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WaysTUP!

VALUE CHAINS FOR DISRUPTIVE TRANSFORMATION OF URBAN BIOWASTE INTO BIOBASED PRODUCTS IN THE CITY CONTEXT

D1.2: Report on urban biowaste composition & physicochemical characteristics

WP1 – Identification of opportunities and barriers to utilisation of urban biowaste sources

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List of Acronyms

Acronyms	Description
AMB	Metropolitan Area Of Barcelona
ASP	Alicante Science Park
Cel	Cellulose
Cl^-	Chlorides
CV	Coefficient Of Variation
d.b.	Dry Basis
DEDISA	Inter-Municipal Solid Waste Management Company Of Chania
DEYAX	Municipal Enterprise For Water And Sewage
EC	Electrical Conductivity
FA profile	Lipid Profile
FEK	Government Gazette Issue
GC-FID	Gas Chromatography - Flame Ionization Detector
GOD-PER method	Glucose Oxidase–Peroxidase Method
Hem	Hemicellulose
IC	Inorganic Carbon
JMD	Joint Ministerial Decision
Lig	Lignin
MIU	Moisture, Impurities, Unsaponifiable
MSW	Municipal Solid Waste
MSWTP	Municipal Solid Waste Treatment Plant
$N\text{-}NH_4^+$	Ammonium Nitrogen
$N\text{-}NO_3^-$	Nitrate Nitrogen
NREL	National Renewable Energy Laboratory
NTUA	National Technical University Of Athens
Olsen-P	Olsen Phosphorus
P	Protein

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Acronyms	Description
PHA	Polyhydroxyalkanoates
SCG	Spent Coffee Grounds
SO_4^{2-}	Sulfates
TC	Total Carbon
TGA	Thermo-Gravimetric Analysis
TKN	Total Kjeldahl Nitrogen
TN	Total Nitrogen
TOC	Total Organic Carbon
TS	Dry Matter/Moisture
TVC	Total Viable Count
UCO	Used Cooking Oil
UEST	Unit Of Environmental Science And Technology
UKAS	United Kingdom Accreditation Service
UNE	Normalization Quality Procedures
VM	Volatile Matter
VS	Volatile Solids
WAC	Waste Analysis Campaign
WWTP	Wastewater Treatment Plants

Executive summary

Deliverable 1.2 of the WaysTUP! "Value chains for disruptive transformation of urban biowaste into biobased products in the city context" project documents the progress of Task 1.1 "Systematic characterization of urban biowaste". After having established a common laboratory analysis protocol and methodology for executing waste analysis campaigns in deliverable 1.1, deliverable 1.2 aims to report upon urban biowaste composition and physicochemical characteristics based on this common protocol.

All the different types of urban biowaste that will be used as feedstocks for the biobased products within WaysTUP! were characterized according to the methodology developed and presented in deliverable 1.1. Specifically, samples of meat waste, fish waste, coffee waste, source separated biowaste, used cooked oils, coffee oil from spent coffee grounds, cellulosic rejections and carton and paper waste of municipal solid waste, nappies, sewage sludge, olive oil mill waste, compost and sawdust were collected and analysed.

More specifically, PILOT 1 located in Valencia, Spain, shall use three feedstocks (meat waste, fish waste and spent coffee grounds) as raw materials. In case of meat by-products, there is a wide ratio of protein content depending on animal species and body part (skin, bones, blood, visceral mass, liver, lungs, etc.), while for fish by-products, there is also a wide ratio of protein content depending on fish species (e.g. oily fish, white fish, seafood, mollusks etc.) and fish part (e.g. fishbone, head, guts, etc). Regarding spent coffee grounds, it was made obvious that different coffee varieties and coffee processing methods can affect the final composition.

PILOT 2 is located in Cambridgeshire, United Kingdom and shall use spent coffee grounds (SCG) as feedstock. The latter could be either retail spent coffee grounds (both fresh and aged) originating from coffee shops and restaurants with an oil yield up to 12% or industrial spent coffee grounds from instant coffee factories with oil yields of up to 25%. In both cases, the physical state of the SCG collected is powder.

PILOT 4A is located in Prague, Czech Republic with coffee oil as feedstock. Supercritical fluid extraction with CO_2 was applied on spent coffee grounds as coffee oil extraction technique. The coffee oil had a dark yellow colour and its appearance was waxy with solid-like consistency. PILOT 4B is located in Terni (Italy) and shall use cooking oil from restaurants and canteens. The moisture content of the received samples ranged from 0 to 1%. Macroscopically, the samples were liquid oils with brown to reddish colour with characteristic odor.

PILOT 5, located in Athens, Greece shall use source separated biowaste from households as feedstock. The five WACs of pre-dried biowaste studied had similar physicochemical characteristics. Yet, significant fluctuations to the parameters are to be expected, especially if

one takes into account the change of seasons and therefore of the eating habits of people (e.g. seasonality of fruits).

PILOT 6 is located in Valencia, Spain. Cellulosic rejection streams from Municipal Solid Waste and Waste Water Treatment Plants from the Barcelona Metropolitan Area were studied as potential feedstock for the production of bioethanol. Additionally, sanitary textiles and carton and paper rejections of MSW were also investigated as other potential feedstocks. The materials analyzed were very heterogeneous. Regarding the glucan and hemicellulose content, the greater differences between samples were found for WWTP cellulosic rejections, whereas carton and paper samples seem to have a more stable carbohydrate content. Nevertheless, given the high variability of the composition of samples among WACs, the performance of the transformation processes could be significantly affected.

PILOT 7 is located in Crete, Greece. The main feedstock material that shall be used is sewage sludge. In addition, sawdust, olive oil mill waste and compost shall be used as additional feedstocks. The pH of all materials varied from the slightly acidic to the slightly alkaline region. Electrical conductivity was highest in compost as anticipated and lowest in olive mill waste. Sewage sludge presented the highest moisture content followed by olive oil mill waste and compost, while sawdust the lowest. The volatile solids content was high for sewage sludge, olive oil mill waste and sawdust, while it was much lower in compost.

All these characterizations will serve as a basis for the setting up of the pilots' operation. Indicative sampling records are also included in Annex I.

1. Introduction

The concept of WaysTUP! is to propose an integrated approach for the establishment of new sustainable value chains of urban biowaste recycling and valorisation for the production of high-value biobased products, including proteins for food and feed, through a multi-stakeholder approach in line with circular economy. The project will showcase a portfolio of new 'urban biowaste to bio-based products' processes starting from different feedstocks i.e., fish and meat waste, spent coffee grounds, household source separated biowaste, used cooking oils, cellulosic waste derived from municipal wastewater and waste treatment plants and sewage sludge. Value chains are set up and will be tested in an urban context with the active participation of all key players including (i) the feedstock suppliers (municipalities, and waste and wastewater management authorities) who provide a certain amount and quality of urban biowaste for the production of biobased products, (ii) the waste-based biorefineries who are responsible to convert urban biowaste into an array of biobased products, and (iii) the end-users: market and consumers. Thus, the WaysTUP! concept is based on 4 pillars: Feedstock, State-of-the-art Technologies, End-products and Change of mindset that form a virtuous circle for an integrated approach in valorising urban biowaste.

In this context, work package 1 entitled "Identification of opportunities and barriers to utilisation of urban biowaste sources" includes as a first task the systematic characterisation of urban biowaste. After having elaborated a common laboratory analysis protocol and methodology for executing waste analysis campaigns (Deliverable 1.1), the project will focus on the first pillar, feedstock. Thus, the main objective of this deliverable (Deliverable 1.2) is to effectively map the composition of urban biowaste that will be valorized within WaysTUP!. Based on the performed characterisations, the urban biowaste valorisation routes will be made evident.

Since one of the main objectives of WaysTUP! is to demonstrate a diverse range of technologies for urban biowaste utilization (7 Pilot plants), resulting in new end products and value chains, the knowledge of their intrinsic characteristics, especially with respect to evaluating their response to various treatments, and their potential impacts on the environment is essential.

Urban biowaste to be valorized arise in a wide variety of types and points of processing (e.g. during a waste production process, from stockpiles, tanks, drums). More specifically, feedstocks will include:

- Meat and fish by-products and spent coffee grounds (SCG) from Valencia Region, Spain (SAV, VAL);
- SCG from London, UK (BIO-BEAN);

- Source separated biowaste from households located in Attica Region, Greece (SUST, NTUA) and from Valencia Region, Spain (SAV, VAL);
- Used cooking oils from restaurants and canteens in the Czech Republic (NFG, NVMT);
- Coffee oil from SCG produced by BIO-BEAN
- Cellulosic rejection materials from municipal solid waste (MSW) and wastewater treatment plants (WWTP, nappies and carton and paper waste of municipal solid waste from the Metropolitan Area of Barcelona (AMB), and
- Sewage sludge from the WWTP, compost, olive mill waste and sawdust from local producers at Crete, Greece (TUC).

Based on the framework that was set up in deliverable 1.1 to produce standardized Waste Analysis Campaigns (WACs) for use under routine circumstances, and the common laboratory protocol for feedstocks characterization, several kinds of urban biowaste were collected by the feedstock providers and successfully characterised so as to be used within WaysTUP! by the Pilot owners.

Deliverable 1.2 is organised as follows:

In *Chapter 1* entitled *Introduction* (the present chapter), the background and the structure of the deliverable is presented.

In *Chapter 2* entitled *Common protocol for executing WACs*, the laboratory protocol and methodology for biowaste characterization that was established in deliverable 1.1 is summarised.

In *Chapters 3 to 7*, the specific sampling and analysis protocols that were applied for each PILOT (1 to 7) along with the characterization findings and their analysis are provided.

The conclusions are drawn in *Chapter 8* entitled *Conclusions*.

References are provided towards the end of this deliverable.

Finally, one (1) *ANNEX* is included. More particularly, this ANNEX includes indicative sampling records from all PILOTS.

2. Common protocol for executing WACs

As it was mentioned above, the first objective of work package 1 entitled "Identification of opportunities and barriers to utilisation of urban biowaste sources" was to set up a common laboratory analysis protocol and methodology for executing waste analysis campaigns. This was accomplished in Deliverable 1.1 and is briefly reviewed in this section.

At first, the parties involved in the Waste Analysis Campaigns (WACs) were promptly identified and mainly consisted of the feedstock provider and pilot owner, but sometimes also the waste producer and the process partner. More specifically:

For PILOT 1- Food & Feed, meat, fish and coffee waste are the feedstocks to be used, SAV is the feedstock provider and pilot owner and BIOPOLIS is the process partner.

For PILOT 2- Coffee Oil, coffee waste is the feedstock to be used, BIO-BEAN is the feedstock provider, pilot owner and process partner.

For PILOT 3- Insect protein, by-products of meat and fish waste are the feedstocks to be used, SAV is the feedstock provider and UA is the pilot owner and process partner.

For PILOT 4A- Bioplastics, coffee oil is provided by BIO-BEAN, while NFG is the pilot owner and process partner.

For PILOT 4B- Bioplastics, used cooking oil will be used as feedstock, NFG is the feedstock provider and NVNT the process partner.

For PILOT 5- Biosolvents, meat, source separated biowaste is the feedstock to be used, SUST is the feedstock provider and NTUA the pilot owner and process partner.

For PILOT 6- Perseo, the cellulosic rejections of MSWTP will be provided by WWTP-Besos while the nappies and carton and paper rejections of MSW by AMB. IMECAL is the pilot owner and IMECAL and Ciemat the process partners.

For PILOT 7- Sludge Biochar, the Sewage sludge is provided by the municipal wastewater treatment plant of Chania, Greece, the olive oil mill waste by olive mill owners, Chania, Greece, the sawdust by carpenters, Chania, Greece and the compost by Inter-Municipal Solid Waste Management Company of Chania (DEDISA). TUC is the pilot owner as well as the process partner.

In view of improving the quality of the WACs, most involved parties contributed a fair share of their expertise and background.

For WaysTUP! feedstocks, stratified random sampling was mainly applied, unless otherwise dictated. Specific parameters such as seasonality, collection system, socio-economic influences etc. which could have an influence on the feedstocks' composition, were also evaluated. A case-by-case evaluation was performed and respective WACs were planned and executed accordingly.

The testing and analytical methods that were applied for each parameter were those reported in deliverable D1.1 (Table 7-D1.1), unless otherwise stated. Furthermore, feedstocks such as oil from spent coffee grounds as well as used cooking oils were characterized according to the standards presented in D1.1 (Table 8-D1.1), representing the oily waste of the project.

Apart from the physicochemical characterization of the feedstocks involved, special care was drawn upon the feedstocks that will be valorized into end-products within the food chain such as feed for insects (PILOT 3) and fertilizer (PILOT 7). These feedstocks were also tested in regards to metal content, phenols, chloride, plastic residues and elementary analysis, when necessary.

As far as the solid/slurry feedstocks are concerned, in Table 9 of D1.1 all the analysis that were performed in order to ensure reliable physical and chemical characterization of the examined urban biowaste of WaysTUP! were presented. It should be pointed out that these analyses are indicative and not exhaustive.

Following, the pilot-by-pilot results from the execution of WACs and physicochemical characterization of feedstocks are presented.

3. PILOT 1 – Food & Feed

PILOT 1 is located in Paiporta, Valencia, Spain, in SAV facilities. For PILOT 1, three feedstocks (meat waste, fish waste and spent coffee grounds) were selected as raw materials for the different bioprocesses. Meat and fish by-products are used to produce gelatins and peptides and oil, polyphenols and carotenoids are obtained from spent coffee grounds. Meat by-products include blood, bones, meat trimmings, skin, fatty tissues, horns, hoofs, feet, skull and viscera, while fish by-products consist of heads, tails, skin, entrails, fins and frames.

3.1 Sampling and analysis protocol

Meat and fish waste is mainly collected from MercaValencia (V-30, Salida 1, Valencia, Spain). MercaValencia was selected as supplier due the availability and accessibility to the raw materials in amounts enough to develop the project. The different samples have been provided by SAV and their characterization has been performed by ADM-Biopolis.

The established protocol defines an early pick-up of the raw material. The collection is done precisely after the end of MercaValencia´s market activities around 5:00-6:00 in the morning, because long storage periods of meat and fish waste could trigger decomposition, oxidation etc. changing significantly its characteristics. Thus, collection takes place around 7:00-8:00 am and in the meantime samples are kept in industrial fridges at low temperatures, between 3-4 ^{o}C. SAV team collects the raw material (of different varieties of carcasses of fish and meat species) in plastic containers of variable dimensions and takes them to BIOPOLIS facilities for analysis. The transportation vehicles are not acclimatized, given the short distance that is covered.

Each sample size is 20kg but the availability and accessibility of the raw material are much higher. Meat waste mainly includes pig by-products. The fish waste samples prepared for BIOPOLIS are mixtures of by-products including heads and spines as well as complete fishes retired from the market and mollusks or crustaceans (Figure 1). The samples are kept in the freezer (~-25^{o}C) until their analysis. Although raw materials are not risky for humans, disposable gloves are used by the personnel to avoid contamination in the samples.

Once samples arrive to the laboratory at BIOPOLIS facilities, they are codified in order to assure traceability during the process.

In case of meat and fish by-products, mechanical pre-treatment is performed. Samples are cut in portions of 5-7 cm and chopped in a grinder to assure the homogeneity in the batch.

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Samples can be analyzed directly. If characterization starts within 24h, samples are stored at 4˚C. For longer storage, samples are frozen in order to preserve their composition.

