MUSHNOMICS

Unlocking data-driven innovation for improving productivity and data sharing in mushroom value chain

D3.1 – Report on potential substrates and their lab analyses

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Disclaimer

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1. Introduction

The aim of this report is to study the potential raw materials that are available in urban settings and assess their applicability for oyster mushroom growing based on the company's own know-how. In the report, we discuss the applicability of different urban biowastes from the perspective of being a raw material and also from the substrate formulation process. The mushroom substrate is defined as a composite material prepared based on a recipe that defines the composition and the preparation process. Based on the characteristics and composition of the raw materials, we have developed different recipes, each containing multiple raw materials to reach optimal parameters for mushroom growing.

After the initial assessment of the urban organic waste landscape, we decided to investigate the next legal categories and shortlisted the following specific substrates for testing:

- within legal category "biowaste":
  - mixed vegetable waste from large retail stores and markets,
  - coffee ground,
  - park waste (leaves, branches, etc.).
- within legal category "packaging waste":
  - cardboard.

For the above categories, as detailed in the next chapters, we assessed the European level statistics and identified local stakeholders to obtain samples. From each type, we managed to obtain samples that were analysed in our lab for composition, substrate development and mushroom growing trials.

This report provides a snapshot of our work, mainly focusing on the assessment, selection and analyses of potential raw materials and substrates produced from them. Based on the outcomes reported here, mushroom growing trials have been ongoing continuously with different recipes and parameter settings.

2. Assessment of potential raw materials

Oyster mushrooms (*Pleurotus spp.*) are saprophytic species as they obtain carbon and nutrients by decomposing various biomass streams. Most of the used substrate materials are types of low-value lignocellulosic wastes that are primarily derived from agriculture or agro-industry. Therefore, it is a common industrial practice to grow oyster mushroom on agricultural by-products, however, it is rare to utilize urban organic wastes for oyster mushroom production. Ideally, the substrate for oyster mushroom growth needs to include fibrous, lignocellulosic material with low free sugar content (such as wheat straw in industrial practice) that works as enzyme inducer, carbon source and place for mycelia development, as well as nitrogen source with a total nitrogen content of approximately 1% by weight (in industry different additives are used for this purpose, including corn gluten).

This means that most likely there is no sole raw material which can be used directly as substrate, but rather a recipe needs to be applied with a mixture of different raw materials, each with their pre-defined percentage. In this chapter we investigated the potential raw materials for oyster mushroom substrate recipes which are available in urban settings. For categorization, we used the legal categories of the European waste management legislation.

Biowaste

According to the Waste Framework Directive's definition, biowaste comprises *'biodegradable garden and park waste, food and kitchen waste from households, offices, restaurants, wholesale, canteens, caterers and retail premises and comparable waste from food-processing plants'*. Apart from biowaste, there are other biodegradable wastes, for example paper and cardboard waste, wood waste and natural fibres in textiles.[1]

According to the Eurostat (2019 data) biowaste accounts for more than 34% of the 249 million tonnes of municipal solid waste generated, amounting to 86 million tonnes in 2017 as total of the 28 EU Member States. Hungary, for instance, has one of the lowest levels of biowaste generation per person, which is 75 kg annually (approximately 17% of the total municipal waste generated). As comparison, Figure 1 shows these values also for other European countries, Denmark has the highest per capita biowaste generation (more than 350 kg/person annually), which – probably due to the good overall recycling system – is half of the generated municipal waste. In general, food waste accounts for nearly two thirds (60 %) of all biowaste from households and similar sources.[2]

[1] EEA Report No 4/2020, Bio-waste in Europe — turning challenges into opportunities https://www.eea.europa.eu/publications/bio-waste-in-europe
[2] EEA Report No 4/2020, Bio-waste in Europe — turning challenges into opportunities https://www.eea.europa.eu/publications/bio-waste-in-europe

