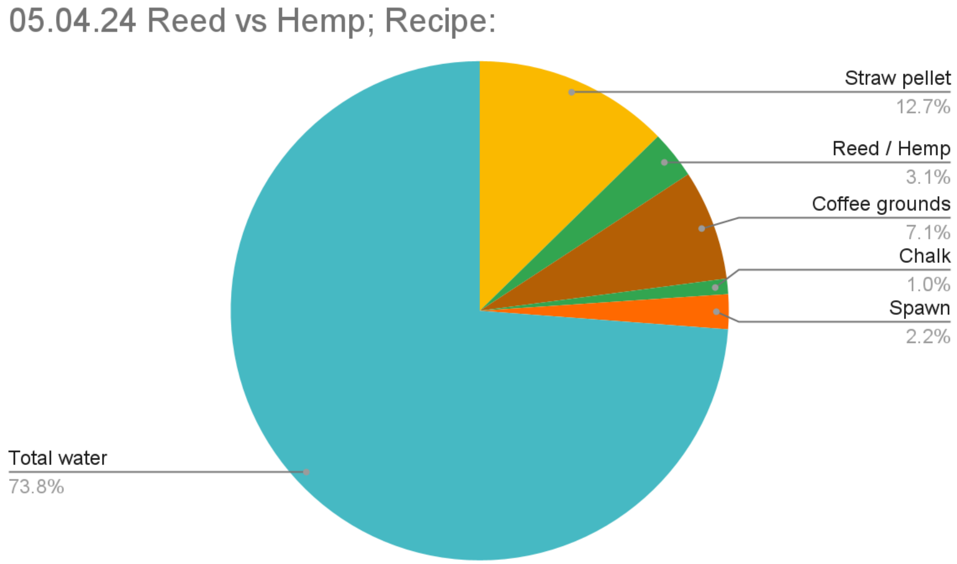
		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	$COGS_€$
Sample #		w/w prod	BE	#days to F1 end	%F1			
64		0.177	0.637	49	75%	0.28	0.38	3.76
65		0.158	0.599	52	67%	0.38	0.40	4.96

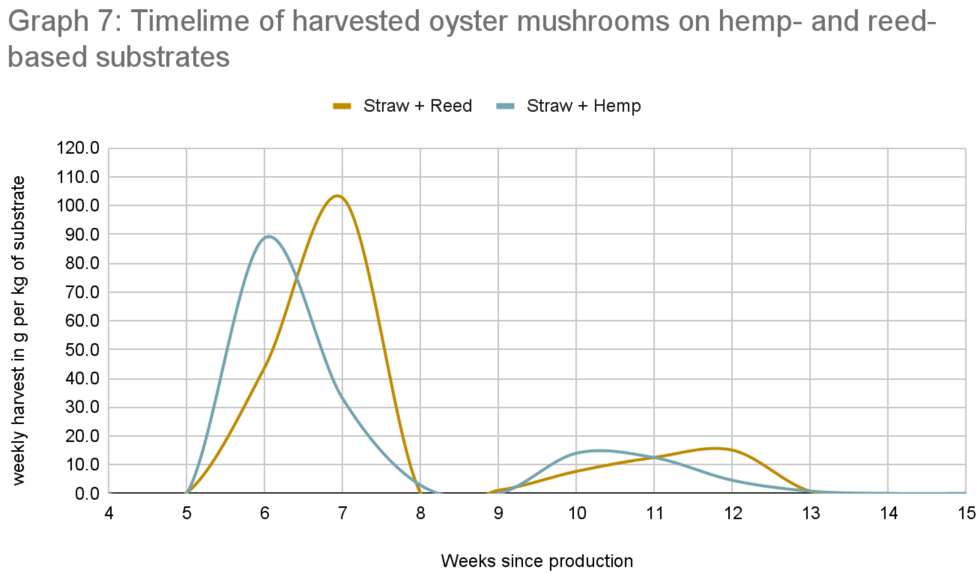
While BE is close for both studied samples (-6% for the fine hemp sample), all other indicators are in favor of the straw+hemp sample: it gives a better wet/wet productivity, a faster first flush and that first flush is bigger.

However, even this finer product is of much coarser consistency than straw pellets. An even finer hemp product would be worth testing but it would require our own refining, as ground hemp shives do not seem to exist on the market.

● HEMP versus REED

Due to quality problems in some batches (Hemparade) and limited availability (Korkeaoja) we have to find an alternative to hemp shives for the second Work Package of this study. We have the opportunity to get enough common reed for the rest of our tests. Its aspect, close in structure to hemp shives, motivates us to compare it with hemp shives to decide whether we can use it in our future control recipe for systematic testing.





		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	$COGS_€$
Sample #		w/w prod	BE	#days to F1 end	%F1			
68		0.184	0.709	49	80%	0.34	0.54	4.83
69		0.156	0.595	52	80%	0.28	0.39	4.30

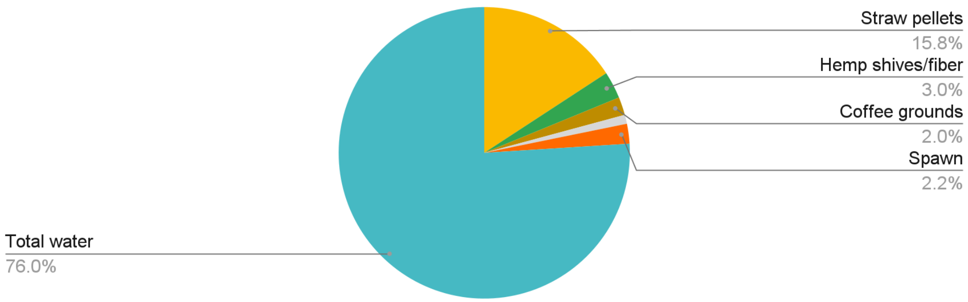
Despite a lower water retention capacity of Treeds (3-4L per kg versus 5-6L per kg for hemp shives) the test we made here gave slightly better results (wet/wet productivity +18%, BE +19%) for our reed-containing sample.

However, the big size of the reeds we collected requires more processing (chopping) and the lack of appropriate machinery gives an unequal result, some remaining long pieces slowing down the bagging. Longer particles might also explain the slower growth of the reed sample.

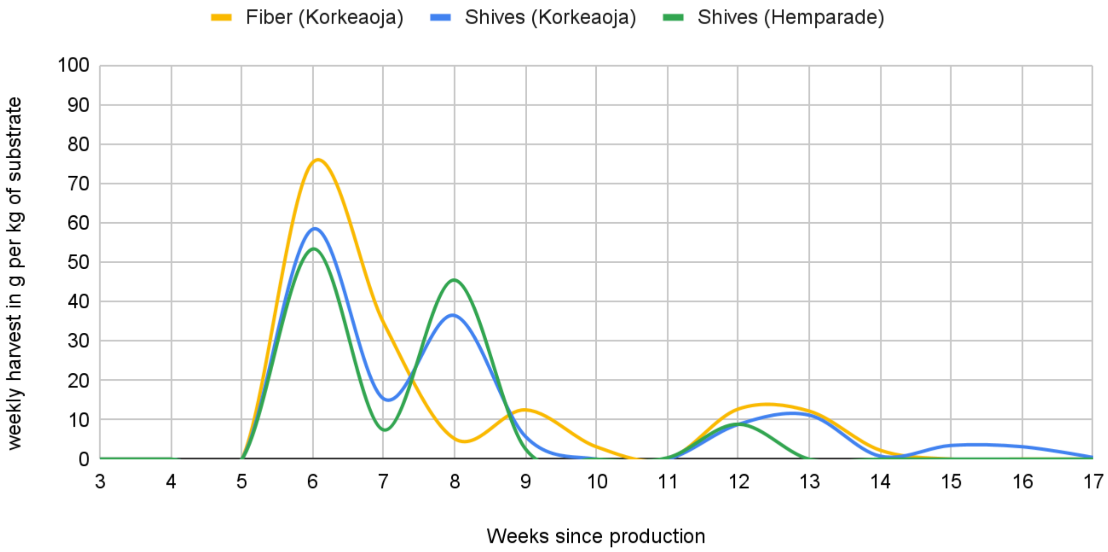
According to this test, reeds appear like an adequate alternative to hemp shives, though less economical at this stage..

- DIFFERENT HEMP PRODUCTS

08.12.23 Hemp fibers vs shives from different origins; Recipe:



Graph 8: Timelime of harvested oyster mushrooms on different hemp products



Production batches

ID #	Prod date	Weight (kg)	Hemp product (brand / provenance)
26	08.12.23	46	Fibers (Korkeaoja)
27	08.12.23	92	Shives (Korkeaoja)
28	08.12.23	127	Shives (Hemparade)

		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	COGS€
Sample #		w/w prod	BE	#days to F1 end	%F1			
26		0.158	0.635	48	71%	0.30	0.40	4.47
27		0.144	0.626	53	71%	0.29	0.40	4.97
28		0.118	0.473	55	86%	0.28	0.41	5.9

Despite their noticeable shape difference (Korkeaoja shives are processed in much longer and thicker pieces than Hemparade), both shives show similar growing patterns, with a moderate first flush and a correct second flush. Hemparade seems to give a smaller first flush and bigger second flush. Korkeaoja gives better results (w/w productivity +22%, BE +32%) than Hemparade.
Fibers in this test give a better wet/wet productivity than shives but the difference is negligible once the water is removed from the calculations (biological efficiencies are almost equal[79]).

According to this test, some chopped fiber mixed with shives can be beneficial to oyster mushroom substrates.

[79] Korkeaoja shives can absorb more water than fibers and Hemparade shives, thus the exact water content of sample 27 is 77% while others' is 75%.

Nitrogen-rich supplementations: Sunflower seed hulls, Oklin compost, coffee

Picture 6: sunflower seed hull pellets

Supplementations are ingredients that are used in minor quantities to improve the physical properties and/or nutritional contents of a mushroom substrate in order to improve its biological efficiency. Most supplements increase nutrient availability for the cultivated species, but also for competing life. They are therefore to be used in well-balanced amounts and might require process and recipe adaptations such as better substrate pasteurisation or spawn rate increase.
Supplementations are usually classified in 3 main groups:

1] Nitrogen-rich: They supply amino-acids that are essential for the mushrooms to build up proteins. Several of them are also rich in lipids, of which mushrooms can benefit as well.
Examples: soy hulls, cereal bran, coffee grounds, beer mash, oil seeds, urea.

2] Starch-rich: They contain easily-available sugars on which mushrooms feed quicker than on longer, more complex molecules (lignin, cellulose, hemicellulose). When spawn is produced from starch-rich grain, it is a starch-rich supplement.
Examples: corn, wheat, barley, rice, millet.

3] Minerals and micro-nutrients: Providing mushrooms with an optimal amount of minerals such as calcium, magnesium, iron, selenium, zinc, manganese, etc. will enable mycelium to access and process a bigger share of the otherwise provided energy. Some minerals like calcium can also increase the pH of substrates to make them less appealing to competitors. On the contrary citric acid can be added to a substrate to lower its pH and make it more suitable to specific mushrooms. Most minerals are contained in other ingredients in variable amounts while some can be specifically added to improve substrates. Examples: calcium carbonate, calcium sulfate.

Here we focus on three locally-available nitrogen-rich supplementation:

- **Coffee grounds**, which have been our main substrate supplementation until now.
- **Sunflower seed hulls**, which may become common in southern Finland in the near future due to climate change. The product we try here is finely chopped and pelletized.
- **Food machine compost**, such as Oklin-branded machine compost, used by some urban restaurants to reduce their organic waste volume. Such a compost is easier to collect from our mushroom customers, store and use than traditional compost because it is dense, dry and mostly sterile.

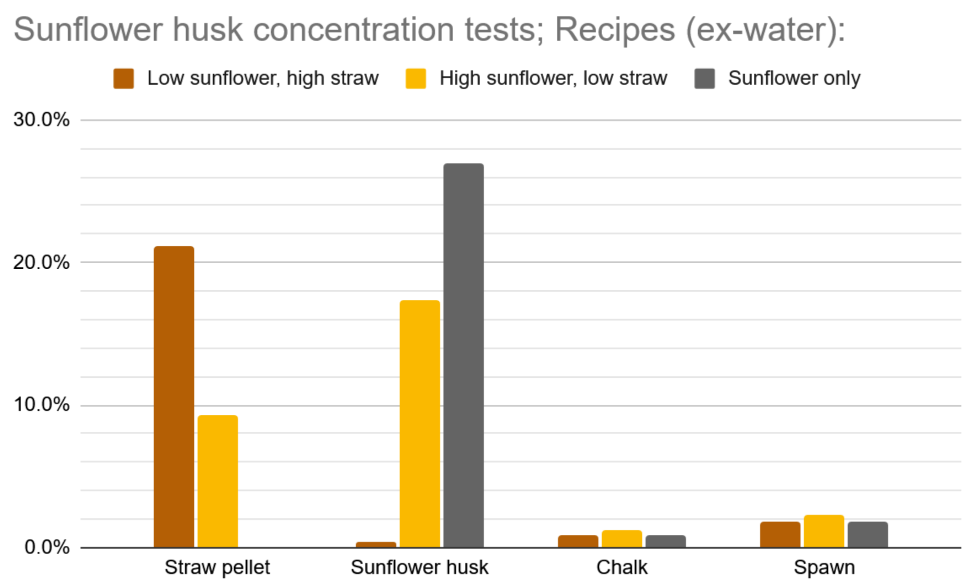
Nitrogen-rich supplementations - List of analysed samples and their recipes:

ID #		Prod date; Weight	Straw pellets (brand)	Hemp/Reed (Product, brand/ provenance)	Sunflower husk	Coffee grounds	Oklin compost	Chalk	Spawn Spoppo	Water content
18[80]		01.06.23 78 kg	Straw pellets (Baltic): 9.3%	-	17.4%	-	-	1.2%	2.3%	69.8%
19[52]		10.07.23 196 kg	Straw pellets (Baltic): 21.1%	-	0.4%	-	-	0.9%	1.8%	75.7%
20[52]		10.07.23 101 kg	-	-	26.9%	-	-	0.9%	1.8%	70.4%
32		29.12.23 137 kg	Straw pellets (Baltic): 13.8%	Hemp shives (Hemparade): 3.0%	-	6.8%	-	1.0%	1.8%	73.7%
34		29.12.23 71 kg	Straw pellets (Baltic): 12.4%	Hemp shives (Hemparade): 2.6%	-	-	8.8%	1.8%	2.9%	71.4%
50		15.02.24 196 kg	Straw pellets (Baltic): 10.7%	Hemp shives (Hemparade): 1.9%	-	12.2%	-	1.8%	1.9%	71.4%
51		15.02.24 58 kg	Straw pellets (Baltic): 14.5%	Hemp shives (Hemparade): 2.2%	-	6.7%	-	1.0%	2.0%	73.6%
52	LATE TRANSFER	15.02.24 121 kg	Straw pellets (Baltic): 14.5%	Hemp shives (Hemparade): 2.2%	-	6.7%	-	1.0%	2.0%	73.6%
53		15.02.24 123 kg	Straw pellets (Baltic): 18.7%	Hemp shives (Hemparade): 2.9%	-	0.0%	-	1.1%	2.4%	74.9%
61		05.03.24 167 kg	Straw pellets (Baltic & Biodela): 15.2%	Hemp shives (Hemparade): 2.9%	5.5%	-	-	2.2%	2.2%	72.1%

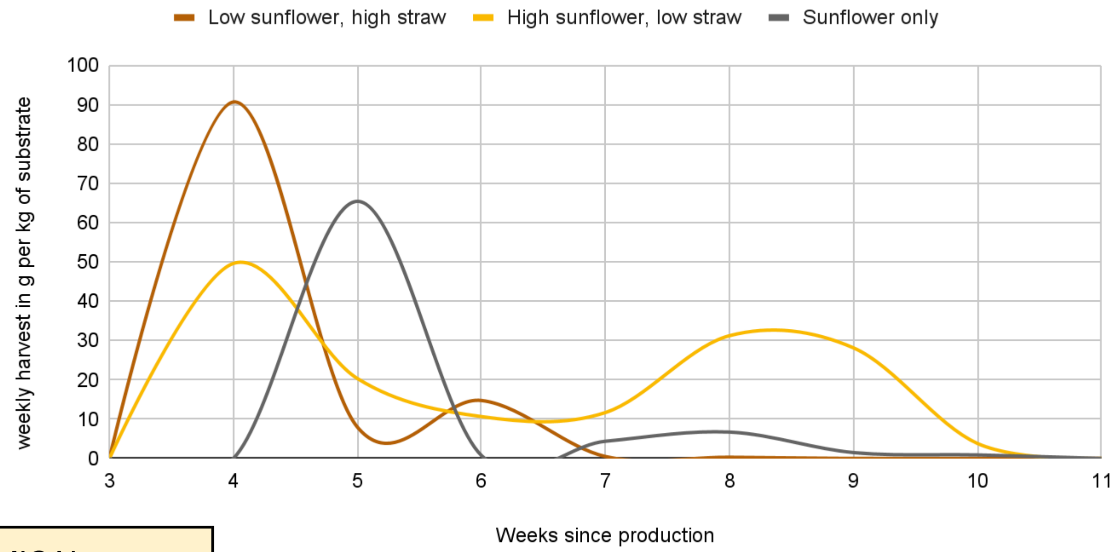
[80] These tests were realised before the start of this project.

- SUNFLOWER HUSK CONCENTRATION

We initially thought of sunflower husk as a carbon source like straw or wood. However, like seen in our desktop study above, literature generally considers it a nitrogen-rich supplement. We tried it at different concentrations to make our own idea of it.



Graph 9: Timelime of harvested oyster mushrooms on different sunflower husk concentrations



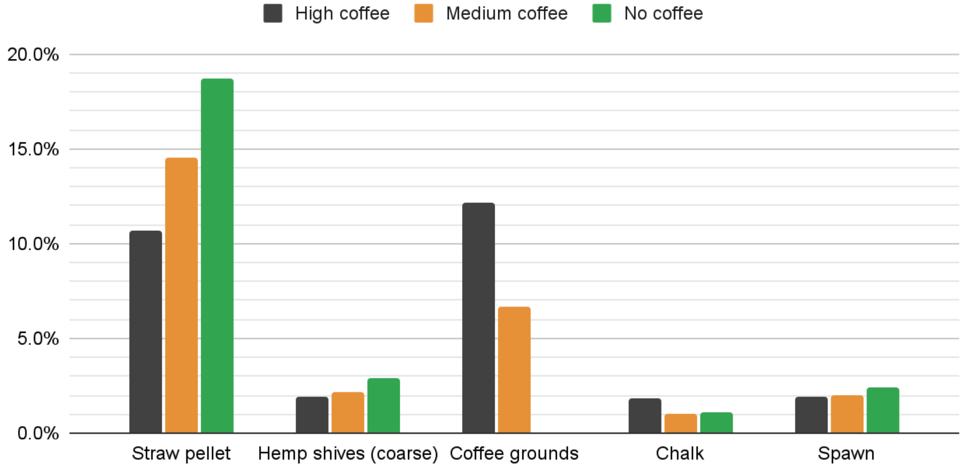
		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	COGS€
Sample #		w/w prod	BE	#days to F1 end	%F1			
19		0.114	0.464	31	86%	0.27	0.41	5.87
18		0.155	0.508	30	45%	0.33	0.44	4.94
20		0.080	0.266	33	82%	0.29	0.42	8.89

According to these tests, mushroom can grow in sunflower husk alone and they give a nicely structured substrate but the contamination rate (50% of #20 sample bags showed traces of mold contaminations) is typical for over-nitrogen containing substrates. Even on healthy bags, the productivity is low for that sunflower-only sample.

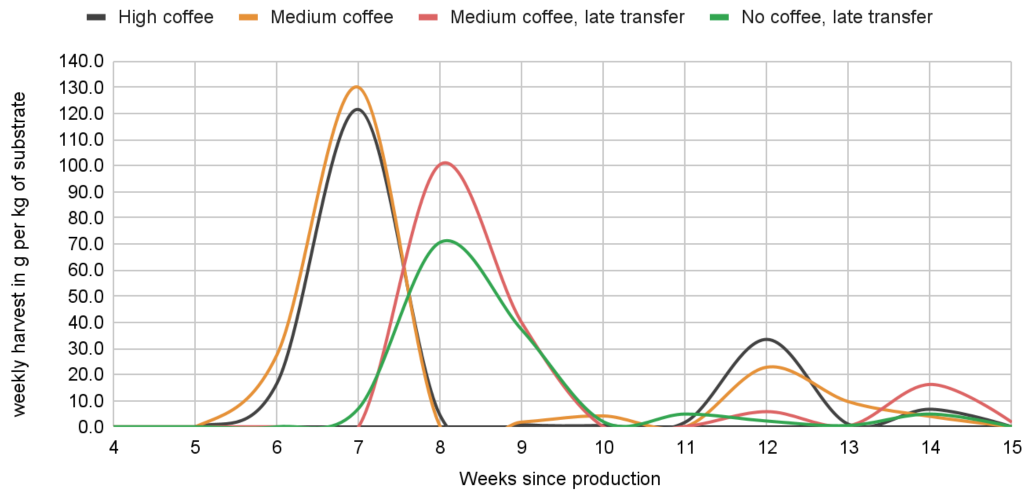
As a supplement, sunflower husk is noticeably improving productivity (+36% productivity) but because of a poor water retention capacity it is only a moderate improvement when considering biological efficiency (+9%). The high-sunflower substrate was also much slower growing than the low-sunflower substrate, produced on the same date and grown in the same conditions. We decide that further testing in WP2 will be done with lower sunflower concentrations (<5% of the wet weight)

- COFFEE GROUND CONCENTRATION

15.02.24 Coffee concentration tests; Recipes (ex-water):



Graph 10: Timelime of harvested oyster mushrooms on different coffee ground concentrations



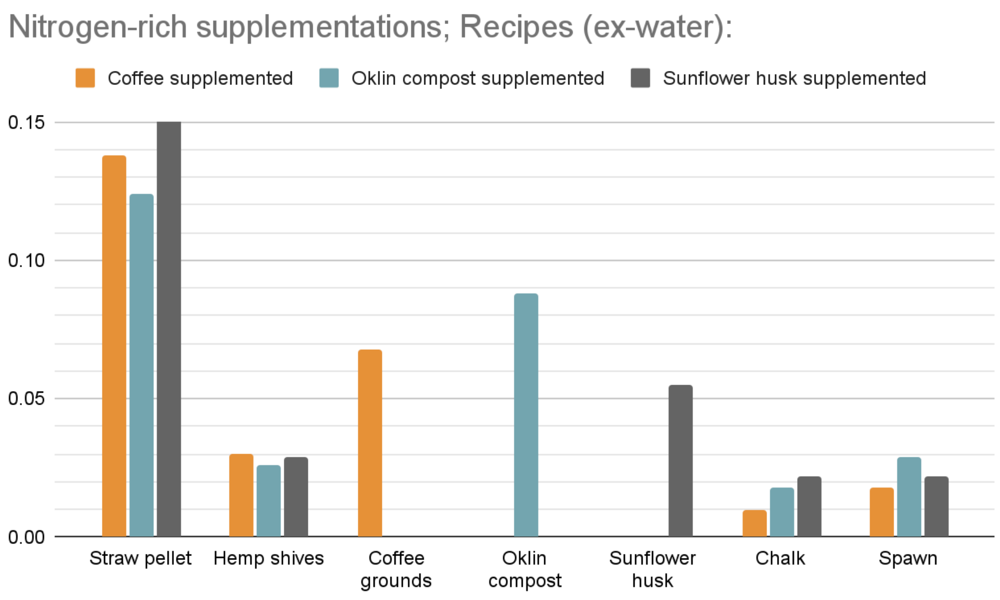
Note: Because of a manipulation mistake, samples 52 & 53 were transferred one week later than 50 & 51 (more than 5 weeks after production). This explains the offset in curves above but also gives us important output on how schedule is important: with exactly the same recipe and produced on the same day, #52 produced 18% less mushrooms than #51. The mistake makes it difficult to compare #50 (high coffee) and #53 (no coffee) but we compare them both to #51/#52 (medium coffee).

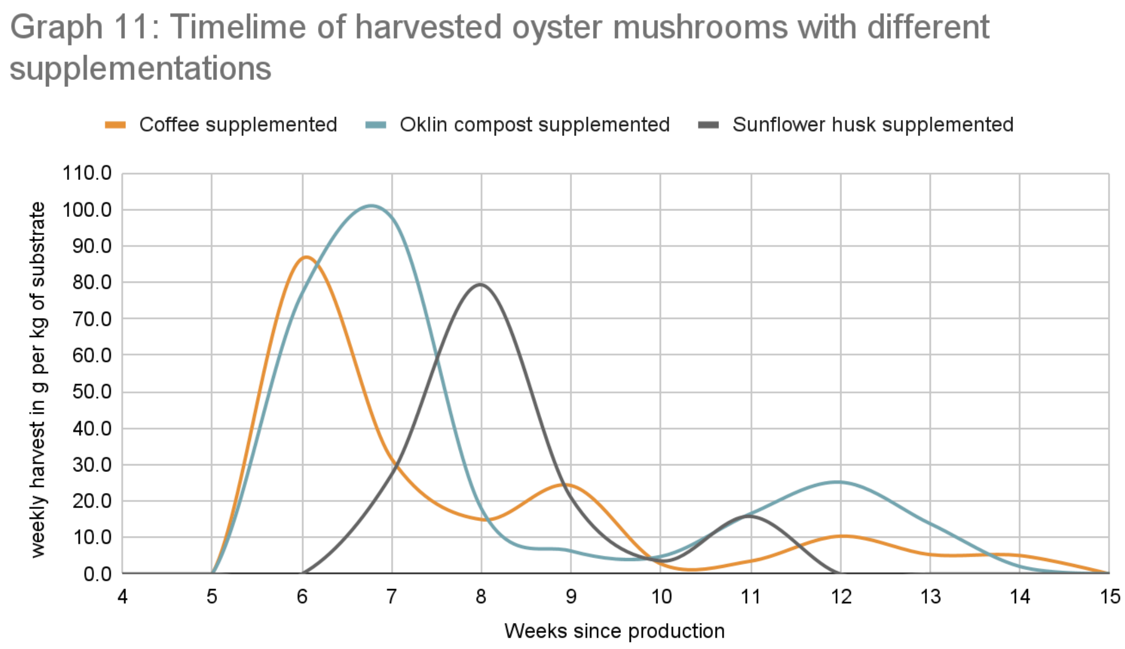
Unlike in former analysis, we observe here an effect of coffee ground supplementation. The high-coffee sample was less productive (weight prod: -7%, BE: -14%) and slower (+1 week for a smaller first flush) than the medium-coffee sample. The unsupplemented substrate produced less (weight prod: -22%, BE: -17%) than the medium-coffee sample. Coffee also brings homogeneity in substrates (preventing clumps).

This preliminary test seems to confirm previous research showing how coffee grounds are a good supplementation in moderate amounts. To be analysed further in the second part of this project.

		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	$COGS_€$
Sample #		w/w prod	BE	#days to F1 end	%F1			
50		0.187	0.652	55	76%	0.24	0.38	3.48
51		0.200	0.757	48	79%	0.26	0.39	3.25
52		0.165	0.623	60	86%	0.26	0.39	4.03
53		0.129	0.515	61	88%	0.30	0.42	5.60

- COFFEE GROUNDS versus OKLIN COMPOST versus SUNFLOWER HUSK





		BIOLOGICAL PERFORMANCE				ECONOMICAL PERFORMANCE		
		Productivity		Growing speed		supply €/kg	process €/kg	COGS €
Sample #		w/w prod	BE	#days to F1 end	%F1			
32		0.184	0.700	50	66%	0.25	0.39	3.48
34		0.264	0.923	50	74%	0.34	0.39	2.80
61		0.147	0.528	60	84%	0.29	0.42	4.82

The poor homogeneity of tests with different supplementation rates calls for a careful result interpretation. However, the results we get for samples #32 are in the average of what we get with coffee supplementation in the range 5-10% on hemp+straw substrates. Sunflower husk was also tried at different concentrations in several recipes without greater results.

On the contrary, the performance we measure on sample #34 (productivity above 26%, BE close to 100%), produced on the same day and grown in the same conditions as #32, has never been seen before on our farm.

Compost composition might differ according to what the machine was fed with. However, these results show a very high potential for machine compost supplementation.

WP1 T3.3: Material processing

Most materials require processing before use. Here is a non-exhaustive list of them:

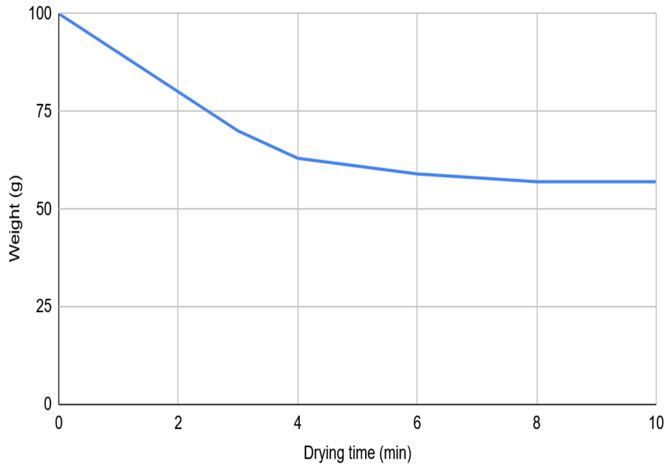
Humidity and water retention measurements

When making up a substrate recipe, one of its crucial properties is its water content. As the water content of mushrooms is 85%-90% of the total mass while substrate ingredients can only hold 50-85% of it, water is one of the main limiting factor for a substrate to produce more mushrooms. Overdry substrates will thus produce less mushrooms. On the other hand, overwet substrates will limitate or block gas transfers (O_2 in, CO_2 out) and they can nest anaerobic bacterial growth which can produce toxins that will slow down or block mushroom growth. This is why for each ingredient we need to know:

- Its water retention capacity: how much water can be held by this ingredient
- Its water content (humidity) at reception: how much water it already holds in storage
- Its water content at use: how much water it carries after pre-processing (pasteurisation/ rinsing / …)

Water retention can only be calculated from a dried product and the most effective way to measure humidity is to dry a wet product. This is why we need to have a simple process to measure humidity. The process we choose is incremental humidity measurements:

- We place 100g of the product we want to test in the microwave
- We heat up the product for 1 minute
- We weigh the product after heating and leave it cool down for 5 minutes[81]
- We repeat the operation until the weight no longer decreases. The final weight is the dry weight and the initial water content in % is:
    - 100 - Final Weight (in grams)



Pictures 7, 8: Incremental humidity measurement and typical drying curve

Once we know how wet an ingredient is, we can measure its water retention capacity by over-hydrating it and then draining it until no water leaks. Water retention capacity can be influenced by the temperature of hydration and the compression of ingredients within the substrate. A final value is admitted after observing the behaviour of several substrate tests. The main benefit of using micro-perforated bags as we do is that we can hydrate ingredients to their limits: if we slightly overcome it, water will leak through micro-perforations and no anaerobic pouch will be left.