Figure 1. Sample containers of fish waste from MercaValencia

Regarding spent Coffee Grounds (SCG), they are collected from the Italian restaurant group "Pecado" Valencia, Spain.

SAVs collection team picks up the raw material in plastic containers and takes it to BIOPOLIS facilities for analysis. In case of coffee, the sampling and storage times aren't so critical, as they can be kept longer at room temperature.

Each sample size is 5 kg, but the availability and accessibility for raw material are much higher.

Once samples arrive to the laboratory at BIOPOLIS facilities, they are codified in order to assure traceability during the process. Samples can be analyzed directly. If characterization starts within 24h, samples are stored at 4˚C. For longer storage, samples are frozen in order to preserve their composition.

For all three feedstocks (fish and meat waste, spent coffee grounds), three WACs per feedstock were performed by the associated partners to ensure the accuracy of the feedstocks' characterization and variability. Table 1 presents the dates when WACs were performed.

Table 1. WACs performed for meat and fish waste, spent coffee grounds for PILOT 1

	Date		
WAC	**Meat Waste**	**Fish Waste**	**SCG**
1st	16/01/20	19/11/20	16/01/20
2nd	17/06/20	17/12/20	17/06/20
3rd	17/12/20	19/11/20	17/12/20

In order to get a representative sample of raw feedstock, sampling was repeated over time to analyze source variations, variability in lots and seasonality. In case of meat by-products and spent coffee ground, samples were taking every 6 months.

In case of fish, samples were taken from different waste containers; one with heads, backbones and guts and the other with mollusks, crustaceans and seafood. From the mixture of heads, backbones and guts, another sample was collected at a different time.

For PILOT 1 processes, Table 2 presents all the parameters that have been considered in order to ensure reliable physical and chemical characterization of the different samples examined. These parameters have been chosen by considering the interest for characterization and final products of the processes.

Table 2. Parameters for PILOT 1 feedstocks characterization

Parameter	Meat by-products	Fish by-products	Spent coffee grounds
Ash	•	•	•
Insoluble fiber	•	•	•
Fats	•	•	•
Carbohydrates	•	•	•
Moisture	•	•	•
Protein	•	•	
Sugars			•

The analytical methods used for the characterization of samples are standard methods. These methods have been chosen by considering the physical state of each feedstock, parameters of interest and required detection limits. Most of them are in accordance with the Spanish normalization quality procedures (UNE). In Table 3, the reference methods used in each analysis are presented.

Table 3. List on standards methods used for the characterization of feedstocks of PILOT 1

Parameter	Reference	Description
Ash	UNE EN13039	Standard test method for ash in biomass
Insoluble fiber	AOAC 985.29	Fibers are measured by a gravimetric enzymatic method
Fats	UNE EN ISO 11085:2015	Fats are extracted by Randall method
Carbohydrates	UNE-EN 16640:2017	Determination of bio-based carbon content
Moisture	ISO 6496	Total solids dried at 105°C
Protein	UNE EN16634-1	Nitrogen content of the biomass sample is measured by combustion
Sugars	UNE 34199:1984	Determination of sugars by Luff-Schoorl method

3.2 Characteristics and Analysis

The structural composition of meat waste for the 3 WACs along with the mean values are presented below (Table 4) in comparison with similar waste from literature. Differences in meat content were observed and the 1st WAC seems to be a mix of meat without visceral biomass. In case of 2nd and 3rd WAC, meat by-products mainly derive from pigs. The main waste contents are fats and protein. In case of 1st WAC a higher fat content is observed in comparison with the 2nd and 3rd WAC. The latter present high protein content, indicating their potential as feedstocks for gelatin and peptides production.

Fluctuations in the values of these parameters have been reported. These can be attributed to the differences in composition depending on the source (poultry, pig, sheep or bovine) and the animal part analyzed (skin, bones, blood, visceral mass, liver or lungs). Focusing on protein content, the higher amount that has been reported is in bovine blood 90%. For pig liver, the protein content is almost 70% [1]. In case of lungs, in beef lungs the protein content is 64.1 % and in pork lungs 69.6% [2]. Given that the samples analysed have derived from mixtures of animal parts, they can be considered representative.

Table 4. Composition of meat waste

Parameter	1st WAC		2nd WAC		3rd WAC		Mean Value	Literature range
	Amount (g/100g) Wet base	Amount (g/100g) Dry base	Amount (g/100g) Wet base	Amount (g/100g) Dry base	Amount (g/100g) Wet base	Amount (g/100g) Dry base	Amount (g/100g) Dry base	
Ash	1.02 ± 0.2	2.83	1.3 ± 0.2	4.14	0.96 ± 0.15	3.26	3.41±0.67	8.16-14.31 [1, 3, 4]
Insoluble fiber	1.61±0.3	4.47	<0.1	<0.32	<0.1	<0.34	1.71±2.4	
Fats	15.5±1.2	43.06	8.75 ± 0.35	27.86	6.27 ± 0.27	21.32	30.75±11.15	1.3-38.79 [3, 4]
Monounsaturated fat	7.66 ± 0.1	21.28	1.63 ± 0.085	5.2	1.827 ± 0.093	6.21	10.89 ± 9	
Polyunsaturated fat	2.11 ± 0.02	5.86	0.246 ± 0.017	0.78	0.149 ± 0.011	0.5	2.38 ± 3.01	
Saturated fat	5.73 ± 0.077	15.92	1.721 ± 0.089	5.48	2.14 ± 0.11	7.28	9.56 ± 5.58	
Trans fat	0.13 ± 0.001	0.36	<0.01	0.032	<0.01	0.034	0.142 ± 0.188	
Carbohydrates	1.19±0.3	3.31	<0.1	<0.32	<0.1	<0.34	1.32±1.7	0.36 [1, 3, 4]
Moisture	64±2.9	177.78	68.6 ± 2.6	218.47	70.6 ± 2.7	240.14	212.1±31.7	
Protein	16.3±0.5	45.27	21.3 ± 1.8	67.8	22.2 ± 1.8	75.5	62.9±15.7	18.1- 90 [1–4]
Salt	0.2 ± 0.013	0.56	0.246 ± 0.017	0.78	0.198 ± 0.014	0.67	0.67 ± 0.11	

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As far as fish waste is concerned, the first two WACs collected a mixture of different parts from different fish at different times while the 3rd WAC was a mixture of mollusks, crustaceans and seafood. The structural composition of fish waste is presented below (Table 5) in comparison with similar waste from literature. In case of the 1st WAC and 2nd WAC, the main contents are fats and proteins while a higher ash content was measured for the 2nd WAC (Table 5) possibly due to a higher content in inorganic matter. The samples of the 1st WAC presented a higher fat content, indicating a higher percentage of oily fish.

In comparison with the samples of the 3rd WAC (Table 6), the protein content of mollusks samples is lower, which is favorable for PILOT 1 processes.

Fluctuations in the values of these parameters have been reported. Based on literature, there is a wide range in the composition depending on the fish classification (oily or white) and the animal part analyzed (head or fishbones). Focusing on lipids and protein content, comparing both types of fish, oily and white, salmon presents the higher content of lipids (50%) and cod has a higher content in proteins (68%). In salmon, the protein content is high in fishbones, however in cod, the protein content is high in the head [5]. For tilapia, a protein content about 42% has also been reported [1]. Table 5 presents values reported in literature for fish products. The values reported in this deliverable, in general, are in accordance with literature.

Given that the samples analysed have derived from mixtures of fish parts and fish by-products, the fat and protein contents can be considered representative.

Table 5. Composition of fish waste

	1st WAC		2nd WAC		Mean value	
Parameter	**Amount (g/100g) Wet base**	**Amount (g/100g) Dry base**	**Amount (g/100g) Wet base**	**Amount (g/100g) Dry base**	**Amount (g/100g) Dry base**	**Literature range** [1, 5–8]
Ash	1.75±0.3	5.93	3.52 ± 0.47	17.17	11.55±7.95	3.55-19.60
Insoluble fiber	1.384±0.3	4.69	<0.1	<0.48	2.59±2.98	0
Fats	11.96±0.2	40.54	2.24 ± 1.1	10.92	25.73±20.94	0.52-52.50
Monounsaturated fat	5.22 ± 0.22	17.69	0.852 ± 0.049	4.15	10.92 ± 9.57	
Polyunsaturated fat	2.94 ± 0.09	9.97	0.0727 ± 0.006	0.35	5.16 ± 6.8	
Saturated fat	3.8 ± 0.17	12.88	1.315 ± 0.071	6.41	9.64 ± 4.57	
Carbohydrates	<0.1	0.34	<0.1	<0.48	0.41±0.10	0.6
Moisture	70.5±3.7	238.98	79.5 ± 2.7	220.83	229.91±12.83	

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	1st WAC		2nd WAC		Mean value	
Protein	15.6±0.5	52.88	14.8 ± 1.2	72.19	62.54±13.65	35.9-94.79
Salt	0.316 ± 0.045	1.07	0.535 ± 0.033	2.6	1.835 ± 1.08	
Total sugars	<0.1	0.34	<0.1	0.488	0.414 ± 0.1	

Table 6. Composition of the mollusks samples of the 3rd WAC

	3rd WAC	
Parameter	**Amount (g/100g) Wet base**	**Amount (g/100g) Dry base**
Ash	37 ± 4.1	63.90
Insoluble fiber	4.98 ±0.5	8.60
Fats	1.269 ±0.3	2.192
Monounsaturated fat	0.216	0.37
Polyunsaturated fat	0.362	0.63
Saturated fat	0.449	0.78
Carbohydrates	6.84 ±0.3	11.81
Moisture	42.1 ±3.2	72.71
Protein	7.8 ±0.5	13.47
Salt	1.392	2.404
Total sugars	0.143	0.25

In case of spent coffee grounds, the samples were more homogeneous than meat and fish by-products. The composition of spent coffee grounds to be used in PILOT1 is presented below (Table 7) in comparison with similar waste from literature.

Table 7. Composition of SCG

Parameter	1st WAC		2nd WAC		3rd WAC		Mean Value	Literature range
	Amount (g/100g) Wet base	Amount (g/100g) Dry base	Amount (g/100g) Wet base	Amount (g/100g) Dry base	Amount (g/100g) Wet base	Amount (g/100g) Dry base	Amount (g/100g) Dry base	
Ash	0.6±0.01	1.67	0.7 ± 0.11	1.93	0.47 ± 0.07	1.3	1.63±0.32	1.3-2.2 [9–11]
Insoluble fiber	21.11±2.8	58.80	22.64 ± 0.79	62.37	24.71 ± 0.85	68.64	63.27±4.98	22.2-51 [9, 11, 12]
Fats	5.25±0.9	14.62	5.99 ± 0.26	16.5	5.71 ± 0.25	15.86	15.66±0.96	7-30 [9, 13]
Monounsaturated fat	0.862 ± 0.033	2.40	0.729 ± 0.043	2	0.597 ± 0.036	1.658	2.02 ± 0.37	
Polyunsaturated fat	1.926 ± 0.2	5.36	2.38 ± 0.12	6.56	2.05 ± 0.1	5.69	5.87 ± 0.62	
Saturated fat	2.46 ±0.018	6.85	2.93 ± 0.14	8.07	3.23 ± 0.15	8.97	7.96 ± 1.064	
Trans fat	0.02 ± 0.001	0.06	<0.01	0.027	<0.01	0.027	0.038 ± 0.019	
Carbohydrates	4.16±0.15	11.59	1.877 ± 0.096	5.17	2.22 ± 0.18	6.16	7.64±3.46	40.3-57 (Cellulose+Hemicellulose) [9, 11]
Moisture	64.1±3.1	178.55	63.7 ± 2.4	175.68	64 ± 2.4	177.78	177.34±1.49	
Protein	<0.06	<0.17	5.08 ± 0.82	13.99	5.11 ± 0.82	14.19	9.45 ± 8.03	13.0-17.54 [9]
Salt	0.08 ± 0.002	0.22	0.029 ± 0.0028	0.079	0.03 ±0.003	0.083	0.12 ±0.08	
Total sugars	1.88±0.07	5.24	1.506 ± 0.079	4.14	1.75 ± 0.32	4.86	4.75±0.56	6.7-14 [9–11]

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Fluctuations in the values of these parameters have been reported mainly due to the coffee variety and origin of the sample (retail or instant). Focusing on oil content, the dry weight oil content of the instant SCG samples ranged from 24.2 to 30.4%, while the retail SCG samples contained considerably lower amounts of lipids with their oil content ranging between 13.4 and 14.8% [13]. Table 7 also presents the values reported in literature for spent coffee grounds. Based on the published literature, some significant deviations on the physicochemical characteristics of SCG have been observed (Table 7). For example, the carbohydrates and protein contents are too low, whereas the insoluble fiber content is too high. Nevertheless, for PILOT 1 processes the total sugars and fat contents are of interest.

To sum up, as far as the feedstocks of PILOT 1 are concerned, in case of meat by-products, there is a wide ratio of protein content depending on animal species and body part (skin, bones, blood, visceral mass, liver, lungs, etc.). Compositional analysis has been performed during a year and different protein and fat contents were found. Wastes with high amount of pig source seem to have a higher protein content and thus they are more suitable for gelatin production.

Regarding fish by-products, there is a wide ratio of protein content depending on fish species e.g. oily fish, white fish, seafood, mollusks etc.) and fish part (e.g. fishbone, head, guts, etc). Compositional analysis has been performed to different mixtures and different protein and fat contents were found. Waste originating from a fish mixture has a higher protein content than that from a mixture of seafood, mollusks and crustaceans, so it is more suitable for gelatin production.

The proteins contents between meat and fish by-products are quite similar so, both of them can stand as a suitable feedstock for PILOT 1.

Regarding spent coffee grounds, the WACs performed resulted in similar composition as far sugars and fat is concerned. Although the sugar content of the samples analysed was lower than that reported in literature, the samples collected from the 3 WACs are suitable for PILOT 1. It is obvious that different coffee varieties and coffee processing methods can affect the final composition.

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4. PILOT 2 – Coffee oil

PILOT 2 is located in Alconbury, Cambridgeshire, United Kingdom. The pilot's feedstock is spent coffee grounds (SCG), provided by BIO-BEAN which is the first company in the world to industrialise the process of recycling SCG into advanced biofuels. PILOT 2 end-product will be coffee oil which will be also used as feedstock for synthesis of long chain dicarboxylic acids and polyhydroxyalkanoates (PHAs) which will ultimately be used to produce biodegradable bioplastics in PILOT 4A and potential use in many other chemical processes.

4.1 Sampling and analysis protocol

BIO-BEAN collects two main raw materials to process in order to be used as feedstock for PILOT 2 (Figure 2):

- Retail spent coffee grounds (both fresh and aged) originating from coffee shops and restaurants
- Industrial spent coffee grounds from instant coffee factories

Retail SCG were obtained at the Bio-bean Alconbury factory. These grounds are delivered several times per week and contain coffee from several types of coffee shops all over the UK. In this study grounds from Costa Coffee, UK were isolated and used to preserve sample heterogeneity. Retail SCG must be processed before extraction; that is, they must be decontaminated, screened and dried, since they may contaminants such as glass etc. They mainly include Arabica beans, brewed with espresso machines. An oil yield up to 12% is expected. Industrial SCG are also delivered to the BIO-BEAN site. They come from factories making instant coffee granules. Industrial SCG on the other hand can be dried directly as they are free from contaminants. They mainly include Robusta beans, brewed with industrial methods. Oil yields of up to 25% can be achieved. In general, dried spent coffee grounds in a powder state, may produce dust while handled. Coffee oil can be produced from both of these types of spent coffee grounds. Figure 2 presents the different feedstocks at the Alconbury factory, as they arrive to the factory and Figure 3. Retail coffee sample before and after extraction and the oil producedFigure 3 presents the extracted samples as well as the SCG before and after.