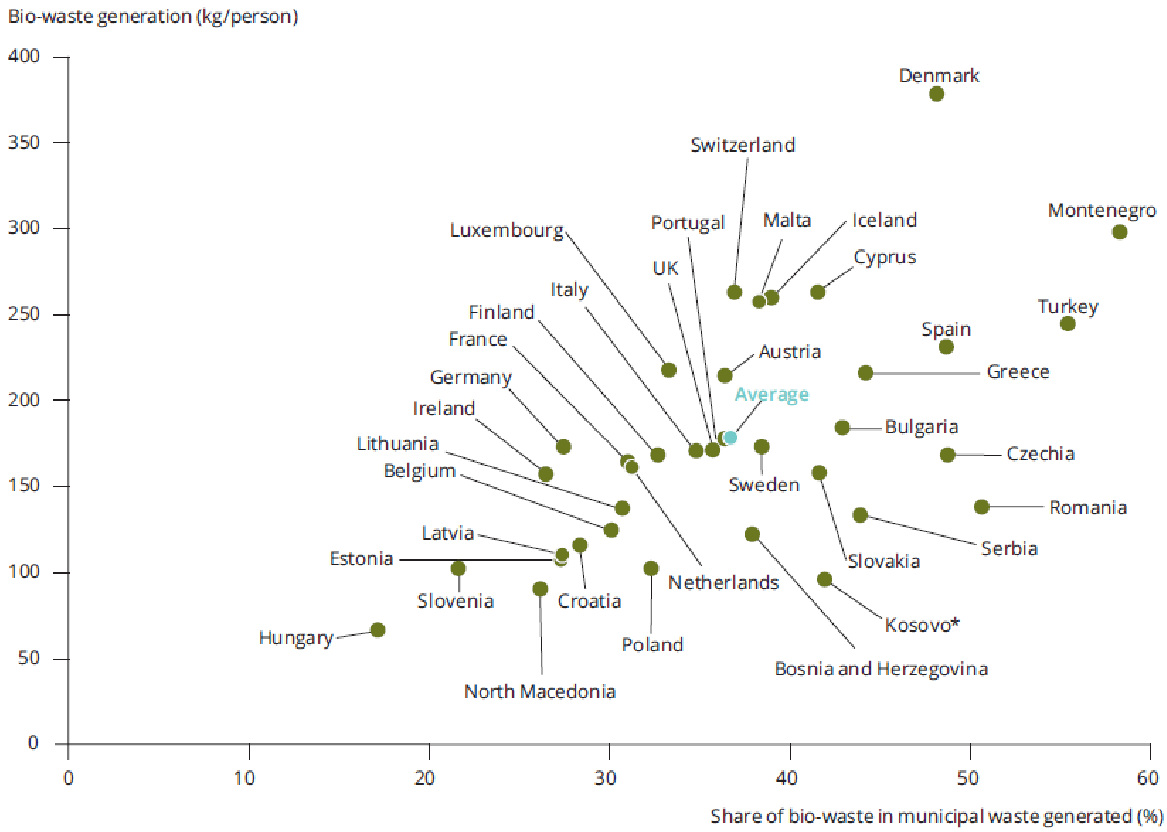

Figure 1: Municipal bio-waste generation per person and share of bio-waste in municipal waste, 2017, source: EEA Report No 4/2020, Bio-waste in Europe — turning challenges into opportunities

Food waste, a key component of biowaste, can be edible (e.g., food purchased but not eaten, leftovers from meals) or non-edible (e.g., banana peel or bones). The edible part is targeted by food waste prevention measures. In the European Union legal environment, food waste was defined as *‚any food substance, raw or cooked, which is discarded, or intended or required to be discarded*' between 1975 and 2000 when the old directive was repealed by Directive 2008/98/EC, which has no specific definition of food waste.

The sectors contributing the most to food waste are households (47 million tonnes ± 4 million tonnes) and the processing sector (17 million tonnes ± 13 million tonnes). These two sectors account for 72% of EU food waste, although there is considerable uncertainty around the estimate for the processing sector. In addition, and as previously mentioned the uncertainties for the production sector is probably underestimated. Of the remaining 28 percent of food waste, 11 million tonnes (12%) come from food service, 9 million tonnes (10%) come from production and 5 million tonnes (5%) comes from wholesale and retail.[3]

[3] FUSIONs (2016) Estimates of European food waste levels
https://www.eu-fusions.org/phocadownload/Publications/Estimates%20of%20European%20food%20waste%20levels.pdf