[81] The cooling down period is very important to avoid fire hazard by autoignition of the product.

Chopping / grinding

Every ingredient comes with different particle sizes, homogeneous or not. Some are available on the market with different particle sizes, some not.

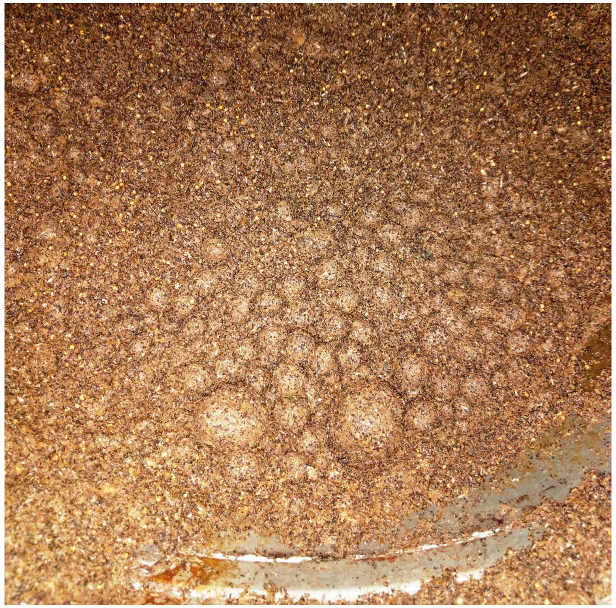
Several issues occur when mixed particles are too big:

- The substrate is too airy and thus too light (inefficient use of space)
- The particles can pierce through the growing bags (risk of external contamination)
- Bagging the substrate becomes difficult and slow
- Water absorption is usually reduced

Pictures 9, 10: Long particles in substrate mixing make bag filling more difficult.

On the contrary, other issues occur when mixed particles are too small:

- The substrate is too packed, worsening gas exchange (oxygen in, CO_2 out) and slowing growth down.
- The particles agglomerate at the bottom of the mixer, making the bagging difficult.
- The particles agglomerate into balls, making mixing ineffective and growth slower.




Pictures 11, 12: Small particles agglomerating into balls

To avoid mixing trouble, it is important to find the appropriate chopping / grinding / shredding process for each material. Literature usually shows that a mixed particle size is more productive than with a single calibrated particle size.
However, it would be hazardous to invest in any machinery before we know what material(s) we will use as a standard because not all machines can work with all ingredients. Therefore, we rather use materials that already exist, when impossible we usually rely on outsourced processing (using machinery that the supplier already has). Only when both do not show satisfactory results, we process ourselves with “low-tech” methods (malt hand-grinder, lawnmower, scissors…), as shown below:

Pictures 13, 14: attempts to chop reeds with different existing tools and machines

Picture 15: chosen method to chop reed or cereal straw: a mulching lawnmower.

Pictures 16, 17, 18: Processing spent shiitake substrate to use it as oyster mushroom substrate

Hydration, pre-incubation and rinsing

Most ingredients are stored dry and need to be hydrated before use. For low-nitrogen ingredients, overnight hydration in a bath of lukewarm water is working well. For nitrogen-rich ingredients it is not suitable, because anaerobic digestion will start to degrade their nutrients and may produce toxins that can inhibit mushroom growth. In our farm we usually hydrate dry ingredients in the pasteurisation process (see below).
When the contaminant load is too big to be gotten rid of with our pasteurisation process and because we do not have an autoclave for sterilisation (105-123°C), we can pre-incubate hydrated ingredients or simply rinse them for 5 min with cold water.

- Pre-incubation means 3-5 days[82] of aerobic growth at room temperature (alternatively outside, if the temperature is >15°C). During this period, fungi spores and bacteria endospores will start to grow. In this state they will be destroyed most effectively in the following pasteurisation process.
- Rinsing can be consecutive to pre-incubation or a quick alternative to it for moderately-hazardous ingredients. It removes a large share of spores and endospores.

Picture 19: Reeds hydration

Picture 20: Hydrated reeds pre-incubation

Picture 21: hemp shives rinsing

[82] In this project we incubated for 2-4 days. We later learned that 2 days is too short for this process.

Pasteurisation

- **High-temperature pasteurisation (99-100°C)**
  We boil the most contamination-hazardous ingredients in a bath of hot water. We heat until reaching the boiling point and then remove the heat source, resulting in a total pasteurisation time (temperature >60°C) of 4-8h. We then leave them cool overnight.
- **Quick 80°C pasteurisation**
  We hydrate the least contamination-hazardous ingredients with an adequate amount of 80°C water and leave them cool down overnight in closed containers. The main advantages of this method are its simplicity (no need for another heat source than a standard water boiler, no need for a heating tank/room) and its perfect adjustment of water amounts to the ingredients' water retention capacities (no further drainage required). In this case pasteurisation time >60°C is 0,5-1h

Picture 22: Quick 80°C pasteurisation (here: straw pellets).

Picture 23: 100°C pasteurisation

Drainage


Picture 24: Coffee ground drainage to remove excess water

After high-temperature immersed pasteurisation , ingredients are too wet for mixing. We drain them in pierced buckets for about 20 minutes before adding them to the blender.

Picture 25: Substrate mixing

All ingredients are mixed per batch of 40-55 kg in a cement mixer.
Bags are filled by hand with a scoop and hanged on danish trolleys with fruiting-compatible spacings (enough space for airflow between bags).

Control batch

After collecting data and refining it with preliminary tests, we could define a well-performing recipe that we would reproduce every week and use as a control batch until all tests are done.

All our standard recipes so far had contained straw pellets and spent coffee grounds. Lately we had added hemp shives[83] due to its excellent water absorption and longer chunks that prevent straw pellets from agglomerating into balls[84].
However, it was impossible to order hemp shives from our new local supplier (Korkeaoja farm) because the new harvest had not occurred, and the batch of Hemparade-branded shives we received from the wholesales was of too poor quality. Thus we decided to replace hemp shives with reed straw of which our stock was enough for all tests.

The recipe for the control batch is as follow:

- **Reed straw (dry)** **1.0** ± 0.1 **kg**
  Roughly chopped into pieces of 1-10 cm. To eliminate most contaminations, the reeds are soaked in water and pre-incubated for 2-4 days. Thus foreign spores germinate[84]. Then, the reeds are boiled in 100°C water for at least 30 minutes and cooled down overnight.
- **Biodela straw pellets (dry)** **8.7** ± 0.5 **kg**
  Humidified with 80°C water
- **Tap water** **24** ± 2 **L**
  Embedded in soaked reeds and pellets
- **Coffee grounds (wet)** **12** ± 2 **kg**
- **Chalk[85] (dry)** **0.5** ± 0.1 **kg**
- **Sylvan Spoppo oyster mushroom spawn[86]** **2.8** ± 0.2 **kg**

TOTAL per mixed batch **49** ± 5 **kg**

[83] See above WP1 T1.5: Hemp shives
[84] see above WP1 T3.3 : Material processing
[85] Calcium carbonate ($CaCO_3$)
[86] About 60% higher spawn rate than our usual recipe. Reason: we want to use the same spawn rate in tests and control batches but some of the test batches may be contamination sensitive and therefore require higher spawn rates.

Bag repartition

Bags of each tested recipe are shared between top and bottom shelves to smoothen the possible influence of temperature between levels[87]. Control batches are shared in two halves[88] that are installed on both sides of the container, to compensate for potential airflow/humidity asymmetries.

Picture 26: Bag repartition

Incubation

All bags incubate at 19 - 22°C, 60-70% relative humidity until they start pinning[89], usually after 3-4 weeks in the case of Spoppo oyster mushrooms.

Fruiting

Fruiting happens together with our normal production, at 80-90% RH and 10-20°C depending on the season.
Fruiting ends when bags look weak, dry or contaminated.

[87] About 2°C difference between top and bottom shelves influence KPI, especially speed-related.
[88] Therefore control batches have 2 batch numbers in test results below. See WP2 T1.3 for a thorough analysis of our fruiting room asymmetries.
[89] Pinning is when the first small mushrooms show up under the plastic.

WORK PACKAGE 2: Production tests on different substrates

Test scheduling

We produced 1 or 2 different test recipes at least every second week. When producing 2 different test recipes in the same week and when possible[90], only one ingredient (or process) differs between them so we can compare one parameter at a time. Every couple of test batches (= every test) stood on a cart next to a couple of control batches, so we can compare each of them with this reference. When enough raw material was available, each couple of tests were produced on 2 different production weeks. Thus we get a solid bank of data and lots of possible comparisons.

- Test schedule:
  - 2.5.2024: 2x Birch, 1x Shiitake spent substrate, 2x Control.
  - 10.5.2024: 2x Birch, 2x Birch+Straw, 4x Control.
  - 17.5.2024: 2x Alder+Straw, 2x Birch+Straw, 4x Control.
  - 31.5.2024: 2x Birch, 2x Birch+Coffee, 4 x Control.
  - 7.6.2024: 1x Apple-tree+Straw, 3x Alder+Straw, 4x Control.
  - 14.6.2024: 1x Chopped reed, 2x Sunflower seed husk, 4x Control.
  - 28.6.2024: 2x Alder, 2 x Alder+Straw, 4x Control.
  - 12.7.2024: 2x Ground oat husk, 2x Hemp shives+Straw pellets, 4x Control.
  - 26.7.2024: 2x Flax shives, 2x LBP[91], 4x Control.
  - 3.8.2024: 2x Flax shives, 2x Hemp shives+Straw pellets, 4x Control.
  - 7.8.2024: 2x Flax shives, 2x LBP, 4 x Control.
  - 14.8.2024: 2x Ground oat husk+Alder pellets, 2x Ground oat husk+Straw pellets, 4x Control
  - 21.8.2024: 2x Chopped straw+Straw pellets, 2x Chopped straw, 4x Control.
  - 29.8.2024: 2x Coffee silverskin, 2x Coffee silverskin+Straw pellets, 4x Control
  - 4.9.2024: 2x Apple-mash, 2x LBP, 4x Control
  - 18.9.2024: 4x Coffee silverskin+Birch, 4x Control
  - 26.9.2024: 2x Reed straw, 2x Hemp shives, 4x Control

[90] It not always possible if the studied parameters have very different densities or if one requires more spawn/ more chalk to prevent a higher risk of contamination.
[91] LBP = Linseed By-Product for the rest of this document

Density and water retention capacity of tested materials

We gathered in the following table data that was collected during this research project. It is of importance when designing a test recipe to know how dense materials are when dry (how much space will be required for humidification) and when wet (how much will they use space in the mixer). It is also important to know how much water they can hold, so we know how much water is embedded in each ingredient after pasteurisation and/or how much extra water must be added to a recipe to maximise its amount while limiting/avoiding leakage.

Note: Calculating a final recipe density cannot be achieved by averaging its ingredient densities. Indeed, smaller particles will lodge in between bigger ones and thus final density will be higher than "expected".

Material	Humidity at reception	Density at reception	Humidity at saturation (= water retention capacity)	Density at saturation
	% H_2O_{dry}	kg/L_{dry}	% H_2O_{wet}	kg/L_{wet}
Chopped common reed[92]	10-20%	0.08	75%	0.33
Chopped wheat straw[92]	15%	*N.A.*	79%	0.33
Straw pellets[93]	10%[94]	0.55	75-77%	0.66-0.72
Alder pellets	10%	0.65	74%	0.53
Coarse birch sawdust	15-25%	0.18	65%	0.43
Hemp shives[93]	10%	0.08	82-85%	0.40-0.48
Flax shives	10%	0.09	80%	0.28
Linseed By-product	10%	0.63	69%[95]	1.05
Ground oat husks	10%	*N.A.*	57%	0.62
Coffee silverskin	10%	0.03-0.04	68%	0.54
Coffee grounds[93]	65-75%	*N.A.*	70%	0.80-1.05

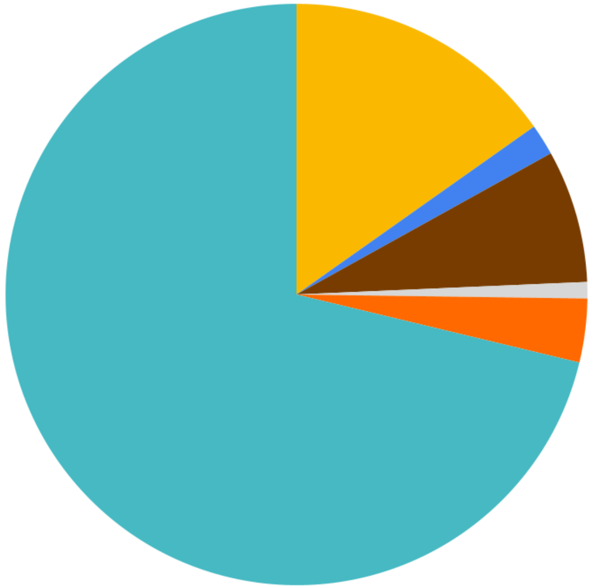
[92] 1-10 cm sized particles
[93] Water retention capacity and wet density depends on brand and batch. Dry density can also slightly vary depending on the pelletising pressure and/or particle size.
[94] Humidity of very dry materials depends on surrounding air's relative humidity. We consider that it is on average 10%.
[95] Boiled linseed changes water into a slimy liquid so it is very hard to drain out. 69% water seems to be a limit above which the slimy liquid will slowly exit ready substrate bags

Task 1. Control Batch

We compare here control batches produced along the study. For various reasons ranging from mistakes to temporary ingredient shortage, a few of the control batches were entirely contaminated before fruiting or were made with a non-compliant recipe (out of the standard recipe tolerance margins defined in WP1 T3.4). They do not appear in this comparative study.

WP2 T1.1: Recipe[96]

INGREDIENT	Weight (% of wet total)	Water content[97] (% per ingredient)	Dry weight (% of dry total)	Dry weight[98] (% of wet total)
Straw pellets	16.9 %	*10 %*	52.9 %	15.2 %
Reed straw	6.7 %	*74 %*[99]	6.0 %	1.7 %
Coffee grounds	24.6 %	*70 %*	25.7 %	7.4 %
Chalk	1.0 %	*10 %*	3.2 %	0.9 %
Spawn	5.9 %	*40 %*	12.3 %	3.5 %
Water	45.0 %	*100 %*	-	*71.2 %*



WP2 T1.2: KPI analysis[100]

[96] Weighted average for all compliant control batches produced.
[97] Humidity content of dried-stored ingredients like pellets and chalk depend on air humidity but we cannot afford measuring it for every single test batch. 10% is a good approximation of their average water content. Other humidity contents were extrapolated from a few measured samples or copied from suppliers datasheets.
[98] Except water: total water weight. All recipes given in WP2 will are written this way.
[99] Water content after pasteurisation and drainage.
[100] See KEY PERFORMANCE INDICATORS

			TOTAL average	79	81	83	85	87	89	91	92	94	96	98	100
SUBSTRATE DATA		**TEST BATCH NUMBER**	**TOTAL average**	**79**	**81**	**83**	**85**	**87**	**89**	**91**	**92**	**94**	**96**	**98**	**100**
		PRODUCTION DATE		17/05/24	17/05/24	31/05/24	31/05/24	07/06/24	07/06/24	14/06/24	14/06/24	28/06/24	28/06/24	12/07/24	26/07/24
		Substrate quantity produced (kg)	2541	99	99	96	96	147	49	98	99	99	99	166[101]	98
		Substrate quantity produced (bags)	156.8	5.3	6.3	5.5	6	9	3	6	6	6.4	6	10.3	6
		Average substrate bag weight (kg)	16.2	18.7	15.7	17.4	16.1	16.3	16.3	16.4	16.4	15.4	16.5	16.1	16.3
		Fruiting temperature (°C)[102]		17.7	17.7	19.3	19.3	19.0	19.0	19.3	19.3	19.1	19.1	18.1	17.1
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.191	0.120	0.190	0.144	0.164	0.233	0.251	0.123	0.159	0.132	0.183	0.164	0.204
		Wet/wet productivity, 70 days	0.164	0.118	0.147	0.115	0.153	0.187	0.167	0.120	0.139	0.132	0.128	0.138	0.186
		BE (wet mushrooms / dry substrate)	0.663	0.416	0.657	0.499	0.569	0.811	0.875	0.432	0.556	0.458	0.638	0.568	0.713
		Bag productivity (kg/bag)	3.08	2.24	2.98	2.50	2.63	3.79	4.09	2.02	2.62	2.03	3.01	2.64	3.33
		Space productivity (kg/bag, 70 days**)**	2.66	2.20	2.32	2.01	2.46	3.04	2.72	1.97	2.28	2.03	2.10	2.23	3.02
	SPEED	**Days to end of first flush**	38	46	44	38	39	37	37	43	51	39	41	34	34
		First flush / total (%)	64.6	65.5	62.4	61.1	68.7	65.5	55.7	70.3	84.0	49.7	36.2	54.5	61.7
	CONT	**Partial contamination** (%)	19.0	0	63	0	0	0	0	50	33	78	0	10	0
		Total contamination (%)	1.3	0	0	0	0	0	0	0	0	0	0	16	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost**[103] (€/**kg** of substrate)	0.41	0.41	0.41	0.42	0.42	0.40	0.40	0.37	0.39	0.40	0.40	0.41	0.39
		Supply cost[104] (€/**bag** of substrate)	6.60	7.71	6.49	7.37	6.81	6.49	6.49	6.08	6.42	6.10	6.50	6.65	6.42
		Processing cost (€/**kg** of substrate)	0.51	0.51	0.51	0.52	0.52	0.51	0.51	0.50	0.51	0.51	0.51	0.51	0.51
		Processing cost (€/**bag** of substrate)	8.30	9.57	8.05	9.00	8.36	8.30	8.30	8.21	8.32	7.83	8.35	8.25	8.27
		COGS$_{€}$ (cost$_{€}$ / kg produced mushrooms)	5.10	7.71	4.88	6.54	5.76	3.90	3.62	7.08	5.63	6.86	4.93	5.65	4.42

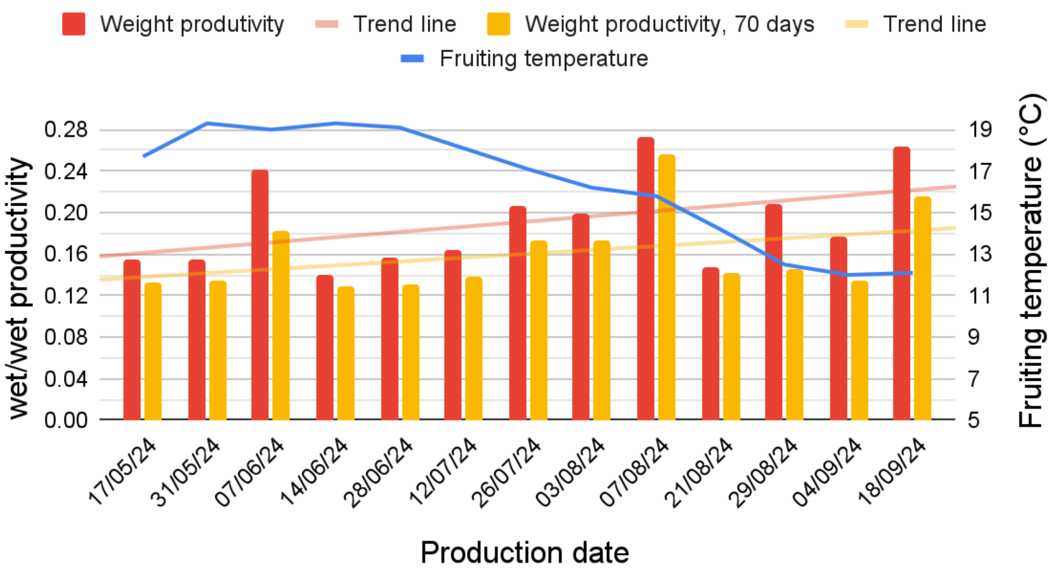
[101] All data and KPI is given excluding fully contaminated substrates (removed before fruiting)
[102] Average temperature from fruiting stage starting date to 70 day after production
[103] Small weight cost variations are due to small recipe variation within the tolerance margins of the standard recipe.
[104] Bag cost variations are mainly due to different bag densities.

Group	Subgroup	TEST BATCH NUMBER	TOTAL average	101	106	107	110	111	115	116	119	120	122	123	128
SUBSTRATE DATA		**PRODUCTION DATE**		26/07/24	03/08/24	03/08/24	07/08/24	07/08/24	21/08/24	21/08/24	29/08/24	29/08/24	04/09/24	04/09/24	18/09/24
SUBSTRATE DATA		**Substrate quantity produced** (kg)	2541	101	101	101	110	110	99	99	90	97	97	101	192
SUBSTRATE DATA		**Substrate quantity produced** (bags)	156.8	6.3	6	6.4	7	7	6	6	5	6	6	6.3	13
SUBSTRATE DATA		**Average substrate bag weight** (kg)	16.2	16.0	16.8	15.7	15.7	15.7	16.5	16.5	18.1	16.2	16.2	16.0	14.8
SUBSTRATE DATA		**Fruiting temperature** (°C)		17.1	16.2	16.2	15.8	15.8	14.2	14.2	12.5	12.5	12.0	12.0	12.1
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.191	0.210	0.195	0.203	0.287	0.259	0.145	0.151	0.227	0.188	0.146	0.177	0.264
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity, 70 days**	0.164	0.159	0.161	0.185	0.271	0.241	0.136	0.148	0.214	0.152	0.138	0.134	0.216
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**BE** (wet mushrooms / dry substrate)	0.663	0.729	0.675	0.703	1.004	0.905	0.503	0.523	0.790	0.654	0.507	0.613	0.917
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Bag productivity** (kg/bag)	3.08	3.36	3.27	3.19	4.50	4.06	2.40	2.49	4.11	3.04	2.37	2.84	3.91
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Space productivity** (kg/bag, 70 days**)**	2.66	2.54	2.70	2.91	4.24	3.78	2.25	2.44	3.87	2.46	2.24	2.16	3.20
KPI: BIOLOGICAL PERFORMANCE	SPEED	**Days to end of first flush**	38	33	33	39	33	32	36	39	38	31	32	40	40
KPI: BIOLOGICAL PERFORMANCE	SPEED	**First flush / total** (%)	64.5	37.5	68.7	71.3	62.2	63.1	70.8	88.0	77.5	42.6	73.3	63.4	81.7
KPI: BIOLOGICAL PERFORMANCE	CONT	**Partial contamination** (%)	19.0	48	0	0	14	0	67	50	0	17	0	48	0
KPI: BIOLOGICAL PERFORMANCE	CONT	**Total contamination** (%)	1.3	0	0	0	0	0	0	0	0	0	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.41	0.39	0.41	0.41	0.38	0.38	0.41	0.41	0.43	0.42	0.4	0.41	0.42
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**bag** of substrate)	6.60	6.32	6.84	6.42	5.98	5.98	6.81	6.81	7.81	6.80	6.79	6.51	6.26
KPI: ECONOMICAL PERFORMANCE		**Processing cost** (€/**kg** of substrate)	0.51	0.51	0.51	0.51	0.50	0.50	0.51	0.51	0.52	0.52	0.52	0.51	0.52
KPI: ECONOMICAL PERFORMANCE		**Processing cost** (€/**bag** of substrate)	8.30	8.09	8.56	8.02	7.79	7.79	8.45	8.45	9.46	8.41	8.35	8.15	7.66
KPI: ECONOMICAL PERFORMANCE		**COGS$_€$** (cost$_€$ / kg produced mushrooms)	5.10	4.29	4.72	4.53	3.06	3.39	6.37	6.13	4.20	5.01	6.40	5.16	3.56

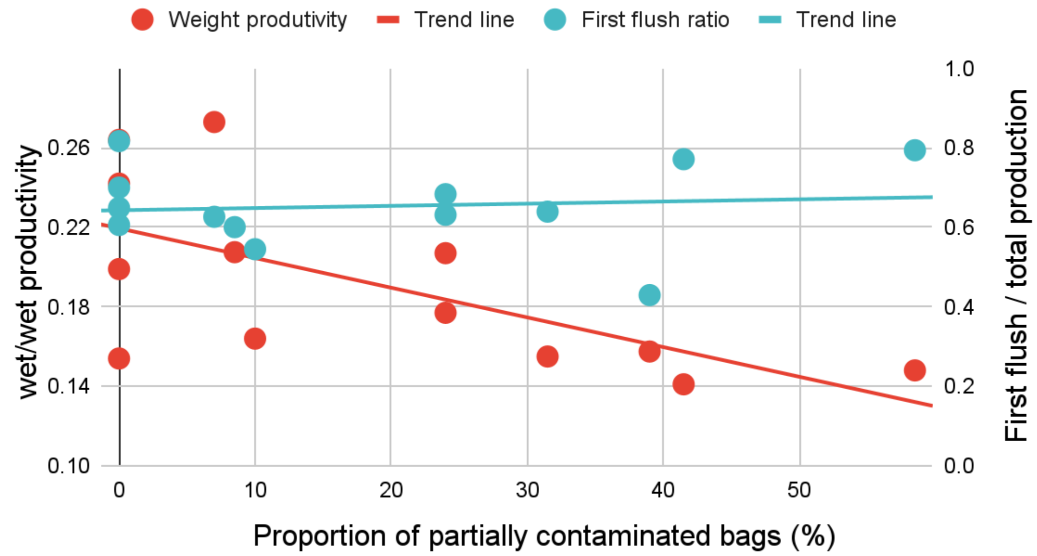
We observe great variations in productivity between all tests even though their recipe and production process are homogeneous. We conclude that we can only compare data if produced the same day. For the batches #110 and #111 (7/8/24), which performed exceptionally, we identified the probable cause: on

that day coffee was pasteurised together with LBP in the same tank. Separators did not let solids mix with each other, but water moved freely within the tank and we noticed the jelly-like consistency of coffee on that day.
We studied more closely the influence of a few other factors:

Graph 12: Evolution of temperature and productivity over time



Graph 13: Effect of contamination on substrates performance



Fruiting temperature[105] is here ranging from 12°C to 19°C depending on the outside temperature, to save on energy costs. In this range, temperature decrease seems to improve productivity (Graph 12, trend lines). This result is surprising in regard to our experience. It is possible that we improved processing and mixing this recipe over time, especially with reeds which were a new ingredient for us. Less surprising, we notice a slight increase of the gap between total productivity (red bars) and 70-day productivity (yellow bars) when temperature drops under 13°C. Most likely because under this limit bags cannot make a third flush within 70 days (lower fruiting temperature slows mushroom growth down).

We then analysed the effect of contamination on substrate performance. For each produced test, we had noted the share of bags with visible contamination spots (molds, bacteria…) at the time of their transfer to the fruiting room. Only slight contaminations are accepted in the fruiting room but even these small strains give a clue on the general health of the produced substrate.
The relative size of the first flush is not significantly affected by the contamination rate. It slightly increases with the most contaminated batches because the most contaminated bags need to be disposed of after the first or second flush to avoid contamination to spread throughout the farm. However productivity is largely affected by contamination[106]

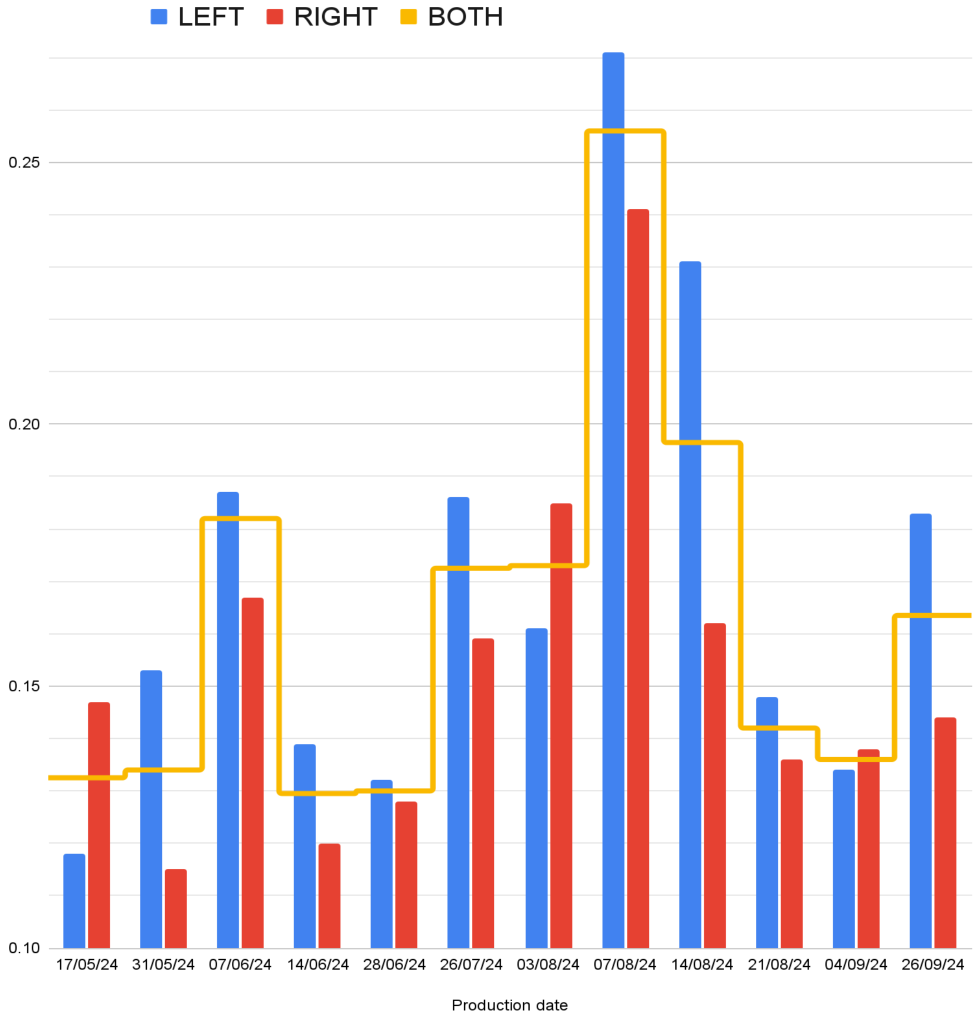
[105]Average temperature for each analysed batch, from transfer to the fruiting room (approx 25-day since production) to the end of the normal production cycle (70-day since production). T

[106] Because results on Graph 12 were unexpected we also checked if there was a (non-causal) correlation between fruiting temperature and contamination. There was none.

WP2 T1.3: Fruiting room side

Control batches were systematically separated in 2 halves that grew in opposite sides of the fruiting room. This enables a systematic comparison, to see if/how our slightly asymmetrical airflow and humidity setup affects results[107].