Figure 2. Retail and industrial coffee to be used as feedstock for PILOT 2 at the Alconbury factory

Figure 3. Retail coffee sample before and after extraction and the oil produced

The 1st WAC took place on 05/12/2019 and retail SCG were collected, while the 2nd WAC took place on 11/09/2020 and industrial SCG were collected.

As far as the physicochemical characterization of the SCG is concerned, the following analyses were performed.

- Particle Size Distribution

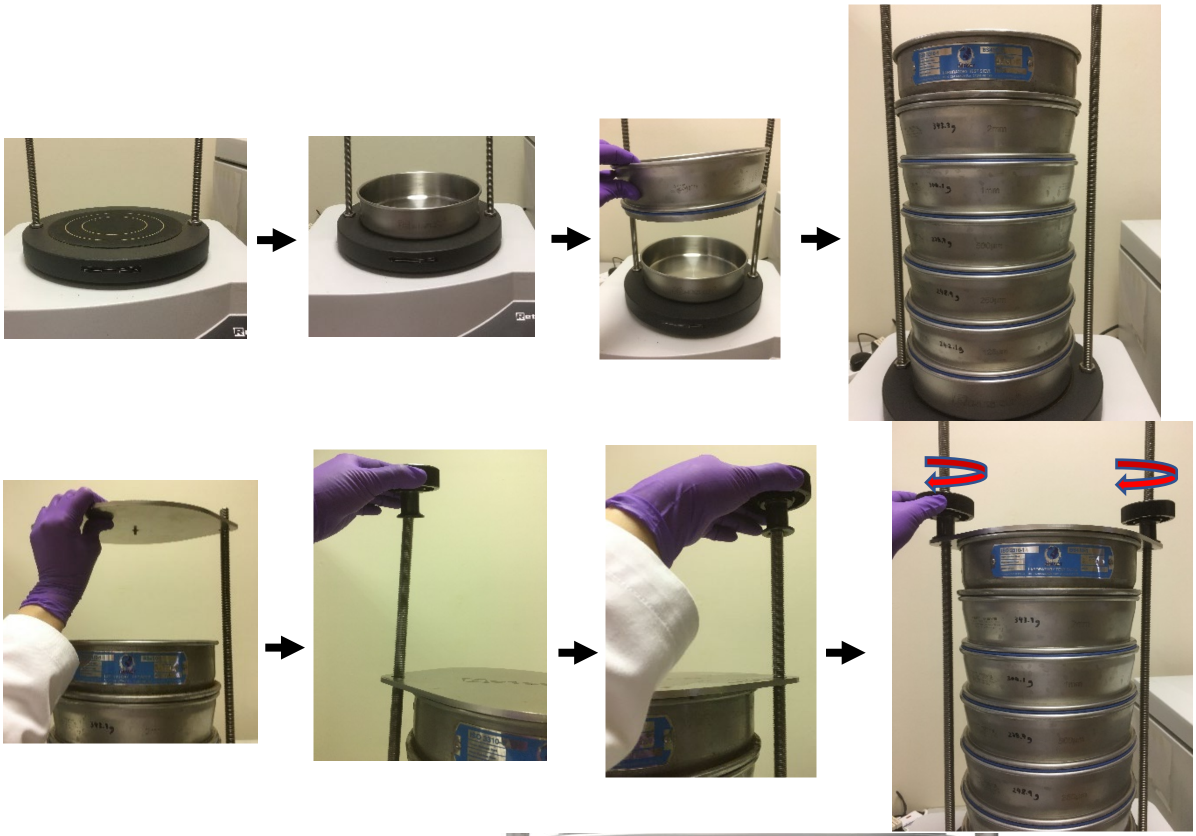
Particle size distribution was determined by sieve shaker analysis. The apparatus used is presented in Figure 4. The stack of sieves include:

- 3.15 mm
- 2.00 mm
- mm
- 500 mm
- 250 mm
- 125 mm
- Pan

Figure 4. Retsch Vibratory Sieve Shaker AS200 Basic

The procedure is as follows (Figure 5):

i. Weigh each sieve empty and record.
ii. Place the pan on the rubber disc and the sieve stack with increasing mesh size on the collection pan. Make sure each test sieve attached with the O-ring.




Mains switch
Mains connection

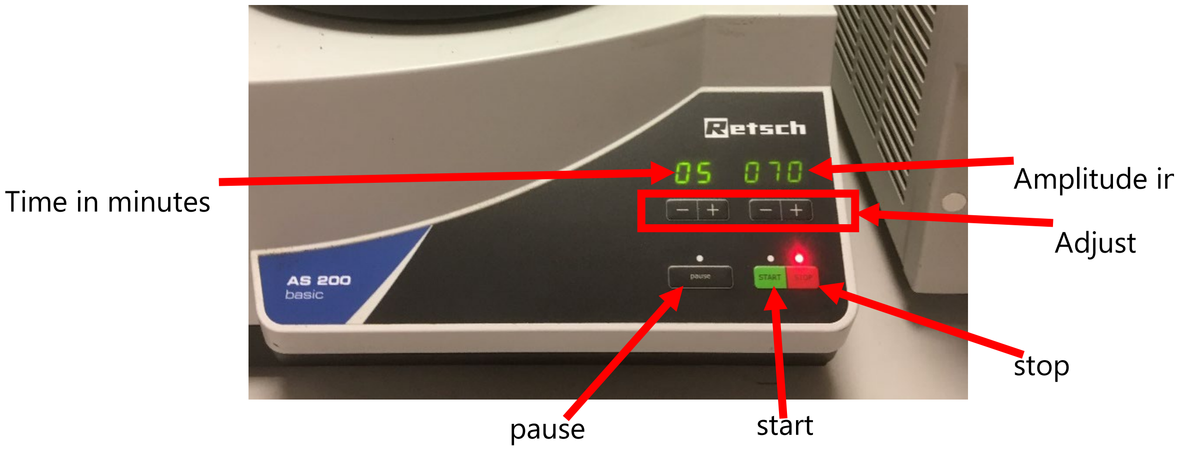


Figure 5. Stages of experimental procedure of sieving

iii. Weigh out at least 100g sample for test.
iv. Pour 100g sample on the uppermost test sieve (biggest mesh size).
v. Place the complete sieve stack centrally on the device and clamp the sieve stack tightly.
vi. Make sure the cable connected at the back and turn the switch on.
vii. Set the time and Amplitude (currently default in 5 min and 70%). To optimise time and amplitude please refer to Operation Manual P.30-34.
viii. Press START and wait until it stopped.
ix. Weigh each sieve and subtract empty pan weight to get amount per sieve (Table 8).

Table 8. Template for data record for sieving

Particle size	Sieve	Empty sieve weight (g)	Sample + Weight (g)	Amount per sieve (g)	Sample size (g)	%
>3.15mm	3.15 mm	579.5				
3.15-2mm	2.00 mm	343.3				
2mm-1mm	1.00 mm	304.1				
1mm-500µm	500 mm	278.7				
500-250µm	250 mm	248.9				
250-125µm	125 mm	242.1				
<125µm	Pan	349.2				

x. Divide each by total amount used (100g) and x 100 for percentage, present in Table 9.

Table 9. Template for data collection for sieving

Particle size	Sieve	Trial 1 %	Trial 2 %	Mean %
>3.15mm	3.15 mm			
3.15-2mm	2.00 mm			
2mm-1mm	1.00 mm			
1mm-500μm	500 mm			
500-250μm	250 mm			
250-125μm	125 mm			
<125μm	Pan			

xi. Repeat this a total of 2 times and calculate their mean of percentage. If two trials differ substantially, increase the sieving time for another 5 minutes.

In Figure 6, an example of particle size distribution data for retail and industrial spent grounds is presented.

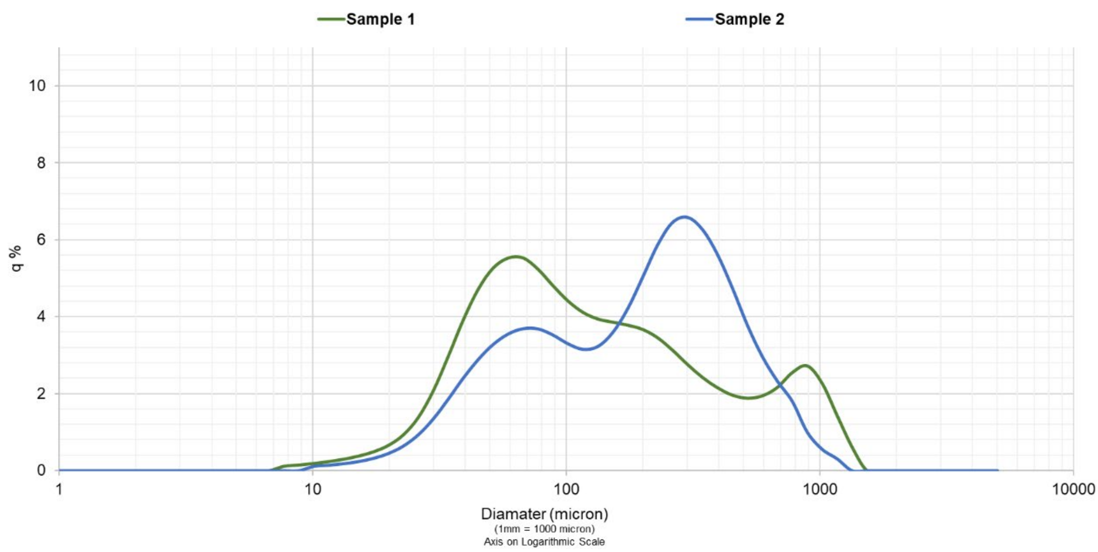


Figure 6. Example of particle size distribution data for retail and industrial spent grounds

Moisture

Moisture is analysed periodically by a benchtop moisture meter. The meter is regularly calibrated against oven drying at 105°C for 4h or until mass change equals zero.

- Micro

Microbiological loads are calculated at an accredited third-party lab. The samples are plated and observed for a number of days after which metrics such as total viable count (TVC), coliforms and other bacteria are counted and reported.

4.2 Characteristics and Analysis

The physical state of the SCG collected is powder. In Table 10, Table 11, Table 12 and Table 13, the characteristics of retail and industrial SCG are presented.

Table 10. Characteristics of retail and industrial SCG

Parameter	Amount (g/100g) Wet base		Amount (g/100g) Dry base		Amount (g/100gVS)	
	Retail SCG	Industrial SCG	Retail SCG	Industrial SCG	Retail SCG	Industrial SCG
Ash	1.6	0.9	1.8	0.9		
Volatile Matter	-	80.3	-	83.9	-	84.7
Fixed Carbon	-	14.5	-	15.2	-	15.3
Total Sulphur	0.12	0.05	0.14	0.05	0.14	0.05
Chlorine	0.01	0.02	0.01	0.02	0.01	0.02
Carbon	46.66	55.7	53.39	58.2	54.38	58.7
Hydrogen	6.3	7.23	7.21	7.55	7.34	7.62
Nitrogen	2.06	1.72	2.36	1.8	2.4	1.82
Oxygen by Difference	-	30.1	-	31.5	-	31.8

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In Table 11, the gross calorific value along with the net calorific value of both feedstocks are presented (retail and industrial SCG).

Table 11. Calorific Values of retail and industrial SCG

Parameter	Wet base		Dry base	(g/100gVS)
	Gross Calorific Value (kJ/kg)	Net Calorific Value kJ/kg	Gross Calorific Value (kJ/kg)	Gross Calorific Value (kJ/kg)
Retail SCG	20223	18552	23138	23570
Industrial SCG	25285	23623	26421	26661

In Table 12, the metal content of retail SCG is presented.

Table 12. Metal content of retail SCG

Metals	Concentration (mg/kg)
Cd	<0.1
Zn	17
Va	<0.6
Pb	0.8
Co	18.4
Cr	1
Ni	1.2
As	<0.3
Hg	<0.1

In Table 13, the physical properties of both retail and industrial SCG are presented.

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Table 13. Physical properties of retail and industrial SCG

Parameter	Units	Retail SCG	Industrial SCG
Bulk Density – Tapped	kg/m^3	450	388
Bulk Density - Poured	kg/m^3	420	
Sieve >3.15mm	w/w%	0.0%	0.6%
Sieve 3.15-2mm	w/w%	15.8%	3.9%
Sieve 2mm-1 mm	w/w%	14.9%	44.8%
Sieve 1mm-500µm	w/w%	16.9%	30.2%
Sieve 500-250µm	w/w%	38.4%	12.1%
Sieve 250-125µm	w/w%	13.3%	7.0%
Sieve <125µm	w/w%	0.2%	1.8%
Average Particle Size			
D10%	mm	0.3	
D50%	mm	0.47	
D90%	mm	0.8	

5. PILOT 3 – Insect Protein

PILOT 3 is located in Alicante, Spain in the Alicante Science Park (ASP). Although in the original proposal the idea for PILOT 3 was to process exactly the same feedstock as PILOT 1, in order to facilitate logistics and obtain homogeneous end products in PILOT 3, this was changed. It was decided that the feedstock of the plant would be the by-products derived from PILOT 1 or at least that PILOT 1 would carry out a pre-treatment of these by-products based on its own production process. In this way, it is possible to implement the circular economy philosophy within the framework of the project and to work with homogeneous development media for the growth of the insect larvae. Another reason for this decision was to facilitate the production of a homogeneous end product (insect protein meal) that can be more feasibly incorporated into WP5 (partner VAL (IVIA) - chicken feeding trials). Given this change, characterization of these products is not included in this deliverable since the operation of PILOT 1 has not started and thus the analysis of the by-products derived was not possible.

6. PILOT 4A – Bioplastics

PILOT 4A is located in Prague (Czech Republic). Feedstock for PILOT 4A is coffee oil provided by BIO-BEAN and will be used for the production of polyhydroxyalkanoates (PHAs).

6.1 Sampling and analysis protocol

Coffee oil was delivered to Martin Kryl in Nafigate laboratories in Institute of Microbiology of the Czech Academy of Sciences in September 2020 by BIO-BEAN. BIO-BEAN collected spent coffee grounds from retail coffee shops in London, UK from January to July 2020. Supercritical fluid extraction with CO_2 was applied on spent coffee grounds as coffee oil extraction technique. More specifically, Nagifate received 5 kg of coffee oil from retail aged spent cofee grounds on July 2020 from BIO-BEAN as 1st WAC. The laboratory samples (10g) were collected from the middle of the coffee oil container with a spatula and stored in sealed glass tubes and dark and cool place before being analysed. The lipid profile of the samples is analysed by a third party lab that operates to the highest UKAS standards. GC-FID is used following esterification of each sample sent. Free Fatty Acid is analysed by Titration where Acid value is the number of milligrams of potassium hydroxide required to neutralize the free fatty acids present in 1 g of fat.

6.2 Characteristics and Analysis

The coffee oil had a dark yellow colour (Figure 7) and its appearance was waxy with solid-like consistency.