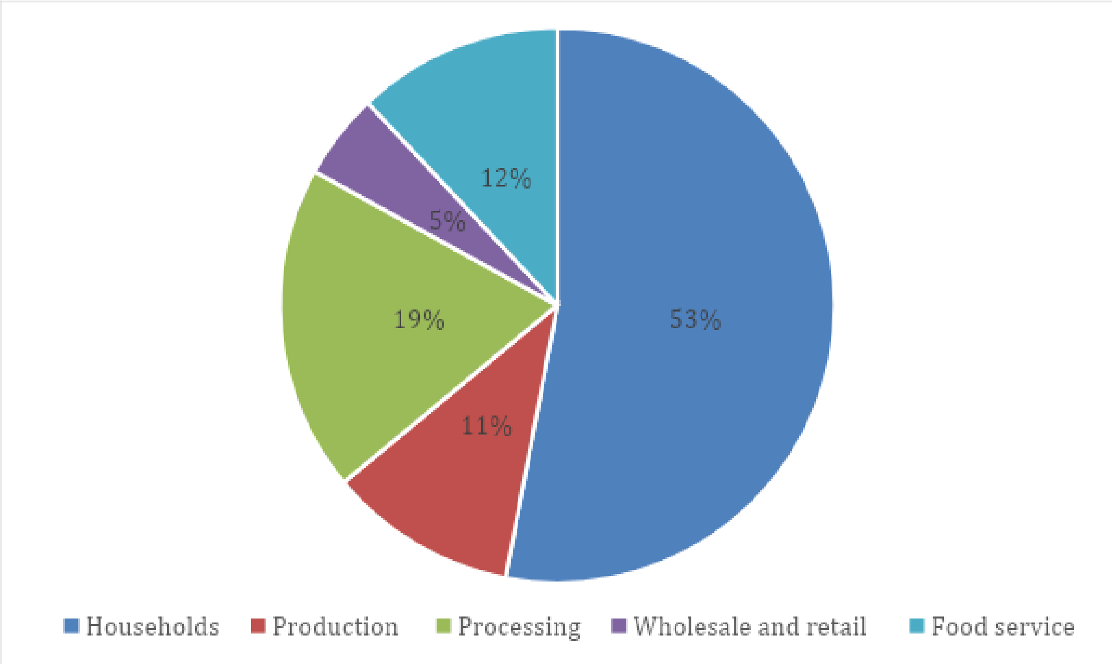

Figure 2: Origin of food waste within the EU, source: FUSIONs, 2016

Plant-based food waste

From the point of mushroom growing, unprocessed (uncooked) and plant-based food waste is of interest. Losses of horticultural produce are a major problem all along the food supply chain, from primary production right through to food processing and consumer. Post-harvest activities include on-farm handling, storage, processing, packaging, transportation, and marketing. Post-harvest losses of vegetables and fruit occur at all stages points in the value chain from agricultural production to the food being placed on a plate for consumption. They can be caused by a wide variety of factors, ranging from growing conditions to handling at retail level, as well as irresponsible and non-sustainable patterns of consumption (e.g., buying more than needed).

Practically this means that the main sources and potential local partners of this kind of 'fresh mixed vegetable food waste' can be the following categories:

- Retailing: hyper- and big supermarket chains.
- Short food supply chains e.g., farmers' markets, community-supported agriculture), on-farm sales, off-farm schemes
- Hospitality and food service e.g., canteens and restaurants.

For the first trials in the project, we have obtained samples of mixed vegetable waste from nearby (i.e., Kecskemét, Hungary) Tesco and METRO supermarkets, as local partners.

Though not applied in large scale for oyster mushroom production, the literature suggests that vegetable waste can be a good raw material to grow oyster mushroom as suggested by the following studies e.g., Shashitha et al. 2016[4] and Ling Ma et al. 2020[5]:

Notably, vegetable waste is not applied alone but mixed with other raw materials, mainly fibrous ones that can act as carbon source and provide structure to the oyster mushroom substrate.

<u>Coffee grounds</u>

Another important raw material within the food waste category is coffee grounds. Using coffee grounds also provides a unique opportunity to reuse urban generated waste. Used coffee grounds are an unconventional and relatively underutilised growth substrate for oyster mushrooms, however, there is not yet a clear recipe for substrate composition and growth conditions at a larger scale. Fresh coffee grounds are pasteurized by the way of coffee making, but its high moisture content and favourable composition make it also a good substrate for moulds. Therefore, there are challenges related to its storage and substrate preparation process to prevent contamination.

Coffee grounds are available in coffee shops, gas stations, office buildings and most of restaurants. However, they are (from a commercial perspective) usually collected in small amounts requiring extensive logistics planning. Due to this, it is better to choose innovative waste management companies, as local partner that manages office buildings and thus can organize selective collection of coffee ground. Daily collection routes would be necessary, as well as relationships with coffee shops to ensure that the used grounds are properly treated.

This is the reason we have teamed up with Recobin (https://recobin.hu/). Recobin is a Hungarian company that offers selective and integrated collection of waste materials to different establishments, such as offices, universities, and shopping malls. The separate collection of waste materials could mean that the establishments partnered with Recobin are stable sources of coffee grounds (and other types of usable biowastes, too). Collection, transportation and logistics of used coffee grounds are important steps. It involves storing the used grounds in a clean container on site, collecting them as soon as possible and then storing them in a freezer until use.