Graph 14: Wet/Wet productivity (70-day production cycle) on both sides of the fruiting room



		KPI ANALYSIS ID	LEFT	RIGHT
SUBSTRATE DATA		TEST BATCH NUMBER	79, 85, 87, 92, 94, 100, 106, 110, 113, 116, 123, 131	81, 83, 89, 91, 96, 101, 107, 111, 114, 115, 122, 130
		Substrate quantity produced (kg)	1226	1126
		Substrate quantity produced (bags)	76.8	69.8
		Average substrate bag weight (kg)	16.0	16.2
		Fruiting temperature (°C)	16.4	16.4
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.190	0.183
		Wet/wet productivity, 70 days	0.170	0.154
		BE (wet mushrooms / dry substrate)	0.660	0.635
		Bag productivity (kg/bag)	3.01	2.95
		Space productivity (kg/bag, 70 days)	2.69	2.47
	SPEED	**Days to end of first flush**	39	37
		First flush / total (%)	67.4	59.3
	CONT.	**Partial contamination** (%)	24.2	25.1
		Total contamination (%)	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.41	
		Supply cost (€/**bag** of substrate)	6.58	6.66
		Processing cost (€/**kg** of substrate)	0.52	
		Processing cost (€/**bag** of substrate)	8.24	8.32
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	5.18	5.33

[107] Batch selection for this study slightly differ from previous WP2 T1.1 and WP2 T1.2 in such, that here recipes can be non-compliant with the standard recipe defined in WP1 T3.4 but opposite batches must not have experienced any other variation than their position difference.

The graphic and table above show a small but significant excess performance on the left side: out of 12 studied sample pairs, only 3 right samples overperformed (compared KPI: **Wet/wet productivity, normal cycle**). KPI averages show performance excess for almost all KPI on the left side, resulting in a COGS (Cost Of Goods Sold) 3% higher for mushrooms produced on the right side of the container.
The performance gap is small enough to neglect it in the coming tests (we will not take in consideration the fruiting side for each test). But we will take this reality into account when updating our protocol in PART II.

Task 2. Hardwood

We tested 2 different types of hardwood products: alder pellets (supplier: Greenfull, Estonia) and coarse birch sawdust from a local sawmill. We also seized opportunities to make 2 additional tests with apple-tree pellets (same supplier, same price as alder pellets) and shiitake spent substrate[108].

Coarse birch sawdust

Pictures 27, 28: A big bag of birch sawdust emptied into an indoor silo for drying

We found this product from a local sawmill. It is a low-grade mixed-size product with most particles in a size range of 5-10 mm. These are the result of firewood sawing. Available for pickup undried (50-60% water content) directly from the factory.

[108] Shiitake substrate mainly contains hardwood, but that wood has already been digested by shiitake mushrooms.

Birch coarse sawdust, Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - Amount of potential suppliers in Finland is virtually **INFINITE**, but with variations in the product. Every new supplier requires a series of testing to check and adapt their product to our recipe.
    - Periodicity: Our local supplier can supply us **12 months a year**.
  - Scalability:
    - Supply Cost: 10€ for a big bag of 500 kg at 30-60% humidity, equivalent to a use cost at 70% humidity below **15€/ton**.
    - Processing cost: We evaluate it for this study at **1.22€/kg** (1220€/ton) which is higher than most ingredients we use, mainly because the logistics and drying were self-organised and non efficient. This cost can be reduced a lot in routinising the process.
    - If this product was used alone and without supplementation, 1 big bag would be enough to produce about 600 kg of substrate. Our local supplier produces about 2 big bags a week, which is **at least twice more than what we would need at full capacity**.

- ENVIRONMENTAL IMPACT
  - Localness: Available at **4 km** from our farm. It is the most local of all our supplies so far.
  - Circularity: We identify **1 competing use** for the raw material: energy because the product is not clean/standardised enough to comply with more noble uses. However, the product is available with a high rate of humidity which decreases the energy efficiency of its burning. Drying would not be necessary for mushroom production as long as the storage time remains short. Circularity is obvious here: the firewood would be sawn anyway, sawdust would be composted or burnt with poor efficiency for a low-value output
  - Organicity: **NO easy organic alternative**. Organic certified wood chips / sawdust are usually obtained by cutting trees from certified forests. It is difficult if not impossible to obtain when using existing side-streams.

Alder pellets

Pictures 29, 30: Grey alder pellets

<u>Alder pellets, Supply and Environmental KPI:</u>

- SUPPLY
  - <u>Availability</u>:
    - The availability of this product is high in quantities (more than what we will ever need) but since the imports from Russia have stopped, we have not found more that **one reliable supplier.**

In most cases this product is too expensive to be used in animal bedding and energy production but as we have seen during the Covid pandemic and when war started in Ukraine, such pellets become an alternative to cheaper products (softwood pellets, straw pellets) when those run out of stock. In such extreme cases they become unavailable within a few weeks.

- Periodicity: Unless supplier shortage, this product is available **12 months a year**.

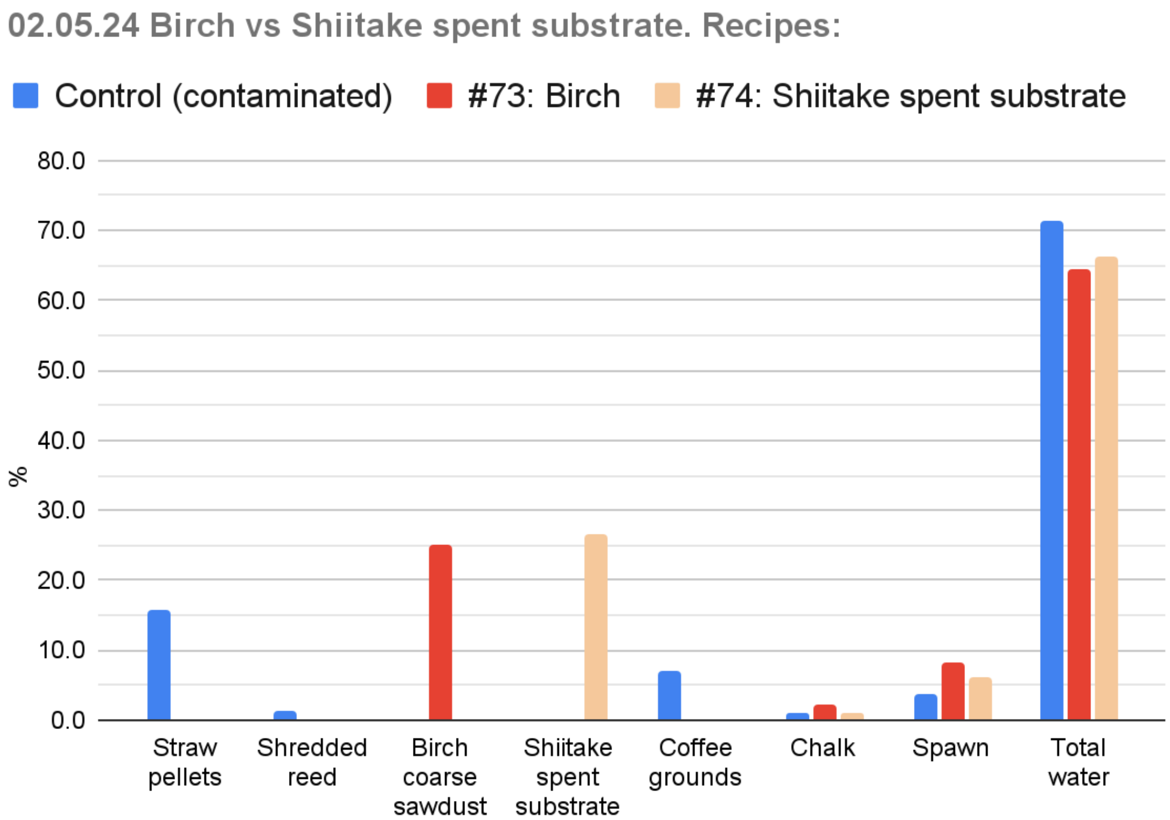
- <u>Scalability</u>:
  - Supply Cost: 640€ for a delivered pallet of 900 kg at 10% humidity, equivalent to a use cost at 70% humidity below **250€/ton**. It is almost twice as expensive as straw pellets.
  - Processing cost: We evaluate it for this study at 0.78€/kg (780€/ton), as much as for straw pellets which require the same low-temperature pasteurisation. Processing cost is highly scale-dependent.
  - If this product was used alone and without supplementation, 1 pallet would be enough to produce **2700 kg** of substrate (300 - 600 kg of mushrooms).

- ENVIRONMENTAL IMPACT
  - <u>Localness</u>: Available in sipa, Estonia, **235 km** from our farm.
  - <u>Circularity</u>: We identify **3 competing uses** for the raw material: **food-smoking, animal bedding and energy**. Food-smoking is a high-value use but it is a seasonal niche with very predictable volumes. <u>Organicity</u>: **NO identified organic alternative**.

 02.05.2024: Birch and Shiitake spent substrate

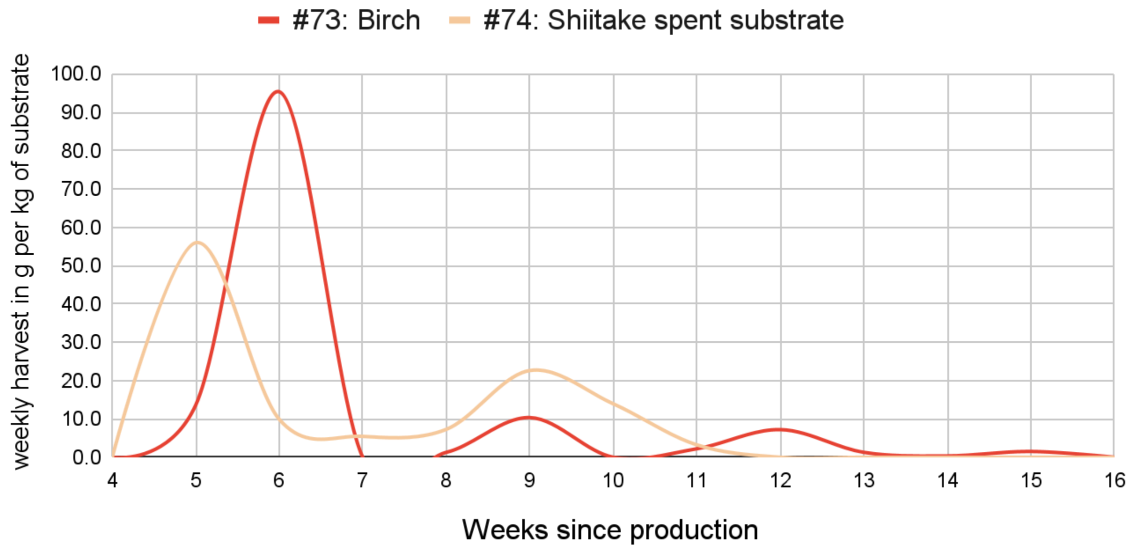
Here we compare birch coarse sawdust with shiitake spent substrate as only substrates, without other supplementation than chalk and spawn.

Section	KPI ANALYSIS ID	Birch	Spent shiitake
SUBSTRATE DATA	**TEST BATCH NUMBER**	**73**	**74**
SUBSTRATE DATA	**Substrate quantity produced** (kg)	43	25
SUBSTRATE DATA	**Substrate quantity produced** (bags)	5	1.6
SUBSTRATE DATA	**Average substrate bag weight** (kg)	8.6	15.5
SUBSTRATE DATA	**Fruiting temperature** (°C)	17.1	17.1
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity**	0.133	0.118
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity, 70 days**	0.121	0.115
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**BE** (wet mushrooms / dry substrate)	0.374	0.352
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Bag productivity** (kg/bag)	1.15	1.83
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Space productivity** (kg/bag, 70 days)	1.04	1.78
KPI: BIOLOGICAL PERFORMANCE – SPEED	**Days to end of first flush**	41	42
KPI: BIOLOGICAL PERFORMANCE – SPEED	**First flush / total** (%)	81.8	55.6
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Partial contamination** (%)	0	38
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Total contamination** (%)	0	0
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/**kg** of substrate)	0.68	0.50
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/**bag** of substrate)	5.82	7.66
KPI: ECONOMICAL PERFORMANCE	**Processing cost** (€/**kg** of substrate)	1.25	1.25
KPI: ECONOMICAL PERFORMANCE	**Processing cost** (€/**bag** of substrate)	10.71	19.27
KPI: ECONOMICAL PERFORMANCE	**COGS$_{€}$** (cost$_{€}$ / kg produced mushrooms)	14.44	14.71



Our control batch was entirely contaminated and had to be disposed of before fruiting. Therefore no performance was measured from it.

Graph 15 **Timelime of harvested oyster mushrooms**
02.05.24 **Birch vs Shiitake spent substrate**

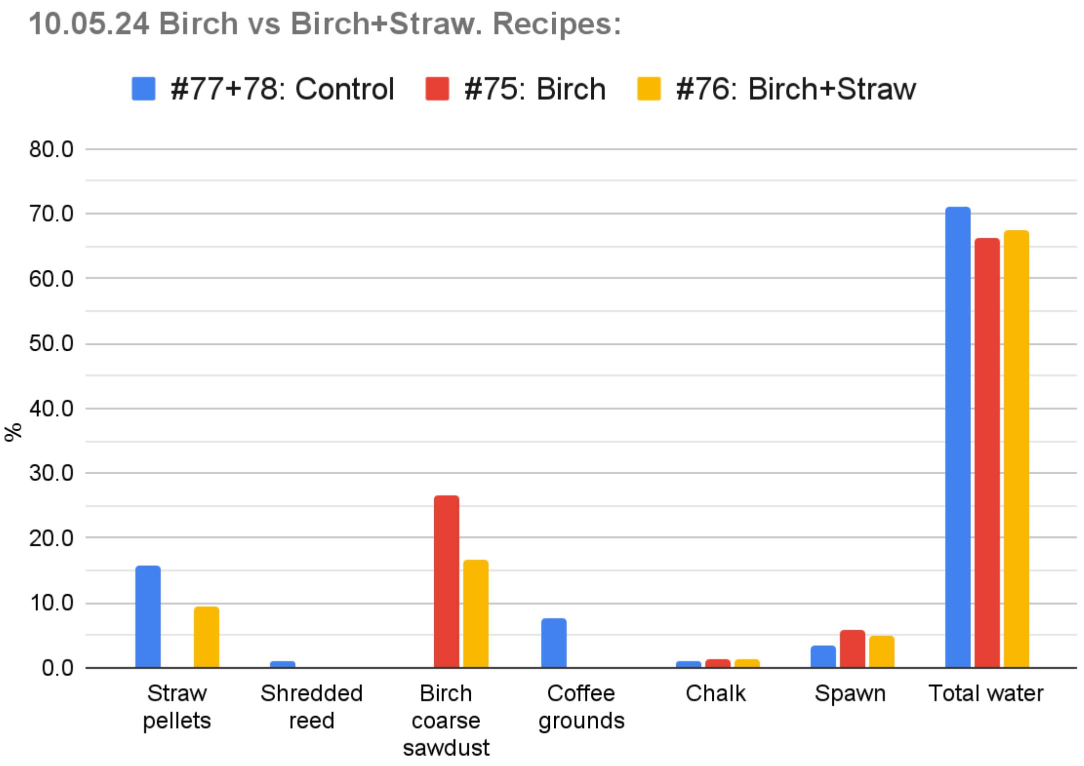


Pictures 31,32: Mushroom growth on birch and shiitake spent subs.

According to the KPI and graph above, the substrate made out of birch performs slightly better than the one made of shiitake spent substrate. However, this birch sawdust is too light to use space efficiently, as bags do not contain enough material. Shiitake might be an interesting circular economy story to work on but we decide to leave it apart for the time being, as its storage and processing is too difficult for us at the moment.
As such both recipes are commercially unusable: their output cost (almost 15€/kg of mushroom produced) would not leave us any sales margin.

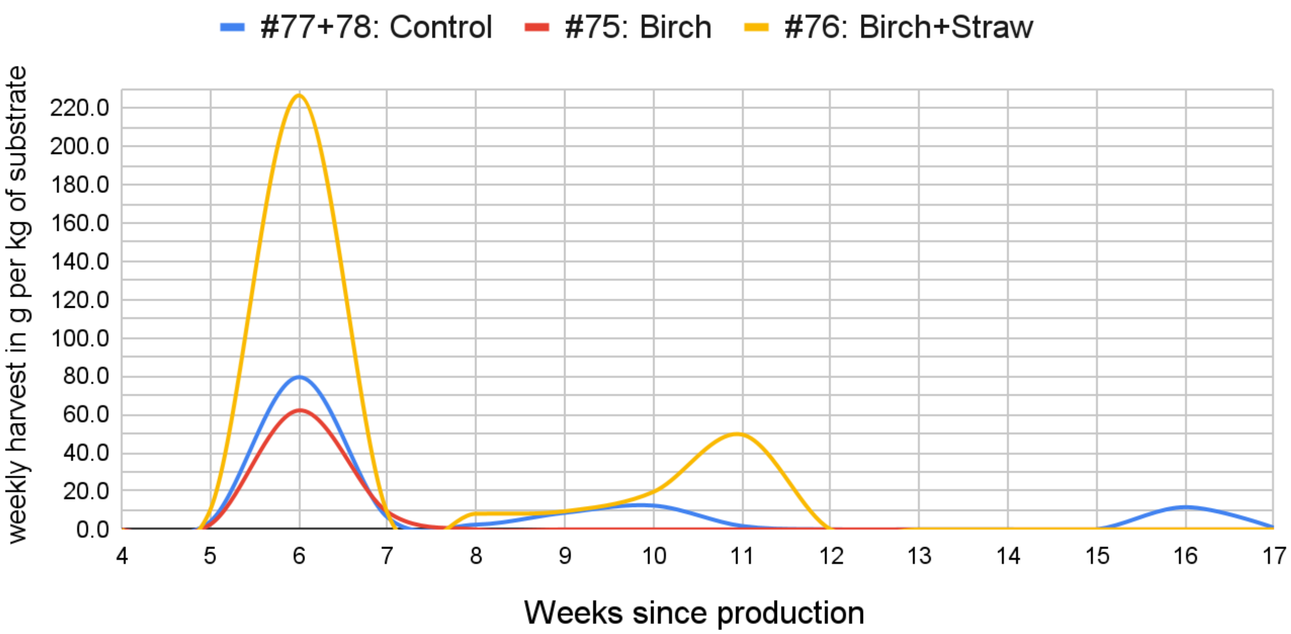
		KPI ANALYSIS ID	Control	Birch	Birch + Straw
SUBSTRATE DATA		TEST BATCH NUMBER	77+78[109]	75	76
		Substrate quantity produced (kg)	210	63	75
		Substrate quantity produced (bags)	13	5.3	6
		Average substrate bag weight (kg)	16.2	11.9	12.5
		Fruiting temperature (°C)	17.2	17.2	17.2
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.128	0.074	0.332
		Wet/wet productivity, 70 days	0.114	0.074	0.283
		BE (wet mushrooms / dry substrate)	0.443	0.218	1.025
		Bag productivity (kg/bag)	2.07	0.88	4.15
		Space productivity (kg/bag, 70 days)	1.86	0.88	3.53
	SPEED	**Days to end of first flush**	41	46	41
		First flush / total (%)	56.6	99.8	71.4
	CONT.	**Partial contamination** (%)	0	0	0
		Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.38	0.47	0.43
		Supply cost (€/**bag** of substrate)	6.22	5.58	5.39
		Processing cost (€/**kg** of substrate)	0.48	1.24	0.95
		Processing cost (€/**bag** of substrate)	7.79	14.72	11.86
		COGS€ (cost€ / kg produced mushrooms)	6.78	23.14	4.16

Here we compare birch coarse sawdust with a birch+straw pellet blend. No nitrogen-rich supplementation.



[109] Reed ratio is lower than standard controle recipe tolerance range

Graph 16 Timelime of harvested oyster mushrooms
10.05.24 Birch vs Birch+Straw



While birch alone performed less well than the control (and less than in the previous test one week earlier), today's birch+straw recipe has been the most productive of all produced test batches in this whole study:

- The only recipe that overcame 0.30 in weight productivity of which already 0.28 after 70 days
- One of the very few that overcame 100% BE

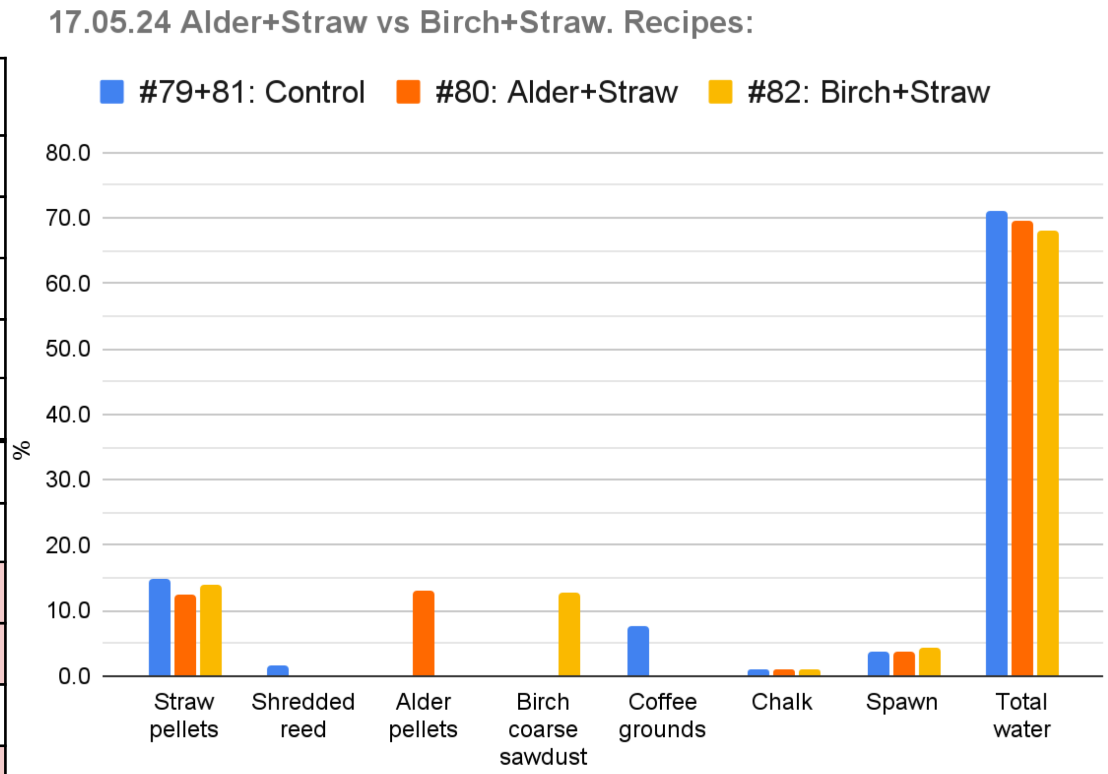
It seems birch and straw pellets work well together. To be continued.

WP2 T2.3 17.05.2024: Alder+Straw vs Birch+Straw

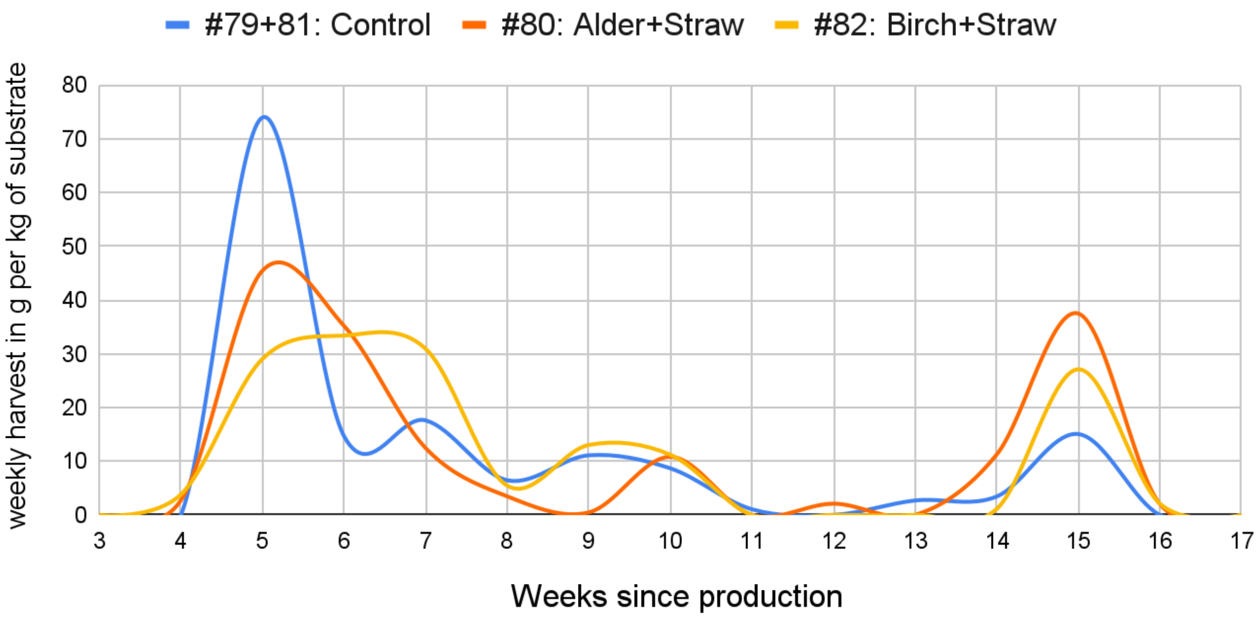
Here we compare blends of alder+straw pellets and birch+straw pellets. No nitrogen-rich supplementation.

From this week onwards, the control recipe was slightly modified with +30% of reed straw to reduce straw pellet clumps in mixing.

		KPI ANALYSIS ID	Control	Alder + Straw	Birch + Straw
SUBSTRATE DATA		TEST BATCH NUMBER	79+81	80	82
		Substrate quantity produced (kg)	198	95	85
		Substrate quantity produced (bags)	11.6	6.5	6.2
		Average substrate bag weight (kg)	17.2	14.6	13.7
		Fruiting temperature (°C)	17.7	17.7	17.7
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.155	0.163	0.157
		Wet/wet productivity, 70 days	0.133	0.111	0.127
		BE (wet mushrooms / dry substrate)	0.537	0.537	0.493
		Bag productivity (kg/bag)	2.61	2.39	2.16
		Space productivity (kg/bag, 70 days)	2.26	1.62	1.74
	SPEED	**Days to end of first flush**	45	44	45
		First flush / total (%)	63.9	56.3	57.4
	CONT.	**Partial contamination** (%)	32	0	0
		Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.41	0.45	0.40
		Supply cost (€/**bag** of substrate)	7.10	6.65	5.51
		Processing cost (€/**kg** of substrate)	0.51	0.47	0.83
		Processing cost (€/**bag** of substrate)	8.81	6.84	11.35
		$COGS_€$ ($cost_€$ / kg produced mushrooms)	6.30	5.63	7.80



Graph 17 Timelime of harvested oyster mushrooms
17.05.24 Alder+Straw vs Birch+Straw



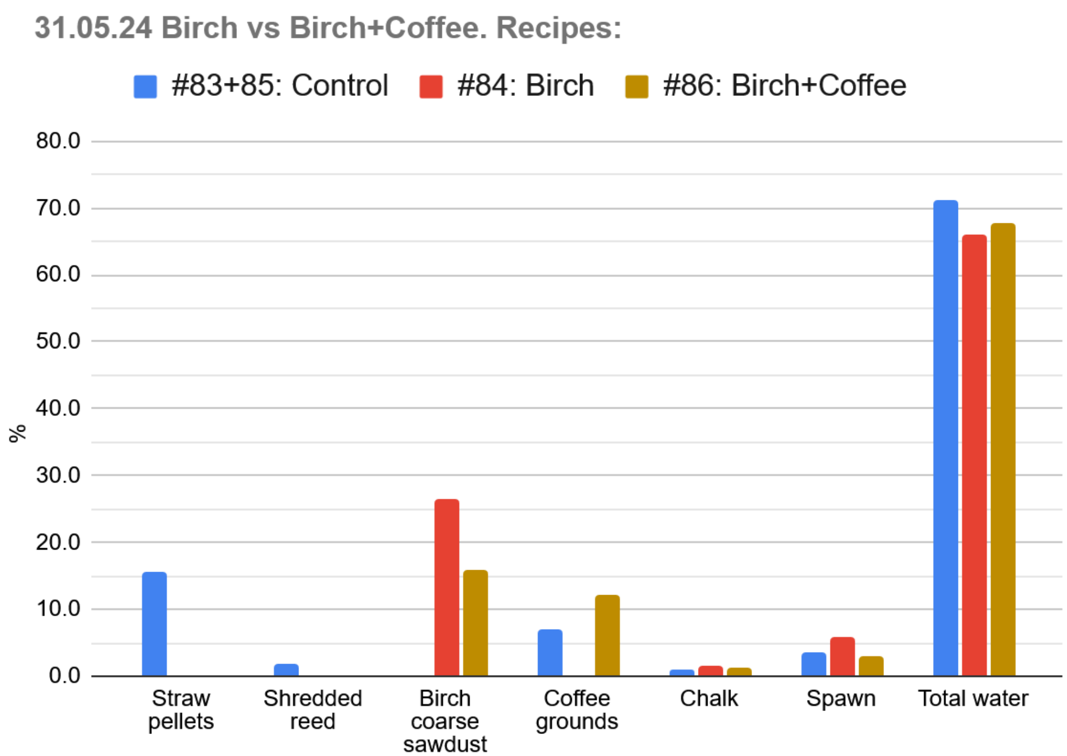
All recipes from today's tests give comparable performance indicators (see table above) but the control was slightly faster, with a bigger first flush. Wood-containing substrates looked very healthy after third flush and were thus kept 5 more weeks, giving a remarkable fifth flush, especially the alder+straw substrate.

The striking result here is BIrch+Straw (#82, yellow) which with a recipe close to the one tested one week earlier in WP2 T2.2, gave twice a smaller productivity. We do not have an explanation for this difference.

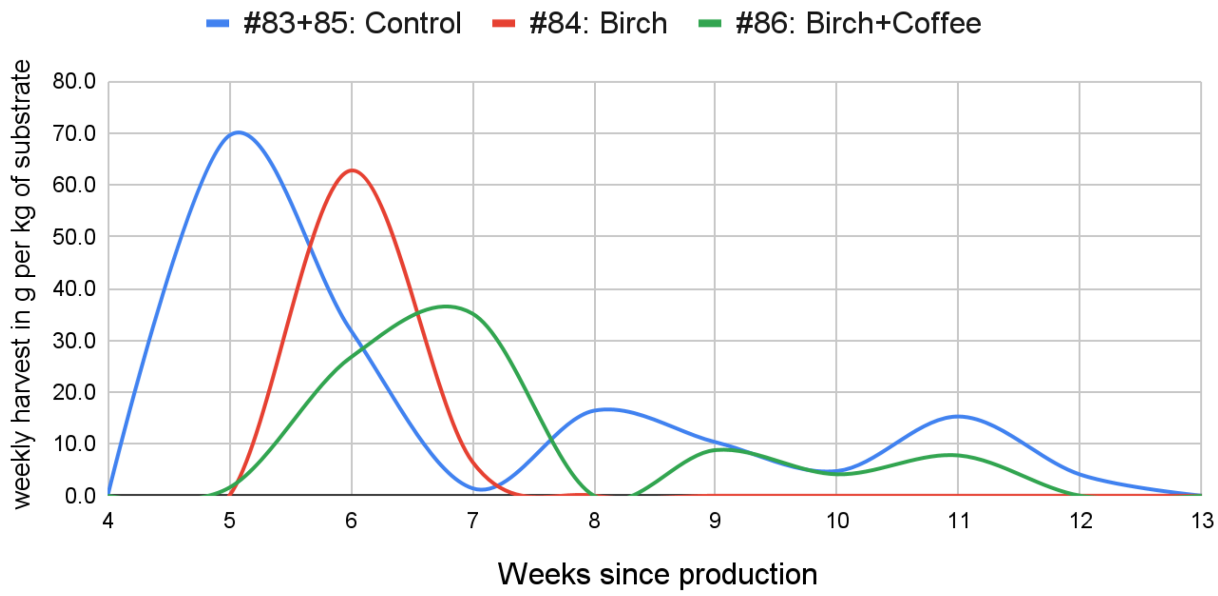
WP2 T2.4 31.05.2024: Birch vs Birch+Coffee

Here we study coffee supplementation on birch-based substrates.