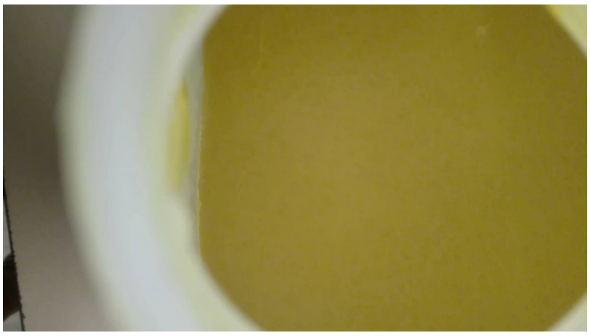

Figure 7. Coffee oil sample

The physicochemical characteristics of coffee oil that will serve as feedstock for PILOT 4A are presented in Table 14.

Table 14. Physicochemical characteristics of coffee oil

Parameter	1st WAC
Saponification (mg KOH/g)	101.4±6.1
Peroxide value (mEqO_2/kg)	0.8098±0.0405
Linoleic acid	44.92
Palmitic acid	24.56
Oleic acid	10.51
Stearic acid	7.28
Arachidic acid	3.39
a-Linoleic acid	1.31
Behenic acid	0.80
Vaccenic acid	0.56
11-Eicosenoic acid	0.45
Lignoceric acid	0.36
Heneicosanoic acid	0.13
Palmitoleic acid	0.11
Tricosylic acid	0.12

7. PILOT 4B – Bioplastics

PILOT 4B is located in Terni (Italy). Feedstock for PILOT 4B will be used cooking oil (UCO) from restaurants and canteens. Nafigate (NFG) is responsible for the provision of feedstock, aiming to produce long chain dicarboxylic acids through fermentation.

7.1 Sampling and analysis protocol

Used cooking oil was collected by Nafigate and delivered to Novamont Biotechnology Research Center in Piana di Monte Verna (ITALY). The samples were filtered mixtures of used vegetable oils and delivered in tanks. Table 15 presents the dates when WACs were performed as well as the quantities received.

Table 15. WACs performed for used cooking oil for PILOT 4b

WAC	Date	Quantity (kg)
1st	December 2019	5
2nd	June 2020	25
3rd	June 2020	25

An indicative sampling record is included in ANNEX I.

Aiming to receive representative laboratory samples, tanks were extensively shaken and sample was picked up by means of an aspiration pipette from the middle of the liquid batch. The size of each laboratory sample was 10-20 g per sample, stored in falcon and triplicate analysis was performed.

Determination of main fatty acid profile of used cooking oil by GC-MS method was performed.

7.2 Characteristics and Analysis

The moisture content of the received samples ranged from 0 to 1%. Macroscopically, the samples were liquid oils with brown to reddish colour with characteristic odor.

Table 16 presents the fatty acid profile of UCO from the 3 WACs, along with their mean values.

Table 16. Fatty acid profile of used cooking oil for the 3 executed WACs along with their mean values

Fatty acid	1st WAC	2nd WAC	3rd WAC	Mean Value
Palmitic acid	14.5±0.13	15.3±0.11	15.4±0.10	15.1±0.20
Stearic acid	3.1±0.03	5.3±0.05	5.3±0.05	4.6±0.08
Oleic acid	55.3±0.18	56.5±0.20	56.2±0.18	56.0±0.32
Linoleic acid	27.0±0.08	23.0±0.07	23.1±0.05	24±0.12

8. PILOT 5 – Biosolvents

PILOT 5 is located in the premises of NTUA, Athens, Greece. The PILOT aims to the biochemical conversion of biowaste to ethanol. Feedstock material is source separated biowaste from households. SUST is responsible for the provision of feedstock material.

8.1 Sampling and analysis protocol

Source separated biowaste collected from the Municipality of Vari - Voula - Vouliagmeni by the responsible cleaning service were delivered to the National Technical University of Athens (NTUA), School of Chemical Engineering, Unit of Environmental Science and Technology (UEST). The sampling protocol was established by Sustainable City. More specifically, Table 17 presents the dates when WACs were performed as well as the quantities received. In all cases, the organic waste was transported to the NTUA facilities after a special permit which had been granted by the competent services of the institution in a municipal truck placed in orange and brown plastic bags (Figure 8).

Figure 8. Source separated food waste delivered to NTUA by the transportation truck of Vari-Voula-Vouliagmeni cleaning service

In Figure 9, the delivered samples at the laboratory of UEST are presented. As it can be observed, the appearance of the samples is very heterogeneous, as expected for this kind of waste materials.

Figure 9. Delivered samples of source separated food waste from households

Upon receipt, the waste was placed in 250L plastic bins inside the Environmental Science and Technology Unit, where it was dried, homogenised and turned into powder (≈1-5 mm in diameter). Drying took place in a commercial Gaia GC-100 decentralized drying system. Food waste Gaia dryer is a simple automated, electrically powered system that dries food and other organic waste by the use of heat and mechanical mixing. This dehydration system reduces significantly (up to 90%) the organic biowaste volume and simultaneously homogenizes the feedstock and ceases the biological degradation of the organic substrates. As organic and food wastes have a high moisture content (up to 98%), the weight and volume reduction is achieved through the evaporation of the water, leaving the operator with a dry, manageable end product that has been sterilized and stabilized so that it doesn't smell and can be stored for many weeks without degrading. By heating and shredding the waste, an accelerated dehydration process is activated, driving off the moisture which is condensed, collected and disposed as wastewater. The possibility of recycling the condensate as process water in the downstream processes of PILOT 5 will be investigated. Furthermore, GAIA GC-100 includes a proprietary control system to minimize odors along with a series of filters cleaning the exhaust air emissions prior to the release in the atmosphere. This dehydration step, apart from being essential for the implementation of feedstock analysis and characterization, it could be incorporated as a pretreatment step in the proposed valorization scheme of PILOT 5. In view of scaling up on industrial level, there exist similar commercial products of industrial scale but a rotary drum dryer could also be designed and constructed for the specific needs of source-separated biowaste. The drying of the received feedstock in GAIA GC-100 ensured the milling and homogenization of large amounts of feedstock and favored reproducible analysis results.

Table 17. WACs performed for source separated biowaste for PILOT 5

WAC	Date	Quantity (kg)
1st	30/6/2020	252.35
2nd	14/07/2020	219.06
3rd	15/9/2020	129.00
4th	29/9/2020	153.72
5th	13/10/2020	90.00

An indicative sampling record is included in ANNEX I.

The dried organic waste, after being homogenized, was stored in ordinary high-strength waste bags for further treatment and then characterized for the following parameters according to the standard analysis methods, described in Deliverable D1.1.:

- pH
- residual moisture
- total solids
- ash
- total volatile solids
- starch
- cellulose
- hemicellulose
- acid soluble lignin
- acid insoluble residue
- fats and oils
- total organic carbon
- total Kjeldahl nitrogen
- proteins.

NREL laboratory analytical processes were adopted for the measurement of moisture, ash, fat, extractives, cellulose, hemicellulose, proteins, acid insoluble residue and acid soluble lignin. The protein content is estimated using an appropriate Nitrogen Factor (6.25) All the samples were centrifuged for 6 min at 4500 rpm and were filtered before further characterisation. The glucose oxidase–peroxidase (GOX–PER) method was applied for the glucose concentration measurement by using a commercially available kit (Biosis S.A., Athens, Greece).

The Total Starch (AA/AMG) test kit (e.g. MEGAZYME) was used for the measurement and analysis of total starch according to AACC Method 76-13.01. This kit contains an improved α -amylase that allows the amylase incubations to be performed at pH 5.0 (as well as pH 7.0).

Soxhlet method was chosen as the conventional oil extraction technique by use of n-Hexane 95% as solvent, by applying the 12 cm Filter Fioroni's extraction thimble in a 500 mL Soxhlet extractor apparatus. After the end of the oil extraction process, the solvent was evaporated by applying the BÜCHI Rotavapor (R KRvr 65/45) at 55 °C, under vacuum 275 mbar. Subsequently, the recovered oil was heated in the drying oven at 105 °C for 1 h in order to remove the residual moisture and solvent content, then it was weighed.

In addition, carbon (TC/IC/TOC), nitrogen (TN/TKN), pH and conductivity of the dried food waste were measured by applying the Solid Sample Module (SSM-5000A) of the SHIMADZU TOC-VCSH (total organic carbon analyser), the KJELDATHERM block digestion unit and the Gerhardt–Vapodest 30s device, and the Mettler–Toledo MPC227 pH/Conductivity Metre, respectively.

All analyses were performed in triplicate.

8.2 Characteristics and Analysis

Macroscopic examination of the samples showed that they are "good quality" samples with very low percentages of impurities of foreign substances. In other words, they are food / kitchen waste from households without significant amounts of other waste such as plastic, glass, etc. In the first two WACs (1st WAC and 2nd WAC), the presence of used coffee capsules was significant, whereas after communication with the responsible authorities, in the following WACs such impurities were negligible. Nevertheless, the presence of paper bags and newspapers is important, which contributes significantly to the percentage of cellulose and hemicellulose in the waste. In general, however, sorting at source and collection network can be considered quite satisfactory.

The moisture content of source separated biowaste upon delivery was 73.12% in the 1st WAC, 75.43% in the 2nd WAC, 75.71% in the 3rd WAC, 77.98% in the 4th WAC and 76.19% in the 5th WAC, presenting a mean value of 75.69±1.74%. Similarly, for the pH in the 1st WAC it was 5.43 ± 0.12, 5.61 ± 0.15 in the 2nd WAC, 5.65 ± 0.13 in the 3rd WAC, 4.80 ± 0.21 in the 4th WAC and 5.65 ± 0.13 in the 5th WAC, presenting a mean value of 5.52±0.35%.

The main characteristics of the samples of the 5 WACs performed are presented in the following table (Table 18).

Table 18. Composition of source separated biowaste in dry basis (d.b.) for the 5 executed WACs along with their mean values

Parameter	1st WAC (% d.b.)	2nd WAC (% d.b.)	3rd WAC (% d.b.)	4th WAC (% d.b.)	5th WAC (% d.b.)	Mean Value
Residual Moisture	4.32±0.12	5.06±0.12	4.27±0.12	4.05±0.13	5.89±0.14	4.74±0.23
Dry Matter	94.67±0.11	95.35±0.12	95.72±0.12	95.94±0.13	94.10±0.14	95.25±0.23
Fats and Oil	8.75±1.07	8.59±0.70	11.02±0.72	12.53±0.63	12.99±0.76	10.78±1.77
Ash	11.40±1.52	7.89±2.22	11.72±0.42	13.24±1.74	11.77±0.25	11.20±3.24
TOC	50.23±2.01	51.18±1.66	50.92 ± 1.96	50.04±2.13	51.32±1.77	50.76±3.39
Total Kjeldahl Nitrogen	1.52±0.02	1.61±0.03	1.67 ± 0.02	1.49±0.03	1.54±0.04	1.57±0.05
Volatile Solids	88.60±1.52	92.11±2.22	88.27±0.42	86.75±1.74	88.22±0.25	88.79±3.24
Cellulose	17.31±2.60	18.75±2.40	15.32±1.78	22.48±5.32	21.80±3.77	19.13±7.63
Hemicellulose	23.95±8.51	24.66±7.77	8.00±1.10	7.71±4.40	4.53±0.34	13.77±12.39
Starch	4.18±0.74	3.56±0.76	3.98±0.49	3.26±0.92	5.56±1.55	4.11±2.15
Acid Insoluble residue	17.69±2.52	16.42±0.11	10.29±1.15	13.53±2.94	13.08±1.32	14.20±4.25
Acid Soluble Lignin	0.99±0.09	1.07±0.11	1.38±0.33	1.55±0.20	1.59±0.13	1.32±0.43
Proteins	9.50±0.20	10.06±0.14	10.44 ± 0.13	9.31 ± 0.19	9.63 ± 0.25	9.62±0.42

It is obvious that the five WACs of pre-dried biowaste studied have similar physicochemical characteristics. Specifically, cellulose and starch, which are the parameters of interest for bioethanol production (PILOT 5), showed concentrations similar to all 5 examined WACs. Hemicellulose which also is of interest, presented a higher content in the first two WACs. This can be mainly attributed to the presence of spent coffee grounds from the coffee capsule which are of high hemicellulose content. Nevertheless, significant fluctuations to the parameters are to be expected, especially if one takes into account the change of seasons and therefore of the eating habits of people (e.g. seasonality of fruits). In general, however, the literature states that household biowaste varies greatly in composition according to Barampouti et al. [14]. For example, Alamanou et al. [15] and Matsakas et al. [16] used in their research household food waste with much lower hemicellulose content (7.55 ± 0.39% w / w) and no starch. Yan et al. [17] used food waste with extremely high starch content and very low cellulose (63.87 ± 2.03 and 1.98 ± 0.36% w/w respectively).

9. PILOT 6 – PERSEO

PILOT 6 is located in L'Alcúdia (Valencia, Spain), in the biotechnological demonstration plant of IMECAL (PERSEO Bioethanol® plant). Cellulosic rejection streams from Municipal Solid Waste (MSW) and Waste Water Treatment Plants (WWTP) from the Barcelona Metropolitan Area will be studied as potential feedstock for the production of bioethanol in PILOT 6. Additionally, sanitary textiles and carton and paper rejections of MSW will also be investigated as other potential feedstocks.

9.1 Sampling and analysis protocol

Cellulosic rejections along with sanitary textiles and carton and paper rejections were obtained from Metropolitan Area of Barcelona (AMB) and IMECAL sent the materials to Biofuels Unit's laboratory at CIEMAT. Sanitary textiles and carton and paper rejections were collected from the municipal solid waste stream and weren't source separated. CIEMAT received and analyzed three (3) batches of samples, corresponding to the WACs of 6th November 2019, 26th June 2020 and 3rd September 2020. Indicative sampling records are presented in ANNEX I. These three campaigns allowed to study the variability of the composition of the selected wastes and its potential for ethanol production depending on the season.

In Figure 10, the delivered samples at the laboratory of CIEMAT are presented. As it can be observed, the appearance of the samples is very heterogeneous, as expected for this kind of waste materials.

Sanitary textiles | Carton & paper | Cellulosic rejections

Figure 10. Delivered samples of sanitary textiles, carton and paper and cellulosic rejections

The samples were homogenized, air dried and milled to small particles. The samples were chemical analyzed for dry matter/moisture, volatile solids/ash, nitrogen, cellulose, hemicellulose, lignin, sugars, C, H, S, N and metal content Table 23 presents the methods used for each analysis.

Table 19. Analytical methods adopted for feedstocks characterisation of PILOT 6

Parameter	Method
Moisture	NREL/TP-510-42621
Ash	NREL/TP-510-42622
Total Kjeldahl Nitrogen	APHA-AWWA-WEF
Extractives content	LAP NREL/TP-510-42619
Cellulose	NREL/TP-510-42618
Hemicellulose	NREL/TP-510-42618
Lignin	NREL/TP-510-42618
Sugars	NREL/TP-510-42623
Elemental analysis: C, H, N	ISO 16948
Chlorine and sulfur	European Standard EN 15289
Inorganic elements in biomass ash	European Standard EN 15290

The delivered samples to CIEMAT laboratory were analysed according to the methodology described below.

pH was measured following the standard EPA Method 9045 for soil and waste. Since the wastes to be measured are highly hygroscopic, the dilution of the sample was adjusted to 1:6 with reagent water. Suspensions were filtered to separate the aqueous phase, except in the case of sanitary textile, where centrifugation was necessary.