While there are no exact statistics on the amount of annual coffee ground production in Europe, this waste stream has been already recognized by urban farmers. Although there are no traditional mushroom farms that use coffee grounds, there are urban mushroom start-ups who experiment with it and use them as substrate in growing kits. These kits are colonized with mycelium at the producer's location and are then sold to consumers so that they can grow the mushrooms at their home.

[4] Shashitha KN, Komal Shlini P, Singh KG (2016) Vegetable Waste-A Potent Substrate for Cultivation of P. Ostreatus. International Journal of Research Studies in Biosciences 4(6), 5-9.

[5] Nyuk Ling Ma, Shing Ching Khoo, Wanxi Peng, Chia Min Ng, Chin Hoe Teh, Young-Kwon Park, Su Shiung Lam (2020) Green application and toxic risk of used diaper and food waste as growth substitute for sustainable cultivation of oyster mushroom (Pleurotus ostreatus). Journal of Cleaner Production, 268:122272.

Some of such initiatives across Europe are presented below

- Permafungi, Brussels, Belgium: https://www.permafungi.be/en/organic-mushrooms
- Let's grow together, Amsterdam, the Netherlands: https://gro-together.com/en
- Beyond coffee, Copenhagen, Denmark: http://www.beyondcoffee.eu/
- Nam-mushroom, Lisbon, Portugal: https://nammushroom.com/
- Hut und Stiel, Vienna, Austria: https://www.hutundstiel.at/
- Espresso mushroom company, Brighton, UK: https://espressomushroom.co.uk/

Nevertheless, exact production volumes, frequency of operation and supposed use of other raw materials are not reported on the above websites. As coffee grounds are also available in households, there are oyster mushroom growing kits specialized for coffee grounds, for example from the above companies, as well as many articles found on the internet discussing this topic[6]. Despite this, there are challenges associated to this raw material. Many of the non-scientific papers suggest using fresh coffee grounds (which is sterile due to previous brewing) plus uses non-economical spawn ratio. Hence, logistics, storage, pasteurization and spawning need to be managed for industrial scale-up.

Garden and park waste

Apart from food related wastes, the biowaste category includes garden and park waste, too. These are generated during maintenance of private gardens and public parks with a variety of components including grass clippings, hedge cuttings, pruning, leaves, branches, and wood, as well as containing undesirable inorganic (e.g., soil and stones) materials. Because of its origin, garden waste is expected to have variable generation rates and composition, depending on factors such as seasons and location (e.g., climate, urbanization, and waste management strategies). By definition, garden waste exists only when collected. Little data and statistics are available regarding garden waste generation but estimates state that approximately 35% of the total municipal biowaste is garden waste. In many European countries garden waste is collected and mixed with communal and organic waste, or composted on-site or separately, without entering the waste management system. Therefore, key local partners are public companies dealing with park and public spaces maintenance, as well as forestry service companies for forestry by-products.
Garden and park wastes are composed of lignocellulosic materials, thus from this aspect they are very similar to wheat straw or most other agricultural by-products. Underpinned by our previous experiences with a variety of agricultural by-products and publications about using agricultural by-products to grow oyster mushrooms, our hypothesis is that garden and park waste can be also a proper raw material.

Packaging waste

6 https://thegreengardenlife.com/growing-mushrooms-in-coffee-grounds/ and https://www.apieceofrainbow.com/grow-mushrooms-coffee-grounds/

The definition of packaging waste concerns containers and packaging as products that are assumed to be discarded the same year the products they contain are purchased. Packaging can come in all shapes and forms ranging from delivery boxes to soda cans that are used to store, transport, contain, and protect goods to keep customer satisfaction. The type of packaging materials includes glass, aluminium, steel, paper, cardboard, plastic, wood, and other miscellaneous packaging. Packaging waste is a dominant contributor to waste generation in today's world and is responsible for around half of the waste in the globe.

From this wide spectrum of different kind of waste, our interest is focused on cardboard. Cardboard is widely used as a packing material and is found in large quantities all over the globe. Cardboard waste contains at least 50% of cellulose, and the physical properties of cardboard make it a favourable candidate for biodegradation and bioproduction.