		KPI ANALYSIS ID	Control	Birch	Birch + Coffee
SUBSTRATE DATA		TEST BATCH NUMBER	83+85	84	86
		Substrate quantity produced (kg)	192	61	107
		Substrate quantity produced (bags)	11.5	5	15.3
		Average substrate bag weight (kg)	16.7	12.2	15.3
		Fruiting temperature (°C)	19.3	19.3	19.3
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.154	0.069	0.084
		Wet/wet productivity, 70 days	0.134	0.069	0.076
		BE (wet mushrooms / dry substrate)	0.534	0.204	0.261
		Bag productivity (kg/bag)	2.57	0.85	1.29
		Space productivity (kg/bag, 70 days)	2.24	0.85	1.17
	SPEED	**Days to end of first flush**	39	47	47
		First flush / total (%)	64.9	100	74.9
	CONT.	**Partial contamination** (%)	0	0	43
		Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.42	0.48	0.27
		Supply cost (€/**bag** of substrate)	7.09	5.91	4.06
		Processing cost (€/**kg** of substrate)	0.52	1.24	0.85
		Processing cost (€/**bag** of substrate)	8.68	15.11	13.00
		$COGS_€$ ($cost_€$ / kg produced mushrooms)	6.15	24.85	13.24



Graph 18 31.05.24 **Timelime of harvested oyster mushrooms Birch vs Birch+Coffee**



As for similar recipes in WP2 T2.1 and WP2 T2.2, unsupplemented birch (#84) colonised smoothly, gives a healthy aspect after incubation but produces poorly. In this case performance is similar as batch #75 in WP2 T2.2 with a mediocre first flush and no second flush (probably just coming too slow to observe within 15 weeks). We observe that batch #73 in WP2 T2.1 performed noticeably better than the other two. One reason might be its higher spawn rate (+45% compared to both others) which in such quantities can be assimilated to a decent starch supplementation.

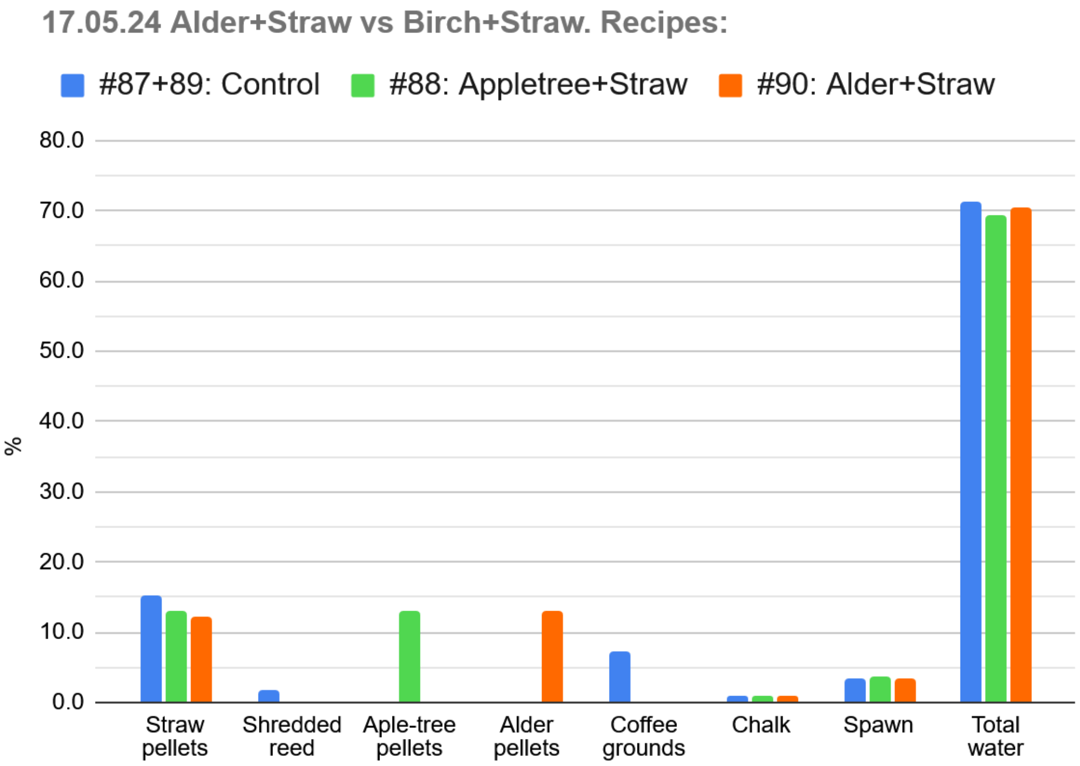
Coffee supplementation improved the consistency of the substrate, making it more dense. The coffee ratio might have been too high in this case especially in regard of the spawn rate and contamination was clearly visible on almost half of the produced bags, probably partly explaining the poor results. However, this recipe is the only one tested that contains (except for chalk and spawn) only very local, low-value and well-available materials with high potentials in sustainability and supply-shortage resilience. However it cannot be economical with such poor performance. Worth further investigation and developments[110].

[110] In the continuation project, Clean & Local Mushroom Substrate Development PART II

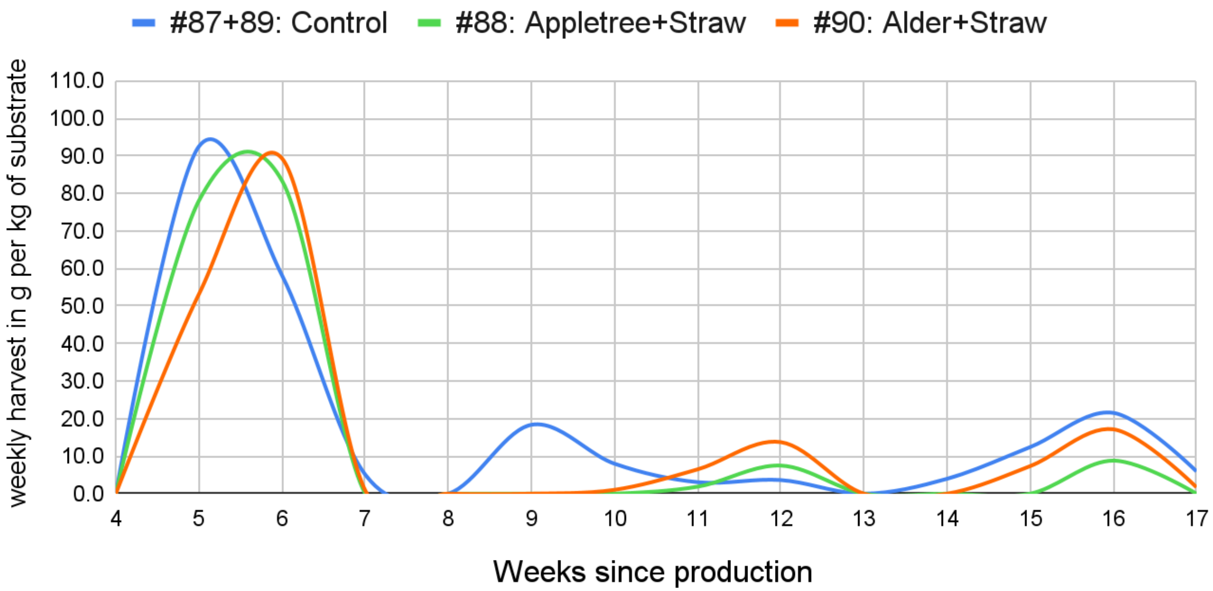
WP2 T2.5 07.06.2024: Apple-tree+Straw vs Alder+Straw

We compare here performances of apple-tree and alder pellets, both in association with straw pellets

		KPI ANALYSIS ID	Control	Appletree + Straw	Alder + Straw
SUBSTRATE DATA		TEST BATCH NUMBER	87+89	88	90
		Substrate quantity produced (kg)	195	45	145
		Substrate quantity produced (bags)	12	3	9.5
		Average substrate bag weight (kg)	16.3	15.1	15.3
		Fruiting temperature (°C)	19.0	19.0	19.0
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.238	0.194	0.193
		Wet/wet productivity, 70 days	0.182	0.161	0.144
		BE (wet mushrooms / dry substrate)	0.827	0.632	0.652
		Bag productivity (kg/bag)	3.87	2.94	2.95
		Space productivity (kg/bag, 70 days)	2.96	2.44	2.20
	SPEED	**Days to end of first flush**	37	37	40
		First flush / total (%)	63.0	83.0	73.7
	CONT.	**Partial contamination** (%)	0	0	0
		Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.40	0.45	0.42
		Supply cost (€/**bag** of substrate)	6.49	6.78	6.38
		Processing cost (€/**kg** of substrate)	0.51	0.47	0.46
		Processing cost (€/**bag** of substrate)	8.30	7.06	6.94
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	3.83	4.71	4.52



Graph 19 07.06.24 **Timelime of harvested oyster mushrooms Alder+Straw vs Apple-tree+Straw**



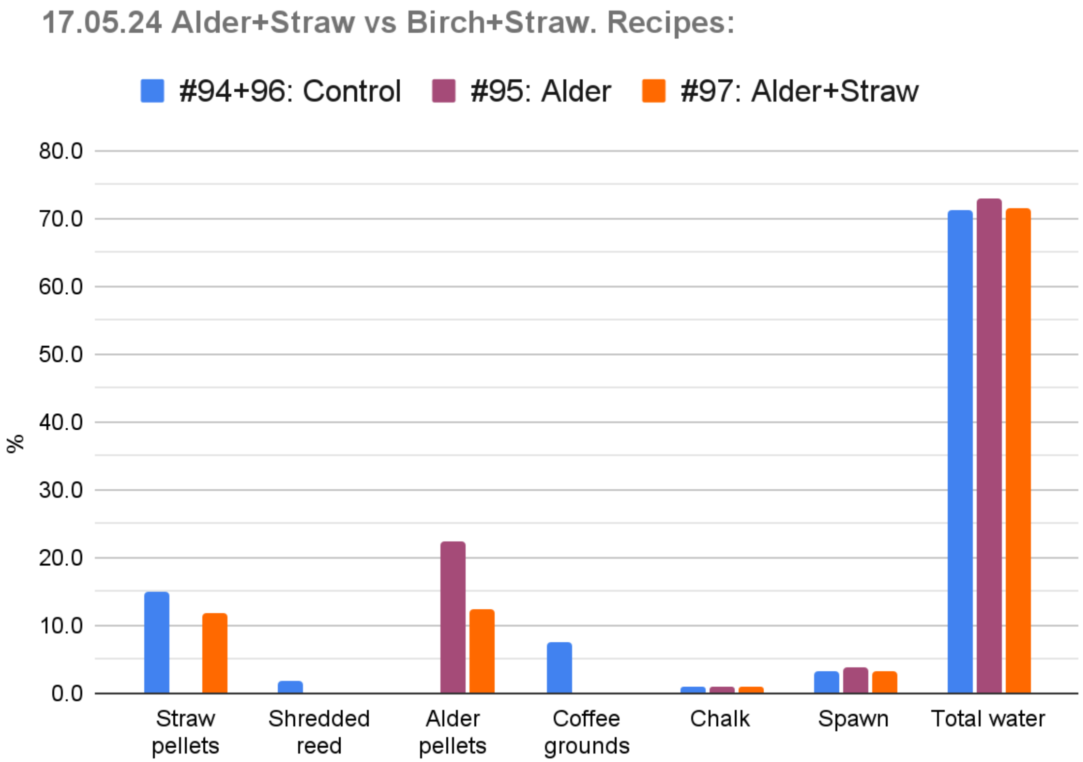
As most wood-containing substrates, apple-tree and alder containing substrates are slow-growing and give their second flush very late. There is no significant difference between apple-tree and alder tests. However, alder is a more available resource than apple-tree so we do not need to continue testing apple-tree.

All of these substrate batches gave good results but out of the 3 recipes, the control recipe performed significantly better. To be continued with nitrogen-rich supplemented wood-based substrates.

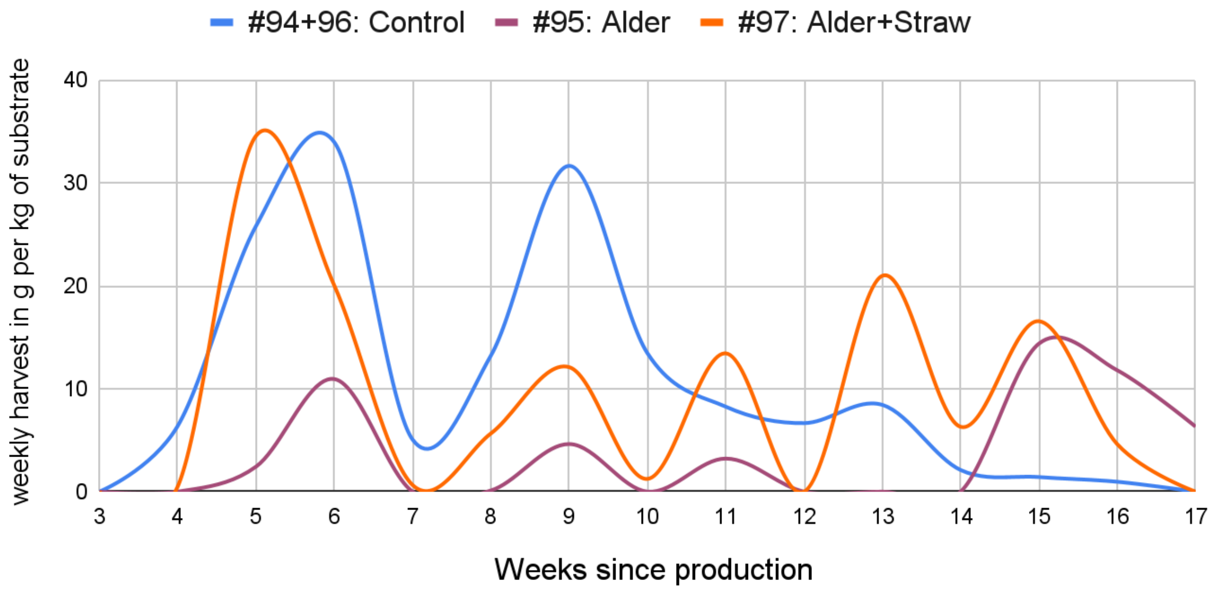
WP2 T2.6 28.06.2024: Alder vs Alder+Straw

We are here doing the same tests as on 10.05.2024 (WP2 T2.2) but with alder pellets instead of birch sawdust.

		KPI ANALYSIS ID	Control	Alder	Alder + Straw
SUBSTRATE DATA		TEST BATCH NUMBER	94+96	95	97
		Substrate quantity produced (kg)	198	89	101
		Substrate quantity produced (bags)	12.4	5.5	6.5
		Average substrate bag weight (kg)	15.9	16.1	15.5
		Fruiting temperature (°C)	19.1	19.1	19.1
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.158	0.054	0.137
		Wet/wet productivity, 70 days	0.130	0.018	0.075
		BE (wet mushrooms / dry substrate)	0.548	0.198	0.479
		Bag productivity (kg/bag)	2.52	0.87	2.12
		Space productivity (kg/bag, 70 days)	2.06	0.29	1.16
	SPEED	**Days to end of first flush**	40	38	42
		First flush / total (%)	42.9	24.8	40.3
	CONT.	**Partial contamination** (%)	39	0	54
		Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.40	0.47	0.41
		Supply cost (€/**bag** of substrate)	6.30	7.63	6.36
		Processing cost (€/**kg** of substrate)	0.51	0.45	0.45
		Processing cost (€/**bag** of substrate)	8.09	7.19	6.95
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	5.90	17.09	6.28



Graph 20 **Timelime of harvested oyster mushrooms**
28.06.24 **Alder vs Alder+Straw**



These tests gave a surprisingly small first flush, even our control which otherwise performed normally. Something must have happened in the climate conditions before/during first flush but we have not identified it.

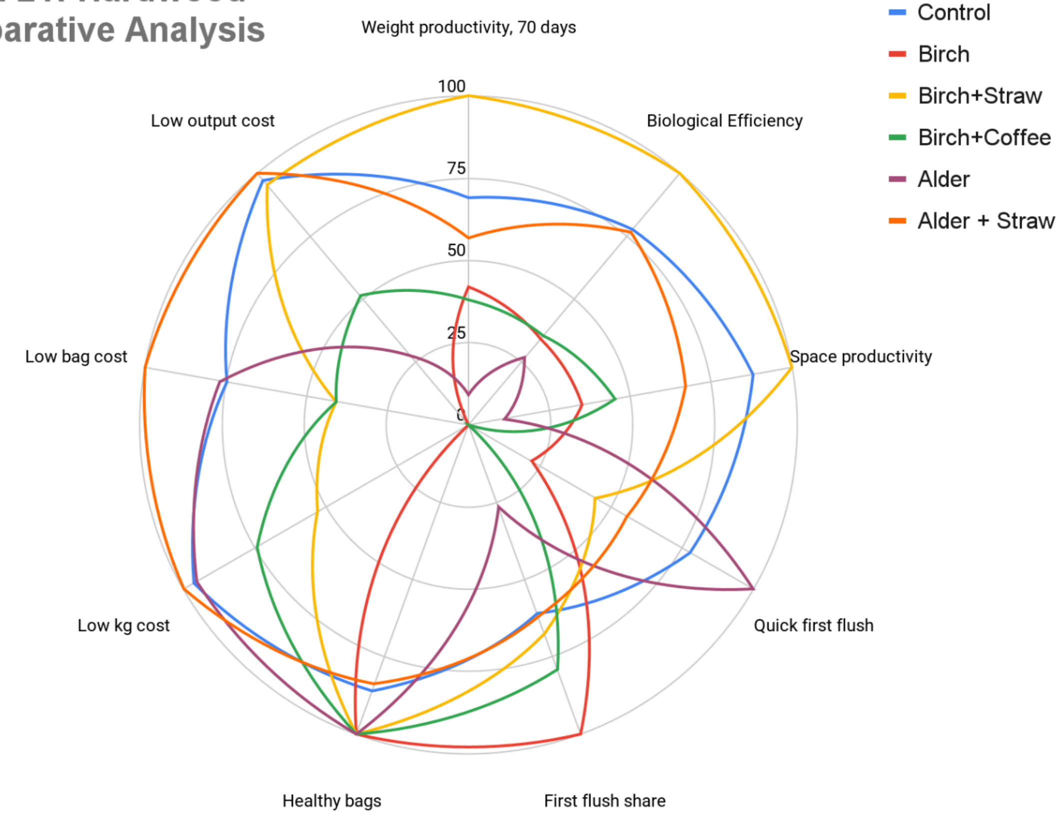
Despite this, alder alone gave even worse results than birch alone (WP2 T2.1, T2.2, T2.4). As in WP2 T2.5, alder+straw performed less well than our control but here also, we have not supplemented our tests with any nitrogen-rich ingredient.

WP2 T2.7 Hardwood comparative study

We compare here KPI from all wood-based recipes tested in WP2 T2. We do not include our spent shiitake recipe (WP2 T2.1) and apple-tree recipe which were small-sized additional (opportunistic) tests.
KPI analysis below contains relevant sums / weighted averages of comparable recipes for each KPI.

		KPI ANALYSIS ID	Control	Birch	Birch+Straw	Birch+Coffee	Alder	Alder + Straw
SUBSTRATE DATA		**TEST BATCH NUMBER**	**77,78,79,81,83,85, 87,89,94,96**	**73,75,84**	**76,82**	**86**	**95**	**80,90,97**
		Substrate quantity produced (kg)	992	200	160	107	89	341
		Average substrate bag weight (kg)	16.5	11.2	13.2	15.3	16.1	15.2
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.166	0.087	0.239	0.084	0.054	0.168
		Wet/wet productivity, 70 days	0.138	0.084	0.200	0.076	0.018	0.114
		BE (wet mushrooms / dry substrate)	0.576	0.253	0.742	0.261	0.198	0.569
		Bag productivity (kg/bag)	2.72	0.93	3.09	1.29	0.87	2.55
		Space productivity (kg/bag, 70 days)	2.27	0.91	2.58	1.17	0.29	1.73
	SPEED	**Days to end of first flush**	40	45	43	47	38	42
		First flush / total (%)	58	95	64	75	25	59
	CONT.	**Partial contamination** (%)	14	0	0	0	0	16
		Total contamination (%)	0	0	0	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Total substrate cost** (€/**kg** of substrate)	0.91	1.77	1.30	1.11	0.92	0.88
		Total substrate cost (€/**bag** of substrate)	14.96	19.59	17.04	17.06	14.82	13.37
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	5.80	21.52	6.10	13.24	17.09	5.35

Graph 21: Hardwood Comparative Analysis



The spider chart below shows a selection of these KPI. Best performances are towards the outside of the chart, lowest performances are towards the middle.

Optimums in this type of graph are figures with a maximal area. Here the best group for biological performance (towards the right of the chart) is **Birch+Straw** recipes, at least when talking about productivity. At the same time birch sawdust is a local, low-value and well-available resource that would also increase sustainability and circularity in our farm.
The best groups for economical performance (towards the left of the chart) are the **Control** recipe and the **Alder+Straw** recipes**.** Economical performance of birch chips may be greatly improved with appropriate logistic and production chains as the resource itself is almost free.

Alder is a more standardised and therefore more homogeneous product with a more reliable quality over time than our local birch sawdust. Its potential added value compared to straw-only recipes will be studied further in the second part of the project. As far as we could observe here, alder can hold slightly less water than straw but gives a less sticky consistency to the mix, enabling a more homogeneous mixing and an easy bagging.

Hardwood alone is of no interest according to this study. However, proper ratios of ingredients to associate with birch or alder is to be determined in the second part of the project. Coffee does not appear to be (at least alone) the most adapted supplement for birch-based substrates.

Task 3. Chopped Straw

Cereal straw is probably the most-used ingredient in oyster mushroom substrates across Europe. Here we study its feasibility in our operations.

Picture 33: Manual pickup of chopped cereal straw from a harvested field

Chopped straw, Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - Several farms in our region produce grain and are therefore potential straw suppliers. However, some of them also have animals and use their entire straw productions. Most others that we contacted usually chop straw directly at grain harvest and leave it on the field to fertilize their soil. Having a few hectares of straw baled is possible, but only on dry years, otherwise humid bales will eventually heat up until all available nutrients are burnt or worse, auto-ignite at risk of burning entire storage facilities. No farmer in our neighbourhood uses small-size straw balers, meaning we would need motorised machinery to move straw around our storage and production facilities. Straw bales also take 2 to 3 times more space per weight than our currently in-use straw pellets. Whole straw is not a product we can use, as it will not mix properly with other ingredients. What we need is chopped straw, but in this region **we have not found suppliers for it**. Chopping whole straw is possible on site but requires investments and space. Once chopped, straw takes even more space than baled.
    - Straw is a **seasonal product** (produced at the end of summer / early autumn) and depending on the year, available stocks might run out before the next harvest. Therefore the needed quantities would require a one-year overview of our needs and enough space to store that one-year needed amount.

  - Scalability:
    - Supply Cost: We cannot evaluate it due to the lack of supply. Our self-organised manual pickup of chopped straw scattered on the field cost us **10€/kg** (10000€/ton) in driving and work time. No scaling is possible at this cost.
    - Processing cost: We evaluate it for this study at **1.63€/kg** (780€/ton) in hydration and pasteurisation work. This could be improved if scaled.
    - For our current needs and if we used unsupplemented straw only, we would need about 5 tons of it per year. This is the average seasonal straw production on **2 hectares of cereal fields**.

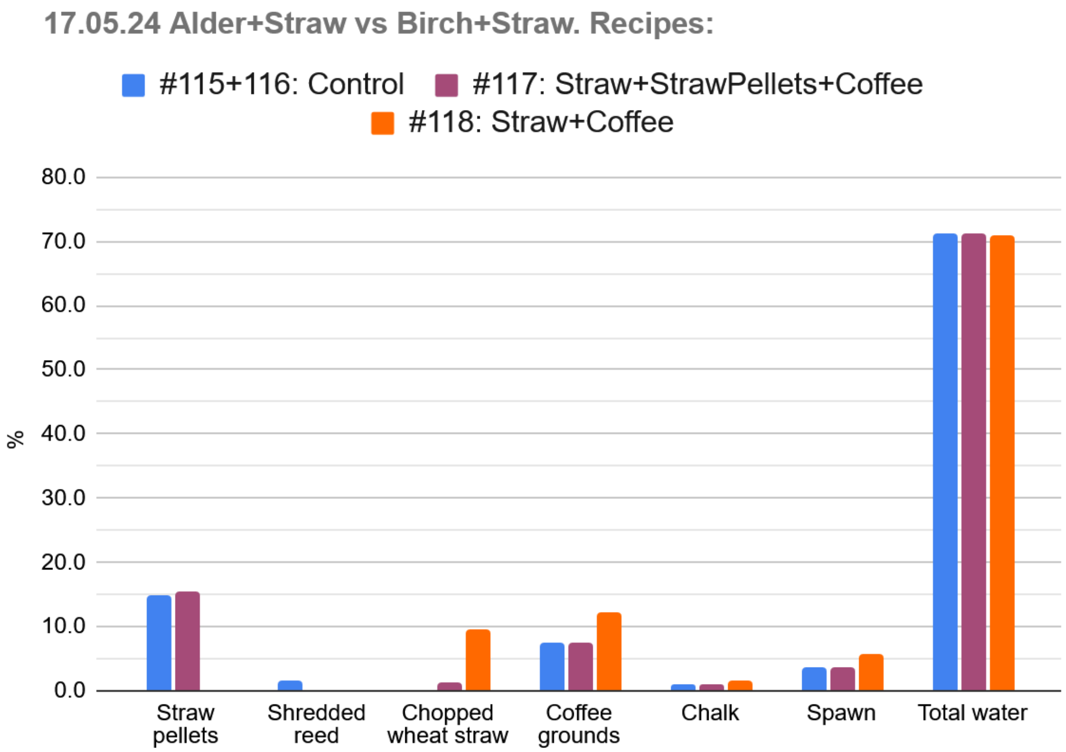
- ENVIRONMENTAL IMPACT
  - Localness: No obvious local supplier found.
  - Circularity: Straw is considered a by-product for cereal agriculture but it has in real life lots of well-established uses ranging from high-value insulation to lower-value fertilizing. Most of harvested straw is used for animal bedding and farmers tend to harvest less and less to save on fertilizing and work.
  - Organicity: Organic cereal farms are common in our region, all being potential organic straw suppliers. But making the product “chopped organic dry straw” available is beyond our reach for now.

WP2 T3.1 21.08.2024: Straw+Coffee vs Straw+StrawPellets+Coffee

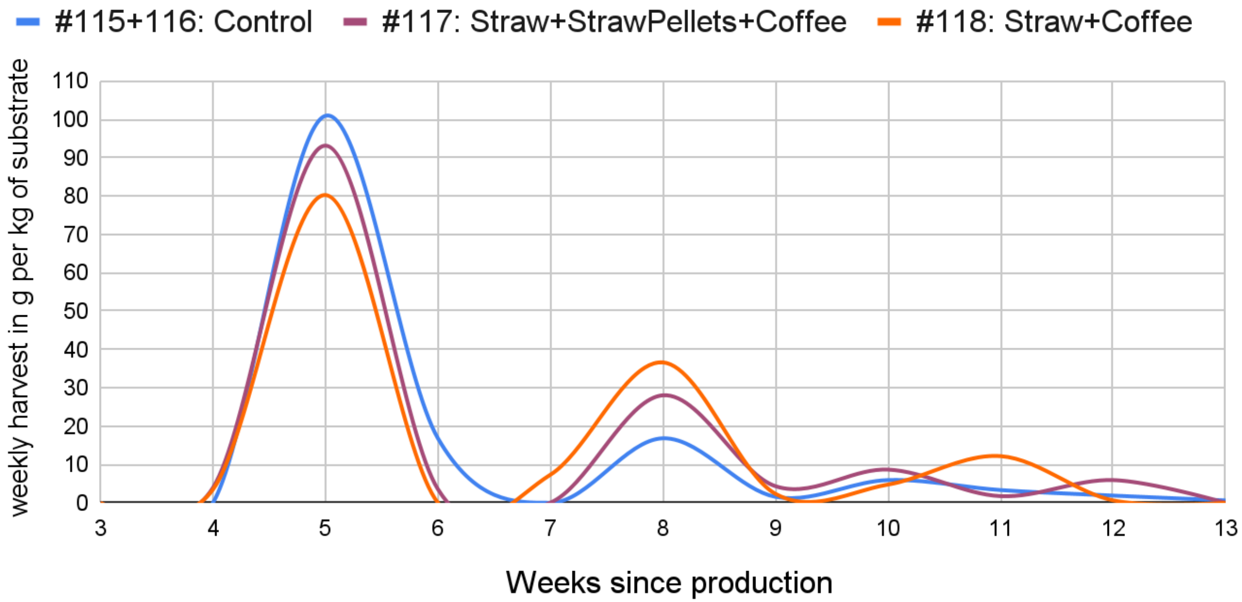
Birch recipe in WP2 T2 taught us that light materials are not adapted to our small-scale operations if not associated with denser ingredients. Therefore, and because our straw supply was difficult and costly, we skipped straw-only recipes to focus on two higher-potential recipes, supplemented with coffee. The second recipe was designed so as to copy the control recipe with chopped wheat straw instead of chopped common reed.

Due to the low density of chopped straw compared to other ingredients, it was difficult to homogenize our third recipe in proportions with other recipes

		KPI ANALYSIS ID	Control	Str., Pellets, Coffee	Straw + Coffee
SUBSTRATE DATA		TEST BATCH NUMBER	115+116	117	118
		Substrate quantity produced (kg)	198	100	62
		Substrate quantity produced (bags)	12	5.7	4
		Average substrate bag weight (kg)	16.5	17.6	15.5
		Fruiting temperature (°C)	14.2	14.2	14.2
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.148	0.149	0.148
		Wet/wet productivity, 70 days	0.142	0.142	0.135
		BE (wet mushrooms / dry substrate)	0.513	0.516	0.510
		Bag productivity (kg/bag)	2.44	2.63	2.29
		Space productivity (kg/bag, 70 days)	2.35	2.50	2.09
	SPEED	**Days to end of first flush**	38	36	34
		First flush / total (%)	79.4	67.4	56.7
	CONT.	**Partial contamination** (%)	59	53	25
		Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.41	0.52	1.61
		Supply cost (€/**bag** of substrate)	6.81	9.17	24.89
		Processing cost (€/**kg** of substrate)	0.51	0.44	0.54
		Processing cost (€/**bag** of substrate)	8.45	7.80	8.44
		COGS€ (cost€ / kg produced mushrooms)	6.25	6.45	14.57



Graph 22 Timelime of harvested oyster mushrooms
21.08.24 Supplemented Straw+Straw pellets vs Straw



Picture 34: Aspect of Batch #118 after mixing, before bagging

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Results of these tests give very homogenous biological performance indicators despite the very different aspect and recipe of batch #118. The most interesting observation concerns the size of the first flush, decreasing with the amount of chopped straw. On the contrary, the second flush of the recipe which does not contain pellets is noticeably bigger than with other recipes. However, the variability in recipe proportions (especially coffee grounds and spawn) as well as mediocre health of many bags do not allow more precise conclusions.

As we noticed since we started to use straw pellets many years ago, straw is a decent carbon base for any oyster mushroom recipe. However a lack of local suppliers for chopped straw leads us to stick to pellets for now. Processing cereal straw ourselves into a mushroom substrate friendly product is not out of the question but would require a long work on the whole supply chain, from the field to the factory.

Task 4. Ground Oat husk

Oat husk was the base of our oyster mushroom substrates until we found out that straw pellets performed better, especially thanks to a far higher water retention capacity. We later made the hypothesis that ground oat husks may hold more water than whole husks, maybe even as much as straw. This is the type of product we want to study here.

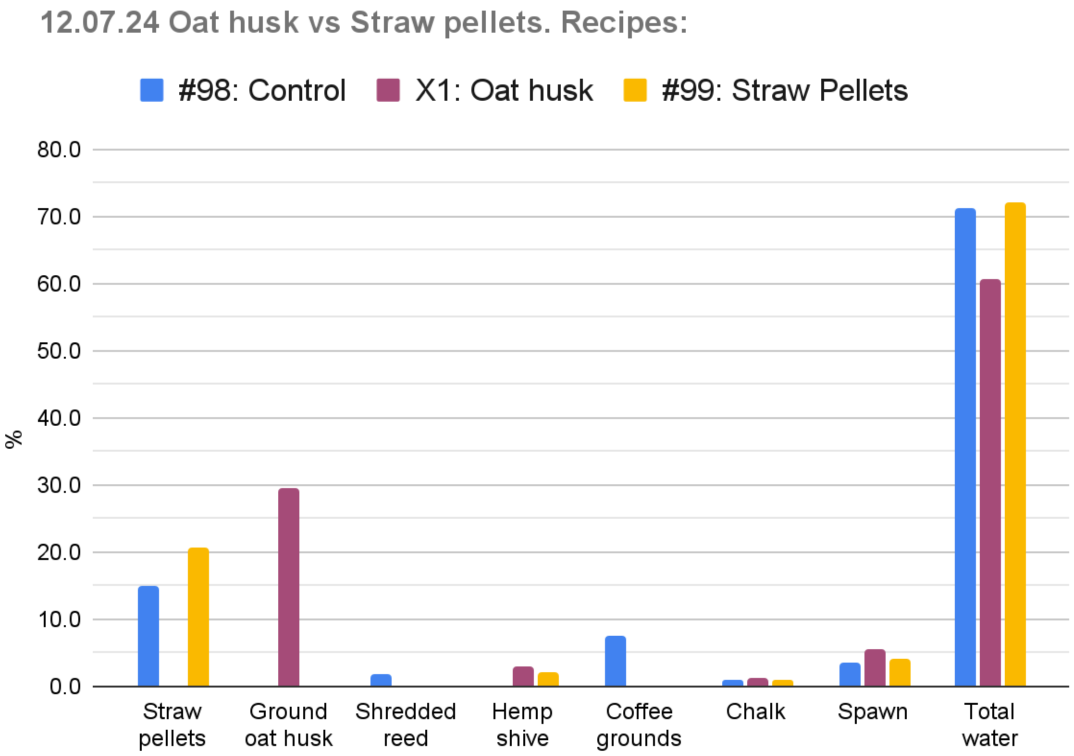
Picture 35: Dry ground oat husk

Picture 36: Wet ground oat husk

Ground oat husk, Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - Oat husks is a very common side-product in local cereal farms. However, grinding it requires the use of a mill, where it competes with more valuable products (grains). We found **one local supplier** who can grind oat husk on demand.
    - Periodicity: As wheat straw, oat husk is a seasonal product and requires thorough planning and storage to ensure yearly availability.
  - Scalability:
    - Our tests below show such poor results that no scalability is considered possible at this stage.
- ENVIRONMENTAL IMPACT
  - Localness: Our identified supplier is located only **19 km** from our farm.
  - Circularity: Oat husks are a semi-low value side-product for which we identified **2 competing uses**: it is somewhat used for animal feeding, especially reindeers. But its production is bigger than what animals are fed with so most of it is burnt. Mushroom production would be a great added value for this product.
  - Organicity: **The local supplier we found is an organic farm/mill**, so as the one with whom we worked earlier (non-ground product). Their husks are therefore organic.

WP2 T4.1 12.7.2024: Ground oat husk vs Straw pellets

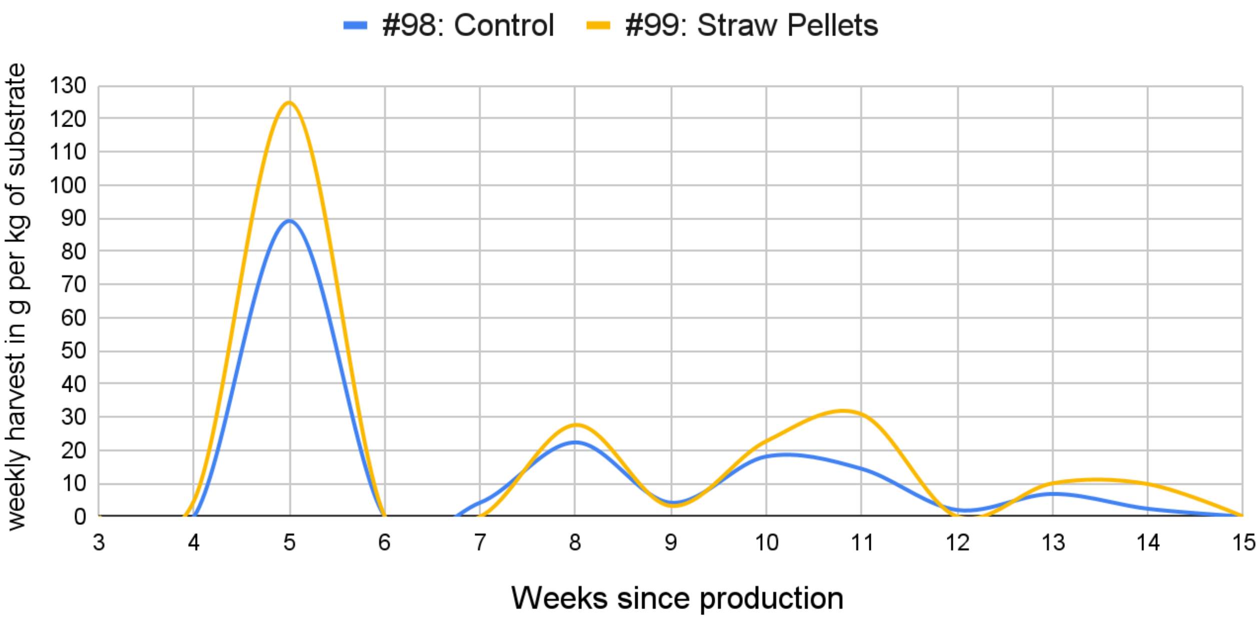


Despite a very fine particle size, oat husk has a poor water retention capacity (1.5L/kg versus 2.5L/kg). Therefore we needed to adapt the recipe (otherwise common with the straw pellet recipe) to lower the water ratio but heavy water dripping was still observed. Ground oat husks were here treated with our low-temperature pasteurisation method as were straw pellets. It is clearly not sufficient to kill the load of spores they contain. Every bag was contaminated with trichoderma molds within 10 days.

Section	KPI ANALYSIS — ID	Control	Oat husk	Straw pellets
SUBSTRATE DATA	**TEST BATCH NUMBER**	**98**	**X1**	**99**
SUBSTRATE DATA	**Substrate quantity produced** (kg)	166[111]	66	87
SUBSTRATE DATA	**Substrate quantity produced** (bags)	10.3[111]	4	6
SUBSTRATE DATA	**Average substrate bag weight** (kg)	16.1	16.5	14.5
SUBSTRATE DATA	**Fruiting temperature** (°C)	18.1		18.1
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity**	0.164		0.234
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity, 70 days**	0.138		0.183
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**BE** (wet mushrooms / dry substrate)	0.568		0.837
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Bag productivity** (kg/bag)	2.64		3.40
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Space productivity** (kg/bag, 70 days)	2.23		2.66
KPI: BIOLOGICAL PERFORMANCE – SPEED	**Days to end of first flush**	34		32
KPI: BIOLOGICAL PERFORMANCE – SPEED	**First flush / total** (%)	54.5		55.3
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Partial contamination** (%)	10[111]		0
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Total contamination** (%)	16[112]	100	0
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/**kg** of substrate)	0.41		0.45
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/**bag** of substrate)	6.65		6.48
KPI: ECONOMICAL PERFORMANCE	**Processing cost** (€/**kg** of substrate)	0.51		0.46
KPI: ECONOMICAL PERFORMANCE	**Processing cost** (€/**bag** of substrate)	8.25		6.70
KPI: ECONOMICAL PERFORMANCE	**COGS$_€$** (cost$_€$ / kg produced mushrooms)	5.65		3.87

[111] After contaminated bags deduction
[112] Before contaminated bags deduction

Graph 23 Timelime of harvested oyster mushrooms
12.07.24 Straw pellets



Batch number #99, initially produced to compare straw pellets with oat husk (X1), gave much higher KPI than the control. Every single of the 4 flushes we harvested was more productive, as visible on the graph (left). It was also more healthy than the control.

It is possible that hemp shives perform better than reed in association with straw pellets. This would however contradict a preliminary test we made earlier[113].

It is also possible that the control recipe's coffee was not properly pasteurised (unlikely) or contained improper chemicals, like bacteria toxins (more likely[114]).

Picture 37: Pleasant texture of test #99 after mixing.

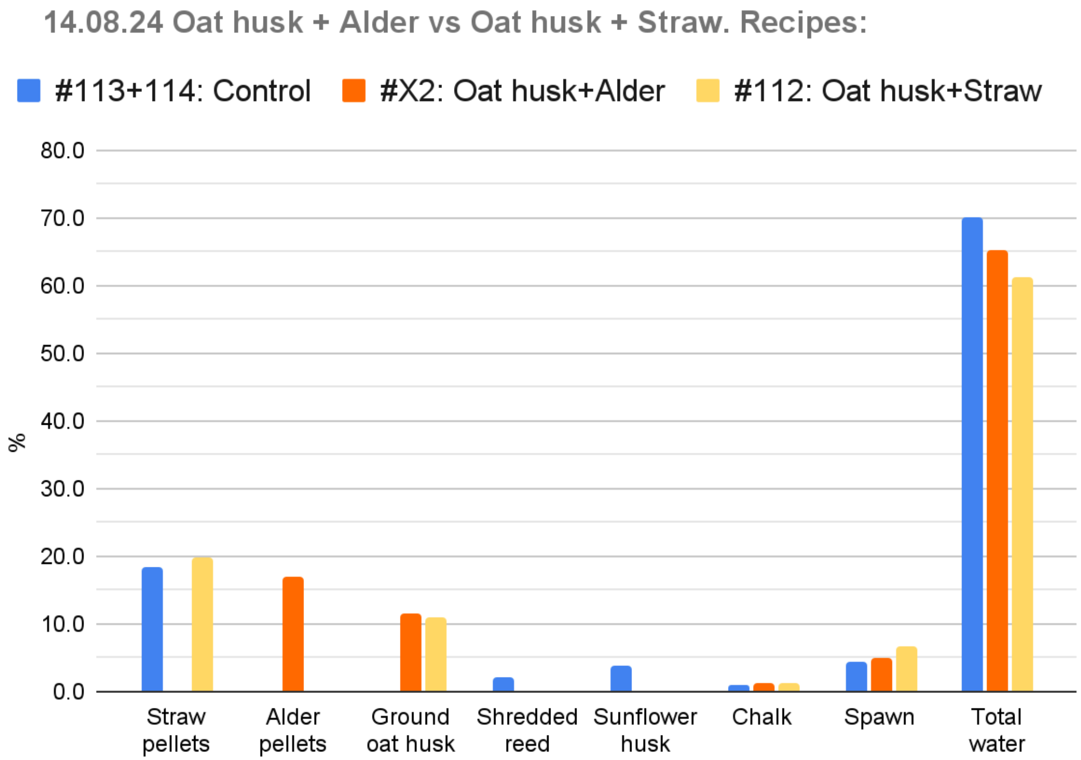
Picture 38: #99 (right) fruiting slightly earlier than control recipe (left)

[113] WP1 T3.2. Hemp shives: HEMP versus REED

[114] Our pasteurisation protocol is always the same and has proven to work consistently. But coffee pre-ferment easily before pasteurisation and we do not always detect it.

Section	KPI ANALYSIS / ID	Control[115]	Oat husk + Alder	Oat husk + Straw
SUBSTRATE DATA	**TEST BATCH NUMBER**	**113+114**	**X2**	**112**
SUBSTRATE DATA	**Substrate quantity produced** (kg)	162	74	29[111]
SUBSTRATE DATA	**Substrate quantity produced** (bags)	11.3	5	2[111]
SUBSTRATE DATA	**Average substrate bag weight** (kg)	14.3	14.8	14.7
SUBSTRATE DATA	**Fruiting temperature** (°C)	14.7		14.7
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity**	0.199		0.234
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity, 70 days**	0.197		0.231
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**BE** (wet mushrooms / dry substrate)	0.664		0.706
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Bag productivity** (kg/bag)	2.82		3.41
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Space productivity** (kg/bag, 70 days)	2.79		3.41
KPI: BIOLOGICAL PERFORMANCE – SPEED	**Days to end of first flush**	34		31
KPI: BIOLOGICAL PERFORMANCE – SPEED	**First flush / total** (%)	69.8		45.2
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Partial contamination** (%)	62		100
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Total contamination** (%)	0	100	50[112]
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/**kg** of substrate)	0.51		0.45
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/**bag** of substrate)	7.26		6.63
KPI: ECONOMICAL PERFORMANCE	**Processing cost** (€/**kg** of substrate)	0.57		0.43
KPI: ECONOMICAL PERFORMANCE	**Processing cost** (€/**bag** of substrate)	8.21		6.39
KPI: ECONOMICAL PERFORMANCE	**COGS$_€$** (cost$_€$ / kg produced mushrooms)	5.62		3.82

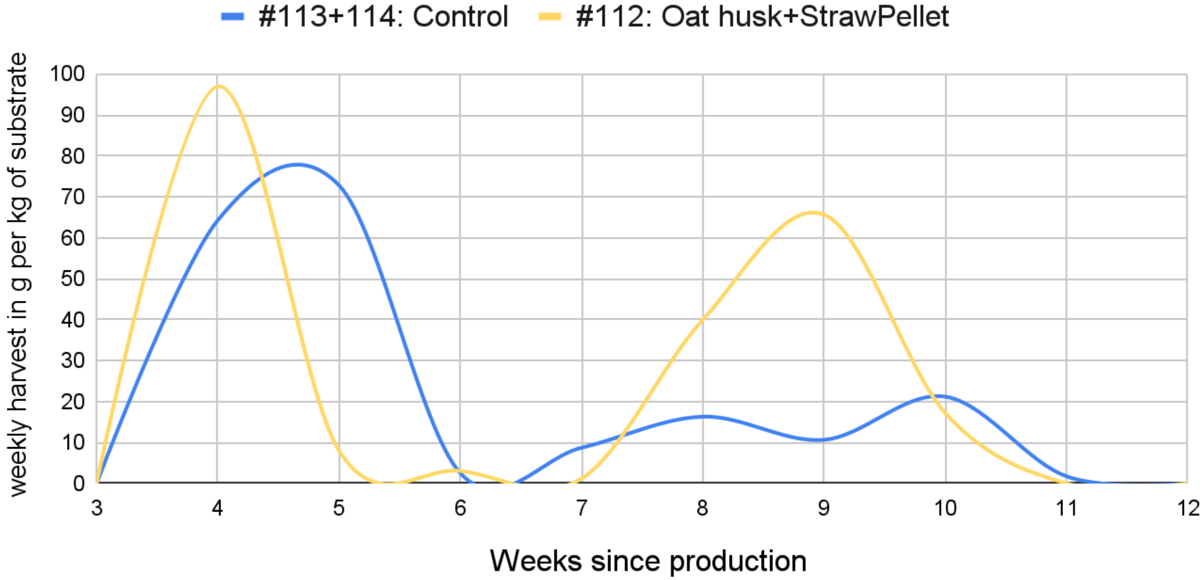
We tried again growing mushrooms of ground oat husk, this time with a careful high-temperature pasteurisation process[116]



Dry pellets were added to oat husk to increase water retention and reduce the substrate dripping observed in WP2 T4.2/ X1. Both alder and straw recipes were designed identical but straw pellets were added last minute to the second recipe (#112) after observing that the alder recipe ("X2") was too wet.

[115] Control was produced with sunflower husk instead of coffee grounds today, due to a supply shortage.
[116] WP1 T3.3. Pasteurisation

Graph 24 Timelime of harvested oyster mushrooms
14.08.24 Oat husk+Straw



Even in smaller proportions and properly pasteurised, all substrates we produced with ground oat husks are at least partially contaminated. In total from this test ¾ of the produced oat husk bags could not be fruited.
However, the few remaining bags, even with poor health, performed significantly better than the control.

These results remind us of tests we did with wheat bran a few years ago rather than what we observed when we worked daily with oat husks back in time. It probably means that this oat husk product contains more nutrients than the ones we have used before. Even after killing all mold spores the product contained, these nutrients are available for competitors such as molds. This oat husk product is not usable as a main ingredient but it could be considered a supplement, either in small quantities in our process or in bigger quantities with a different (more sterile) process.

This product will not be studied further for the time being, not for its lack of potential but for its difficult adaptation to our production system.

Task 5. Common reed

Due to its local availability and rare industrial uses, on top of a biological content which matches with the oyster mushroom needs, reed is a material we want to test thoroughly.

Common reed, Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - Common reed is to be found on nearly every wetland in Finland. It is commonly harvested to remove the nutrients it contains from the waters. However, reed is slow to harvest in its semi-wet growing environment. With the help of the owner of a harvested patch and a specialised NGO (John Nurminen foundation), We were able to identify **one local supplier** (Pikkalan Kaislanleikkuu oy) for reed harvesting and chopping.
      Another supplier making reed pellets (Nine Voices) was identified in Lithuania but their reseller in Finland discontinued its sales due to unreliable storage ability: some batches of the product were covered with mold before use. Molding pellets are not acceptable in a mushroom farm due to cross-contamination risks. We thus decide not to test this second product.
    - Periodicity: Common reed can be harvested **round the year**. However, different seasons require different harvesting processes (due to different water levels and frozen/unfrozen shores). As found in WP1 Task 2: Desktop Study, summer reeds are likely to have higher nutrient contents than winter reeds, making them potentially proper for mushroom substrates without nitrogen-rich supplementation. On the other hand, summer-harvested reeds are more wet, requiring quicker use or additional drying. We have not identified a relevant supplier for summer reed at this stage.
    - Depending on the weather, the season of harvest and the storage facilities, reed can be colonised by other mushrooms, making it improper for our use or more costly to process
  - Scalability:
    - Supply cost: Coarsely chopped reed that we picked up from the harvest place for this study cost us 0.75€/kg (**750 kg/ton**) in time and travel expense.
    - Processing straw (extra chopping, hydration, pasteurisation, mixing) is evaluated here at 1.63€/kg (**1630€**/ton) making reed an expensive supply at this stage.
    - Once better organised and systematic, the supply can easily reach our need (<**5 ton/ year**).

- ENVIRONMENTAL IMPACT
  - Localness: Our identified supplier is located **41 km** from our farm. Reeds are widely available around us.
  - Circularity: Not only reeds are an under-used abundant resource but their removal from the waters actually reduces eutrophication. It is a perfect example of a virtuous circular economy, in this case indirectly transforming pollution into a resource. There is **no well-developed industrial use** for this product at the moment.
  - Organicity: We have not asked the relevant authorities yet but certifying reeds might be difficult.

Picture 39: Reed harvest on the frozen river.

Picture 40: Pick up from a harvested patch

Some challenges arise during the tests:

¤ Our first tests, with a quick 80°C pasteurisation were semi successful but the following were entirely contaminated, probably due to moulds that may have developed in storage due to a relatively high material humidity (25%). We then decided to boil reeds for 30 minutes but this did not show to be enough, making it clear that the reed is heavily contaminated. We choose an alternative method: fermenting the reed for a few days so the spores can germinate before pasteurisation. With these combined processes we could conduct somewhat successful tests.

Picture 41: Chopped reed transport

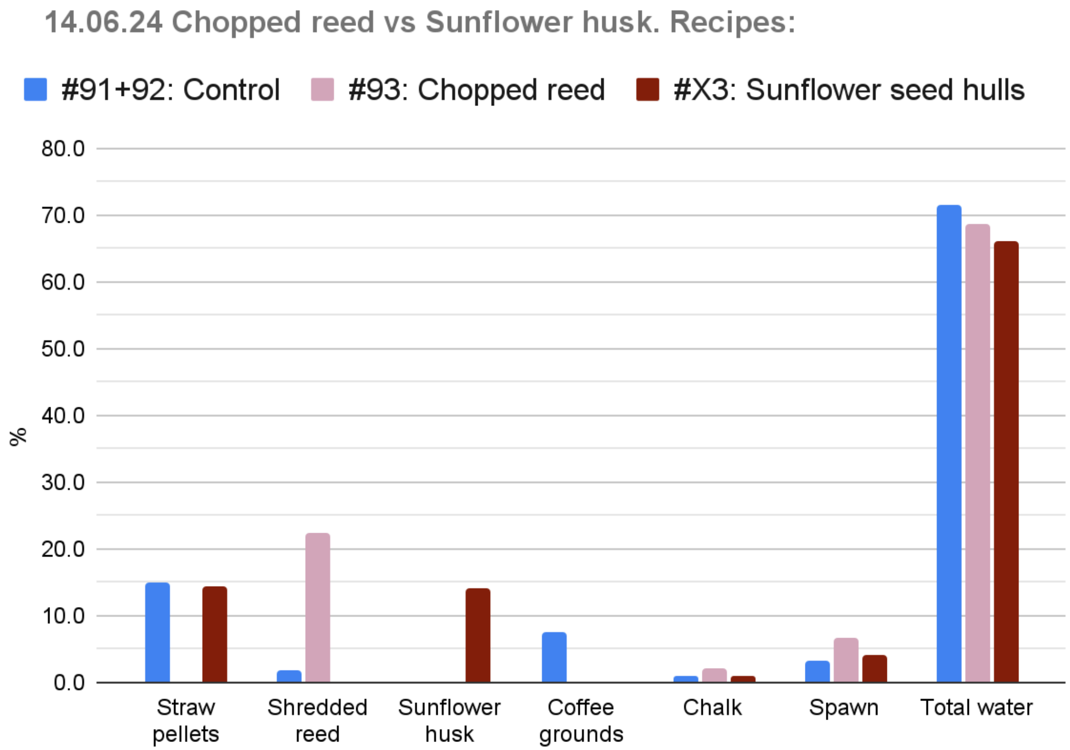
Picture 42: Chopped reed drying & storage (indoor silo)

¤ The particle size of the material we received is slightly too big, making the substrate too light and airy, on top of being difficult to manipulate with our current tools. We tried various methods to chop it, without great success. We finally received a finer material sample from the harvester but quantities only allowed one test (see below, batch #93). The bigger material we picked up could only be used in limited quantities, mixed with other ingredients.

WP2 T5.1 14.06.2024: Chopped reed vs Sunflower seed hulls

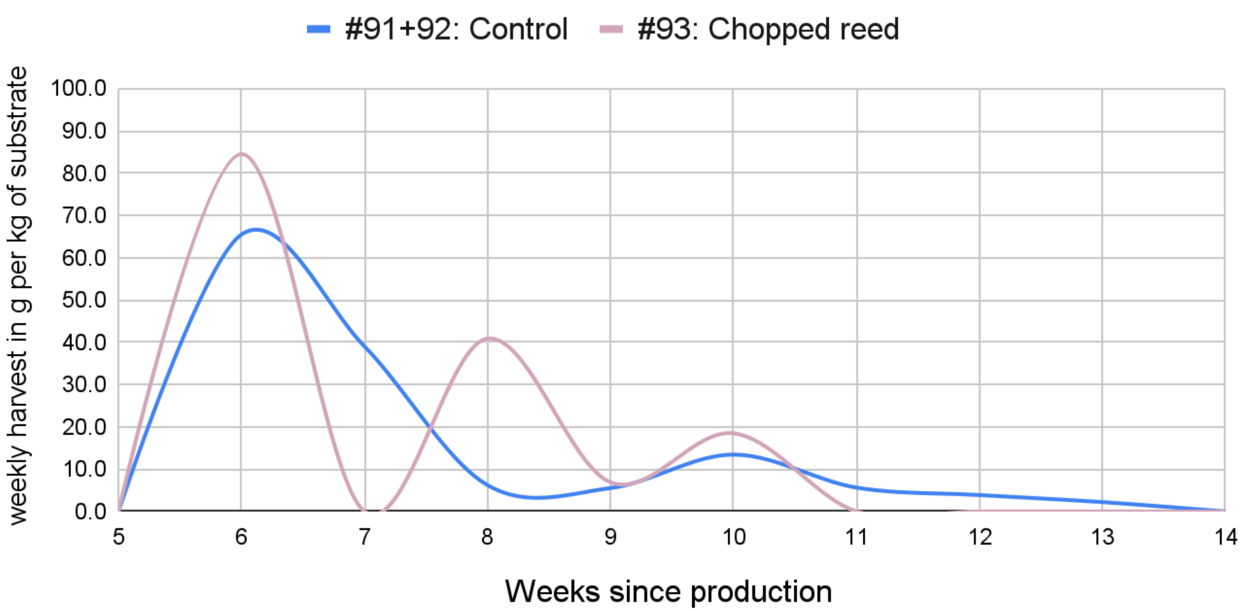
Here we tried 2 recipes which do not share lots of characteristics but we wanted to have them both compared with our control recipe.
However, our quite high-sunflower recipe did not give results as all bags were removed due to trichoderma contamination.The sunflower husk rate we used (14% of the total weight = 42% of the dry weight) is seemingly too high in our context of non-sterile substrates.

		KPI ANALYSIS **ID**	**Control**	**Reed**	**Sunflower**
SUBSTRATE DATA		**TEST BATCH NUMBER**	**91+92**	**93**	**X3**
		Substrate quantity produced (kg)	197	20	86
		Substrate quantity produced (bags)	12	1.7	6
		Average substrate bag weight (kg)	16.4	11.9	14.4
		Fruiting temperature (°C)	19.3	19.3	
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.141	0.151	
		Wet/wet productivity, 70 days	0.130	0.151	
		BE (wet mushrooms / dry substrate)	0.494	0.480	
		Bag productivity (kg/bag)	2.32	1.80	
		Space productivity (kg/bag, 70 days)	2.13	1.80	
	SPEED	**Days to end of first flush**	47	42	
		First flush / total (%)	77.2	56.0	
	CONT.	**Partial contamination** (%)	42	100	
		Total contamination (%)	0	0	100
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.38	1.18	
		Supply cost (€/**bag** of substrate)	6.25	14.09	
		Processing cost (€/**kg** of substrate)	0.50	1.59	
		Processing cost (€/**bag** of substrate)	8.27	18.95	
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	6.35	18.36	



Test batch number 93 was produced with more finely chopped reed than the one we use for controls. It was a sample of a yet-to-be-industrialised product that we obtained from our local reed supplier. It consists in mix-sized particles and makes a substrate of low density (11.9kg/bag = 25% less than most controls), with an acceptable water retention capacity for such a coarse product. Due to the presence of longer fibers, bags were difficult to fill.

Graph 25 Timelime of harvested oyster mushrooms
14.06.24 Chopped reed



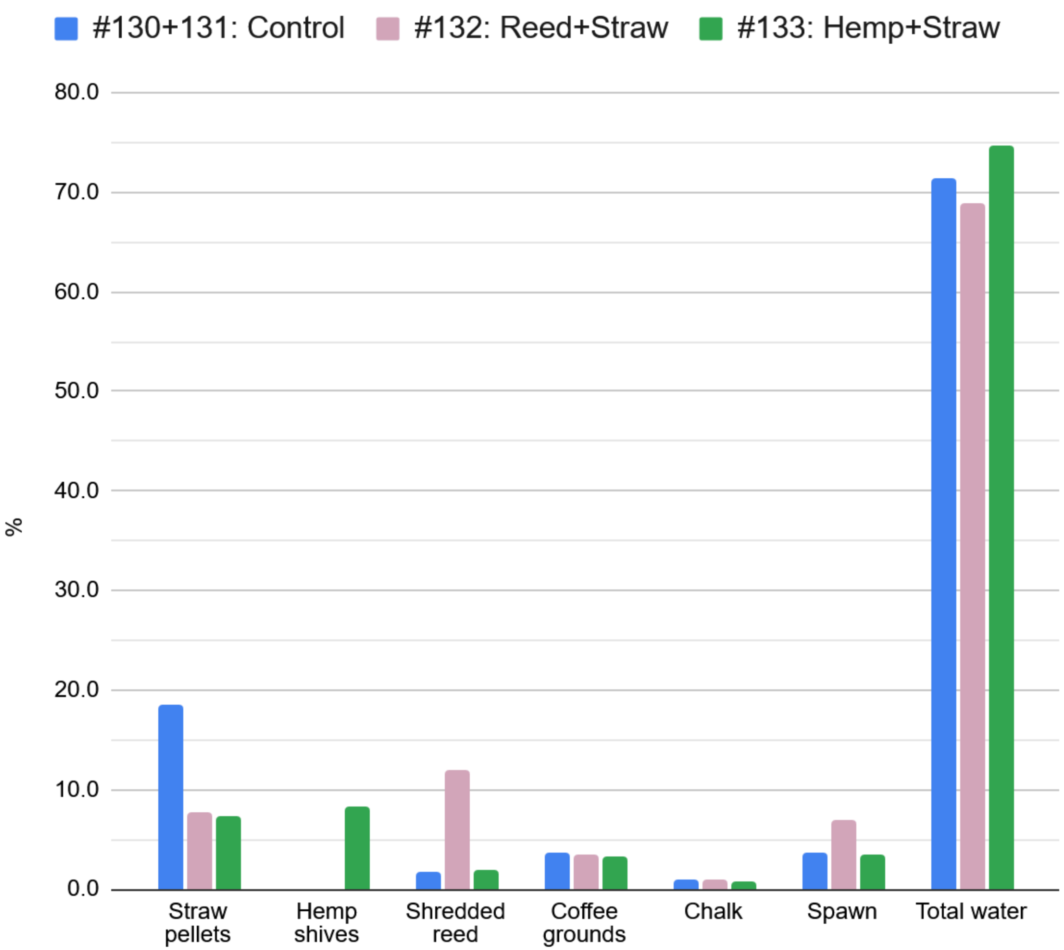
Pictures 43, 44: Chopped reed dried (left) and pasteurised (right)

Though unsupplemented, the chopped reed recipe gave similar productivity as the coffee-supplemented control. The most noticeable difference is the lower bag productivity due to the low density (bags are not carrying “enough” substrate). Probably due to a more efficient gas exchange in the core of bags, typical for low-density recipes, growth is more homogeneous in time and gives three clearly identified flushes while the control gives a more dispersed harvest. Time between each flush is exactly 2 weeks in warm summer conditions (19.3°C is our highest reached average fruiting temperature during summer 2024).

Reed can probably give much better results with a proper supplementation but it already gives acceptable results on its own (with a high spawn rate in this case), showing good potential for further research, provided that we manage to get its cost radically down.

		KPI ANALYSIS ID	Control	Reed + Straw	Hemp + Straw
SUBSTRATE DATA		TEST BATCH NUMBER	130+131	132	133
		Substrate quantity produced (kg)	195	52	80[118]
		Substrate quantity produced (bags)	12.8	3	5.7[118]
		Average substrate bag weight (kg)	15.3	17.3	14.0
		Fruiting temperature (°C)	12.2	12.2	12.2
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.204	0.129	0.124
		Wet/wet productivity, 70 days	0.164	0.097	0.088
		BE (wet mushrooms / dry substrate)	0.714	0.416	0.492
		Bag productivity (kg/bag)	3.09	2.23	1.74
		Space productivity (kg/bag, 70 days)	2.49	1.67	1.24
	SPEED	**Days to end of first flush**	41	39	50
		First flush / total (%)	50.2	47.2	64.6
	CONT.	**Partial contamination** (%)	8	100	35[118]
		Total contamination (%)	0	0	26[119]
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/kg of substrate)	0.43	0.93	0.45
		Supply cost (€/**bag** of substrate)	6.51	16.03	6.34
		Processing cost (€/**kg** of substrate)	0.53	1.09	0.53
		Processing cost (€/**bag** of substrate)	8.04	18.76	7.37
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	4.74	15.61	7.86

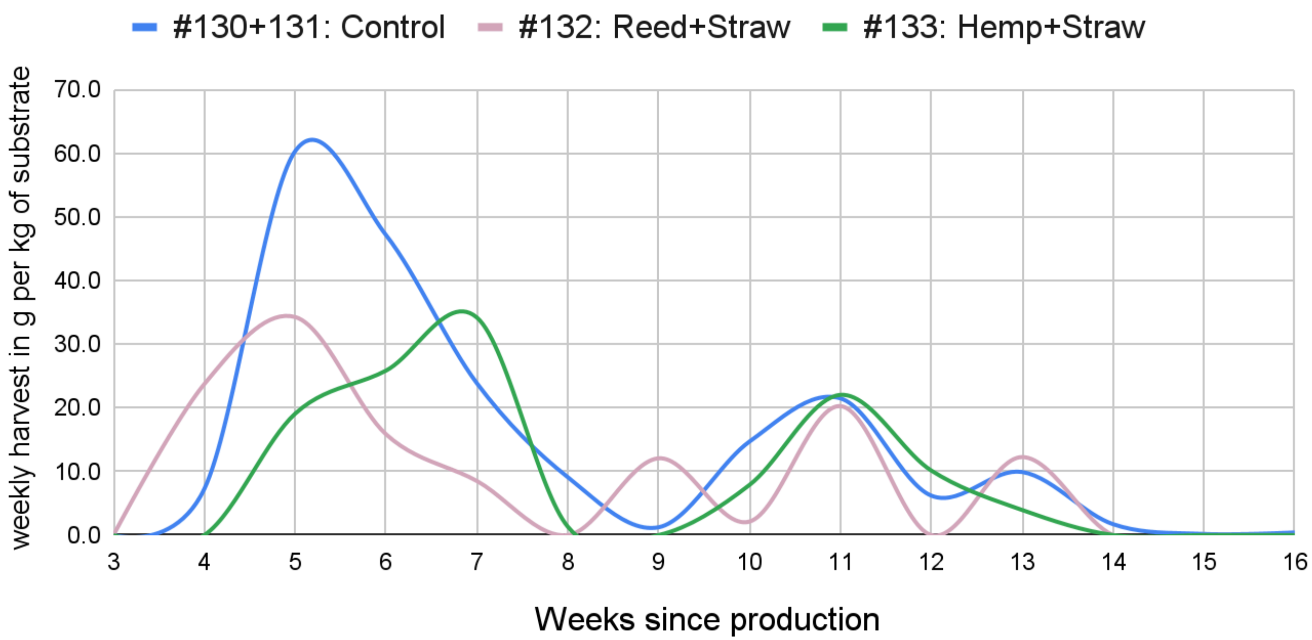
26.09.24 Reed vs Hemp. Recipes:



We tried to compare reed and hemp in otherwise very similar recipes, in association with straw pellets. The test is close to our preliminary test[117] with higher reed/hemp concentrations, higher spawn rates and lower coffee supplementations.

[117] WP1 T3.2 Hemp shives, Hemp versus REED
[118] After contaminated bags deduction
[119] Before contaminated bags deduction

Graph 26 Timelime of harvested oyster mushrooms
26.09.24 Reed vs Hemp



This test was an important one to understand differences between reed-based and hemp-based recipes.
Despite low fruiting temperatures, low nitrogen supplementations and high spawn rates, test batches 132 and 133 were too heavily contaminated to give proper results. As the control batch produced with the same ingredients on the same day gave good results, it is likely that other tests failed due to:
- Improper reed processing / pasteurising (#132)
- Too wet and therefore unbreathing substrate (#133)

This test came too late in our research schedule to be reproduced once more with more success.
To be continued in PART II.

Task 6. Coffee silverskin

Coffee silverskin is the main organic by-product of coffee roasteries. It consists of the peel which separates from beans during roasting. It is of very light consistency, making it difficult to transport, store and process. However, it is used by some mushroom growers in Europe and we want to test it once again here.

Picture 45: Coffee silverskin

Coffee silverskin, Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - Silverskin can be found in every coffee roasterie of which **about 50** of various sizes can be found in Finland[120], usually as a free-to-pickup side-stream. We have partnered for these tests with Helsingin kahvipaahtimo but we know we could find the product in many other places in southern Finland.
    - Silverskin is available **round the year**.
  - Scalability:
    - Supply cost: Silverskin can be picked up for free. However, due to its very low density (<40g/L), it is expensive to transport. For this tests we evaluate transport costs at 0.74€/kg (**740€/ton**). It could be reduced if scaled but will always remain high unless processed into a denser form before transport (bales / pellet / briquettes…)
    - Processing cost: Due to its low density, silverskin is slow to hydrate and pasteurise. We evaluate processing costs for these tests at 1.22€/kg (**1220€/ton**).
    - The supply of any big roastery would meet our maximum yearly needs (**<5 tons/year**). Some of the small-sized ones would also meet our needs.

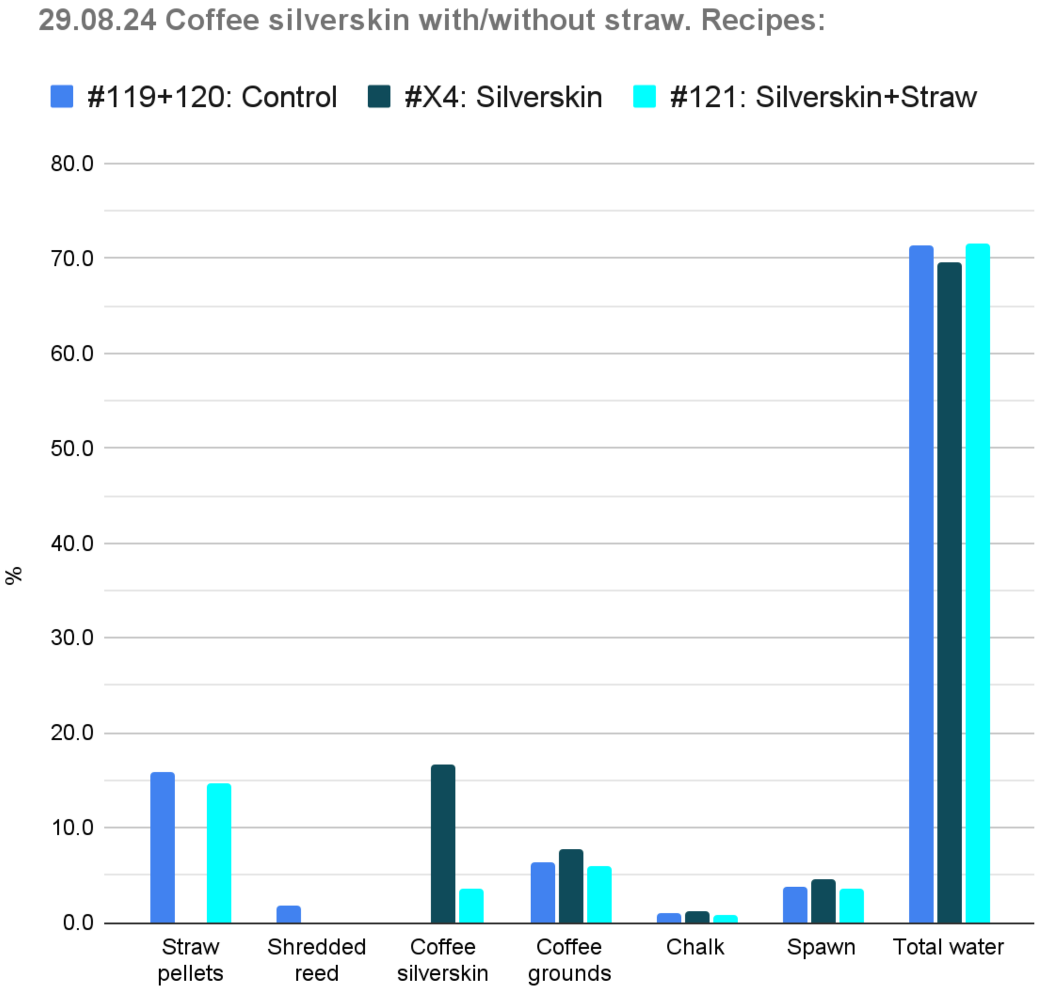
- ENVIRONMENTAL IMPACT
  - Localness: The closest coffee roaster (Cafetoria roastery Oy) is located in Lohja, **42 km** from our farm. We have not contacted them as we used for this project silverskin from a roaster we have worked with for years: Helsingin kahvipaahtimo, 81 km from our farm.
  - Circularity: We have not identified any competing uses for this material. Its light density does not even make energy production relevant.
  - Organicity: Most roasteries produce both organic and non-organic coffee. The supply of organic material is therefore probably possible but not the easiest to certify as it needs to be carefully separated from the non-organic product.

120 https://uuttaja.fi/lista-kotimaisista-pienpaahtimoista/

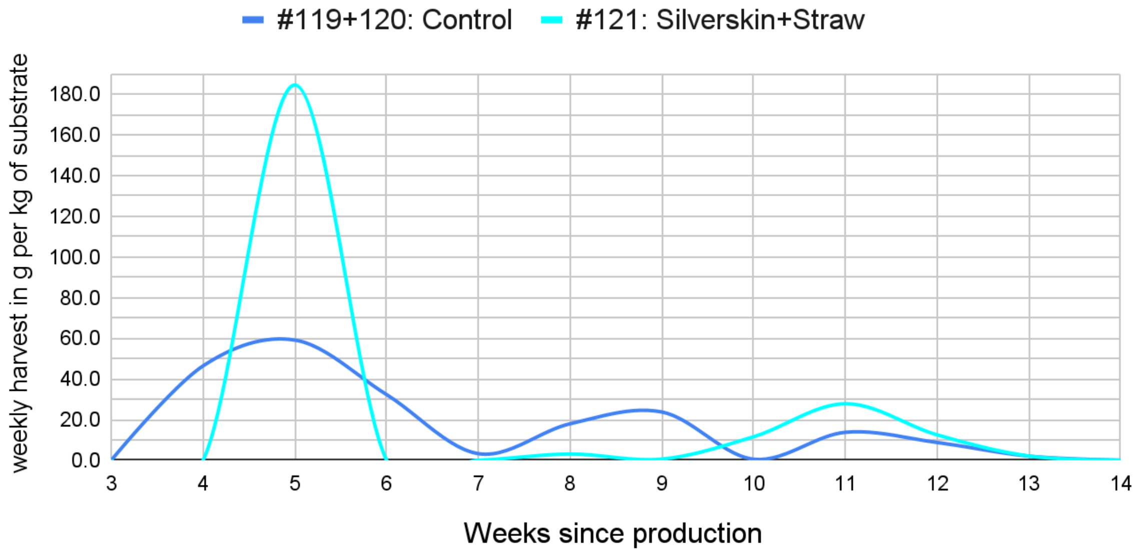
29.08.2024: Coffee silverskin with/without straw pellets, supplemented with coffee grounds

	KPI ANALYSIS	ID	Control	Silverskin	Silverskin + Straw
SUBSTRATE DATA	TEST BATCH NUMBER		119+120	X4	121
	Substrate quantity produced (kg)		188	75	102
	Substrate quantity produced (bags)		11		6
	Average substrate bag weight (kg)		17.1		16.9
	Fruiting temperature (°C)		12.5		12.5
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.207		0.242
		Wet/wet productivity, 70 days	0.182		0.200
		BE (wet mushrooms / dry substrate)	0.720		0.848
		Bag productivity (kg/bag)	3.56		4.09
		Space productivity (kg/bag, 70 days)	3.14		3.38
	SPEED	**Days to end of first flush**	34		35
		First flush / total (%)	59.4		76.3
	CONT.	**Partial contamination** (%)	9		0
		Total contamination (%)	0	100	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.43		0.38
		Supply cost (€/**bag** of substrate)	7.29		6.44
		Processing cost (€/**kg** of substrate)	0.52		0.45
		Processing cost (€/**bag** of substrate)	8.92		7.59
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	4.62		3.43

We aimed to compare here recipes containing different ratios of silverkin. Unfortunately the recipe containing unmixed silverskin quickly contaminated with trichoderma and needed to be removed from the farm. It is likely that one of the bags of silverskin we received had been stored in humid conditions because it was denser than others. The low-temperature pasteurisation process we used to treat silverskin was not enough to inactivate the big load of spores it must have contained.



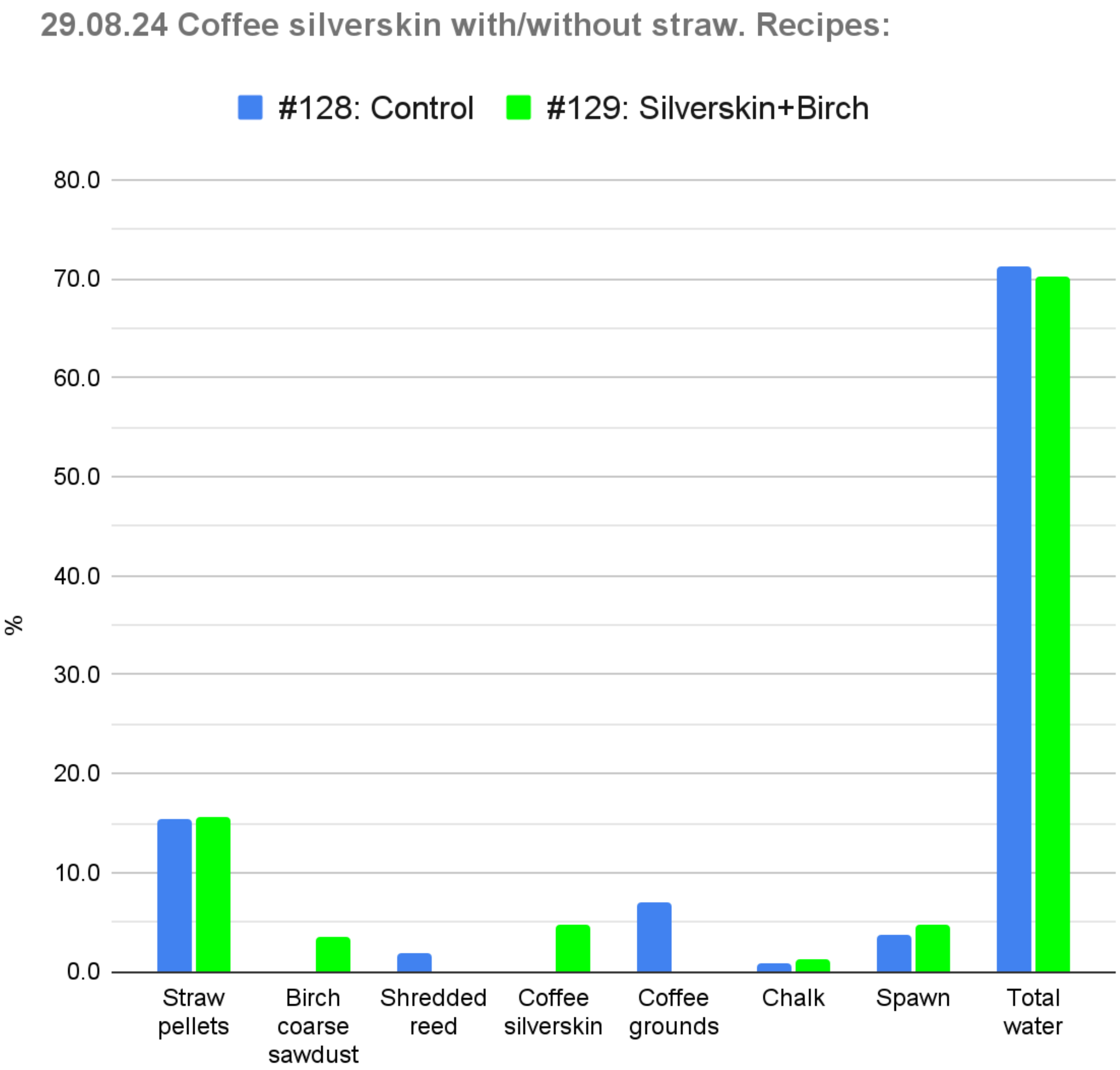
Graph 27 Timelime of harvested oyster mushrooms
29.08.24 Coffee silverskin + straw pellets



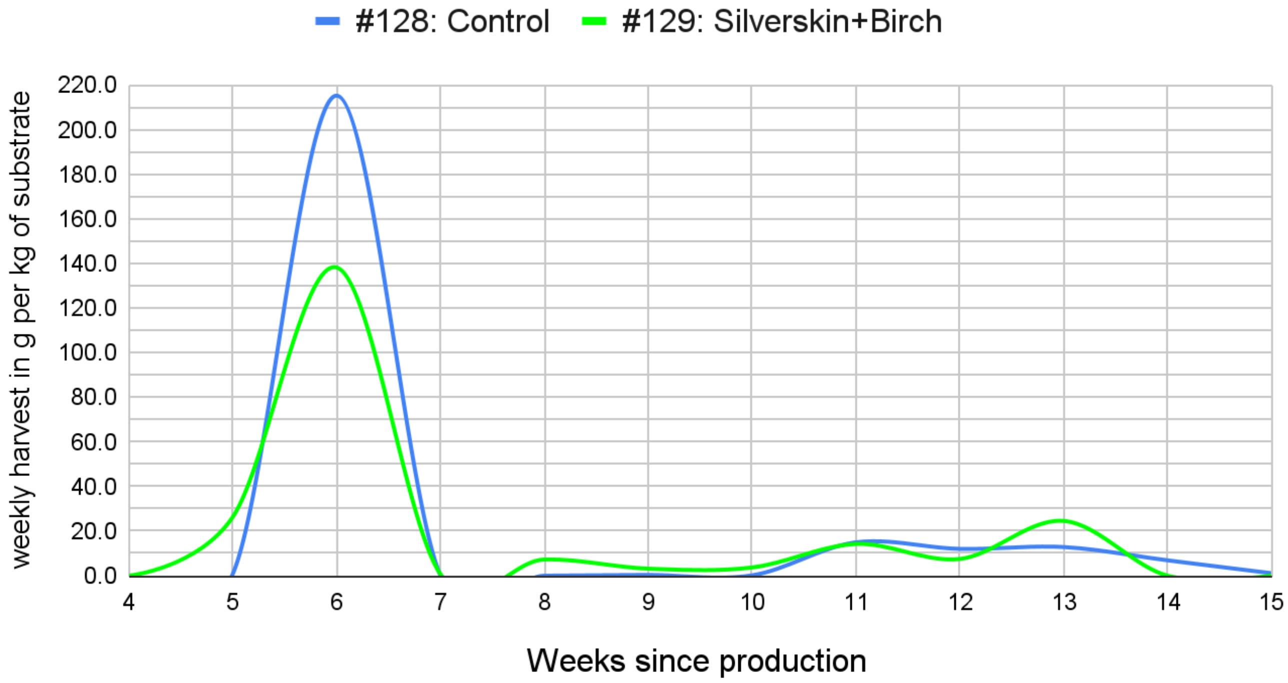
Batch #121 is very close in recipe to the control. The only significant difference is the replacement of reed by coffee silverskin. The amount of silverskin is the maximum that can be easily used with our current process. Unlike the removed silverskin X4 batch, #121 grew healthy. Even though today's control was one of the most productive ever produced, #121 was clearly performing better.

Silverskin is not easy to manipulate. However, good results show this recipe as a good potential base for further research. It might be tested together with other performing recipes in PART II.

	KPI ANALYSIS ID	Control	Silverskin + Birch
SUBSTRATE DATA	**TEST BATCH NUMBER**	**128**	**129**
	Substrate quantity produced (kg)	192	152
	Substrate quantity produced (bags)	13	11.6
	Average substrate bag weight (kg)	14.8	13.1
	Fruiting temperature (°C)	12.1	12.1
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity**	0.264	0.226
	Wet/wet productivity, 70 days	0.216	0.179
	BE (wet mushrooms / dry substrate)	0.917	0.760
	Bag productivity (kg/bag)	3.91	2.96
	Space productivity (kg/bag, 70 days)	3.20	2.35
KPI: BIOLOGICAL PERFORMANCE – SPEED	**Days to end of first flush**	40	44
	First flush / total (%)	81.7	73.2
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Partial contamination** (%)	0	0
	Total contamination (%)	0	0
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/**kg** of substrate)	0.42	0.48
	Supply cost (€/**bag** of substrate)	6.26	6.28
	Processing cost (€/**kg** of substrate)	0.52	0.59
	Processing cost (€/**bag** of substrate)	7.66	7.74
	COGS€ (cost€ / kg produced mushrooms)	3.56	4.74



Graph 28 Timelime of harvested oyster mushrooms
18.09.24 Coffee silverskin + Birch coarse sawdust



This recipe is a replicate of our best-ever batch #76 but with part of the birch replaced with silverskin. It performs well but not quite as good as #76, nor overcomes the control.

Silverskin seems to improve straw-based recipes more than wood-based recipes.

Task 7. Hemp shives

Hemp shives' high potential was identified in WP1 T1.5 and widely analysed in WP1 T3.2. Hemp shives were therefore used in many recipes throughout this project. Moreover, local hemp shives were hardly available when we conducted WP2 tests (see below). For all these reasons we decided not to design more specific tests with Hemp shives.

<u>Hemp shives, Supply and Environmental KPI:</u>

- SUPPLY
  - <u>Availability</u>:
    - We were reached by one motivated hemp grower, who was our only local supplier for this project: Aapo Korkeaoja. However, he decided to join a consortium of hemp growers who are currently building fiber hemp processing factories in Finland (Hemka Oy). Due to project postponing and slow industrial tuning, **local hemp shives are hardly available at the moment**. We can find in Finland imported hemp shives from a few European suppliers. The one we have used is a Dutch product: Hemparade. This product has shown dramatic quality changes between received batches and cannot be considered a long-term reliable supply.
    - Fiber hemp is harvested seasonally (in Finland during spring) but it is stored to be industrially processed year round. We can expect a continuous local supply once Hemka's factories are up and running.
  - <u>Scalability</u>:
    - Supply cost: Korkeaoja's hemp supply cost was evaluated at 1.35€/kg (**1350€/ton**) while imported Hemparade was evaluated at 0.92€/kg (920€/ton). These prices include transport. We hope to see local prices decrease significantly when Hemka factories start to supply us.
    - Processing cost: Due to its low density, hemp shives are slow to hydrate and pasteurise. We evaluate processing costs for these tests at 0.94€/kg (**940€/ton**).
    - Preliminary tests show that hemp shives cannot be used alone so our needs will not exceed **2 tons**, which we expect to supply easily in the near future.

- ENVIRONMENTAL IMPACT
  - <u>Localness</u>: We are distant from the Korkeaoja farm of **220 km**.
  - <u>Circularity</u>: The current **one main use** of hemp shives is animal farm bedding, thanks to its high water retention capacity.
  - <u>Organicity</u>: Korkeaoja's product was organic but we do not know yet if Hemka will build an organic production line. No imported product is certified organic as far as we know.

Task 8. Sunflower seed hulls

Sunflower seed hulls' were used in 5 tests above: batches 18,19,20, 61[121] and X3[122] with low to moderate success. Contamination rates were higher than average while performance was not remarkable. We thus decided to stop here sunflower seed hull testing and developments.

Sunflower seed hulls, Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - Sunflower seed hull supply was an opportunity that we seized when a local store sold imported pellets made of it, a few years ago. It is not a standard product in Finland and local sunflower seeds are still rare. **No local supplier** was identified and we do not know any constant importer of such a product. We however wanted to test this resource in anticipation for quick climate changes that will bring more sunflower fields to southern Finland in the foreseeable future. Sunflower cultivation's attractivity greatly depends on vegetable oil global prices but we can already note a trend for cultivation increase in Germany[123]and Poland[124] and a slow but noticeable decrease in southern european countries like Spain[125], Italy[126] or Greece[127].
    - Sunflower seed hulls are seasonal, they come with the harvest of sunflowers at the end of the summer.
  - Scalability:
    - Supply cost: We supplied imported sunflower husk pellets for 0.40€/kg (400€/ton)
    - Processing cost: Sunflower pellets processing costs (pasteurisation, mixing) were evaluated at 0.72€/kg (720€/ton) for this project.
    - No scalability is possible due to the unreliable supply at the moment. Our current process does also not seem to be compatible with this product: it brings contamination to our farm. We can test the product again when/if local farmers start to process sunflower seeds.

- ENVIRONMENTAL IMPACT
  - Localness: **No local availability** at the moment. Imported sunflower seed husks that we found came from southern-central Europe.
  - Circularity: sunflower seed hulls are currently used for similar application as any wood by-product (litter, insulation, …) but due to their higher nutritive value, mushroom cultivation might be a potential higher value use for them.
  - Organicity: **No organic product is available** in Finland at the moment.

[121] WP1T3.2: Preliminary studies
[122] WP2 T5.1: Chopped reed vs Sunflower seed hulls
[123] https://tradingeconomics.com/germany/area-under-cultivation-of-sunflower-seed-eurostat-data.html
[124] https://tradingeconomics.com/poland/area-under-cultivation-of-sunflower-seed-eurostat-data.html
[125] https://tradingeconomics.com/spain/area-under-cultivation-of-sunflower-seed-eurostat-data.html
[126] https://tradingeconomics.com/italy/area-under-cultivation-of-sunflower-seed-eurostat-data.html
[127] https://tradingeconomics.com/greece/area-under-cultivation-of-sunflower-seed-eurostat-data.html

Task 9A: Flax shives

Flax shives are somewhat similar to hemp shives[128] though thinner. They share hemp shives main benefit: a very high water retention. But in lower proportions[129]. The product we got is difficult to blend due to the non-neglictable proportion of long fibers mixed with the shives.

Flax shives, Supply and Environmental KPI:

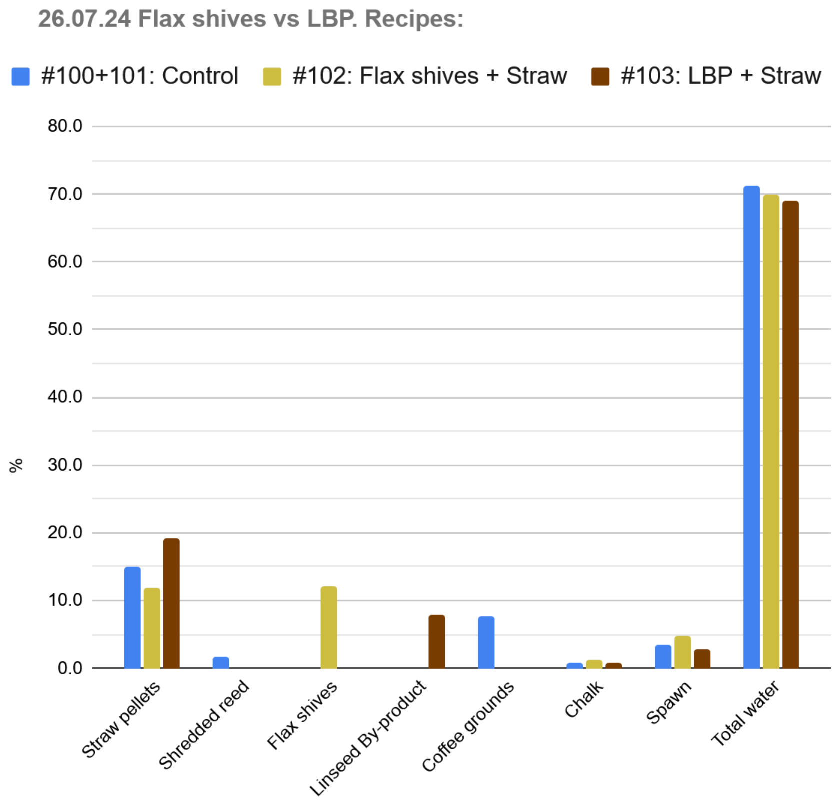
- SUPPLY
  - Availability:
    - The Fiber flax industry is residual/ experimental in Finland. The **one and only supplier** we identified for local shives is *Linen Stories*, a fiber flax activist who tries to gather knowledge to facilitate a potential industrial revival. Flax shives do not seem to be much more developed in neighbouring countries at the moment except for Russia and Belarus, with whom trade is hardly possible at the moment.
    - Flax shives are **harvested seasonally** in the autumn together with fibers. Their low relative humidity at harvest make it easy-drying and almost ready-for-storage to be used year round.
  - Scalability:
    - Supply cost: We supplied our local flax shives for 0.87€/kg (**870€/ton**)
    - Processing cost: Sunflower pellets processing costs (pasteurisation, mixing) were evaluated at 0.94€/kg (**940€/ton**) for this project.
    - Scalability is currently difficult due to the unreliable supply. Supply depends on the local fiber flax experiment dynamics with a production that is often less than what we could use if we designed a flax-based recipe.

- ENVIRONMENTAL IMPACT
  - Localness: Finnish fiber flax cultivation mostly takes place in Tavastia, less than **200 km from our farm**. However, flax shives were scutched (separated) from fibers in Ostrobothnia, about **350 km** from our farm.
  - Circularity: Fiber flax can be used in animal bedding but because the current processing line is not adapted for this, there is currently **no competitive use** for fiber flax. It is used for crop cover and dry toilet litter, which are low value uses for which alternatives are abundant.
  - Organicity: **All shives we received were organic** but the future is unsure for local organic flax.

[128] See WP1 T1.5, WP1 T3.2: Hemp shives, WP2 T7
[129] See WP2: Density and water retention capacity of tested materials

		KPI ANALYSIS ID	Control	Flax shives + straw	LBP + straw
SUBSTRATE DATA		TEST BATCH NUMBER	100+101	102	103
		Substrate quantity produced (kg)	199	67	44[130]
		Substrate quantity produced (bags)	12.3	6.5	3[126]
		Average substrate bag weight (kg)	16.1	10.3	14.6
		Fruiting temperature (°C)	17.1	17.1	17.1
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.207	0.196	0.022
		Wet/wet productivity, 70 days	0.172	0.186	0.007
		BE (wet mushrooms / dry substrate)	0.721	0.651	0.071
		Bag productivity (kg/bag)	3.35	2.03	0.32
		Space productivity (kg/bag, 70 days)	2.78	1.92	0.10
	SPEED	**Days to end of first flush**	34	35	61
		First flush / total (%)	49.6	61.2	32.0
	CONT.	**Partial contamination** (%)	24	0	100[126]
		Total contamination (%)	0	0	57[131]
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.39	0.55	0.35
		Supply cost (€/**bag** of substrate)	6.37	5.70	5.07
		Processing cost (€/**kg** of substrate)	0.51	0.50	0.42
		Processing cost (€/**bag** of substrate)	8.18	5.17	6.08
		COGS€ (cost€ / kg produced mushrooms)	4.35	5.37	34.49

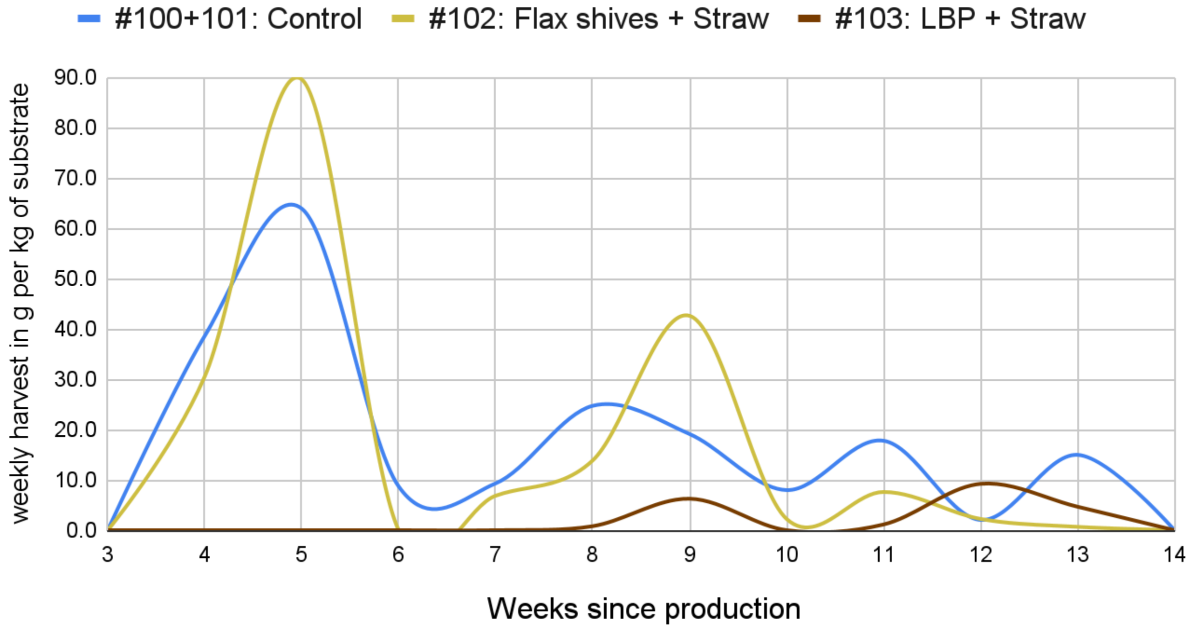
As LBP was first considered a potential carbon base for mushroom substrates[132], we tried to compare it with flax shives.



As flax shives are of very low density and LBP of very high density, it was very difficult to keep recipe proportions similar. Without the addition of straw pellets, batch #102 would have been to light and #103 too dense.

[130] After contaminated bags deduction
[131] Before contaminated bags deduction
[132] See WP2 Task 9B below

Graph 29 Timelime of harvested oyster mushrooms
26.07.24 Flax shives vs Linseed By-Product

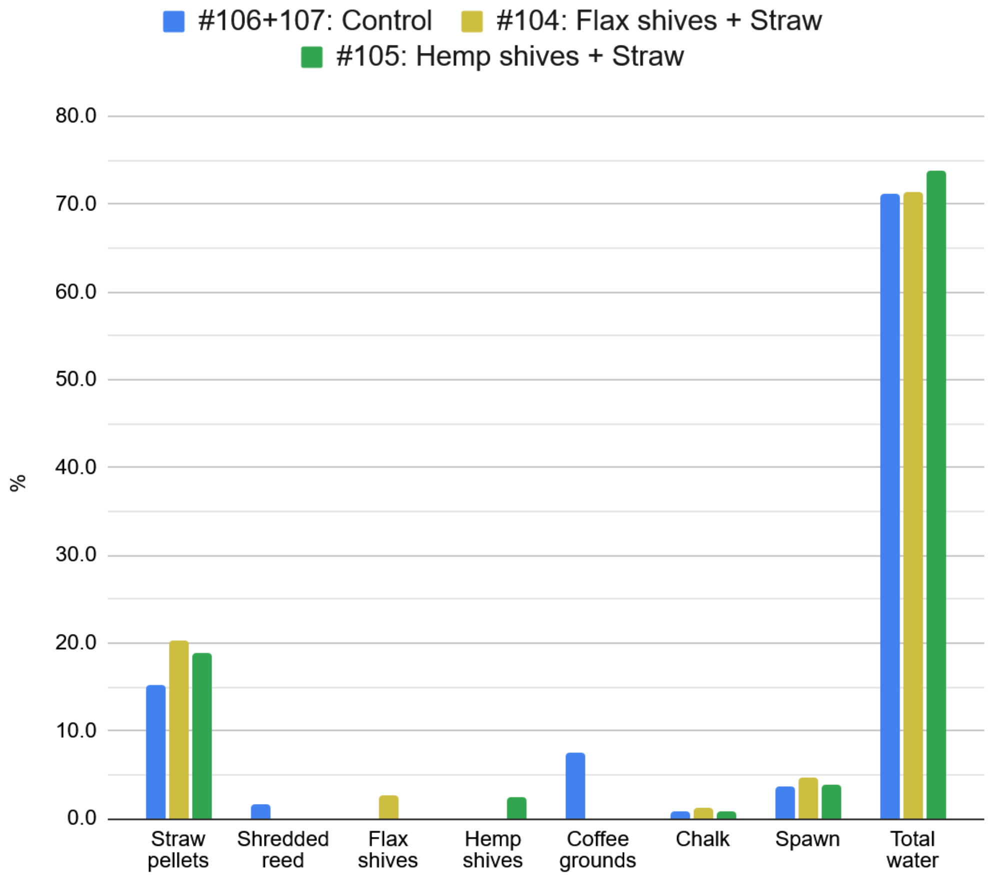


Though unsupplemented, #102 (Flax shives + straw pellets) gave results that were comparable to the control in performance. Growing bags are so light though, that such a high-flax recipe is not quite space efficient.

Because LBP is a low-cost (free product, easy-transport) resource, it makes a cheaper substrate. But the very high contamination rate of batch #103 did not enable any further conclusion at this stage. LBP seems to be a sensitive product that seems to be used in smaller ratios and with the most efficient pasteurisation process.

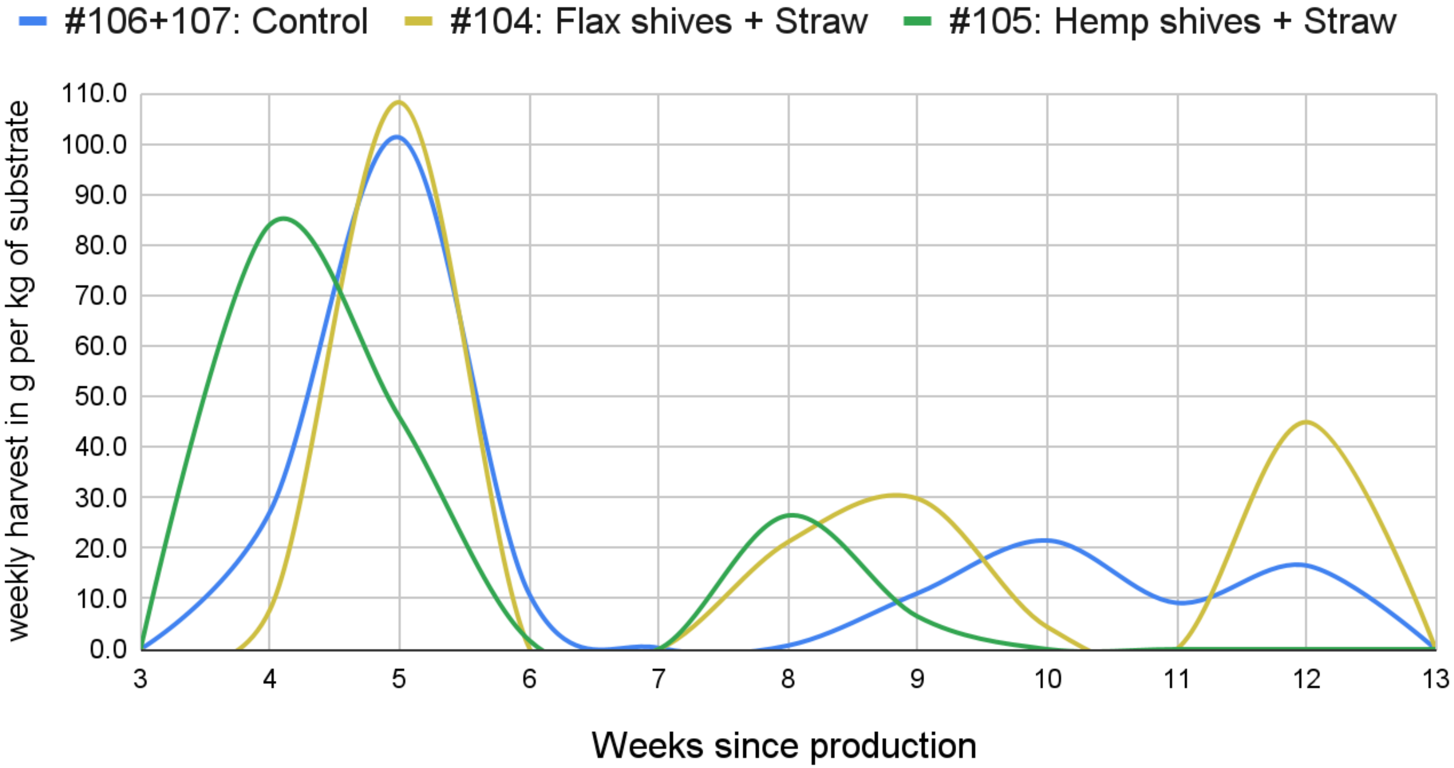
	KPI ANALYSIS ID	Control	Flax shives + straw	Hemp shives + straw
SUBSTRATE DATA	**TEST BATCH NUMBER**	**106+107**	**104**	**105**
	Substrate quantity produced (kg)	202	78	95
	Substrate quantity produced (bags)	12.4	5	5.6
	Average substrate bag weight (kg)	16.7	15.6	17.0
	Fruiting temperature (°C)	16.2	16.2	16.2
KPI: BIOLOGICAL PERFORMANCE – PRODUCTIVITY	**Wet/wet productivity**	0.199	0.217	0.165
	Wet/wet productivity, 70 days	0.173	0.172	0.165
	BE (wet mushrooms / dry substrate)	0.689	0.756	0.630
	Bag productivity (kg/bag)	3.23	3.39	2.80
	Space productivity (kg/bag, 70 days)	2.80	2.69	2.80
KPI: BIOLOGICAL PERFORMANCE – SPEED	**Days to end of first flush**	36	33	36
	First flush / total (%)	70.0	53.6	79.9
KPI: BIOLOGICAL PERFORMANCE – CONT.	**Partial contamination** (%)	0	0	0
	Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE	**Supply cost** (€/kg of substrate)	0.41	0.47	0.42
	Supply cost (€/**bag** of substrate)	6.63	7.39	7.08
	Processing cost (€/**kg** of substrate)	0.51	0.47	0.45
	Processing cost (€/**bag** of substrate)	8.29	7.38	7.57
	COGS€ (cost€ / kg produced mushrooms)	4.62	4.36	5.24

03.08.24 Flax shives vs Hemp shives. Recipes:



Unlike in previous tests, recipes were easy to homogenise here thanks to the similar properties of hemp and flax shives. Their main difference being their water retention capacity, batch#105 is more wet than #104. Both recipes contain a big amount of straw pellets to reach an acceptable density.

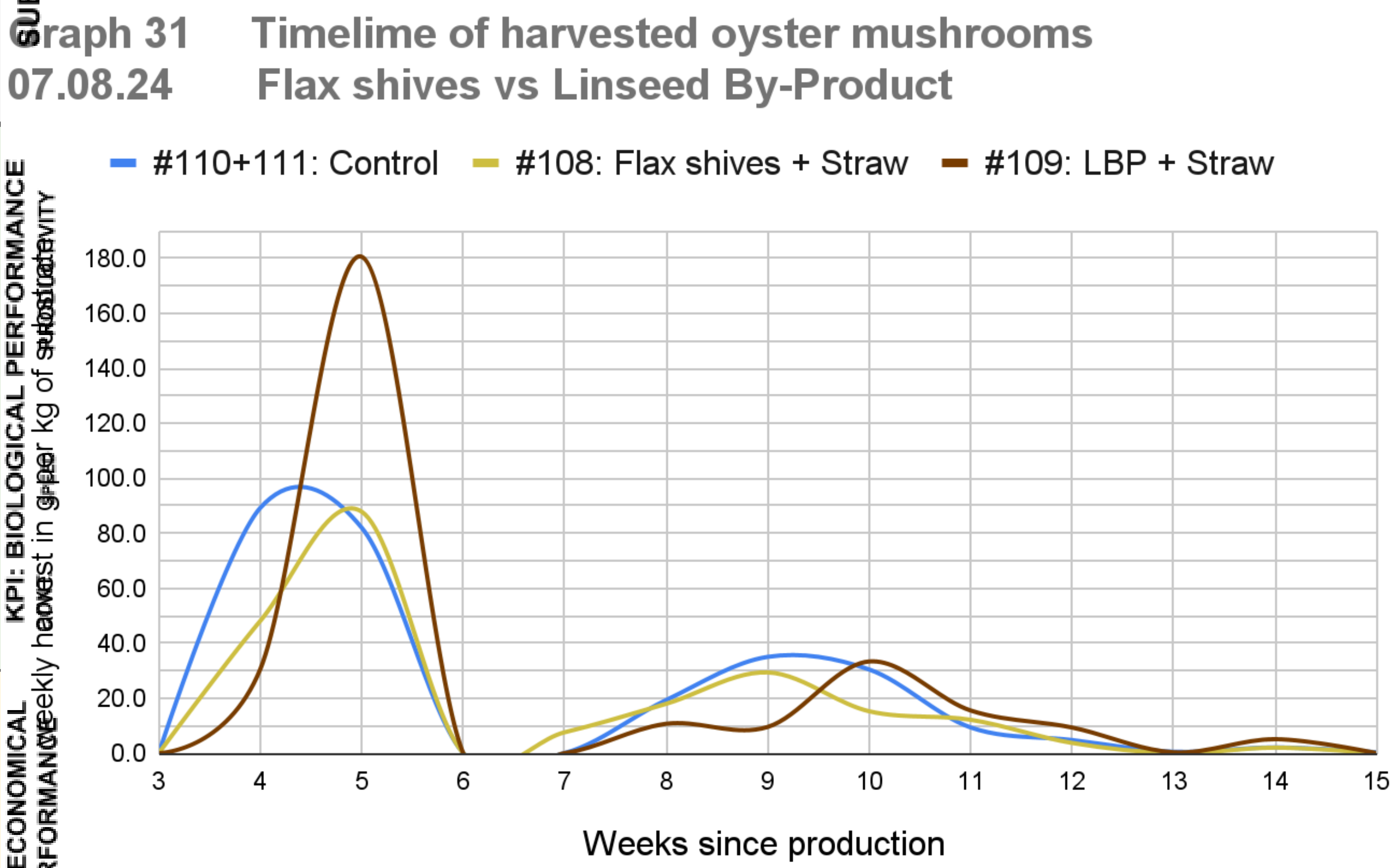
Graph 30 Timelime of harvested oyster mushrooms
03.08.24 Flax shives vs Hemp shives

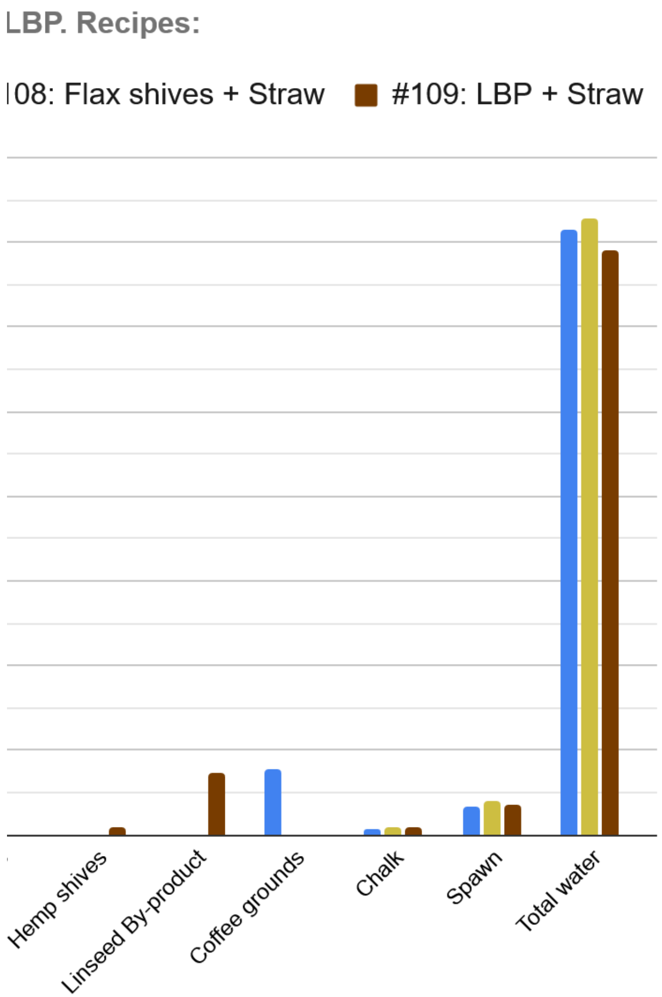


All recipes performed comparably. Batch #104 (Flax shives) gave the best productivity but was slightly slower than the control and hemp shives (smaller first flush, later second flush). On the contrary batch #105 (Korkeaoja hemp shives) gave a slightly lower productivity but produced quicker. As a result, productivity after 70 days was almost identical for all 3 recipes.

SUBSTRATE DATA	KPI ANALYSIS ID	Control	Flax shives + straw	LBP + straw
	TEST BATCH NUMBER	**110+111**	**108**	**109**
	Substrate quantity produced (kg)	220	90	99
KPI: ECONOMICAL PERFORMANCE	**Processing cost** (€/**bag** of substrate)	7.79	6.34	7.14
	COGS€ (cost€ / kg produced mushrooms)	3.23	3.97	2.85

Recipes from WP2 T9A.1 were re-engineered to solve contamination problems and pasteurisation issues. LBP was boiled for a longer time (<1h at 100°C) together with coffee grounds[133]. A small amount of hemp shives was added in batch #109 to improve gas exchange.





[133] Both were separated in the tank by a cloth that avoided solids to mix up. However, liquids were communicating and as a result coffee grounds became slimy as their water mixed up with linseed's pectins.

The changes made to the LBP recipe were successful and batch #109 was one of the most productive ever produced, its performance being close to our best-ever produced batch #76[134], even surpassing it in terms of space-use (best-ever bag productivity and space productivity with more than 4kg/bag produced in a 70-day production cycle).
With more straw pellets and less flax shives than in batch #102[135], batch #108 was slightly improved, especially in terms of space-use due to a higher density. However, it performed clearly under both other recipes.

The most surprising result here is the high performance of the control, the best ever produced with this recipe. After closer production data analysis, we note that for the first time coffee was pasteurised together with LBP and their boiling liquids mixed up. It is therefore likely that pectins contained in boiling LBP waters is a great supplement for our oyster mushroom substrates.

Task 9B: Linseed by-products ("LBP")

Linseed and flax by-products were initially considered a common topic with flax shives (see above). When searching for flax by-products, we mainly thought of flaxseed hulls or other carbon-rich subproducts coming together with linseed and fiber flax main productions. However, when researching the market we identified a locally-available by-product of the linseed industry of a very different nature: **refused linseeds.** They actually contain multiple elements: oversized/undersized/broken linseeds, weed seeds, small flax shive pieces, fibrous weed particles, organic dust… However most of it is linseed, making it a potential supplementation rather than a carbon source. Supplements were not supposed to be analysed at this stage of the project but because results with this product gave very promising results above[136], we decided to pursue one more test.

Linseed By-Product (LBP), Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - The product we tried here is specific to the process of linseed selection, uncommon in Finland. We identified **one supplier** for this resource (Vihervakka Oy). Alternative suppliers might exist but we have not searched at this stage.
    - Our supplier selects linseed seasonally in winter-spring. LBP must be collected at this moment, otherwise they are sent to a local heat plant.

[134] See WP2 T2.2
[135] See WP2 T9A.1
[136] See above WP2 T9A

- Scalability:
    - Supply cost: LBP has been given to us for this project. Its cost was the transport cost: 0,39€/kg (**390€/ton**). It can become much less if transported in bigger amounts.
    - Processing cost: LBP processing costs (pasteurisation, mixing) were evaluated at 0.31€/kg (**310€/ton**) for this project. LBP is not easy to use due to the pectins it contains but it is very dense so each kilogram is only a small amount to process.
    - Vihervakka processes 50-300 tons of linseed every year, of which 5-25% is refused. This makes a total amount of available product ranging from 2,5 to 75 tons. Because LBP is dense and creates a sticky juice, it can only be used as a limited supplement: we can scale up our production a lot before reaching a need of 2,5 ton/ year.

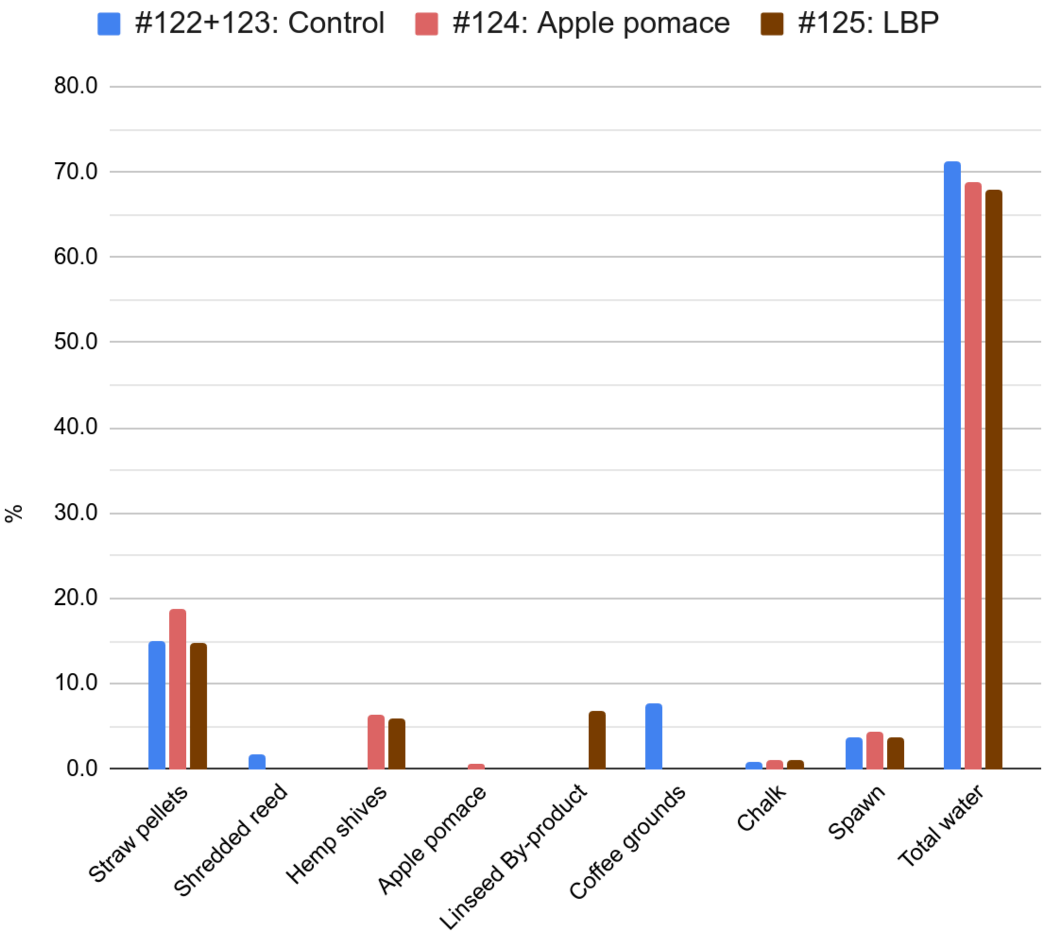
■ ENVIRONMENTAL IMPACT

- Localness: Vihervakka sorts out linseeds in Pöytyä, **133 km** from our farm.
- Circularity: Refused linseed **are currently burnt (only identified use)**. It is a low-value use considering the embedded labour and energy it contains.
- Organicity: **No organic certification** is available for this resource.

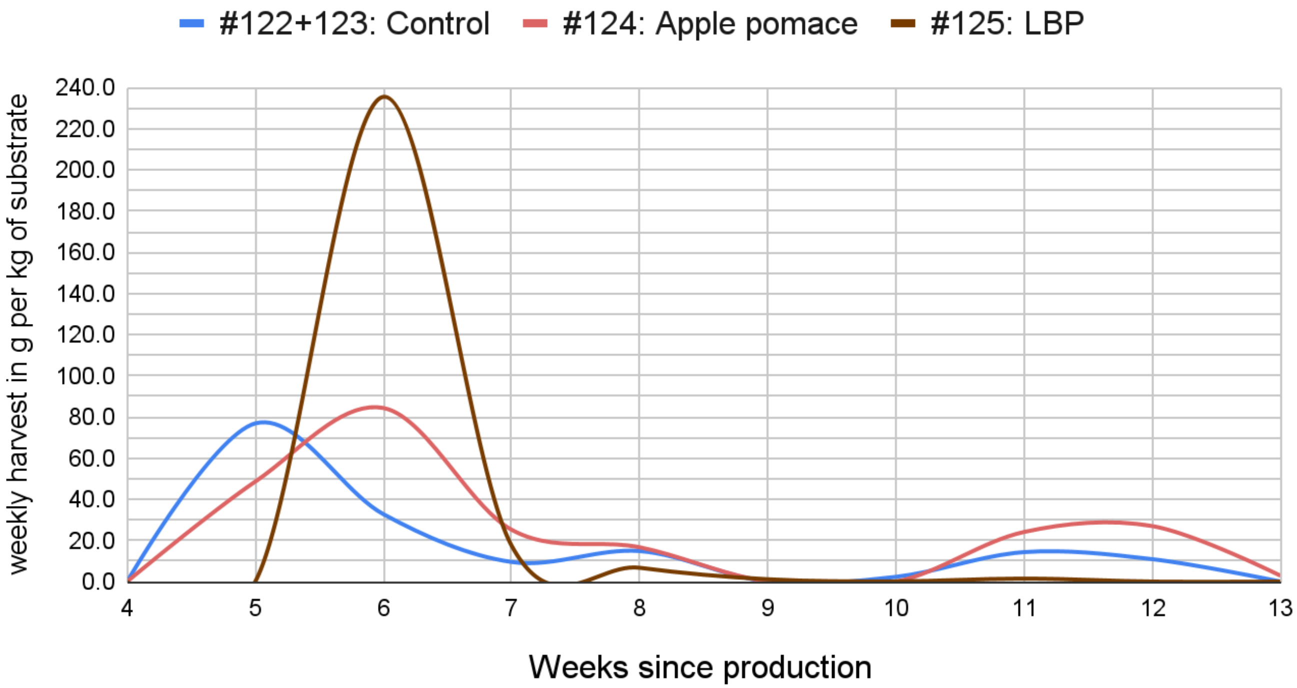
We seized the opportunity of the seasonality of this test to compare LBP with another abundant high-nitrogen resource: apple pomace. Apple trees are very common in our region and apples are pressed into juice or cider along the fall. Both LBP and apple pomace can only be used as supplements. The tested substrate was based on straw pellets and hemp shives.

		KPI ANALYSIS ID	Control	Pomace	LBP
SUBSTRATE DATA		**TEST BATCH NUMBER**	**122+123**	**124**	**125**
		Substrate quantity produced (kg)	198	85	107
		Substrate quantity produced (bags)	12.3	6.7	7.5
		Average substrate bag weight (kg)	16.1	12.6	14.3
		Fruiting temperature (°C)	12.0	12.0	12.0
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.162	0.229	0.262
		Wet/wet productivity, 70 days	0.136	0.175	0.261
		BE (wet mushrooms / dry substrate)	0.561	0.734	0.818
		Bag productivity (kg/bag)	2.61	2.89	3.76
		Space productivity (kg/bag, 70 days)	2.20	2.21	3.74
	SPEED	**Days to end of first flush**	36	47	47
		First flush / total (%)	68.2	68.5	96.4
	CONT.	**Partial contamination** (%)	24	30	27
		Total contamination (%)	0	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.41	0.48	0.44
		Supply cost (€/**bag** of substrate)	6.65	6.04	6.25
		Processing cost (€/**kg** of substrate)	0.51	0.49	0.45
		Processing cost (€/**bag** of substrate)	8.25	6.14	6.42
		COGS$_€$ (cost$_€$ / kg produced mushrooms)	5.77	4.22	3.37

04.09.24 Apple pomace vs LBP. Recipes:



Graph 32 Timelime of harvested oyster mushrooms
04.09.24 Apple pomace vs Linseed By-Product



To avoid mixing of LBP pectins in coffee as observed above[137], we isolated them in the pasteurisation process. As a possible consequence we observe a bigger performance divergence between #125 and control as what we then observed. Apple pomace is difficult to uncompact and though particles are small, they are stuck together and do not mix easily in the blender. It gave satisfactory results but we do not see how to use it in bigger quantities. Storing pomace would also require freezing as its high humidity content makes it storage-unstable.

#125 here shows the same high potential as #109 above[134] with an impressive first flush. It is another possible game changer that we will analyse in PART II. The question we need to address then is: in what quantities can/should we use LBP to maximise performance while keeping contamination rates acceptable.

[137] See WP2 T9A.3

Task 10: Broad bean husk

We identified in our desktop study board bean hulls as a potential high-performance ingredient for mushroom substrate preparation[138]. However, after contacting many farmers, we did not find any producer of such a product. As far as we understood, broad bean husks are not collected and left behind harvesters as fertilizer for the next season. Their small size makes it difficult to collect manually, even for a test.
We found a pea producer who was ready to bale pea straw[139] for a test but the harvest occurred after a rainy week and the straw was improper for baling. According to the producer, straw started to mold and ferment straight away on the field and we decided not to go collect it.

Broad bean and pea by-products do not seem to be potential resources for us at this stage.

Task 11: Cellulose fibre sidestreams

After preliminary studies we decided not to test cellulose fibre side streams because we cannot easily guarantee their compatibility with food safety requirements.

[138] See WP1 T2.1: Carbon sources literature review
[139] Pea straw and broad bean hulls may be comparable in organic content

Task 12: Oklin machine compost

Oklin composting machines transform biowaste into a fine dry powder that resembles coffee grounds. It is not a resource we identified ourselves but one we were asked to try by Perho culinary college in Helsinki. After a few tuning tests[140] we noticed the high potential of "Oklin compost" and decided to integrate it in our final testing series.

Picture 46: Oklin compost machine

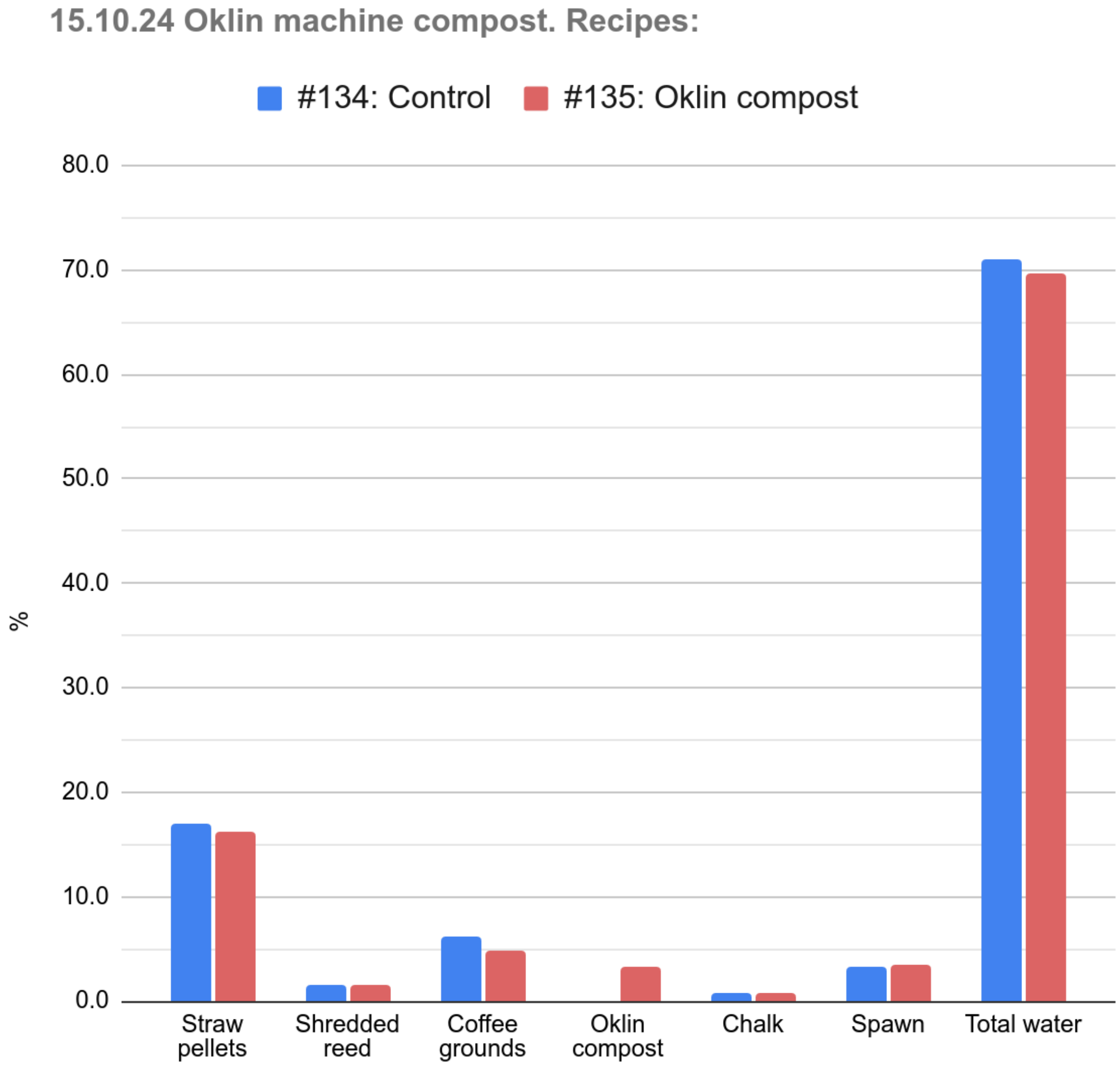
Oklin machine-compost, Supply and Environmental KPI:

- SUPPLY
  - Availability:
    - We identified **3 local suppliers** for this ingredient but there are potentially as many as Olkin machines in use.
    - The product is available **round the year.**
  - Scalability:
    - Supply cost: Oklin compost is a free resource for pickup, its cost only depends on logistics. We evaluate it here at 0,28€/kg (**280€/ton**). That cost cannot be reduced greatly due to relatively small quantities available at a time.
    - Processing cost: Olkin compost processing costs (pasteurisation, mixing) were evaluated at 0.31€/kg (**310€/ton**) for this project. Oklin compost can be processed as coffee grounds: it can be added to or used instead of them.
    - After careful logistic planification as we have made for coffee grounds, the collection of Oklin machine compost could reach any need we would face. It is difficult though to estimate how homogeneous the compost is, depending on what biowaste it is fed with.

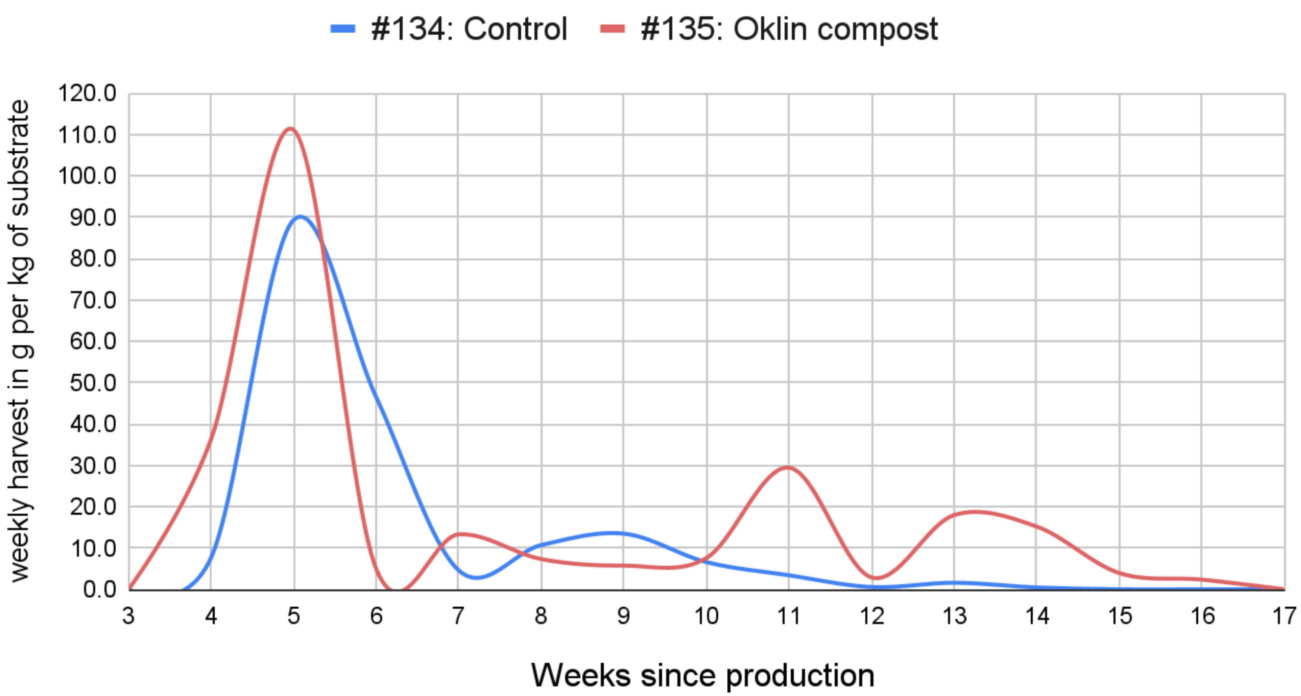
- ENVIRONMENTAL IMPACT
  - Localness: The currently used Oklin compost came from of of our regular mushroom customer (Nolla ravintola, **78 km).**
  - Circularity: The **only current use** of Oklin compost is soil fertilising. That use is still possible after adding the step of growing mushrooms from it (mushroom substrate can also be composted and used as fertiliser).
  - Organicity: **Organic certification is likely to be hard for such a resource.** The only foreseeable solution would be to find an organic food processor who uses this type of machine in their production.

[140] Such as batch #34 analysed above in WP1 T3.2

		KPI ANALYSIS ID	Control	Oklin compost
SUBSTRATE DATA		TEST BATCH NUMBER	134	135
		Substrate quantity produced (kg)	202	201
		Substrate quantity produced (bags)	13.4	14
		Average substrate bag weight (kg)	15.0	14.3
		Fruiting temperature (°C)	11.9	11.9
KPI: BIOLOGICAL PERFORMANCE	PRODUCTIVITY	**Wet/wet productivity**	0.185	0.258
		Wet/wet productivity, 70 days	0.179	0.186
		BE (wet mushrooms / dry substrate)	0.637	0.852
		Bag productivity (kg/bag)	2.78	3.70
		Space productivity (kg/bag, 70 days)	2.69	2.67
	SPEED	**Days to end of first flush**	41	40
		First flush / total (%)	77.5	59.1
	CONT.	**Partial contamination** (%)	0	7
		Total contamination (%)	0	0
KPI: ECONOMICAL PERFORMANCE		**Supply cost** (€/**kg** of substrate)	0.39	0.41
		Supply cost (€/**bag** of substrate)	5.90	5.81
		Processing cost (€/**kg** of substrate)	0.51	0.50
		Processing cost (€/**bag** of substrate)	7.64	7.19
		COGS€ (cost€ / kg produced mushrooms)	4.87	3.52



Graph 33 Timelime of harvested oyster mushrooms
15.10.24 Oklin machine compost



Oklin compost show high performance, reminding those of LBP-supplemented recipes[141]. However the production shape and speed features are different: Batch #135 does not give a huge first flush but rather 4 decent flushes, the global performance being more remarkable than any single flush productivity.

It is a high-potential supplement that needs to be tested further and compared with other supplements in PART II.

Picture 47: Dry Oklin machine compost

Picture 48: Wet Oklin maching compost

[141] See WP2 T9A and T9B.

Task 13: Comparative Study

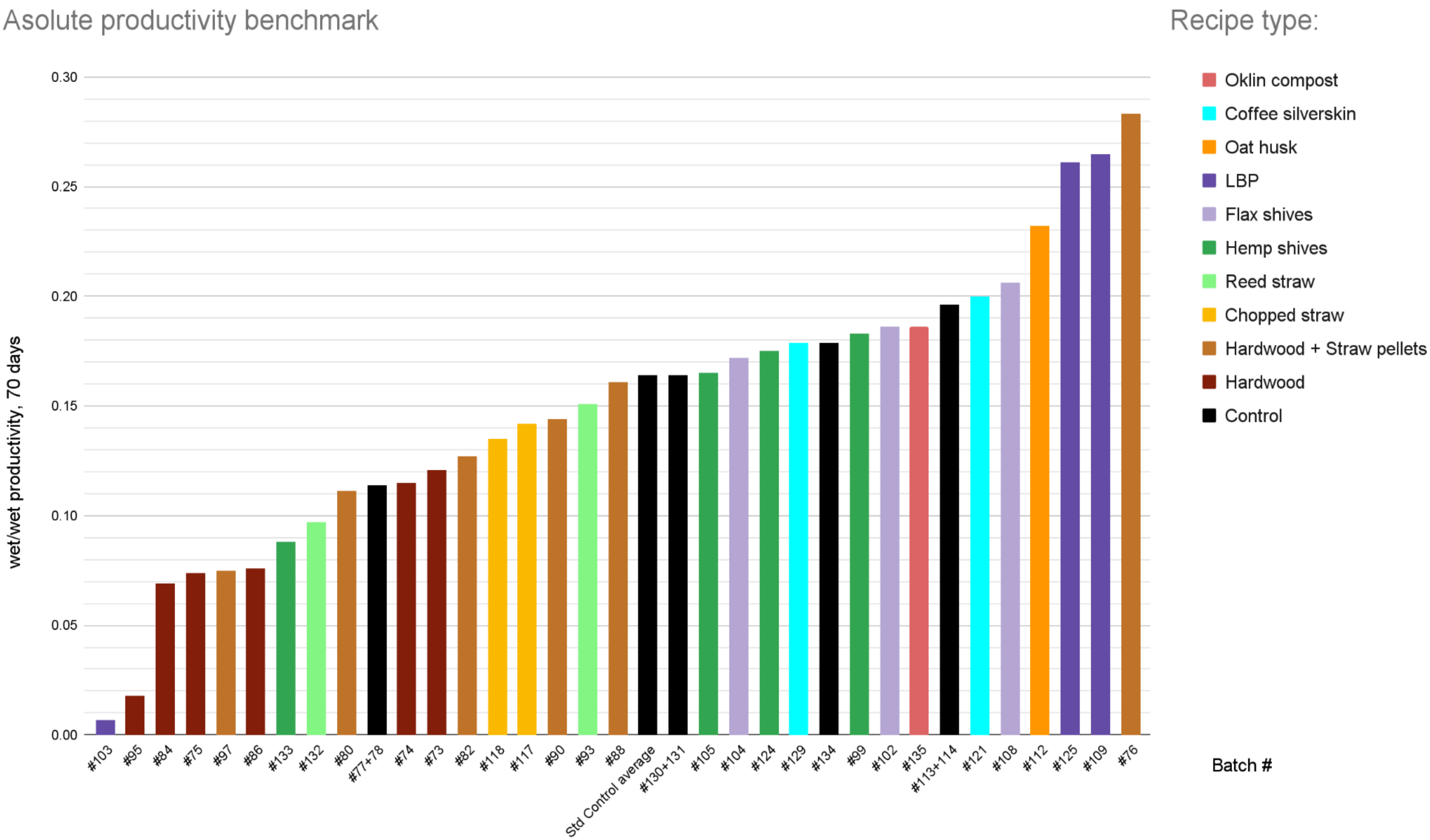
WP2 T13.1: Recipe List of all test batches analysed in WP2 in dried ingredient ratio % / total wet weight

Batch #	Straw pellets	Alder pellets	Apple -tree pellets	Birch coarse sawdust	Spent shiitake subs.	Sunflower seed hull pellets	Hemp shives Hemparade	Hemp shives Korkeaoja	Flax shives	Ground oat husk	Chopped wheat straw	Common reed (winter)	Coffee silver -skin	Linseed By-Product	Oklin comp -ost	Apple poma -ce	Coffee groun ds	Chal k	Spawn	H_2O [142]
Control [143]	**15,2**											**1,7**					**7,4**	**0,9**	**3,5**	**71,2**
73				25,1														2,1	8,4	64,4
74					26,7													0,9	6,1	66,4
75				26,7														1,4	5,7	66,2
76	9,6			16,8														1,2	4,8	67,6
77, 78	15,9											1,1					7,5	0,9	3,4	71,1
80	12,5	13,2																0,9	3,8	69,5
82	14,0			12,7														1,1	4,2	68,1
84				26,6														1,5	5,9	66,1
86				15,8													12,3	1,3	2,9	67,7
88	13,1		12,9															1,0	3,7	69,3
90	12,3	13,0																0,9	3,4	70,4
93												22,4						2,2	6,8	68,6
95		22,3																1,0	3,8	72,8
97	11,8	12,5																0,9	3,3	71,5
99	20,7							2,1										1,0	4,1	72,0
102	11,8								12,1									1,3	4,9	69,9

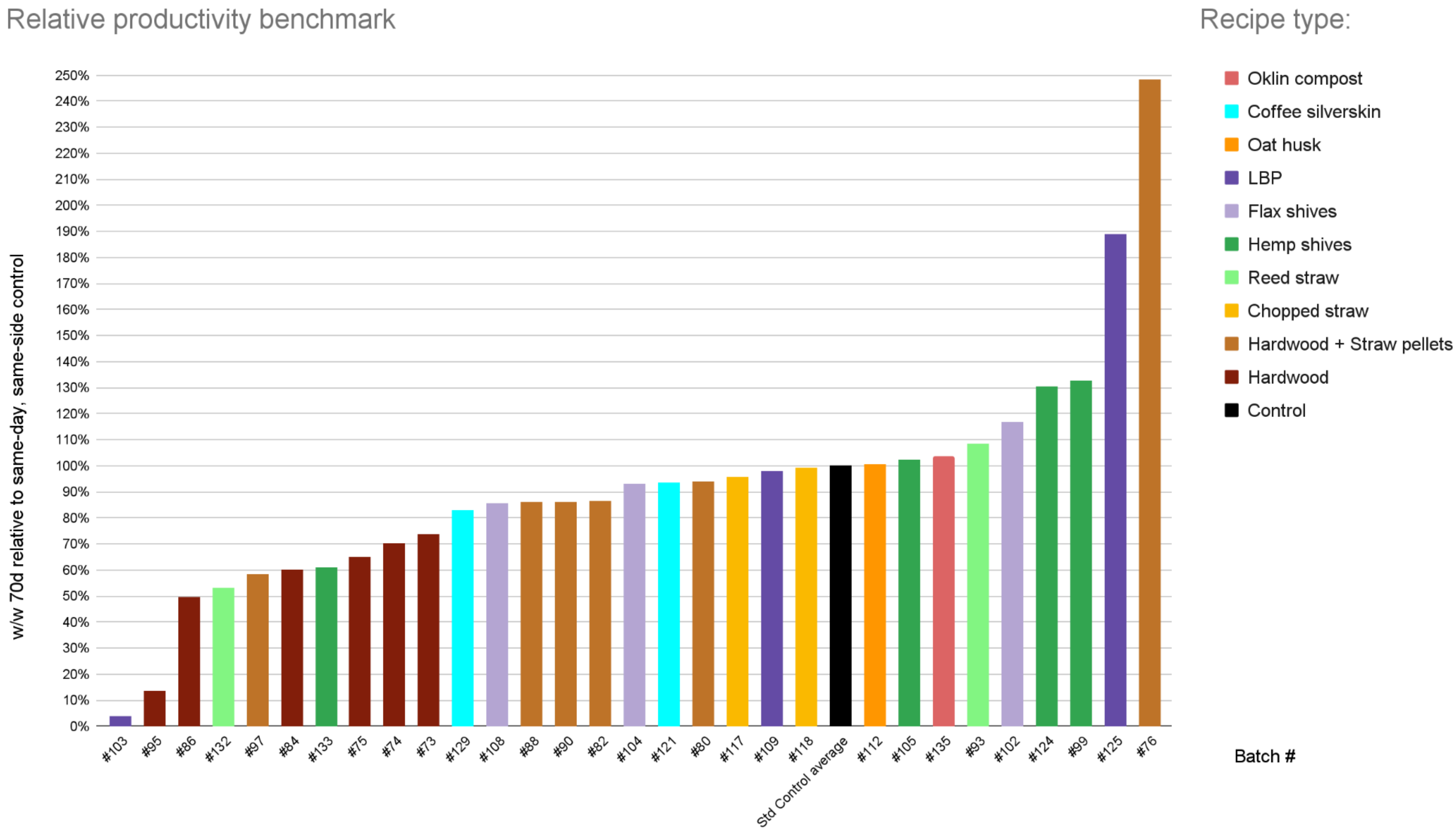
[142] Total water content, including other ingredients embedded water
[143] All batches that comply to the defined standard: 79, 81, 83, 85, 87, 89, 91, 92, 94, 96, 98, 100, 101, 106, 107, 110, 111, 115, 116, 119, 120, 122, 123, 128

Batch #	Straw pellets	Alder pellets	Apple -tree pellets	Birch coarse sawdust	Spent shiitake subs.	Sunflower seed hull pellets	Hemp shives Hemparade	Hemp shives Korkeaoja	Flax shives	Ground oat husk	Chopped wheat straw	Common reed (winter)	Coffee silver -skin	Linseed By-Product	Oklin comp -ost	Apple poma -ce	Coffee groun ds	Chal k	Spawn	H_2O
103	19,3													7,9				0,9	2,9	69,0
104	20,3								2,6									1,2	4,6	71,3
105	18,9							2,5										0,9	3,8	73,8
108	17,6								4,6									1,0	4,0	72,8
109	18,2							1,0						7,3				0,9	3,6	69,0
112	18,0									9,8								1,0	4,1	67,1
113,114	18,4					3,8						2,1						1,1	4,5	70,1
117	15,6										1,4						7,5	0,9	3,6	71,1
118											9,6						12,1	1,5	5,8	71,0
121	14,6												3,5				5,9	0,9	3,5	71,5
124	18,7						6,4									0,7		1,1	4,3	68,8
125	14,8						5,9							6,7				1,1	3,6	67,9
129	15,6			3,4									4,7					1,2	4,7	70,3
130,131	18,5											1,8					3,7	0,9	3,7	71,5
132	7,7											12,0					3,5	0,9	6,9	69,0
133	7,3						8,3					1,9					3,3	0,8	3,5	74,7
134	17,0											1,6					6,2	0,8	3,3	71,0
135	16,3											1,6			3,3		4,9	0,8	3,5	69,7
X1								3,0		29,5								1,4	5,5	60,7
X2		17,0								11,7								1,2	4,9	65,3
X3	14,4					14,2												1,1	4,2	66,2
X4													16,7				7,8	1,2	4,7	69,7

We first compared the absolute 70-day wet/wet productivity of all above analysed batches, grouped in recipe types. Only visible control batches here are those which do not comply with the standard control recipe as defined in [WP1 T3.4](WP1 T3.4).



We then decided to do the same analysis relatively to each test batch's control. When no control was available/healthy (batch #73 and #74), the used reference was the standard control average value (0,164).



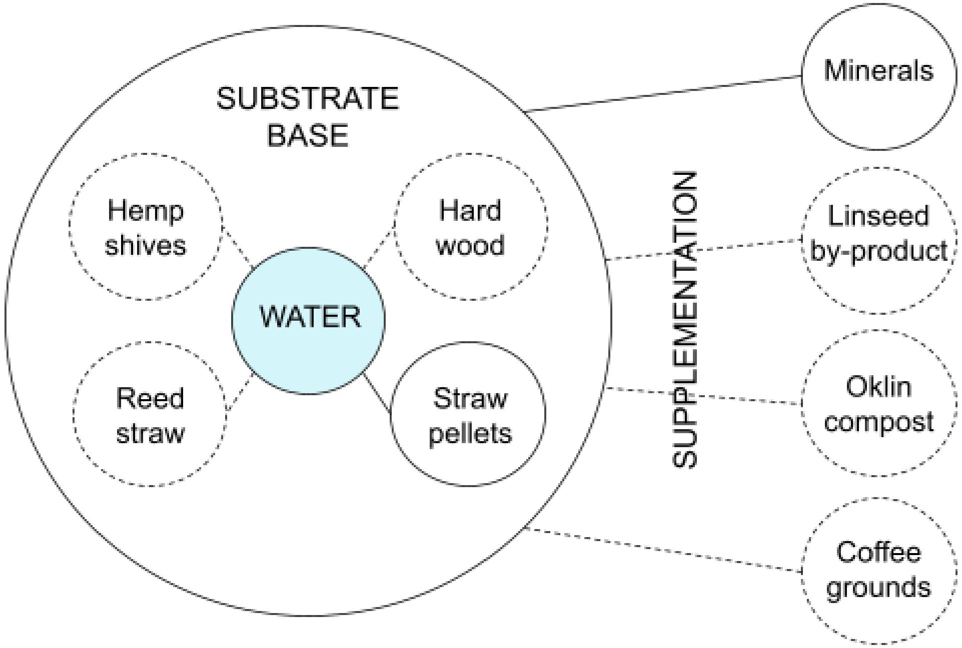
WORK PACKAGE 3: Preliminary Results and project continuation

Productivity benchmarks above as well as previously reported WP1 and WP2 test results give us precious information about ingredients potential:

- **Hardwood**-based and hardwood-mixed recipes are generally less performing than any other substrate type. However, batch #76 (Birch sawdust + straw pellets) overperformed any other tests in absolute and relative productivity. Its performance is so high (250% of the corresponding control) and so different to all other mixes containing hardwood, that we have to consider a possible mistake in data collection. However, without proof of such a mistake and considering that hardwood is more locally abundant than straw, we will continue researching hardwood in PART II. The question we will need to answer are:
    - In what proportion can hardwood be used without reducing a recipe's productivity?
    - Can hardwood be supplemented to compete with straw?
- **Chopped straw** performs as well as straw pellets. However, the difficult local supply and storage push us to push it aside until further notice. We will not test it further in part 2.
- **Common reed** seems to be a potential substitute for straw: batch #93 shows it can perform unsupplemented as well as straw, its water retention capacity is almost as good and its consistency alike. Our local supplier sounds motivated to develop new processed products out of it and if he manages to make available a finely chopped product that can compete in price with straw pellets, we could use it as a local and circular alternative to imported straw pellets. Using common reed in our control recipe has helped us to understand how to process and use it. If appropriate products are available, we will pursue its testing in PART II.
- **Hemp shives** have shown a high potential when mixed with denser materials, as visible in the benchmark. Hemp shives benefits in a substrate seem to be an increased water retention capacity and better gas exchange. They would have been integrated in the control recipe if not for supply troubles. We hope to supply local hemp shives to be tested in various proportions and with various supplements in PART II.
- **Flax shives** do not seem to improve our recipes in the same proportions as hemp shives. It might be due to a poorer water retention capacity or because of the unwanted fiber mixed in the shives we used that prevented a proper blending. Flax can be considered an alternative to hemp in case we cannot find an easy supply of hemp shives.
- **LBP** (refused linseeds) was the divine surprise in this project. Not only is it the perfect circular economy case (high-potential resource that ends up burnt) but in relatively small proportions it seems to greatly improve oyster mushroom substrates productivity. As all highly nutritive supplements it is however uneasy to master with a non-sterile production process, as irregular results and occasional failure (such as batch #103) show. We will study it further in PART II.
- **Oat husks**, ground or whole, do not seem to be usable as a carbon base due to their low water retention capacity. The local ground product we supplied for this project could be considered a potential supplement but its potential is too hazardous for us to research further at this stage.
- **Coffee silverskin** is a difficult resource to transport and store, due to its very low density. It will not be extensively researched in PART II . However, batch #121 performed well and used a moderate amount of silverskin that was still reasonable (and scalable). Its production process was also fluid, silverskin being low-temperature pasteurised together with straw pellets. A close adaptation of that recipe might be side-tested in PART II.
- **Oklin machine compost** is with LBP the other new supplementation that requires further research. It shows a high potential but maybe not in 70 days. To be determined in PART II.

- **Coffee grounds** were not treated as a separate topic here as they have been in most of our substrate recipes for 9 years. Most present and past tests show their humble potential: coffee-supplemented recipes perform more or less as well as unsupplemented recipes. However we still consider of interest for many reasons:
    - Their small particle size can help increasing substrate density when working with very low-density / coarse materials;
    - They are available everywhere and mostly considered waste, unlike straw
    - They can be used in big amounts, up to ⅓ of the dry weight without increase dramatically contamination risks
    - They are slightly oily and help other ingredients not to agglomerate in balls/ make bagging easier
    - They can be used in smaller amount with other supplements to improve their consistency / make them less sticky
    - Mixed with LBP in boiling water, they improve its fluidity and help avoid sticky linseeds burning at the bottom.
- **Broad bean and pea side-products** cannot be easily supplied in Finland at this stage.
- **Cardboard, paper and other cellulose fiber** side streams cannot be guaranteed as safe to produce food without (deeper) research.

At this stage of the project, this is how our substrate looks like:



The next phase will consist in finding out how to articulate these ingredients (supply, proportions, pasteurisation, mixing and bagging) to get the best compromise between biological, economical and environmental performances.

OVERVIEW OF PROJECT PART II OBJECTIVES

Part II will be conducted as a separate project 2024-2025. Here is an overview of its objectives.

Work package 4: Refined tests on selected substrates

Optimal proportions
Supplementation
Outsourced tests (different growing conditions, same recipe)

Work package 5: Other mushroom species on selected/adapted substrate

Cerioporus Squamosus
Pholiota Microspora

Work package 6: Biology

Organic composition
Nutrition facts

Work package 7: Product lifecyle

Mushroom production in private gardens
Composting
Energy production
Biogas production

Work package 8: Pre-industrialisation

Organic production (Y/N)
Business plan
Scaling partners research

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  - Nina Långstedt (Svartå)
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  - Korkeaojan luomutila (Kokemäki)
  - Linenstories (Helsinki)
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  - Perho liiketalousopisto (Helsinki)
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It is a pleasure to work surrounded with talented, cooperative and kind partners.

Stéphane Poirié, Helsieni Oy, 27.04.2025