Samples were characterized following NREL's Biomass Compositional Analysis Laboratory Procedures. A diagram of such procedures can be seen in Figure 11.

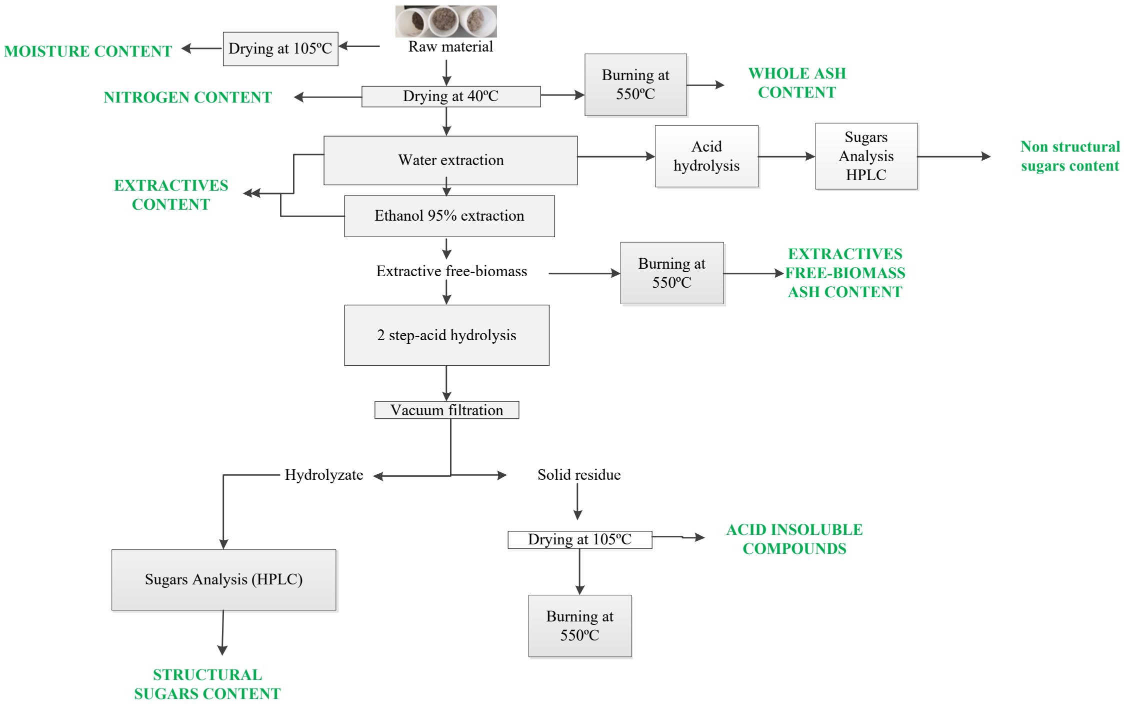

Figure 11. Schematic flowsheet for the characterization of sanitary textile, paper & cardboard and WWTP cellulosic rejections samples

The moisture content was determined by weight difference after drying the samples at 105ºC.

For analysis dry milled (‹1 mm) material at 45 ºC overnight was utilized. The ash content was determined by LAP-NREL procedure "Determination of ash in biomass" (NREL/TP-510-42622). The extractives content was analyzed after exhaustive and ethanol extraction using Soxhlet method (LAP NREL/TP-510-42619 "Determination of Extractives in Biomass") and the structural carbohydrates and acid insoluble residue (AIR) were analyzed following the LAP-NREL "Determination of structural carbohydrates and lignin in Biomass" (NREL7TP-510-42618). Finally, nitrogen content of the samples was measured by Kjeldahl method. Protein content is usually calculated applying a Nitrogen Factor to the value of N determined, but in this kind of materials, the nitrogen may come from different sources other than proteins. Therefore, the protein content is not given in the tables.
Shortly, after extraction, structural sugars content was measured based on monomers content after a two-step acid hydrolysis procedure to fractionate the fiber. A first step with 72 % (w/w) sulphuric acid at 30 ºC for 60 min was used, followed by a second step in which the reaction mixture was diluted to 4 % sulphuric acid and autoclaved at 121 ºC for 1 h. Sugars concentration was determined by high performance liquid chromatography (HPLC) in a Waters 2695 liquid chromatograph with refractive index detector. A CARBOSep CHO-682 LEAD column (Transgenomic, Omaha, NE) operating at 75 ºC with Milli-Q water (Millipore) as mobile-phase (0.5 mL /min) was used. Anhydrous correction was applied to the quantification

results of monomeric sugars to calculate the polymer of carbohydrates. The factor used to convert sugars monomers to anhydromonomers were 0.90 for glucose to glucan, galactose to galactan, mannose to mannan and 0.88 for xylose to xylan, arabinose to arabinan.

Other analysis carried out included both ultimate analysis and major elements in samples ash. These analyses have been determined by Biomass Characterization Laboratory CEDER, CIEMAT.

Carbon (C), hydrogen (H) and nitrogen (N) analyses were carried out by the internal procedure of determination of carbon, hydrogen and nitrogen in biomass using an elemental analyzer TruSpec (Leco). It is based on catalytic combustion of the sample of biomass with pure oxygen and 950 °C to achieve a very rapid combustion. After treatments, the gaseous combustion products are homogenized and determined by infrared detectors (C and H) and thermal conductivity detector (N). The internal procedure is derived from the International Standard ISO 16948 "Solid Biofuels - Determination of total content of carbon, hydrogen and nitrogen - Instrumental method".

The determination of chlorine and sulfur were carried out by ion chromatography, after the recovery in aqueous solution of chlorine and sulfur compounds, which come from the bomb calorimeter. This procedure is based on the European Standard EN 15289 "Solid Biofuels - Determination of total content of sulfur and chlorine." s

Finally, analyses major elements in biomass ash (Al, Ca, Fe, K, Mg, Na, P, S, Si, Ba, Mn, Sr, Ti and Zn) were carried out by internal procedure and is based on digestion in a microwave oven, according to European Standard EN 15290 "Solid Biofuels - Determination of major elements" of the ash obtained at 550 °C. The digested samples are analyzed by atomic emission spectrometry by inductively coupled argon plasma with optical detection by a simultaneous spectrometer THERMO JARRELL ASH, model IRIS.
All the measurements were done in triplicate and the results are presented as percentage on an oven-dry weight basis.
The variability between WACs of the composition of the different samples was analyzed statistically using the software Statgraphics Centurion XVII.I (Statpoint Technologies, Inc., Warrenton, VA, USA).

9.2 Characteristics and Analysis

The physicochemical characteristics of sanitary textiles and their ultimate analysis and inorganic elements content, measured according to the previously referred method, are shown in Table 20 and

Table 21 respectively.

The moisture content of sanitary textiles was 48.34% in the 1st WAC, 44.81% in the 2nd WAC and 70.56% in the 3rd WAC, presenting a mean value of 54.57±11.40. Similarly, the pH of the 1st WAC was measured 7.25, 8.10 for the 2nd WAC and 8.05 for the 3rd WAC, presenting a mean value of 7.80±0.39.

Table 20. Composition of sanitary textiles in dry basis (d.b.) for the 3 executed WACs along with their mean values

Parameter	1st WAC (% d.b.)	2nd WAC (% d.b.)	3rd WAC (% d.b.)	Mean Value
Extractives	16.29	19.40	22.16	19.28 ± 2.40
Aqueous	15.35 ± 0.61	16.43 ± 0.49	19.01 ± 0.18	16.93 ± 1.54
Organic	0.93 ± 0.00	2.97 ± 0.20	3.15 ± 0.11	2.35 ± 1.00
Monomeric sugars	0.04 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.00
Total sugars	0.54 ± 0.02	0.53 ± 0.06	0.07 ± 0.01	0.38 ± 0.27
Structural sugars	53.48	63.84	68.23	61.85 ± 6.18
Glucan	45.23 ± 1.06	48.67 ± 1.32	52.56 ± 0.39	48.82 ± 2.99
Xylan	4.13 ± 0.54	4.22 ± 0.19	4.96 ± 0.12	4.44 ± 0.37
Galactan	0.40 ± 0.05	0.45 ± 0.04	0.49 ± 0.07	0.33 ± 0.02
Arabinan	0.35 ± 0.06	0.30 ± 0.03	0.34 ± 0.04	0.33 ± 0.07
Mannan	3.37 ± 0.11	10.20 ± 0.22	9.88 ± 0.07	7.82 ± 3.15
Acetyl groups	0.64 ± 0.00	0.49 ± 0.19	0.35 ± 0.02	0.49 ± 0.12
Acid insoluble residue	24.15 ± 3.05	16.59 ± 1.68	9.35 ± 1.30	16.70 ± 6.04
Whole ash	7.90 ± 0.33	16.93 ± 0.91	12.48 ± 0.35	12.44 ± 3.69
Extractives-free ash	2.44 ± 0.29	2.71 ± 0.09	3.47 ± 0.00	2.87 ± 0.44
Nitrogen	0.49 ± 0.01	0.52 ± 0.03	0.71 ± 0.01	0.57 ± 0.10

Table 21. Ultimate analysis and inorganic elements content of sanitary textiles in dry basis (d.b.) for the 3 executed WACs along with their mean values

Parameter		1st WAC (% d.b.)	2nd WAC (% d.b.)	3rd WAC (% d.b.)	Mean Value
Ultimate analysis (%, d.b.)	C	48.1	44.7	42.9	45.2 ± 2.6
	H	6.9	6.2	6.2	6.4 ± 0.4
	N	0.45	0.29	0.38	0.37 ± 0.08
	S	0.07	0.11	0.07	0.08 ± 0.02
	Cl	0.41	0.20	0.38	0.33 ± 0.11
Inorganic elements (%, d.b.)	Al	0.7	0.5	0.67	0.6 ± 0.1
	Ba	0.024	0.008	0.006	0.013 ± 0.010
	Ca	5.2	2.8	5.0	4.3 ± 1.3
	Fe	0.49	0.60	0.54	0.54 ± 0.06
	K	4.0	2.6	3.2	3.3 ± 0.7
	Mg	0.70	0.41	0.38	0.50 ± 0.18
	Mn	0.009	0.008	0.008	0.008 ± 0.001
	Na	23.0	27.0	28.0	26.0 ± 2.6
	P	0.99	0.62	0.95	0.85 ± 0.20
	S	1.1	1.0	3.1	1.7 ± 1.2
	Si	5.4	4.9	3.4	4.6 ± 1.0
	Sr	0.013	0.006	0.006	0.008 ± 0.004
	Ti	0.78	0.13	0.12	0.34 ± 0.38
	Zn	0.12	0.063	0.016	0.066 ± 0.052

The physicochemical characteristics of paper & cardboard rejections, and their ultimate analysis and inorganic elements content measured according to the previously referred method, are shown in Table 22 and

Table 23 respectively.

The moisture content of carton and paper samples was 23.80% in the 1st WAC, 30.19% in the 2nd WAC and 29.82% in the 3rd WAC, presenting a mean value of 27.94±2.92. Similarly, the pH of the 1st WAC was measured 7.71, 7.76 for the 2nd WAC and 7.38 for the 3rd WAC, presenting a mean value of 7.62±0.17.

Table 22. Composition of carton and paper samples in dry basis (d.b.) for the 3 executed WACs along with their mean values

Parameter	1st WAC (% d.b.)	2nd WAC (% d.b.)	3rd WAC (% d.b.)	Mean Value
Extractives	7.56	5.58	9.34	7.49 ± 1.53
Aqueous	4.66 ± 0.17	4.72 ± 0.37	7.10 ± 0.08	5.49 ± 1.14
Organic	2.90 ± 0.33	0.86 ± 0.01	2.23 ± 0.04	2.00 ± 0.85
Monomeric sugars	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Total sugars	0.98 ± 0.03	1.29 ± 0.08	0.59 ± 0.02	0.95 ± 0.35
Structural sugars	56.66	64.56	52.20	57.81 ± 5.11
Glucan	44.86 ± 0.57	53.37 ± 0.35	41.23 ± 0.19	46.49 ± 5.09
Xylan	7.28 ± 0.10	7.88 ± 0.15	5.88 ± 0.06	7.01 ± 0.84
Galactan	0.80 ± 0.03	0.63 ± 0.03	0.90 ± 0.03	0.78 ± 0.11
Arabinan	0.43 ± 0.01	0.16 ± 0.01	0.42 ± 0.04	0.34 ± 0.12
Mannan	3.29 ± 0.08	2.52 ± 0.08	3.76 ± 0.12	3.19 ± 0.51
Acetyl groups	0.73 ± 0.00	0.62 ± 0.04	0.51 ± 0.02	0.62 ± 0.09
Acid insoluble residue	10.67 ± 0.42	7.36 ± 0.84	11.75 ± 0.25	9.93 ± 1.87
Whole ash	22.22 ± 0.12	21.74 ± 0.01	20.97 ± 0.53	21.64 ± 0.51
Extractives-free ash	22.20 ± 0.12	20.65 ± 0.11	22.02 ± 0.24	21.34 ± 0.69
Nitrogen	0.16 ± 0.00	0.14 ± 0.02	0.25 ± 0.01	0.18 ± 0.05

Table 23. Ultimate analysis and inorganic elements content of carton and paper samples in dry basis (d.b.) for the 3 executed WACs along with their mean values

Parameter		1st WAC (% d.b.)	2nd WAC (% d.b.)	3rd WAC (% d.b.)	Mean Value
Ultimate analysis (%, d.b.)	C	38.9	35.9	36.8	37.2 ± 1.5
	H	5.2	5.1	5.0	5.1 ± 0.1
	N	0.13	0.12	0.15	0.13 ± 0.02
	S	0.05	0.29	0.10	0.15 ± 0.13
	Cl	0.10	0.06	0.17	0.11 ± 0.06
Inorganic elements (%, d.b.)	Al	4.1	11.0	4.2	6.4 ± 4.0
	Ba	0.009	0.10	0.013	0.041 ± 0.051
	Ca	27.0	19.0	36.0	27.3 ± 8.5
	Fe	0.47	0.50	0.42	0.46 ± 0.04
	K	0.53	0.60	0.75	0.6 ± 0.1
	Mg	1.6	0.79	0.99	1.13 ± 0.42
	Mn	0.013	0.012	0.012	0.012 ± 0.001
	Na	1.0	0.71	1.0	1 ± 0.17
	P	0.094	0.11	0.11	0.10 ± 0.01
	S	0.51	1.8	2.2	1.5 ± 0.9
	Si	9.6	15.0	7.2	10.6 ±4.0
	Sr	0.035	0.042	0.046	0.041 ± 0.006
	Ti	0.098	0.14	0.11	0.12 ± 0.02
	Zn	0.012	0.039	0.015	0.022 ± 0.015

The physicochemical characteristics of WWTP cellulosic rejections and their ultimate analysis and inorganic elements content measured according to the previously referred method, are shown in Table 24 and

Table 25 respectively.

The moisture content of cellulosic rejections was 61.89% in the 1st WAC, 62.52% in the 2nd WAC and 48.69% in the 3rd WAC, presenting a mean value of 57.70±6.38. Similarly, the pH of the 1st WAC was measured 6.25, 5.68 for the 2nd WAC and 6.48 for the 3rd WAC, presenting a mean value of 6.15±0.35.