Between 2008 to 2018, paper and cardboard were the main packaging waste material in the EU (31.8 million tonnes in 2018) followed by plastic and glass (14.8 million tonnes for plastic and 14.5 million tonnes for glass waste materials in 2018). In 2018, packaging waste generated was estimated at 174.1 kg per inhabitant in the EU.[7]

Despite its abundancy, only a very few studies have been carried out using cardboard (and wheat)[8] as a resource for growing oyster mushrooms, such as Tesfay et al. 2020 [9]

[7] https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Packaging_waste_statistics#Waste_generation_by_packaging_material

[8] http://sietalab.com/how-to-grow-grey-oyster-mushroom-pleurotus-ostreatus-on-wheat-straw-and-cardboard-mixture/

[9] TesfayT, Godifey T, Mesfin R et al. (2020) Evaluation of waste paper for cultivation of oyster mushroom (Pleurotus ostreatus) with some added supplementary materials. AMB Expr 10, 15 https://doi.org/10.1186/s13568-020-0945-8

SWOT

To summarize our above research, we prepared a SWOT analysis about the uses of biowastes for oyster mushroom production.

Table 1: SWOT analysis of using urban biowastes as potential raw materials for oyster mushroom substrate

Strengths	Weaknesses
▪ years of experiences in mushroom farming secure an advantage ▪ own know-how to assess applicability of a raw material for oyster mushroom growing ▪ lab and other infrastructure are available for trials ▪ access to partners that can supply biowastes, contribute with know-how and finance	▪ storage and processing technology to be tailored to cope with extreme parameters and not biologically stable matters to minimize risk of contamination (mainly in case of mixed vegetable waste) ▪ need for external partner in collection and logistics which may be difficult due to segmented and small volume production ▪ in many aspects, our development needs to start from scratch as there are significant gaps in the literature and technology, especially for scale-up and container design
Opportunities	**Threats**
▪ large amounts of biowaste available ▪ both nitrogen rich and fibrous materials are available as biowastes providing the opportunity for balanced substrate composition ▪ legal background will push for selective collection and utilisation of biowaste ▪ growing need for utilization of those biowastes	▪ no well-defined composition, may contain harmful materials and non-biodegradable parts ▪ park wastes have seasonal fluctuation ▪ low awareness or non-existing separate collection for certain streams ▪ competing uses and already established market and recycling chain for cardboard that may risk supply ▪ due to increasing needs for those biowastes, owners may request unreasonable prices risking payback time

3. Origin and preparation of the samples

For the analyses and initial trials of mushroom growing samples the following urban waste raw materials were collected, as also shown on Figure 3:

- coffee grounds were collected from the office, from households of colleagues, from the nearby gas station and through Recobin from office buildings – this latter was the largest sources with delivery of approximately 30 kgs of coffee grounds;
- mixed vegetable waste was obtained from nearby Tesco and METRO retail stores;
- fallen leaves and branches were collected within the site of Pilze-Nagy Ltd. in Kecskemét, Hungary;
- cardboard without any painting was obtained from the operation and logistics of Pilze-Nagy Ltd.

Figure 3: Samples of chopped biowaste analysed, from top left clockwise: i) mixed vegetable waste, ii) fallen leaves, iii) cardboard and iv) branches

The samples (except coffee grounds) were chopped by using a commercially available garden shredder (Figure 4).

Figure 4: Chopping of the samples (branches on the figure) and storing the chopped samples in net bags

While fallen leaves, branches and cardboard are easy to store in case they are dry, storage of coffee grounds and vegetable waste under room conditions can be difficult as moulds and other harmful microbes can develop on it. Additionally, in the case of mixed vegetable waste composting will also start with heat formation due to high nitrogen and moisture content, and also flies can place eggs into it followed by quick penetration of the material by larvae. Therefore, coffee grounds were stored in freezer, mixed vegetable waste were obtained on the day of processing (though the collecting bin in the stores was exposed to contamination and flies), while chopped fallen leaves, branches and cardboard were stored in a closed and dry placed within net bags.

This samples were used for composition analyses and also preparation of the substrates for initial oyster mushroom growth trials.

4. Methodology for lab analyses

Moisture content

To effectively store the substrate material, the feedstock must be dry (max. of 15% moisture content, wet basis). If the moisture content is higher, microorganisms (mainly moulds) that can be harmful to the oyster mushroom can easily develop in it during storage. The storage time of the feedstock is determined by moisture content. Wetting must be only provided during pre-treatment of the substrate preparation step, as the substrate needs to have around 70% moisture content.
For this reason, we measured the moisture content of the selected samples (including certain raw materials and the prepared substrate, too) by using an AND-MX-50 type device, as shown on Figure 5 below.