Table 24. Composition of cellulosic rejections in dry basis for the 3 executed WACs along with their mean values

Parameter	1st WAC (% d.b.)	2nd WAC (% d.b.)	3rd WAC (% d.b.)	Mean Value
Extractives	17.75	14.87	23.33	18.65 ± 3.51
Aqueous	7.34 ± 0.30	6.01 ± 0.04	6.47 ± 0.06	6.61 ± 0.55
Organic	10.40 ± 0.87	8.86 ± 0.21	16.86 ± 0.00	12.04 ± 3.47
Monomeric sugars	0.09 ± 0.01	0.74 ± 0.37	0.03 ± 0.00	0.29 ± 0.39
Total sugars	1.02 ± 0.11	2.21 ± 0.32	0.43 ± 0.01	1.22 ± 0.91
Structural sugars	30.47	37.22	21.96	29.88 ± 6.24
Glucan	25.82 ± 0.50	30.92 ± 0.84	19.19 ± 1.05	25.31 ± 4.80
Xylan	2.97 ± 0.05	3.90 ± 0.04	1.68 ± 0.05	2.85 ± 0.91
Galactan	0.50 ± 0.02	0.82 ± 0.04	0.38 ± 0.01	0.57 ± 0.19
Arabinan	0.46 ± 0.02	0.52 ± 0.02	0.34 ± 0.02	0.44 ± 0.07
Mannan	0.72 ± 0.04	1.06 ± 0.04	0.37 ± 0.01	0.72 ± 0.28
Acetyl groups	1.06 ± 0.00	3.40 ± 0.19	3.11 ± 0.67	2.52 ± 1.04
Acid insoluble residue	38.54 ± 0.88	22.73 ± 0.06	27.56 ± 1.20	29.61 ± 6.62
Whole ash	7.55 ± 0.15	6.01 ± 0.02	30.63 ± 2.20	14.73 ± 11.26
Extractives-free ash	7.93 ± 0.17	8.86 ± 0.21	28.93 ± 1.92	15.24 ± 9.69
Nitrogen	1.35 ± 0.03	1.19 ± 0.02	1.13 ± 0.03	1.22 ± 0.09

Table 25. Ultimate analysis and inorganic elements content of cellulosic rejections in dry basis (d.b.) for the 3 executed WACs along with their mean values

Parameter		1st WAC (% d.b.)	2nd WAC (% d.b.)	3rd WAC (% d.b.)	Mean Value
Ultimate analysis (%, d.b.)	C	55.1	51.9	36.0	47.7 ± 10.2
	H	7.0	6.9	3.5	5.8 ± 2.0
	N	1.32	1.18	0.83	1.11 ± 0.25
	S	0.25	0.19	0.25	0.23 ± 0.03
	Cl	0.17	0.08	0.07	0.11 ± 0.06
Inorganic elements (%, d.b.)	Al	2.2	2.7	4.2	3.0 ± 1.0
	Ba	0.059	0.08	0.050	0.063 ± 0.015
	Ca	22.0	24.0	14.0	20.0 ± 5.3
	Fe	2.9	2.7	3.3	2.97 ± 0.31
	K	2.2	2.0	1.7	2.0 ± 0.3
	Mg	1.6	2.0	1.0	1.53 ± 0.50
	Mn	0.040	0.042	0.036	0.039 ± 0.003
	Na	2.2	1.6	0.96	2 ± 0.62
	P	3.3	3.8	0.78	2.63 ± 1.62
	S	1.6	1.6	1.6	1.6 ± 0.0
	Si	10.0	11.0	23.0	14.7 ± 7.2
	Sr	6.4	0.086	0.029	2.172 ± 3.662
	Ti	0.60	0.80	0.36	0.59 ± 0.22
	Zn	0.16	0.27	0.052	0.161 ± 0.109

Samples of sanitary textiles and carton and paper samples showed a neutral-basic pH between 7-8, while the WWTP cellulosic rejections had a slightly acidic pH of around 6. Thus, adjustment of the pH prior to the enzymatic hydrolysis and fermentation steps could be required.

Sanitary textiles and carton and paper samples had the highest content in glucans, with values fluctuating between 41 and 53%. Considering all structural sugars (glucan, xylan, galactan, arabinan and mannan), the amount of carbohydrates in sanitary textiles and carton and paper samples added up to more than 50%, reaching values over 60% in the best cases. Due to the lower glucans and structural sugars content in samples from WWTP cellulosic rejections, testing a pretreatment step is recommended with the aim of increasing the sugars content for subsequent transformation into ethanol.

It is worth to mention that carton and paper samples had a higher ash content compared to the other materials, which is probably due to the inks present in this residue. This high content could affect the yields both the pre-treatment stage and the subsequent stages of hydrolysis and fermentation.

All the samples analyzed had low sugars content in aqueous extract, being the WWTP cellulosic rejections the material with the highest value with as much as 2% of total soluble sugars referred to dry weight basis in the sample from the 2^{nd} WAC.

The high presence of Al and Si in carton and paper samples could be due to the use of kaolin ($Al_2Si_2O_5(OH)_4$) as absorbent to improve ink retention and/or as part of the pigments added to coated paper. Calcium carbonate is also used as pigment, which could explain the high amount of Ca found in this type of residue.

Analysis of the variability of the composition of samples between WACs

The materials analyzed were very heterogeneous; therefore, the compositional data was statistically processed to investigate the variability between WACs of each of the samples. To this end, the coefficient of variation intra-groups and inter-groups and ANOVA analysis for each sample type were calculated. Results of the statistical analysis are shown in Table 26 below.

Variability intra-groups, represented by the CV of WACs 1, 2 and 3, was low to moderate in all cases (<20%). However, the variability inter-groups was higher. This is confirmed by the results of the ANOVA, represented by the p-value in Table 26. These results show that, in general, the composition of the samples differs significantly among WACs for a 95% confidence level. Regarding the glucan and hemicellulose content, the greater differences between samples were found for WWTP cellulosic rejections, whereas carton and paper samples seem to have a more stable carbohydrate content. Nevertheless, given the high variability of the composition of samples among WACs, the performance of the transformation processes could be significantly affected.

Table 26. Coefficient of variation (CV) intra- and inter-groups and ρ-value for the main determined components of each of the samples

	Sanitary textiles					Carton and paper					WWTP cellulosic rejections				
	CV 1st WAC	CV 2nd WAC	CV 3rd WAC	CV Total	p-value	CV 1st WAC	CV 2nd WAC	CV 3rd WAC	CV Total	p-value	CV 1st WAC	CV 2nd WAC	CV 3rd WAC	CV Total	p-value
Aqueous extractives	5.7%	4.2%	1.3%	10.4%	0.0254*	5.3%	11.0%	1.7%	23.3%	0.0086**	5.9%	1.2%	18.0%	38.4%	0.0001**
Ethanol extractives	0.1%	9.5%	4.7%	47.3%	0.0021**	16.1%	2.5%	2.5%	47.9%	0.0107*	11.9%	13.4%	19.4%	23.9%	0.1264
Glucan	2.9%	3.3%	0.9%	6.8%	0.0010**	1.6%	0.8%	0.6%	11.6%	0.0001**	2.4%	3.3%	6.7%	22.9%	0.0000**
Hemicellulose	8.6%	3.2%	1.8%	28.6%	0.0001**	2.3%	2.1%	1.7%	3.8%	0.0105*	3.1%	2.6%	3.1%	36.8%	0.0000**
Acid insoluble residue	15.5%	12.4%	19.0%	43.8%	0.0010**	4.7%	13.9%	2.6%	20.8%	0.0006**	2.8%	0.3%	5.3%	25.2%	0.0000**
Whole ash	5.9%	7.6%	3.9%	32.9%	0.0040**	0.7%	0.1%	3.6%	3.0%	0.1417	2.9%	0.5%	6.7%	67.5%	0.0000**
Extractives-free ash	17.1%	4.4%	0.2%	18.1%	0.0524	0.2%	0.7%	1.5%	4.7%	0.0043**	3.1%	3.3%	5.7%	53.9%	0.0000**

*Significant difference at 95% confidence level

**Significant difference at 99% confidence level

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10. PILOT 7 – Biochar

PILOT 7 is located at Chania (Crete, Greece) at the premises of the Technical University of Crete Campus. The basic feedstock used to produce biochar is sewage sludge, as mentioned in the proposal. In addition, TUC explored the benefits of using alternative feedstocks for the production of biochar, i.e. olive oil mill by-products as well as sawdust from carpentries to act also as bulking agents. Furthermore, it was decided to test municipal solid waste compost as an alternative product that could be combined with the biochar as soil improver and fertilizer. In the FP7 SOILTREC project, TUC used the same compost as a fertilizer to grow tomatoes for 4 consecutive years. So, the objective in this case is to use biochar as a bio stimulant and compost as the fertilizer. TUC believes that the co-valorization of different locally produced agricultural by-products, is more advantageous, in line with the principles of circular economy, generates new knowledge and is added value for the project.

10.1 Sampling and analysis protocol

The feedstocks for the Pilot Plant 7 were collected from the different feedstock providers (Table 27) and placed in the Campus of Technical University of Crete.

Table 27. Feedstocks for Pilot 7

Feedstock	Feedstock provider
Sewage sludge	Municipal wastewater treatment plant of Chania, Greece
Olive Oil mill waste	Kapetanakis olive mill, Akrotiri, Chania, Greece
Sawdust	Local Carpenter, Chania, Greece
Compost	Inter-Municipal Solid Waste Management Company of Chania (DEDISA)

Sewage sludge was obtained from the Wastewater Treatment Plant of Chania and the sample taken was 750L. Sawdust obtained from a local carpentry in Chania, Greece; the sample taken was approximately 100 kg. Olive mill waste was acquired from an organic olive mill in Akrotiri Chania. The material sampled was the dried sludge left after evaporation of the wastewaters, produced from a 3-phase mill in an evaporation pond. The sample taken was approximately 750L. The compost was provided by the Inter-Municipal Solid Waste Management Company of Chania (DEDISA). The sample taken was approximately 100 kg.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no. 818308

Sewage sludge and Olive Oil mill waste were collected and placed in barrels of 75L. The total volume of each one of the two waste streams will be used for the experiments of the production of biochar. The total amount of the materials was homogenized and 8 samples for each one of the 2 aforementioned waste streams were taken. An auger was used for the collection of the samples of the waste streams in order to have representative samples of the whole depth of the barrel (Figure 12a-c).

Moreover, 4 samples of the sawdust and compost were taken for further analysis (Figure 12d).

Figure 12. Sampling procedure of the feedstocks. a, b) Sewage sludge's sampling using auger, c) Olive Oil mill waste sampling using auger, d) Samples of sawdust

An indicative sampling record of the feedstock of PILOT 7 is included in ANNEX I.

The samples were homogenized, air dried and milled to small particles. The samples were analyzed for physical and chemical properties including pH, conductivity, dry matter/moisture, volatile solids/ash, total organic carbon, total major and trace metals, phenols, C, H, S, N, chlorides and sulfates, ammonium and nitrates and phosphorus. Table 28 presents the methods used for each analysis.

All materials have been subjected to thermogravimetric analysis (TGA) using a differential thermogravimetric analyzer TGA-6/DTG of Perkin Elmer (temperature measurement precision of ±2 °C, microbalance sensitivity <5 μg). The rate and % weight loss for each material were determined continuously as a function of time or temperature, under dynamic conditions, in the range of 25-850 °C. The experiment was carried out at atmospheric pressure, under nitrogen/air atmosphere, with flow rate of 45 $mL \cdot min^{-1}$ and linear heating rate of 10 $°C \cdot min^{-1}$. Volatile matter (VM) and char content were also determined based on the TGA. All experiments were carried out in duplicate.

Table 28. Analytical methods adopted for feedstocks characterisation of PILOT 7

Parameter	Method
pH, Electrical Conductivity (pH, EC)	EPA Method 9045D ASTM D4972-19
Dry matter/moisture (TS)	APHA-AWWA-WEF 2540 B. ASTM D2216 - 19
Volatile solids/ash (VS)	APHA-AWWA-WEF 2540 G. ASTM E1755 - 01
Total organic carbon (TOC)	ASTM D6316
Metal content	EPA Method 3051a, EPA Method 6010b
Phenols	EPA Method 1312, DIN 38409-16:1984-06 (HACH: LCK345 kit)
Elemental analysis: C, H, S, N	Thermo Scientific™ FLASH 2000 CHNS/O Analyzers (using BBOT standards (2.5-Bis(5-tert-butyl-2-benzo-oxazol-2-yl) thiophene) containing carbon)
Chlorides, Sulfates (Cl^-, SO_4^{2-})	EPA Method 1312, EPA Method 9038 (HACH: LCK153 kit), EPA Method 9251 (HACH: LCK311 kit)
Ammonium Nitrogen, Nitrate Nitrogen ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$)	ISO/TS 14256-1:2003, ISO 7890-1-2- 1986, EPA Method 350.2 (HACH Method 8038)
Olsen Phosphorus (Olsen-P)	ISO 11263, 1994, EPA Method 365.1 (HACH Method 8048)

The feedstock biowaste of PILOT 7 includes sewage sludge from Municipal waste water, olive mill waste water sludge and sawdust in case it is needed as bulking agent (not necessary so far). All the required quality analysis such as nutrient and metal content, phenols, chloride, and elementary analysis are conducted. Analysis for plastic residues was not performed because none of the processes of waste generation allow the possibility to include plastics. In the wastewater treatment systems pre-screening and pre-treatment processes are included eliminating such possibilities, while in the olive mill the effluent derives from the olive oil plant and fruit residue of olives.

10.2 Characteristics and Analysis

The aforementioned materials have been characterized in terms of their main physico-chemical properties (Table 29), their ultimate analysis and inorganic elements content (Table 30).

Table 29. Composition of sewage sludge, olive mill waste, sawdust and compost in dry basis

Parameter		Sewage sludge	Olive oil mill waste	Sawdust	Compost
pH		8.52 ± 0.24	5.57 ± 0.11	5.16 ± 0.09	7.23 ± 0.18
Electrical Conductivity	mS/cm	2.38 ± 0.22	0.96 ± 0.06	2.66 ± 0.28	6.49 ± 0.36
Moisture	%	67.39 ± 3.61	38.1 ± 12.27	5.11 ± 0.06	25.49 ± 2.02
Dry Matter	%TS	32.6 ± 1.36	61.88 ± 2.22	94.88 ± 3.12	74.5 ± 2.77
Volatile Solids	(% VS)	70.77 ± 2.55	93.22 ± 4.08	97.86 ± 4.23	42.27 ± 2.12
Ash	%	29.23 ± 2.11	6.78 ± 0.25	2.14 ± 0.08	57.73 ± 3.52
TOC	%	38.96 ± 3.55	55.40 ± 4.20	48.70 ± 1.28	19.48 ± 3.44
TN	%	6.00 ± 0.81	6.16 ± 0.67	0.17 ± 0.05	2.15 ± 0.33
N-NO_3^-	mg/kg	35.22 ± 7.18	167.65 ± 52.32	250.46 ± 52.16	612.57 ± 31.57
N-NH_4^+	mg/kg	3257.61 ± 644.36	155.94 ± 39.24	100.84 ± 17.67	50.61 ± 28.31
Olsen-P	mg/kg	376.52 ± 58.21	111.66 ± 27.90	10.13 ± 3.61	108.34 ± 10.98
Cl^-	g/kg	0.64 ± 0.22	2.29 ± 0.83	<0.02	7.02 ± 0.67
SO_4^{2-}	g/kg	12.29 ± 1.89	7.64 ± 6.00	<0.01	5.84 ± 1.25
Phenols	mg/kg	128.88 ± 23.42	460.10 ± 149.92	310.45 ± 94.89	37.07 ± 5.52

Table 30. Ultimate analysis and inorganic elements content of sewage sludge, olive mill waste, sawdust and compost in dry basis (d.b.)

Parameter			Sewage sludge	Olive oil mill waste	Sawdust	Compost
Ultimate analysis	C	%d.b.	31.92 ± 1.86	64.56± 3.52	58.94 ± 3.04	21.41 ± 1.08
	H		4.96 ± 0.18	3.67 ±0.12	5.1 ± 0.20	1.88 ± 0.04
	N		6.05 ± 0.85	6.18 ±0.86	0.18 ± 0.02	2.14 ± 0.09
	S		1.78 ± 0.12	0.44 ±0.04	-	0.49 ± 0.06
Inorganic elements	Al	g/kg	3.24 ± 0.42	0.93 ±0.11	0.03 ± 0.00	6.49 ± 0.25
	As	mg/kg	2.84 ± 0.12	<DL	<DL	<DL
	B	mg/kg	84.85 ± 2.41	54.44 ±3.96	3.53 ± 0.32	63.01 ± 1.57
	Ba	mg/kg	759.72 ± 65.41	4.69 ±0.27	10.43 ± 0.26	282.60 ± 6.86
	Ca	g/kg	52.59 ± 4.72	13.32 ±1.00	1.03 ± 0.07	112.00 ± 4.90
	Cd	mg/kg	<DL	<DL	<DL	<DL
	Co	mg/kg	1.24 ± 0.05	<DL	<DL	4.10 ± 0.44
	Cr	mg/kg	60.20 ± 4.70	2.91 ±0.21	0.64 ± 0.03	55.14 ± 2.86
	Cs	mg/kg	<DL	<DL	<DL	<DL
	Cu	mg/kg	343.20 ± 26.79	84.71 ±8.43	9.40 ± 0.40	328.47 ± 22.92
	Fe	g/kg	35.37 ± 3.05	1.23 ±0.14	0.11 ± 0.00	10.95 ± 0.46
	Hg	mg/kg	2.33 ± 0.08	0.24 ±0.03	<DL	1.24 ± 0.02
	K	g/kg	2.30 ± 0.23	19.79 ±1.42	0.27 ± 0.07	14.29 ± 0.96
	Li	mg/kg	2.09 ± 0.14	1.08 ±0.13	0.46 ± 0.03	4.47 ± 0.31
	Mg	g/kg	10.54 ± 1.10	1.59 ±0.20	0.45 ± 0.03	9.49 ± 0.42
	Mn	mg/kg	201.7 ± 16.87	39.29 ±2.32	54.76 ± 6.93	244.53 ± 3.36
	Mo	mg/kg	11.14 ± 1.01	2.84 ±0.41	1.69 ± 0.09	5.10 ± 0.26
	Na	g/kg	1.23 ± 0.19	1.00 ±0.07	0.05 ± 0.01	9.21 ± 1.02
	Ni	mg/kg	31.68 ± 2.79	3.39 ±0.30	0.86 ± 0.03	37.99 ± 2.71
	Pb	mg/kg	144.79 ± 9.50	<DL	<DL	138.47 ± 3.14
	Rb	mg/kg	2.43 ± 0.30	11.28 ±0.51	0.69 ± 0.29	10.59 ± 0.46
	Sb	mg/kg	4.05 ± 0.09	1.87 ±0.05	1.38 ± 0.03	3.38 ± 0.14
	Se	mg/kg	1.87 ± 0.07	<DL	<DL	<DL
	Si	g/kg	1.65 ± 0.15	0.89 ±0.09	0.43 ± 0.02	2.11 ± 0.29
	Sn	mg/kg	8.78 ± 0.27	2.33 ±0.09	1.72 ± 0.01	5.28 ± 0.42
	Sr	mg/kg	373.11 ± 2.84	24.42 ±2.45	18.75 ± 1.04	298.36 ± 12.36

Parameter			Sewage sludge	Olive oil mill waste	Sawdust	Compost
	Ti	mg/kg	74.29 ± 4.32	6.58 ±0.25	<DL	46.34 ± 3.76
	U	mg/kg	5.81 ± 0.11	<DL	<DL	<DL
	V	mg/kg	12.52 ± 1.07	2.69 ±0.15	0.52 ± 0.01	12.37 ± 1.18
	Y	mg/kg	3.98 ± 0.27	2.04 ±0.22	1.16 ± 0.06	9.34 ± 0.69
	Zn	mg/kg	1408.88 ± 89.84	56.13 ±7.32	11.24 ± 1.35	698.90 ± 75.11

<DL: Below detection limit

From the data presented above (Table 29 and Table 30), it is observed that:

- The pH of all materials varied from the slightly acidic to the slightly alkaline region; sawdust exhibited the lowest pH, 5.2, while sewage sludge the highest, 8.5.
- Electrical conductivity was highest in compost (6.5 mS/cm) as anticipated and lowest in olive mill waste, almost 1 mS/cm.
- Sewage sludge presents the highest moisture content (~67 %) followed by olive oil mill waste and compost, while sawdust the lowest (~5 %).
- The % VS ranged between ~70-98 % for sewage sludge, olive oil mill waste and sawdust, while it was much lower in compost ~42 %.
- The % carbon content varied between ~20 % for compost and 65 % for olive oil mill waste.
- The % sulfur content was very low in all materials; no sulfur was detected in sawdust while the highest content was in sewage sludge (~1.8 %)
- Olive oil mill waste and sewage sludge exhibited the highest % nitrogen content, close to 6 %, while compost and sawdust had much lower content, 2.1 and 0.2 %, respectively.
- The % hydrogen content was lower in compost, ~2 %, and higher in sawdust and sewage sludge, ~5 %.
- Sewage sludge presents high values of N-NH_4^+ (about 3258 mg/kg), while the total nitrogen content is about 6%.
- As far as the heavy metals content, the feedstocks present lower values than the maximum thresholds (Table 31) defined for heavy metals in compost and in sewage sludge used in agriculture according to the Greek guidelines (Joint Ministerial Decision (JMD) 114216, Government Gazette Issue (FEK) 1016/B’ and JMD 80568/4225/91, FEK 641/B/7-08-1991).

Table 31. Greek guidelines for heavy metals in compost and sewage sludge

Parameter	Maximum threshold in compost (mg/kg dry)	Thresholds in sewage sludge (mg/kg dry)
Cr	510	-
Ni	200	300 - 400
Cd	10	20 - 40
Pb	500	750 - 1200
Cu	500	1000 - 1750
Zn	2000	2500 - 4000
As	15	-
Hg	5	16 - 25

The results of the thermogravimentric analysis of the examined feedstocks are depicted in Figure 13.

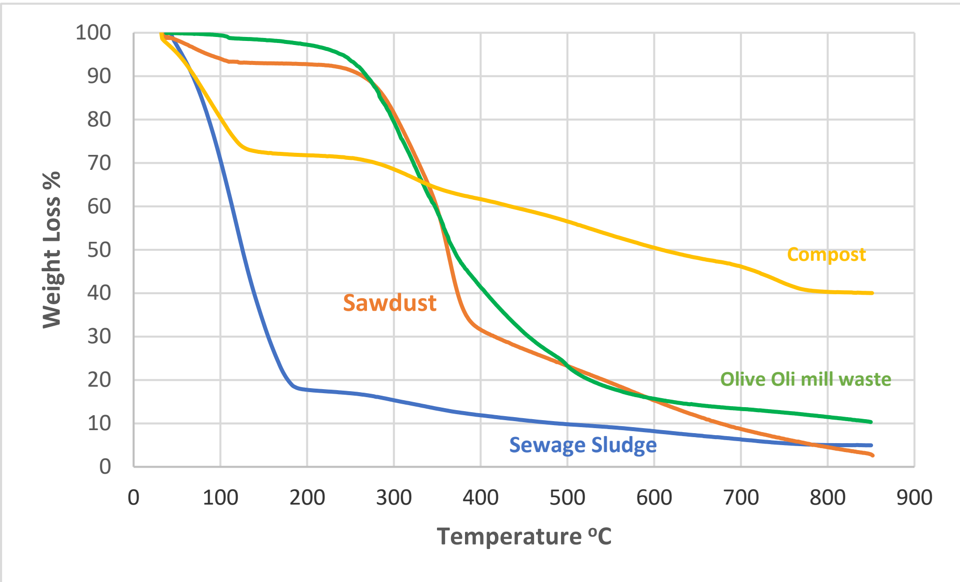

Figure 13. Thermogravimetric analysis of feedstocks of PILOT 7

Based on these curves, it is deduced that:

- Olive oil mill waste and sawdust presented similar thermal behavior in terms of weight loss, while compost and sewage sludge a similar but different one, compared to the other two feedstocks.
- The weight loss between 120 °C and 170 °C, depending on the raw material, is mainly associated to loss of moisture.

- The weight loss recorded above 350 °C, varies for each different material and is mainly attributed to the decomposotion of carbonhydrates and lipids i.e. the decomposition of hemicellulose and cellulose.
- Above 750 °C the weight loss is associated with the decomposition of inorganic compounds.

Finally, the contents of volatile matter and char have been also calculated from the gravimetric analysis and presented in the following Table (

Table 32). Sawdust and sewage sludge presented the highest values of %VM, namely ~97 % and 92 % respectively, and the lowest values of Char, namely ~2.7 and 7 %, repsectively.

Table 32. Volatile matter and char content of feedstocks of PILOT 7 from TGA

Parameter		Sewage sludge	Olive oil mill waste	Sawdust	Compost
Volatile Matter	(%)	92 ± 2.12	59.45 ± 1.39	97.27 ± 2.32	48.08 ± 1.25
Char	(%)	7.99 ± 0.33	40.54 ± 1.21	2.72 ± 0.11	51.91 ± 1.76

Conclusions

In summary, the purpose of this deliverable was to report on the physical and chemical analysis of urban biowaste to be used as feedstock within WaysTUP! project.

This report comes as a result of the application of all the necessary requirements for conducting effective Waste Analysis Campaigns. The quality requirements, defined in deliverable D1.1 ensured that sample collection and laboratory analysis activities generated data which meet the pilot plants and project's requirements, and are technically valid, useable and legally defensible relative to the use for which the data are obtained.

Implementation of the developed methodology in D1.1 ensured the high data quality collection for the successful progress of D1.2 "Report on urban biowaste composition & physicochemical characteristics" and constitutes the stepping stone for the successful initiation of WaysTUP! experimental part.

Samples of meat waste, fish waste, coffee waste, source separated biowaste, used cooked oils, coffee oil from spent coffee grounds, cellulosic rejections and carton and paper waste of municipal solid waste, nappies, sewage sludge, olive oil mill waste, compost and sawdust were collected and analysed.

More specifically, for PILOT 1 three feedstocks (meat waste, fish waste and spent coffee grounds) were collected and analysed. For meat and fish by-products, there is a wide ratio of protein content depending on animal/fish species and body part, while for spent coffee grounds, the different coffee varieties and coffee processing methods may significantly affect the final composition.

For PILOT 2, retail spent coffee grounds (both fresh and aged) originating from coffee shops and restaurants with an oil yield up to 12% or industrial spent coffee grounds from instant coffee factories with oil yields of up to 25% shall be used as feedstocks. In both cases, the physical state of the SCG collected is powder.

PILOT 4A uses coffee oil as feedstock produced after supercritical fluid extraction with CO_2. The coffee oil had a dark yellow colour and its appearance was waxy with solid-like consistency. PILOT 4B shall use cooking oil from restaurants and canteens. The moisture content of the samples ranged from 0 to 1%, while all the samples were liquid oils with brown to reddish colour with characteristic odor.

For PILOT 5 source separated biowaste from households shall be used feedstock. The biowaste studied had similar physicochemical characteristics, although fluctuations to the parameters are expected, especially if one takes into account the change of seasons and therefore of the eating habits of people (e.g. seasonality of fruits).

For PILOT 6, cellulosic rejection streams from the MSW of AMB, sanitary textiles and carton and paper rejections of MSW were also investigated as other potential feedstocks. The materials analyzed were very heterogeneous and thus the performance of the transformation processes could be significantly affected.

In PILOT 7 sewage sludge, sawdust, olive oil mill waste and compost shall be used as feedstocks. Sewage sludge presented the highest moisture content followed by olive oil mill waste and compost, while sawdust the lowest. The volatile solids content was high for sewage sludge, olive oil mill waste and sawdust, while it was much lower in compost.

Conclusively, the results of this deliverable will pave the way for the successful implementation of several urban valorization routes.

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Annex I

PILOT 2

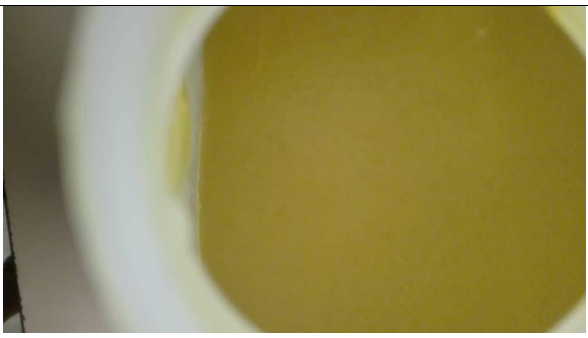
SAMPLING RECORD – SPENT COFFEE GROUNDS
Sample Name: Dry SCG Retail 001 Date:
Sampled By: Signature:
INFORMATION
Waste Source: Alconbury Factory Tip Ticket Number: Date of Tip (Determine Coffee Age): Contact:
Sampling Objective: The objective of sampling is to comply with the D1.1: Laboratory analysis protocol and Methodology for executing WAC and to supply feedstock to the Enfield Lab for oil extraction purposes.
MATERIAL
Type of Feedstock: Spent Coffee Ground (Retail) Dried Bag Moisture content: 10% Source: Coffee Shop In Wellingborough Area Description: Dried Spent Coffee Ground Processing: Dried in Factory Dryer to 10% ± 2%

SAMPLING METHODOLOGY
Describe/define sub-population or consignment sampled: The sample is obtained from the dried coffee bags collected from the outlet of the dried ground sieve at the edge of the dryer. *Place and point of sampling:* Alconbury Factory. Unit 4002, Alconbury Weald, Huntingdon. PE28 4WX, UK *Access problems that affected areas or volumes of feedstock sampled:* Not applicable *Date* and time of sampling: *Persons present (record name and address of witnesses present where appropriate):* Not applicable *Procedure (describe procedure adopted):* *Equipment used:* Spade *Number of increments/samples collected:* 1 *Increment size/Sample size:* 10 kg *Observations diving sampling (e g. gassing out, reactions, development of heat):* Not applicable *Details of on-site determinations:* Not applicable

SUB-SAMPLING AND PRE-TREATMENT
Safety measures taken: Protective gloves and clothes as well as respiratory protection for dusty environments. *Identify location: e.g. on-site or fixed laboratory facility (describe whether open air or enclosed)* Sub sampling is carried out at the Enfield Labs. Procedure: With spade.
PACKAGING. PRESERVATION. STORAGE AND TRANSPORT DETAILS
Packaging: Product is packaged in sealable plastic bags until extraction. *Preservation*: Dried Spent Grounds have a shelf life at ambient of <12 months. *Storage*: Dried Spent Grounds are stored in the laboratory in sealed boxes protected from heat and light.
DEVIATIONS FROM SAMPLING PLAN
Detail: No Deviations