Figure 5: AND-MX-50 device for moisture content determination

This device is suitable for quick determination of moisture content. For the measurement trial 5 g of the sample was weighed in the device. The material was heated with a halogen lamp at 160°C that results in drying of the sample when the weight is measured again (after reaching constant status). The method determines the actual moisture content in % wet basis, by the method of drying to constant weight, using the following formula:

$$Moisture\ content,\ \% = \frac{m_{initial} - m_{dry}}{m_{initial}}$$

Composition (ash and nitrogen content)

The feedstock must not contain waxes, as oyster mushroom is a wood-decomposing fungus, and it makes good use of materials with high lignin and cellulose content while waxes preserve the wood and limit the ability of fungus to digest it. Materials with higher nitrogen content should also be avoided or used only as an additive to reach optimal nitrogen content. Oyster mushrooms can be successfully produced on a mixture with a maximum nitrogen content of 2%, with the optimal value being 0.8-1.2%.
The applied protocol measures Kjeldahl nitrogen (organic nitrogen and ammonium nitrogen forms) of the raw materials. This is done by first using sulfuric acid with a catalysator and heat treatment, which causes the nitrogen content of the feedstock to be digested and converted to $(NH_4)_2SO_4$. The sulfuric acid content of the sample is then neutralized with NaOH added in excess. After this the ammonia content of the mixture is removed by distillation and taken up in boric acid. To measure the ammonium content, we titrate the mixture with a known concentration of acid (sulfuric or hydrochloric acid) using potentiometric endpoint signalling. The N content is corrected for the dry matter content of the sample.
The digestion process is performed in the TurboTherm and TurboSog devices (as shown on Figure 6) which also used blank and control samples, too. After that, the extraction tubes are inserted into the VAPODEST distillation unit, and the titration program is started.

Figure 6: Devices for nitrogen measurement

The nitrogen content of the samples is expressed as a percentage of the dry matter which can be calculated using the following formula:

$$Nitrogen\ content,\ \% = \frac{1.4007 \times 0.1 \times (V - V_{blind})}{m}$$

Where, m is the mass of the sample, V is the volume of acid during the titration and it is corrected with the volume of acid needed to titrate the blind sample.
The crude ash content which is measured is the residue obtained after incineration at 550°C, expressed as a percentage of the initial samples mass. We weighed 5 g to the nearest 0.001 g of the homogenised dried test sample into an ash crucible which is then heated at 550°C for 3

hours (to constant weight) and then cooled in a desiccator to room temperature. The sample is weighed again and ash content calculated and expressed as a percentage of the dry sample, using the formula:

$$Ash\ content,\ \% = \frac{m_{initial} - m_{oven}}{m_{initial}}$$

5. Results of lab analyses

Composition of the raw material

First, here we present the results of the feedstock analysis. From each raw material we performed the measurements with three parallel samples. The results for different analyses are presented in the below tables. The separate analysis of raw materials is important to develop mixtures of raw materials that secure the optimal conditions for the growth of oyster mushroom. Target values and objectives for substrate composition are:

- 0.8 – 1.2 % (of dry matter) nitrogen
- 8 – 10 % (of dry matter) ash
- 60 – 70 % moisture (to reach this, some dry raw materials are soaked for overnight and/or wetted)
- containing fibrous lignocellulosic material which works as carbon source and habitat for the oyster mushroom, as well as provides the structure of the substrate (not having sludge like material after the substrate production process)

We analysed the below results in view of reaching the optimal substrate composition, which, of course, is subject to an expected physical, chemical, and microbiological change during the composting and pasteurization process of substrate production. For this reason, we also analyse the prepared substrates, to be detailed in the next section.

Table 2: Nitrogen content of samples expressed as percentage of dry matter (%)

Sample	1	2	3	4	Average	Std. dev.
Fallen leaves (chopped)	0.83	0.82	0.83		0.83	0.01
Branches (chopped)	0.58	0.57	0.60		0.58	0.02
Cardboard (chopped)	0.19	0.19	0.20		0.19	0.01
Coffee ground	1.87	1.89	1.87		1.88	0.01

Mixed vegetable waste (chopped)	2.58	3.74	2.61	3.06	3.00	0.54

Table 3: Ash content of samples expressed as percentage of dry matter (%)

Sample	1	2	3	Average	Std. dev.
Fallen leaves (chopped)	15.15	15.31	15.18	15.21	0.09
Branches (chopped)	4.68	4.17	4.22	4.36	0.28
Cardboard (chopped)	12.62	13.31	12.94	12.96	0.35
Coffee ground	0.95	0.95	0.95	0.95	0.00
Mixed vegetable waste (chopped)	10.58	11.07	11.24	10.96	0.34

Table 4: Moisture content (%) and pH of samples

Sample	pH	1	2	3	Average	Std. dev.
Fallen leaves (chopped)	5.27					
Branches (chopped)	6.39					
Cardboard (chopped)	7.50					
Coffee ground	5.33					
Mixed vegetable waste (chopped)	4.95	88.57	89.12	89.54	89.08	0.49

Lignocellulosic materials are low in protein, and thus in nitrogen, with the nitrogen content decreasing with the age of the biomass. Green, young (seasonal) biomass has higher nitrogen content than brown biomass (such as stems). In line with this, from the tested materials leaves have the highest as they are only one season old, followed by branches as they can be even a few years old. Cardboard has the lowest nitrogen content, as it is made of timber or recycled paper. Therefore, this demonstrates that alone they cannot meet the optimal nitrogen content for mushroom production, which requires the addition of new raw material with higher nitrogen. For this reason, the addition of mixed vegetable waste and/or coffee grounds can be a good choice to improve the substrate for optimal mushroom production.

In the case of the mixed vegetable waste, its heterogeneity is worth mentioning, as reflected in the results. The reason for this being that it contains a variety of vegetables, even after chopping it is hard to have good samples, which is the reason for four parallel samplings. Also, the composition of mixed vegetable waste depends on the store, season, discount offers, consumption patterns, etc. In general, this biowaste seems to have appropriate moisture levels and nitrogen source, however, with the caveat concerning the extreme risk of contamination due to possible high simple sugar content (mainly fruits) and open-air storage (exposed to flies, high temperature, etc.).
Ash is basically the mineral content of the sample, thus it not only due to the biomass characteristics but production and collection, too (i.e., collection the leaves from a sandy soil). Our results show that it is important to conclude that coffee ground is a good nitrogen source without enhancing the ash content.
Moisture content was only significant in the case of the mixed vegetable waste, which is why it was the only one measured.
The pH values from our study do not indicate any problem. During the substrate preparation process pH is expected to grow significantly due to microbiological activities. Thus, the initial pH of the substrate is more important to support the growth of oyster mushroom and to suppress harmful competitors.
The analysed samples showed a great variety in nitrogen and ash content, indicating that good mushroom growth can be achieved by mixing different materials. This way the optimal nitrogen content of 1% can be achieved. Based on the results above, different mixtures (recipes) were developed to trial oyster mushroom growth.

Initial recipe development

Recipes with balanced composition of different raw materials for optimal nitrogen and fibrous materials contents were developed, as detailed in Table 5.

Table 5: First recipes for oyster mushroom growth trials, mass percentage of each raw material for a given substrate composition

Ratio/recipe	1	2	3	4
Fallen leaves (chopped)	50			20
Branches (chopped)	30		15	20
Cardboard (chopped)		67	55	25
Coffee ground		33	30	10
Mixed vegetable waste (chopped)	20			25

The samples in the above respective ratios were measured together, mixed well, and put in mesh bags. These mesh bags were placed in the large-scale pasteurization chamber. After

pasteurization they were inoculated with oyster mushroom spawn (HK-35 in 2.6 v/m%) and put in buckets with holes in the side. The buckets were closed and placed into the growing houses (as part of the existing industrial scale process without including the theorized container design and technology applied there).
Before inoculating the substrate, a small fraction of each recipe was saved for repeated composition analyses with results shown in the below tables.

Table 6: Nitrogen content of substrates prepared according to different recipes, expressed as a percentage of dry matter (%)

Recipe number	1	2	3	Average	Std. dev.
1	0.81	0.85	0.85	0.84	0.02
2	0.84	0.92	1.17	0.98	0.17
3	0.85	0.86	1.03	0.91	0.10
4	0.99	0.74	0.85	0.86	0.13

Table 7: Ash content of substrates prepared according to different recipes, expressed as percentage of dry matter (%)

Recipe number	1	2	3	Average	Std. dev.
1	10.60	11.58	10.33	10.84	0.66
2	4.83	4.96	4.78	4.86	0.09
3	3.99	5.40	4.83	4.74	0.71
4	8.03	12.94	9.42	10.13	2.53

Table 8: Moisture content (%) and pH of substrates prepared according to different recipes

Recipe number	pH	1	2	3	Average	Std. dev.
1	6.90	65.78	66.27	64.86	65.64	0.72
2	6.35	53.14	52.76	53.63	53.18	0.44
3	6.49	61.89	63.21	60.55	61.88	1.33

4	6.43	65.19	65.43	64.67	65.10	0.39

The composition of the substrates, in terms of nitrogen levels, were close to optimal, and in this respect the recipes are appropriate. However, in case of number 2 and 3 ash content is much lower than expected, thus there is room for improvement in this aspect. More importantly, the moisture content is also low, especially in case of recipe 2. Low moisture content not only limits oyster mushroom growth but can lead to unsuccessful pasteurization, as the main transfer of heat happens through water. Both facts can mean increased risk of microbial contamination. Due to this, advanced soaking of the raw material is necessary to ensure needed amount of water in the substrate – both during and after pasteurization.

Compared to the traditional large scale substrate preparation process, the pH values are too low (versus pH 9 in case of industrial, wheat straw-based substrate). The reason for this may be the lack of pre-composting which can enhance the microbial activities and thus pH. As a conclusion, additives to enhance the pH may be necessary in further experiments and in container-based operation where pre-composting is not possible.

The heterogeneity of the raw materials (compared to wheat straw) can be also an important factor in the inappropriate moisture and pH values as it complicates proper mixing and sampling. Thus, homogenization is important to prevent formation of drier areas with the risk of hotspots for contamination (as mentioned before, effective pasteurization is due to steam and wetness). This indicates that during the design process of the container-based technology, we must consider the proper chopping and mixing of the incoming raw materials.

6. Preliminary trials with oyster mushrooms

The batches of the first recipes were also tested in oyster mushroom growing. Control buckets with industrial, wheat straw-based substrates were also applied to compare growth. For the trials, the HK-35 strain was used which is widely spread in the mushroom industry.

The mushroom production process has two stages, and as the buckets were placed in the large scale growing houses, their growing period also went through the incubation part when the mycelia is colonizing the mass of the substrate followed by the fruit body development. The switch between the two phases is due to the change in environmental conditions: during incubation the temperature is around 19-20°C, while in the next stage is 15-20°C. In the fruit body formation phase low levels of light are also applied. The incubation period usually takes three weeks' time, while in the fruit body formation the first wave of harvest can take place already after one week.

Mushroom yields are generally expressed as a measure of biological efficiency (BE). This is the conversion rate of dry substrate into fresh mushrooms.

$$BE = \frac{Mass\ of\ fresh\ mushroom}{Mass\ of\ dry\ substrate} \cdot 100\%$$

While these experiments are still ongoing, in order to update the recipes and the bucket management, a few conclusions already can be drawn. First, the recipes proved to be successful, we experienced oyster mushroom growth comparable to the wheat straw-based control. However, repetition and finetuning of the experiments are in still progress. In some cases, we have faced issues with contamination which need to be addressed by enhanced bucket management, i.e. better cleaning and sterilization of the inside of the bucket, as well as considering also better mixing and soaking of the substrate before pasteurization. Due to the lower pH of the substrate than in case of the industrial process, the addition of additives (for example lime or $CaCO_3$) can help to increase the pH which may prevent contamination, too.

The following figures show the buckets where fully colonized substrate (inside the bucket) and fruiting bodies of oyster mushroom (outside, through the holes) can be seen. The white, "foamy" colour indicates no contamination (due to the presence of competing, harmful fungi, such as *Trichoderma* the colour of the substrate gets greenish. During cultivation, of course, the bucket is closed by a fitting plastic lid.

Figure 7: Oyster mushroom growing on biowaste-based substrate (recipe 4)

Figure 8: Healthy, colonized substrate in the bucket (recipe 4)

Figure 9: Oyster mushroom growing on biowaste-based substrate (recipe 4) with closed lid

7. Conclusions

During this task, we identified many types of substrates available in urban settings that could be applicable to successfully growing oyster mushrooms. In this task we shortlisted and obtained a few for analyses and testing. The analyses showed that each type of selected biowaste has valuable parameters, and by mixing them (i.e., generating proper recipes), the substrate can have near optimal composition.

While those raw materials can be good sources to produce oyster mushroom substrates, they have associated challenges in logistics, storage, and durability. Nevertheless, with proper technology design and logistics these challenges can be overcome.

The initial recipes prove that by combining the substrates they can work well to grow oyster mushroom; however, for achieving a homogenous substrate and for contamination prevention good soaking of the raw materials and proper pasteurization are necessary. Mushrooms show similar production in biowaste-based substrate as well as on wheat straw-based industrial medium. However, mushroom growing trials are still ongoing with the aim to find the best recipes and develop a proper way to manage buckets-based production (including cleaning and sterilization of the buckets).

This task allows us to conclude that raw materials are thus available and applicable for oyster mushroom growth; however, for optimal parameters they need to be mixed. These measurements and work with the real samples provided us valuable insights into the design process of the containers through which we may avoid some critical errors already sensed in this early phase measurement. This will allow us to achieve successful production in later tasks (WP3).