PILOT 4A Coffee Oil

SAMPLING RECORD	
Sample code: CO-Retail-Aged-5Kg *Nagifate received 1st batch of app. 5 kg of coffee oil from retail aged spent cofee grounds on July 2020 from* BIO-BEAN *who prepared the sample.*	
Date of sampling: **January to July 2020**	
Signature of sampler: **N/A**	
GENERAL INFORMATION	
Waste producer: **Retail coffee shops** Contact: **N/A**	Client (Company): **N/A** Contact: **N/A**
Location of sampling: **London**	Carried out by (Company): BIO-BEAN Sampler: **N/A**
SAMPLING OBJECTIVE	
SAMPLING APPROACH/PATTERN (with justification): **Single grab sampling.** The sample is homogenized due to its production and characterisation analysis are also provided by BIO-BEAN, so interlaboratory comparison is made.	
MATERIAL	
Type of Feedstock: Coffee Oil (**CO**)	Estimated moisture content: **N/A**
Source and origin of the feedstock (e.g. form and nature of arising): **Retail spent coffee ground collected by** BIO-BEAN**.**	
Process/activity producing the feedstock: **Supercritical fluid extraction with CO2.**	
Description: (colour, odour. consistency/homogeneity/grain size - uniform or diverse) **Waxy, solid-like consistency. Dark Yellow colour.**	



SAMPLING METHODOLOGY
Describe/define sub-population or consignment sampled: **Whole sample, appr. 5 Kg.**
Place and point of sampling: **Nafigate laboratories in Institute of Microbiology of the CAS**
Access problems that affected areas or volumes of feedstock sampled: **None.**
Date and time of sampling: **September 2020**
Persons present (record name and address of witnesses present where appropriate): **Martin Kryl, NFG.**
Procedure (describe procedure adopted): **The sample was collected from the middle of the container.**
Equipment used: **Spatula.**
Number of increments/samples collected: **1 samples from the container.**
Increment size/Sample size: **10 g per sample**
Observations diving sampling (e g. gassing out, reactions, development of heat): **None-**
Details of on-site determinations: **None.**
Safety measures taken: **Standard protective work aids.**
SUB-SAMPLING AND PRE-TREATMENT
Identify location: e.g. on-site or fixed laboratory facility (describe whether open air or enclosed)
Procedure: No pre-treatment.
PACKAGING. PRESERVATION. STORAGE AND TRANSPORT DETAILS

Packaging: **Plastic**	
Preservation: **Samples were stored in sealed glass tubes before being analysed**	
Storage: **Dark and cool place.**	
Transport: **N/A**	
DEVIATIONS FROM SAMPLING PLAN	
Detail: **Sampling timeline affected by the receiving date of the samples.**	
DELIVERY TO ANALYTICAL LABORATORY	
Company: Received by:	Delivery date:
	Signature:

PILOT 4B -Used Cooked Oils

SAMPLING RECORD	
Sample code: (Reflect site location, feedstock and date of collection) ***Novamont received 2 batches of UCO from Nagifate***: - 1^{st} **Batch** received in December 2019 (**5 kg** in a **single tank** labeled NFG Batch 17) - 2^{nd} **Batch** received in June 2020 (**2** separate **tanks** containing **25 kg each**)	
Date of sampling: **N/A**	
Signature of sampler: **N/A**	
GENERAL INFORMATION	
Waste producer: **N/A** Contact: **N/A**	Client (Company): **N/A** Contact: **N/A**
Location of sampling: **N/A**	Carried out by (Company): **Nafigate** Sampler: **N/A**
SAMPLING OBJECTIVE	
SAMPLING APPROACH/PATTERN (with justification): **Determination of main fatty acid profile of UCO by GC-MS method**	
MATERIAL	
Type of Feedstock: Used Cooking Oil (**UCO**)	Estimated moisture content: **0-1%**
Source and origin of the feedstock (e.g. form and nature of arising): **Filtered mixture of used vegetable oils**	
Process/activity producing the feedstock: **N/A**	
Description: (colour, odour. consistency/homogeneity/grain size - uniform or diverse) **Liquid oil with brown to reddish colour, characteristic odor**	
SAMPLING METHODOLOGY	
Describe/define sub-population or consignment sampled: **N/A**	
Place and point of sampling: **Novamont Biotechnology Research Center in Piana di Monte Verna (ITALY)**	

Access problems that affected areas or volumes of feedstock sampled: **N/A**	
Date and time of sampling: **1st Batch** sampling in **January 2020** **2nd Batch** sampling of Tank 1 and Tank 2 in **June 2020**	
Persons present (record name and address of witnesses present where appropriate): **N/A**	
Procedure (describe procedure adopted): **Tanks were extensively shaken and sample was picked up by means of pipette from the middle of the liquid batch**	
Equipment used: **Aspiration with pipette**	
Number of increments/samples collected: **3 samples from each single tank**	
Increment size/Sample size: **10-20 g per sample**	
Observations diving sampling (e g. gassing out, reactions, development of heat): **N/A**	
Details of on-site determinations: **N/A**	
Safety measures taken: **Proper DPI utilization**	
SUB-SAMPLING AND PRE-TREATMENT	
Identify location: e.g. on-site or fixed laboratory facility (describe whether open air or enclosed)	
Procedure:	
PACKAGING. PRESERVATION. STORAGE AND TRANSPORT DETAILS	
Packaging: **Plastic tank** (same container received with UCO delivery)	
Preservation: **Samples were collected and stored in Falcon tubes before being analysed**	
Storage: **UCO Batches were stored in a dark and cool place in order to prevent oxidation**	
Transport: **N/A**	
DEVIATIONS FROM SAMPLING PLAN	
Detail: **N/A**	
DELIVERY TO ANALYTICAL LABORATORY	
Company:	Delivery date:

Received by:	Signature:

PILOT 5

SAMPLING RECORD	
Sample code: VVV-BIO-15/09/20	
Date of sampling: 15/09/2020	
Signature of sampler: Lagoudataris Grigoris	
GENERAL INFORMATION	
Waste producer: Households Contact: Nikoletta Maneta (SUST)	Client (Company): Contact:
Location of sampling: Region of Miladeza (Municipality of Vari – Voula – Vouliagmeni in Attica, Greece)	Carried out by (Company): Municipality of Vari – Voula – Vouliagmeni Sampler: Lagoudataris Grigoris
SAMPLING OBJECTIVE Physicochemical characterisation of source separated food waste	
SAMPLING APPROACH/PATTERN (with justification): Composite sampling	
MATERIAL	
Type of Feedstock: Source separated food waste from households	Estimated moisture content: ~75-78%
Source and origin of the feedstock (e.g. form and nature of arising): Households of Vari – Voula – Vouliagmeni in Attica, Greece	
Process/activity producing the feedstock:	
Description: (colour, odour. consistency/homogeneity/grain size - uniform or diverse) High moisture, heterogenity	
SAMPLING METHODOLOGY	
Describe/define sub-population or consignment sampled:	
Place and point of sampling: Region of Miladeza (Municipality of Vari – Voula – Vouliagmeni in Attica, Greece)	
Access problems that affected areas or volumes of feedstock sampled:	

Date and time of sampling: 15/9/2020 5:00a.m.	
Persons present (record name and address of witnesses present where appropriate):	
Procedure (describe procedure adopted):	
Equipment used: Plastic bags are collected manually	
Number of increments/samples collected: 8 plastic bags	
Increment size/Sample size: 129kg	
Observations diving sampling (e g. gassing out, reactions, development of heat):	
Details of on-site determinations:	
Safety measures taken: Gloves, protective glasses and lab coats	
SUB-SAMPLING AND PRE-TREATMENT	
Identify location: e.g. on-site or fixed laboratory facility (describe whether open air or enclosed)	
Procedure:	
PACKAGING. PRESERVATION. STORAGE AND TRANSPORT DETAILS	
Packaging:	
Preservation: Collection and direct delivery	
Storage: Sealed plastic bags	
Transport: Vechile of the cleaning service of municipality of Vari-Voula-Vouliagmeni	
DEVIATIONS FROM SAMPLING PLAN	
Detail:	
DELIVERY TO ANALYTICAL LABORATORY	
Company: NTUA Received by: Passadis Kostas	Delivery date: 15/9/2020 Signature: Πασσάδης Κ.

PILOT 6

Sampling records (11.03.2020, 02.09.2020) for cellulosic rejections from urban waste from Metropolitan area of Barcelona.

Annex 2

Example information Sampling Record

SAMPLING RECORD	
Sample code: *WWTPBCellReject110320*	
Date of sampling: *11.03.2020*	
Signature of sampler:	
GENERAL INFORMATION	
Waste producer: *Urban waste from Metropolitan area of Barcelona* Contact: *Julia Hereza Atienza, jhereza@amb.cat*	Client (Company): *IMECAL* Contact:
Location of sampling: *EDAR del Besòs, Barcelona*	Carried out by (Company): *Aigües de Barcelona* Sampler: *Ester Pastoret*
SAMPLING OBJECTIVE *The objective of sampling is to comply with the D1.1: Laboratory analysis protocol and Methodology for executing WAC as well as to supply the feedstock to IMECAL in order they could do analyisis.*	
SAMPLING APPROACH/PATTERN (with justification):	
MATERIAL	
Type of Feedstock: *Cellulosic Rejections*	Estimated moisture content: *55-60%*
Source and origin of the feedstock: *Cellulosic Rejections from municipal WWTP.*	
Process/activity producing the feedstock:	

Annex 2

Example information Sampling Record

SAMPLING RECORD	
Sample code: *WWTPBCellReject020920*	
Date of sampling: *02.09.2020*	
Signature of sampler: Ester Pastoret Martín	
GENERAL INFORMATION	
Waste producer: *Urban waste from Metropolitan area of Barcelona* Contact: *Julia Hereza Atienza, jhereza@amb.cat*	Client (Company): *IMECAL* Contact:
Location of sampling: *EDAR del Besòs, Barcelona*	Carried out by (Company): *Aigües de Barcelona* Sampler: *Ester Pastoret Martín*
SAMPLING OBJECTIVE *The objective of sampling is to comply with the D1.1: Laboratory analysis protocol and Methodology for executing WAC as well as to supply the feedstock to IMECAL in order they could do analyisis.*	
SAMPLING APPROACH/PATTERN (with justification):	
MATERIAL	
Type of Feedstock: *Cellulosic Rejections*	Estimated moisture content: *55-60%*
Source and origin of the feedstock: *Cellulosic Rejections from municipal WWTP.*	

Sampling record for nappies from urban waste from Metropolitan area of Barcelona.

Annex 2

Example information Sampling Record

SAMPLING RECORD	
Sample code: *ECO2Nappies170620*	
Date of sampling: *17.06.2020*	
Signature of sampler:	
GENERAL INFORMATION	
Waste producer: *Urban waste from Metropolitan area of Barcelona* Contact: *Julia Horeza Atienza, jhereza@amb.cat*	Client (Company): IMECAL Contact:
Location of sampling: *Ecoparc del Besòs. Montcada i Reixac (Barcelona)*	Carried out by (Company): *Ecoparc del Besòs* Sampler: *Vicente Álvarez*
SAMPLING OBJECTIVE *The objective of sampling is to comply with the D1.1: Laboratory analysis protocol and Methodology for executing WAC as well as to supply the feedstock to IMECAL in order they could do analyisis.*	
SAMPLING APPROACH/PATTERN (with justification):	
MATERIAL	
Type of Feedstock: *Nappies*	Estimated moisture content:
Source and origin of the feedstock: *Urban waste from the municipal rest fraction, not separated at source.*	
Process/activity producing the feedstock:	

Sampling record for carton and paper from urban waste from Metropolitan Area of Barcelona.

Annex 2

Example Information Sampling Record

SAMPLING RECORD	
Sample code: *ECO2C&P170620*	
Date of sampling: *17.06.2020*	
Signature of sampler:	
GENERAL INFORMATION	
Waste producer: *Urban waste from Metropolitan area of Barcelona* Contact: *Julia Hereza Atienza, jhereza@amb.cat*	Client (Company): *IMECAL* Contact:
Location of sampling: *Ecoparc del Besòs, Montcada i Reixac (Barcelona)*	Carried out by (Company): *Ecoparc del Besòs* Sampler: Vicente Alvarez
SAMPLING OBJECTIVE *The objective of sampling is to comply with the D1.1: Laboratory analysis protocol and Methodology for executing WAC as well as to supply the feedstock to IMECAL in order they could do analysis.*	
SAMPLING APPROACH/PATTERN (with justification):	
MATERIAL	
Type of Feedstock: *Carton and Paper*	Estimated moisture content:
Source and origin of the feedstock: *Urban waste from the municipal rest fraction, not separated at source.*	
Process/activity producing the feedstock:	

PILOT 7

SAMPLING RECORD	
Sample code: (Reflect site location, feedstock and date of collection) **Sewage sludge (SS) SS1, SS2, SS3, SS4, SS5, SS6, SS7, SS8**	
Date of sampling: **28th May 2020**	
Signature of sampler:	
GENERAL INFORMATION	
Waste producer: **Municipal wastewater treatment plant of Chania** Contact:	Client (Company): **Municipal wastewater treatment plant of Chania** Contact:
Location of sampling: **Akrotiri, Chania, Greece**	Carried out by (Company): **Municipal wastewater treatment plant of Chania** Sampler:
SAMPLING OBJECTIVE	
SAMPLING APPROACH/PATTERN (with justification): **The feedstock was collected and placed in 10 barrels of 75L. The objective of the sampling was the characterization of the feedstock (laboratory analyses) and its use for the experiments of the production of the final product.**	
MATERIAL	
Type of Feedstock: **Sewage sludge**	Estimated moisture content: **70%**
Source and origin of the feedstock (e.g. form and nature of arising):	
Process/activity producing the feedstock:	
Description: (colour, odour. consistency/homogeneity/grain size - uniform or diverse) **Black colour, strong odour, consistent, homogeneous, uniform**	
SAMPLING METHODOLOGY	
Describe/define sub-population or consignment sampled: **10 barrels of 75L. 8 homogeneous samples were taken**	

Place and point of sampling:): **The feedstock was collected and placed in 10 barrels of 75L in the Municipal wastewater treatment plant of Chania. The barrels were transported in the Technical University of Crete. After that, 8 homogeneous samples were taken and placed in plastic bags.**
Access problems that affected areas or volumes of feedstock sampled:
Date and time of sampling:
Persons present (record name and address of witnesses present where appropriate):
Procedure (describe procedure adopted):
Equipment used: **A truck was used for the collection of the feedstock in barrels. An auger was used for the final sampling in plastic bags.**
Number of increments/samples collected: **8 samples**
Increment size/Sample size: **2kg/sample**
Observations diving sampling (e g. gassing out, reactions, development of heat): **no observations**
Details of on-site determinations:
Safety measures taken: **Mask, gloves**
SUB-SAMPLING AND PRE-TREATMENT
Identify location: e.g. on-site or fixed laboratory facility (describe whether open air or enclosed)
Procedure:
PACKAGING. PRESERVATION. STORAGE AND TRANSPORT DETAILS
Packaging: **10 barrels of 75L. 8 samples in plastic bags**
Preservation:
Storage:
Transport:
DEVIATIONS FROM SAMPLING PLAN
Detail:

DELIVERY TO ANALYTICAL LABORATORY	
Company: **Technical University of Crete** Received by: **Maria Liliana Saru, Stella Voutsadaki, Anna Kritikaki**	Delivery date: **28th May 2020** Signature: