BIO SUCCINIC ACID

D2.2 Report on the feedstock composition, variability and requirements

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LIST OF ABBREVIATIONS

ACRONYM	DESCRIPTION
AUA	Agricultural University of Athens
BBEPP	Bio Base Europe Pilot Plant
FCC MA	FCC Medio Ambiente
GA	Gallic acid
HPLC	High-performance liquid chromatography
LAP	Laboratory Analytical Procedure
Monti	Montinutra OY
NREL	National Renewable Energy Laboratory
OFMSW	Organic fraction of municipal solid waste
SA	Succinic acid
TPC	Total phenolic content
WP	Work package

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Publishable summary

Report D2.2 focuses on evaluating the availability, seasonality, and composition of biomass resources, specifically the organic fraction of municipal solid waste (OFMSW) and sawdust (from Spruce fibre), with an emphasis on their variability throughout the year. It aims to identify key challenges and provides a thorough characterization of the feedstocks, analysing their components such as cellulose, hemicellulose, lignin, and other factors that influence biomass conversion processes.

Implementation and Partners Involved:

The work presented in this report is being conducted by a collaborative team of project partners involved in WP2. FCC MA is responsible for investigating the seasonality and availability of OFMSW, examining its logistics and the impact of seasonal fluctuations on biomass supply. MONTI focuses on studying sawdust availability, addressing logistical challenges and collaborating with FCC MA. AUA leads the characterization of the feedstocks, analysing their composition, including key components like cellulose, hemicellulose, lignin, and proteins, while also considering regional and seasonal differences. BBEPP utilizes the composition data and safety datasheets to process both these feedstocks in their pilot plant for processes on pilot scale.

Key Problems and Questions Addressed:

This deliverable addresses several critical issues related to biomass feedstock variability, including:

- How do seasonal and regional variations affect the availability and composition of OFMSW and sawdust as biomass resources
- How can the composition of feedstocks be characterized to optimize biomass conversion processes

Expected Benefits:

The primary benefits of this deliverable are:

- A deeper understanding of biomass variability, which will enhance planning for biomass collection, storage, and processing.
- Obtain data that supports the optimization of biomass conversion processes, increasing efficiency in subsequent project phases.

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Results:

The report provides detailed findings on the year-round availability and composition of OFMSW and sawdust, highlighting seasonal fluctuations. A seasonal characterization was conducted on various organic materials processed at the AD plant Las Dehesas, including fruits and vegetables from the wholesale market of Madrid, “Mercamadrid”, Biowaste from households, Expired food, kitchen waste, and post-consumer waste from supermarkets, as well as sawdust supplied by Monti (brand name: Boreal Bioproducts) in Finland.

Analysis of organic waste from MercaMadrid, households, and supermarkets revealed significant compositional variations. MercaMadrid samples had the highest moisture content in winter (up to 83.9%) and showed higher lipids, free sugars (especially fructose), and starch during autumn and winter, likely due to the higher proportion of fresh produce. Household waste demonstrated a more stable composition year-round, with elevated protein levels, reflecting the variety of waste types. Supermarket waste contained more starch in autumn and spring, likely from packaged foods, with higher organic acids in spring and winter. Pectin was most prominent in MercaMadrid samples, while glucan content remained stable across waste streams. Lignin levels were highest in supermarket waste.

In the case of sawdust, a comparison of hot water extracted sawdust and sawdust (untreated) revealed that the extraction process increases cellulose content by removing non-cellulosic components like hemicellulose and lipids. Both types of sawdust contained minimal starch, protein, organic acids, and free sugars. The higher cellulose content in hot water extracted from sawdust is especially beneficial for enzymatic hydrolysis, improving the efficiency of bioprocesses aimed at fermentable sugar production.

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1. Introduction

This deliverable, **D2.2 Report on Feedstock Composition, Variability, and Requirements**, focuses on assessing and characterizing biomass resources, specifically the organic fraction of municipal solid waste (OFMSW) and sawdust, with an emphasis on their seasonality, composition, and availability. The biomass resources under study are critical for subsequent bioprocesses, such as pretreatment and biobased succinic acid production. The challenge this deliverable addresses lies in understanding the variability of these feedstocks, as fluctuations in availability and composition—driven by factors such as seasonality and regional differences—can significantly impact the efficiency of biomass conversion processes. Ensuring a reliable and sustainable supply of biomass feedstocks requires thorough analysis of these variables, which is the primary focus of this report.

Purpose and target group

The purpose of this deliverable is to provide a detailed analysis of the availability, composition, and variability of OFMSW and sawdust, which will inform the design and optimization of biomass processing systems. The target group for this report includes project partners working on biomass conversion processes, logistics, safety, sustainability assessments, and economic evaluations into feedstock variability and its impact on the overall project.

Contributions of partners – Link with other WPs

The development of this report has seen key contributions from several project partners. FCC MA is responsible for analysing the availability, seasonality, and composition of the organic fraction of municipal solid waste (OFMSW), with a focus on studying its location, quantities, handling, disposal methods, and utilization, particularly in the city of Madrid. They will also address logistical challenges, such as transport systems, collection methods, and safety measures, while providing valuable insights into the seasonal variations in organic waste, which will help understand biomass availability throughout the year. MONTI will contribute by focusing on the seasonality and availability of sawdust as a biomass resource, studying its quantities, handling, and disposal methods, and working with FCC MA to tackle logistical challenges (Linked with D2.1).

AUA will lead the feedstock characterization process, analysing the composition of both OFMSW and sawdust, determining key components like cellulose, hemicellulose, pectin, lignin, and proteins, and assessing how these vary by region and season. Safety datasheets preparation to ensure the materials are compatible with BBEPP's pilot plant processing requirements.

BBEPP’s role involves processing the characterized feedstocks in their pilot plant, using the safety datasheets and composition data to ensure safe and efficient processing of the biomass materials. BBEPP can run FOOD (according to FSSC 22000) and NON-FOOD processes. Within BBEPP in both cases of feedstock, traceability requirements, cleaning/sanitation procedures and validation of cleaning are followed. BBEPP will perform analysis within LUCRA on incoming solid fraction for several food safety parameters, based on risk assessment. Also, further DSP pre-treatment, such as extra pasteurisation/ killing step and filtration will be performed. The biomass will stay labelled as waste until it is treated in the dedicated process hall. Based on outcome of additional analysis, the biomass becomes labelled as safe & technical product and is cleared by quality department to be further processed in the fermentation process hall.

The D2.2 deliverable is closely linked with D2.1 deliverable and several work packages (WPs) in the project, including:

- **WP3 (Bioprocess optimisation for succinic acid production using an electrochemical membrane bioreactor)**: The data collected regarding the composition and variability of biomass will directly feed into WP3, which focuses on optimizing the conversion processes for succinic acid production. Understanding the biomass composition will help to design and fine-tune the conversion processes to maximize yield and efficiency, including the integrated system.
- **WP4 (LUCRA Biorefinery demonstration)**: The safety datasheets and feedstock characterization developed will support WP4, which involves scaling up the processes to pilot trials in the facilities of BBEPP (Belgium). Ensuring that the feedstocks meet processing requirements is essential for smooth implementation in the pilot phase.
- **WP6 (Safety, sustainability and economic assessment)**: As the characterization and analysis of biomass feedstocks play a critical role in ensuring the safety, sustainability, and economic viability of the entire project.

2. Objectives and expected impacts

Objectives

The D2.2 Report on feedstock composition, variability, and requirements provides a comprehensive evaluation of biomass resources, focusing on the OFMSW and sawdust. It examines their availability, seasonal fluctuations, and detailed composition—including cellulose, hemicellulose, pectin, lignin, and proteins—while accounting for regional and seasonal variations. This in-depth characterization aims to identify potential challenges and differences that may affect subsequent project phases. To ensure safe and efficient processing at BBEPP's pilot plant, safety datasheets will also be prepared, confirming that the feedstocks meet all necessary criteria.

Expected impacts

The successful completion will provide crucial insights into the seasonality and availability of biomass feedstocks, enabling more efficient logistics and optimizing biomass collection, handling, and storage strategies. The overall efficiency of the biomass supply chain could be enhanced by addressing potential bottlenecks in transportation and infrastructure. The detailed feedstock characterization will offer a better understanding of the biomass composition, particularly the concentration of fermentable sugars and other key components, which is critical for optimizing biobased succinic acid production with the integrated system. The comparison with previous studies will help identify potential concerns and ensure that regional and seasonal variations in biomass composition are well understood. Additionally, the preparation of safety datasheets, where necessary, will facilitate safe and efficient processing of these materials in the pilot plant, supporting smoother transitions into subsequent stages of the project.

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3. Experimental design

3.1. Analysis of biomass resources availability based on the seasonality

A seasonal characterization was conducted on the different types of organic matter processed at the AUA plant, including the organic fraction of municipal waste, fruits and vegetables from Madrid's central market, biowaste from supermarkets managed by FCC MA in Spain, and sawdust material supplied by Monti, Finland. The compositional analysis was performed in duplicate, and the results are presented as average values. A detailed description of the protocols used for the analysis is provided below.

Figure 1 Samples from FCC MA, Spain

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Figure 2 Sawdust samples provided by Monti, Finland

3.1.1. Moisture and ash content

Moisture content was calculated by weight difference after drying the sample at 60◦C for 24 h until constant weight. Ash determination was carried out via incineration in an oven (550–600◦C) and consequently at 60 ◦C for 24 h.

3.1.2. Lipid content

Dried samples were subjected to hexane extraction for 6 hours using a Soxhlet apparatus to determine their lipid content.

3.1.3. Soluble extractives

Dried samples were mixed with deionized water in a proportion of 1:10 (w/v). The suspension was stirred for 2 h at 40 ◦C and then the extract was separated by filtration. The process was repeated two times, and the resulting extract was analyses for its content via HPLC.

3.1.4. Protein content

To determine the total protein content of the sample, Kjeldahl method was selected by using the Kjeltek TM 8100 distillation unit (Foss, Denmark). The Kjeldahl method involves a three-step process consisting of digestion, distillation, and titration. Dried sample was precisely weighed on rice paper to four decimal places and placed within a digestion tube. Using a bottle-top dispenser, 25 mL of H_2SO_4 was added, along with a Kjeldahl tablet containing Na_2SO_4 (96.5%), $CuSO_4$ (1.5%), and Se (2.0%). The same reagents were incorporated for the blank. Digestion was conducted at 430°C for an hour, and after the tubes returned to room temperature, the distillation process followed. During this step, 30 mL of H_2O and 100 mL of NaOH (40%, w/v) were automatically added in the case of solid samples. In

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the case of liquid sample, 5 g was added directly within the digestion tube along with 10 mL of H_2SO_4 and a Kjeldahl tablet. During the digestion step, 80 mL of H_2O and 50 mL of NaOH (40%, w/v) were automatically added. The distillate obtained was collected in an Erlenmeyer flask, and to this, 50 mL of a boric acid solution containing 40 g of boric acid, 7 mL of methyl red indicator (0.1%), and 10 mL of bromocresol green indicator (0.1%) in one liter of solution were added. Lastly, the solution was titrated using a 0.1 N HCl standard solution. The volume of HCl standard solution consumed during titration was converted into total nitrogen content according to Equation (1).

$$Nitrogen\ (\%) = \frac{(\mathrm{mLsample} - \mathrm{mLblank}) \times 0.1\mathrm{N} \times 14.007 \times 100}{mg\ sample} \quad (1)$$

Where, N is the normality of the HCl solution, mL of sample is the mL of 0.1 N HCl consumed during the titration of the sample, and mL of blank is the mL of 0.1 N HCl consumed during the titration of the blank.

The conversion of %N to % protein was conducted with the Equation (2).

$$Protein\ (\%) = 6.25 \times \%N \quad (2)$$

where 6.25 is the conversion factor of organic nitrogen into proteins because most of the proteins have 16% nitrogen and therefore the conversion factor is 6.25.

3.1.5. Structural carbohydrates and lignin

Analysis of lignocellulosic composition of samples was carried out based on the NREL Laboratory Analytical Procedure (LAP) for the "Determination of structural carbohydrates and lignin in biomass" with slight modifications [1]. Briefly, 0.300 g ± 0.001 g of extractives-free sample were initially hydrolysed with 3 mL of 72 % (v/v) H_2SO_4 under continuous stirring at 30 ◦C for 1 h. Dilution to 4% (v/v) H_2SO_4 was subsequently carried out with the addition of 84 mL dH_2O, and the mixture was placed in an autoclave at 121 ◦C for 2 h. The hydrolysate was then filtered while still hot, under vacuum, using glass microfiber filters (934-AH). The filtrate was collected, and a small portion was used for sugar analysis and the rest for acid soluble lignin determination. The solids were washed with dH_2O and then quantitatively transferred in a crucible and dried at 105◦C until constant weight. When the drying step was completed, the crucibles were placed in a muffle furnace at 575◦C for 24 h. Acid soluble lignin was analysed using a UV–vis spectrophotometer (Shimadzu UV-1900) at 320 nm. The amount of cellulose

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and hemicellulose were finally calculated based on the correction of 0.88 for pentoses and 0.90 for hexoses.

3.1.6. Extraction of phenolic compounds and determination of total phenolic content

The extraction of phenolic compounds was carried out based on the modified method reported by Negro et al. [2] using 70 % (v/v) ethanol acidified with 0.5 % (v/v) HCl (0.1 M). The suspension (1:30 w/v) was ultrasonicated for 20 min and then the extract was separated by filtration. The extract was vacuum evaporated for the recovery of ethanol and re-diluted with analytical grade methanol. The process was repeated 3 times for maximising the extraction of the phenolic compounds.

Total phenolic content (TPC) of the extract was determined by the Folin-Ciocalteu colourimetric method, using gallic acid (GA) as standard [3].

3.1.7. Pectin content

The GA content was measured spectrophotometrically based on the m-hydroxydiphenyl method of Melton and Smith [4].

3.1.8. Starch content

Starch content was measured using the Total Starch Assay Kit (Megazyme, Ireland) based on the use of thermostable α-amylase and amyloglucosidase.

3.2. Analytical methods

Sugars, organic acids and potential fermentation inhibitors (e.g., furfural, 5-hydroxymethylfurfural or 5-HMF) were determined using a Shimadzu HPLC system with a Shimadzu RI detector and a Rezex ROA-Organic acid H^+ column. The temperature of the column was 65°C and the mobile phase was a 10 mM H_2SO_4 aqueous solution at 0.6 mL/min flow rate.

Monosaccharides were also determined with a Shodex SP0810 (8.0 × 300 mm) column using a Shimadzu HPLC system and Shimadzu RI detector. The temperature of the column was 80°C and the mobile phase was HPLC grade water at flow rate 1.0 mL/min.

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4. Results of biomass availability based on the seasonality and composition study

4.1. Organic Fraction of Municipal Solid Waste characterisation

In the LUCRA project, the OFMSW from Madrid is studied by distinguishing between the main OFMSW streams received at the AD plant Las Dehesas:

- Fruits and vegetables from the wholesale market of Madrid, “Mercamadrid.”
- Biowaste from households.
- Expired food, kitchen waste, and post-consumer waste from supermarkets.

Samples from each season and from three different locations were provided by FCC in frozen form. The compositional analysis was conducted at the AUA facilities as described in Section 3.

The compositional analysis of OFMSW during the summer season (Table 1) revealed significant variations across the three different sources: MercaMadrid (fruits and vegetables), household biowaste, and supermarket biowaste.

Moisture content was highest in MercaMadrid samples (66.9 ± 1.9%), consistent with the water-rich nature of fruits and vegetables, while household and supermarket biowaste exhibited lower moisture levels (39.0 ± 5.3% and 46.4 ± 4.1%, respectively). Ash content followed a similar trend, with MercaMadrid waste showing the highest levels (16.5 ± 0.9%). Other components varied significantly; supermarket waste exhibited the highest starch content (17.4 ± 1.3%), while household biowaste was richer in protein (15.4 ± 0.6%). MercaMadrid samples had the highest levels of free sugars (18.0 ± 1.3%), dominated by fructose (9.6%) and glucose (6.8%), reflecting the composition of fruit and vegetable residues. In contrast, supermarkets showed lower free sugar content (9.2 ± 1.6%) but had the highest organic acid content (7.2 ± 1.1%), primarily lactic acid (6.0%).

The compositional analysis also revealed noteworthy trends in lipid and pectin content across the three OFMSW streams. Lipid levels were highest in household biowaste (12.6 ± 0.4%) and supermarket biowaste (11.3 ± 0.3%), likely due to the inclusion of fatty food residues such as oils, dairy, and processed food items. In contrast, MercaMadrid waste exhibited significantly lower lipid content (7.1 ± 0.9%), reflecting the low-fat nature of fruits and vegetables. Pectin content, on the other hand, was highest in MercaMadrid samples (2.5 ± 0.9%), as expected from the fruit and vegetable origins of this

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stream, which are rich in pectic polysaccharides. Household and supermarket waste contained lower pectin levels (1.3 ± 0.6% and 1.1 ± 0.1%, respectively), reflecting the mixed and processed nature of these streams.

Structural carbohydrates, such as glucan, hemicellulose, and lignin, also differed among the streams. MercaMadrid samples had the highest glucan levels (14.7 ± 0.0%) and lignin content (7.2 ± 0.1%), reflecting the fibrous structure of fruit and vegetable waste. Hemicellulose content was relatively consistent across streams, with supermarkets showing slightly higher levels (8.6 ± 0.4%). Within hemicellulose, xylan, galactan, arabinan, and mannan were present in varying proportions, with mannan being most abundant in supermarket waste (2.7%). Additionally, total phenolic content (TPC) was highest in MercaMadrid samples (1.6 ± 0.0%), indicating the presence of antioxidant compounds, while lignin levels were comparable across all streams.

Table 1 Characterization of OFMSW from Madrid, AD plant Las Dehesas, Summer season

Component (%)	MercaMadrid	Biowaste from household	Supermarket
Moisture	66.9 ± 1.9	39.0 ± 5.3	46.4 ± 4.1
Ash	16.5 ± 0.9	10.9 ± 0.7	10.8 ± 0.8
Starch	8.0 ± 0.6	13.1 ± 0.4	17.4 ± 1.3
Pectin	2.5 ± 0.9	1.3 ±0.6	1.1 ± 0.1
Protein	9.8 ± 0.0	15.4 ± 0.6	14.8 ± 0.1
Lipids	7.1 ± 0.9	12.6 ± 0.4	11.3 ± 0.3
Free sugars	18.0 ± 1.3	15.1 ± 1.0	9.2 ± 1.6
Sucrose	*1.6*	*5.6*	*3.3*
Glucose	*6.8*	*3.4*	*2.7*
Fructose	*9.6*	*6.1*	*3.2*
Organic Acids	6.7 ± 1.2	4.2 ± 0.8	7.2 ± 1.1
Citric	*1.1*	*0.9*	*0.9*
Acetic	*0.6*	*0.6*	*0.3*
Lactic	*5.0*	*2.7*	*6.0*
TPC	1.6 ± 0.0	1.1 ± 0.0	1.1 ± 0.1
Lignin	7.2 ± 0.1	6.5 ± 0.1	6.4± 0.9

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Component (%)	MercaMadrid	Biowaste from household	Supermarket
Glucan	14.7 ± 0.0	9.7 ± 0.5	11.0 ± 0.1
Hemicellulose	6.7 ± 0.3	8.5 ± 0.8	8.6 ± 0.4
Xylan	*2.0*	*2.7*	*2.6*
Galactan	*1.6*	*2.2*	*1.8*
Arabinan	*1.3*	*1.2*	*1.5*
Mannan	*1.8*	*2.4*	*2.7*

The compositional analysis of the autumn season organic waste streams revealed also notable differences across the three sources (Table 2).

Moisture content was highest in MercaMadrid waste (75.3 ± 1.0%), consistent with the high-water content of fresh fruits and vegetables, followed by household waste (66.9 ± 1.2%) and supermarket waste (60.5 ± 5.8%). The ash content was highest in household biowaste (17.9 ± 0.1%), while MercaMadrid and supermarket waste contained lower levels of ash (11.1 ± 0.6% and 12.4 ± 0.0%, respectively). Regarding starch, supermarket waste had the highest proportion (20.3 ± 0.3%), likely reflecting the presence of starch-rich processed foods, while household and MercaMadrid waste contained lower levels (14.9 ± 0.1% and 11.7 ± 0.2%, respectively). The pectin content was notably similar between MercaMadrid and household biowaste (9.0 ± 0.2%), consistent with the pectic polysaccharides present in fruit and vegetable waste. However, supermarket waste contained slightly lower pectin levels (7.0 ± 0.3%).

In terms of lipids, MercaMadrid waste showed the highest value (16.8 ± 0.1%). Household and supermarket biowaste had similar lipid contents (14.4 ± 0.7% and 15.9 ± 0.2%, respectively), indicating a significant amount of fat from food residues like oils and dairy products. The free sugars content was highest in MercaMadrid (11.1 ± 0.2%), while both household and supermarket biowaste had lower levels (6.5 ± 0.0% and 5.9 ± 0.7%, respectively).

The organic acids in the streams varied, with MercaMadrid having the highest levels (15.4 ± 0.2%), dominated by citric acid (1.5%), which is typical of fruits. Supermarket waste showed a moderate number of organic acids (9.9 ± 0.2%), primarily acetic acid (1.4%). Household waste had the lowest organic acids content (6.8 ± 0.1%). The lignin content was highest in MercaMadrid waste (5.1 ± 0.0%),

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while household and supermarket wastes had lower lignin values (3.6 ± 0.0% and 2.1 ± 0.5%, respectively), reflecting the differing composition of these streams.

Finally, glucan and hemicellulose were most abundant in MercaMadrid waste (6.2 ± 0.1% and 3.1 ± 0.9%, respectively). Supermarket waste had lower glucan content (3.9 ± 0.4%) but slightly higher hemicellulose (3.4 ± 0.8%). The total phenolic content (TPC) was highest in MercaMadrid (0.7 ± 0.0%), which aligns with the antioxidant compounds found in fruits and vegetables, while supermarket and household biowaste contained lower TPC levels (0.5 ± 0.0% and 0.4 ± 0.0%, respectively).

Table 2 Characterization of OFMSW from Madrid, AD plant Las Dehesas, Autumn season

Component (%)	MercaMadrid	Biowaste from household	Supermarket
Moisture	75.3 ± 1.0	66.9 ± 1.2	60.5 ± 5.8
Ash	11.1 ± 0.6	17.9 ± 0.1	12.4 ± 0.0
Starch	11.7 ± 0.2	14.9 ± 0.1	20.3 ± 0.3
Pectin	9.0 ± 0.2	9.0 ± 0.2	7.0 ± 0.3
Protein	9.9 ± 0.1	14.2 ± 1.1	16.7 ± 0.6
Lipids	16.8 ± 0.1	14.4 ± 0.7	15.9 ± 0.2
Free sugars	11.0 ± 0.2	6.5 ± 0.0	5.9 ± 0.7
Sucrose	*0.1*	*0.1*	*0.2*
Glucose	*8.0*	*3.3*	*2.5*
Fructose	*2.9*	*3.1*	*3.2*
Organic Acids	15.4 ± 0.2	6.8 ± 0.1	9.9 ± 0.2
Citric	*1.5*	*0.7*	*0.4*
Succinic	*1.4*	*0.5*	*0.5*
Acetic	*1.1*	*0.7*	*1.0*
Lactic	*11.4*	*4.9*	*8.0*
TPC	0.7 ± 0.0	0.5 ± 0.0	0.4 ± 0.0
Lignin	5.1 ± 0.0	3.6 ± 0.0	2.1 ± 0.5
Glucan	6.2 ± 0.1	5.4 ± 0.6	3.9 ± 0.4
Hemicellulose	3.1 ± 0.9	3.8 ± 0.4	3.4 ± 0.8
Xylan	*1.2*	*1.6*	*1.1*

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Component (%)	MercaMadrid	Biowaste from household	Supermarket
Galactan	*1.0*	*1.1*	*1.0*
Arabinan	*0.2*	*0.3*	*0.3*
Mannan	*0.7*	*0.8*	*1.0*

The compositional analysis of the winter season organic waste streams from MercaMadrid (fruits and vegetables), household biowaste, and supermarket is presented in Table 3.

Moisture content was highest in MercaMadrid waste (83.9 ± 0.3%), compared to household (69.0 ± 0.7%) and supermarket waste (67.7 ± 0.7%). The ash content was significantly lower in MercaMadrid waste (6.3 ± 0.0%) compared to household biowaste (19.5 ± 0.1%). Supermarket waste had a moderate ash content (8.8 ± 0.3%). Regarding starch, supermarket waste had the highest value (19.1 ± 0.5%), followed by household waste (18.2 ± 0.5%), both reflecting the presence of starchy processed foods. MercaMadrid waste contained much lower starch levels (1.5 ± 0.3%).

The pectin content was highest in MercaMadrid (15.8 ± 0.3%), which is characteristic of fruit waste, followed by household biowaste (10.2 ± 0.4%) and supermarket waste (4.5 ± 0.7%), with the latter showing lower values. Protein content was notably higher in supermarket waste (18.1 ± 0.5%), possibly due to the presence of protein-rich food products like meats and dairy, while household and MercaMadrid waste contained lower levels of protein (11.3 ± 0.1% and 8.3 ± 0.2%, respectively).

In terms of lipids, supermarket waste had the highest lipid content (13.8 ± 0.5%), followed by household waste (11.1 ± 0.1%) and MercaMadrid waste (5.0 ± 0.2%). This is consistent with the higher fat content typically found in packaged or processed foods discarded by supermarkets. The free sugars content was highest in MercaMadrid (41.1 ± 0.1%), reflecting the naturally occurring sugars in fruits and vegetables, followed by supermarket (15.8 ± 0.1%) and household waste (8.9 ± 0.0%), which contained lower levels of free sugars, possibly due to the different types of waste involved.

Organic acids were higher in MercaMadrid waste (5.9 ± 0.4%) compared to household (3.7 ± 0.0%) and supermarket waste (5.2 ± 0.0%), mainly driven by citric acid (5.9%), a characteristic organic acid in fruits. Supermarket waste contained trace amounts of acetic acid (0.2%). Total phenolic content (TPC) was highest in MercaMadrid waste (1.1 ± 0.0%), indicating a higher presence of antioxidants, as expected from the polyphenolic compounds in fresh produce, while supermarket and household waste had lower TPC values (0.9 ± 0.0% and 0.8 ± 0.0%, respectively).

Regarding the carbohydrate components, MercaMadrid waste had the highest glucan (5.8 ± 0.4%) and hemicellulose (4.3 ± 0.1%) contents. Supermarket and household waste contained lower levels of these components, with supermarket waste showing slightly lower glucan (5.6 ± 0.0%) and hemicellulose (4.3 ± 0.1%) content compared to household waste. Additionally, the lignin content was highest in household biowaste (5.1 ± 0.6%) and lowest in MercaMadrid waste (2.6 ± 0.0%).

Table 3 Characterization of OFMSW from Madrid - AD plant Las Dehesas – Winter season

Component (%)	MercaMadrid	Biowaste from household	Supermarket
Moisture	83.9 ± 0.3	69.0 ± 0.7	67.7 ± 0.7
Ash	6.3 ± 0.0	19.5 ± 0.1	8.8 ± 0.3
Starch	1.5 ± 0.3	18.2 ± 0.5	19.1 ± 0.5
Pectin	15.8 ± 0.3	10.2 ± 0.4	4.5 ± 0.7
Protein	8.3 ± 0.2	11.3 ± 0.1	18.1 ± 0.5
Lipids	5.0 ± 0.2	11.1 ± 0.1	13.8 ± 0.5
Free sugars	41.1 ± 0.1	8.9 ± 0.0	15.8 ± 0.1
Sucrose	*0.0*	*0.5*	*1.2*
Glucose	*20.0*	*3.9*	*7.7*
Fructose	*21.1*	*4.5*	*6.9*
Organic Acids	5.9 ± 0.4	3.7 ± 0.0	5.2 ± 0.0
Citric	*5.9*	*2.2*	*2.4*
Acetic	*0.0*	*0.0*	*0.2*
Lactic	*0.0*	*1.5*	*2.6*
TPC	1.1 ± 0.0	0.8 ± 0.0	0.9 ± 0.0
Lignin	2.6 ± 0.0	5.1 ± 0.6	3.7 ± 0.2
Glucan	5.8 ± 0.4	6.0 ± 0.4	5.6 ± 0.0
Hemicellulose	4.3 ± 0.1	5.3 ± 0.6	4.3 ± 0.1
Xylan	*2.2*	*1.6*	*0.8*
Galactan	*1.0*	*1.5*	*1.5*

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Component (%)	MercaMadrid	Biowaste from household	Supermarket
Arabinan	*0.4*	*0.4*	*0.2*
Mannan	*0.7*	*1.8*	*1.8*

The compositional analysis of OFMSW from the spring stream (Table 4) showed variations in key components across samples.

The starch content was highest in supermarket samples (18.6%), followed by biowaste household samples (16.4%) and MercaMadrid samples (1.6%). This suggests that supermarkets contribute significantly to the starch component in the organic waste stream. Glucan content, a key polysaccharide, was similar across all samples, with supermarket samples containing 9.6%, household samples 8.8%, and MercaMadrid samples 8.6%. Pectin content, was highest in MercaMadrid samples (15.2%), followed by household samples (11.5%) and supermarket samples (9.9%).

Protein concentrations were comparable in supermarket (13.3%) and household (13.2%) samples, while MercaMadrid samples had slightly lower protein content (8.8%). The higher protein content in the household and supermarket streams could be linked to the diversity of waste in these categories, including food residues rich in proteins. Lipid content followed a different trend, with MercaMadrid samples having the highest lipid content (16.0%), followed by supermarket (12.4%) and household samples (11.1%).

The free sugar content varied significantly across the samples, with MercaMadrid samples containing the highest concentration (21.7%), followed by supermarket samples (12.1%) and household samples (6.5%). The predominant sugars were glucose and fructose. MercaMadrid samples had the highest levels of both glucose (6.9%) and fructose (13.9%), while supermarket and household samples had lower levels, particularly fructose. Sucrose levels were minimal across all samples (ranging from 0.2% to 0.9%.

Organic acids, primarily lactic acid, were present in all samples, with the highest levels found in supermarket samples (2.4%), followed by household (1.4%) and MercaMadrid samples (0.9%). Lignin content, indicative of plant material, was relatively similar across the samples, with supermarket samples having the highest levels (5.0%), followed closely by MercaMadrid (4.9%) and household samples (4.4%). Finally, the TPC was low in all samples, ranging from 0.8% to 0.9%, which is consistent with the generally low polyphenolic concentration in organic waste from food and vegetables.

Table 4 Characterization of OFMSW from Madrid - AD plant Las Dehesas – Spring season

Component (%)	MercaMadrid	Biowaste from household	Supermarket
Moisture	75.9 ± 1.3	59.8 ± 0.5	62.3 ± 0.7
Ash	11.6 ± 0.1	16.0 ± 0.4	7.9 ± 0.2
Starch	1.6 ± 0.5	16.4 ± 0.2	18.6 ± 0.7
Pectin	15.2 ± 0.3	11.5 ± 0.7	9.9 ± 0.6
Protein	8.8 ± 0.1	13.2 ± 0.5	13.3 ± 0.1
Lipids	16.0 ± 0.3	11.1 ± 0.6	12.4 ± 0.1
Free sugars	21.7 ± 0.1	6.5 ± 0.0	12.1 ± 0.7
Sucrose	*0.9*	*0.2*	*0.2*
Glucose	*6.9*	*2.8*	*5.3*
Fructose	*13.9*	*3.5*	*6.6*
Organic Acids	3.9 ± 0.4	3.3 ± 0.2	4.4 ± 0.2
Citric	*2.8*	*1.7*	*1.5*
Acetic	*0.2*	*0.2*	*0.5*
Lactic	*0.9*	*1.4*	*2.4*
TPC	0.8 ± 0.0	0.9 ± 0.0	0.9 ± 0.1
Lignin	4.9 ± 0.2	4.4 ± 0.4	5.0 ± 0.1
Glucan	8.6 ± 0.0	8.8 ± 0.3	9.6 ± 0.2
Hemicellulose	4.7 ± 0.8	5.1 ± 0.2	5.0 ± 0.5
Xylan	*2.3*	*1.3*	*1.2*
Galactan	*0.8*	*1.5*	*1.4*
Arabinan	*0.7*	*0.4*	*0.4*
Mannan	*0.9*	*1.9*	*2.0*

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BBEPP is responsible for processing the characterized biomass in their pilot plant, relying on technical datasheet, a safety datasheet, and exact description of the origin of the waste and composition data to ensure the materials are handled safely and processed efficiently. Safety Data Sheets are not a requirement for food waste unless there is a presence of hazardous chemicals. Therefore, within LUCRA FCC MA is providing such documentation, on the compositional analysis with an external laboratory of the Marcamadrid stream (Central Market) stream.

Also, the ICP-MS analysis has been performed during WP3 (shared in D3.1). The ICP-MS analysis of crude hydrolysates provides critical insights into their elemental composition, particularly the presence of trace metals and other essential nutrients. This information is pivotal in assessing the suitability of hydrolysates as feedstocks for succinic acid production and identifying the need for additional supplementation to optimize fermentation performance. The first analysis of ICP-MS shows, that there are low levels (within acceptable limits) or undetectable levels of toxic or hazardous chemicals.

Comparison of data from the PERCAL project

The comparison of data from the previous PERCAL project, which analysed two representative organic waste streams collected from an industrial MSW treatment plant in the Valencia Metropolitan Area (Spain), highlights distinct seasonal and location-based trends in biowaste composition. These findings align well with data collected from the analysis of organic waste streams at the AD plant Las Dehesas in Madrid (Spain). The two streams examined during PERCAL project were: (1) “sorted biowaste” from a separate collection of organic waste from hotels, restaurants, markets, and schools (HORECA stream), and (2) “unsorted biowaste” from mixed household waste after mechanical sorting to recover recyclables, prior to entering the composting stage of the plant [5].

The composition of sorted and unsorted biowaste differs notably, with seasonal variations playing a key role. Unsorted biowaste, primarily originating from households, shows higher levels of inert materials and ash. Inert material content reaches 25% during spring/summer and 36% during autumn/winter, while sorted biowaste contains much lower inert material content (4–5%). This is also reflected in moisture content, with unsorted biowaste consistently exhibiting higher moisture levels (43.3% in spring/summer and 51.61% in autumn/winter) compared to sorted biowaste (31.36% in spring/summer and 16.14% in autumn/winter).

In terms of organic components, sorted biowaste has a higher glucan (cellulose and starch) content, with levels around 39% in both seasons, while unsorted biowaste contains significantly lower glucan, especially in autumn/winter (25.06%). Pectin, primarily sourced from fruit waste, is more abundant in sorted biowaste (15.87%–18.25%) compared to unsorted biowaste (10.1%–12.19%). Fat content is notably higher in unsorted biowaste (4.59%–5.86%), whereas sorted biowaste contains more protein (8.75%–10.15%). Lignin content shows variability in both types of biowaste, with sorted biowaste having moderate levels (8.00%–9.47%), while unsorted biowaste displays a broader range (5.64%–11.02%).

These results emphasize the significant impact of seasonal variations, source-specific factors, and collection system differences on biowaste composition. These insights are crucial for refining waste management strategies and optimizing resource recovery processes, ensuring more efficient and sustainable handling of organic waste.

4.2. Sawdust (spruce fibre) characterisation

Sawdust, a byproduct of wood processing, is a lignocellulosic material commonly utilized in industrial applications. The composition of sawdust can be modified through treatments like hot-water extraction, which selectively removes water-soluble components and enhances its structural constituents. This section compares the chemical composition of untreated sawdust and hot-water extracted sawdust, focusing on key components like cellulose, hemicellulose and lignin to assess the changes induced by the extraction process.

The composition of both hot-water extracted sawdust, and sawdust is presented in Table 5, highlighting key differences and similarities between these two materials.

The moisture content of hot-water extracted sawdust was with 39.1 ± 0.6%, slightly lower than that of regular sawdust, which had a moisture content of 41.4 ± 0.7%. In terms of ash content, both materials exhibited low levels: hot-water extracted sawdust had 0.2 ± 0.1%, and sawdust had 0.3 ± 0.2%. This indicates that neither material contains significant amounts of inorganic matter, making both suitable for applications such as bioenergy production where a low ash content is desirable to avoid excessive residue.

The starch content in both materials was negligible, with hot-water extracted sawdust showing no detectable starch (0.0 ± 0) and sawdust having a trace amount (0.1 ± 0.1%). This aligns with the general understanding that sawdust is primarily composed of lignocellulosic materials as in comparison with other biomass types like e.g. grains.

Pectin levels were notably lower in hot-water extracted sawdust (0.2 ± 0.0%) compared to sawdust (1.0 ± 0.6%). The reduction in pectin after hot-water extraction could be attributed to its solubility in hot water, which may leach out some of the pectin from the cell walls, further altering the material's composition and making it potentially more suitable for certain applications, such as those requiring reduced pectin content.

Both materials showed no detectable levels of protein (0.0 ± 0.0%), organic acids (0.0 ± 0.0%), or free sugars (0.0 ± 0.0%), suggesting that both hot-water extracted sawdust and regular sawdust contain negligible amounts of these compounds, which is typical for sawdust in its raw form.

The lipid level was higher in sawdust (7.1 ± 0.0%) compared to hot-water extracted sawdust (3.3 ± 0.9%), indicating that the extraction process likely removed some of the lipids, which could affect the energy content or suitability for certain applications that rely on higher lipid levels, such as biodiesel production.

In terms of polysaccharides, glucan ratio was significantly higher in hot-water extracted sawdust (52.0 ± 1.0%) compared to sawdust (43.2 ± 0.2%). This suggests that hot-water extraction may increase the cellulose content by removing non-cellulosic materials, such as hemicellulose and pectin, making it a more cellulose-rich material. Similarly, hemicellulose content was lower in hot-water extracted sawdust (11.0 ± 0.9%) compared to sawdust (20.4 ± 0.2%), further supporting the idea that the extraction process removed a portion of the hemicellulose.

Xylan, a major component of hemicellulose, was slightly lower in hot-water extracted sawdust (7.6%) than in sawdust (10.0%), which further indicates the removal of hemicellulose material during the extraction. Both materials contained similar levels of galactan (0.9%) and arabinan (0.0%), suggesting that these specific hemicellulose sugars were not significantly affected by the extraction process.

The mannan content was significantly lower in hot-water extracted sawdust (2.5%) compared to sawdust (9.5%), further reinforcing the idea that hot-water extraction removes some of the hemicellulose sugars. The TPC of hot-water extracted sawdust was 1.0 ± 0.0%, which is higher than that of sawdust (0.5 ± 0.1%).

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Finally, lignin level was higher in hot-water extracted sawdust (31.7 ± 0.0%) than in sawdust (27.2 ± 0.1%), suggesting that the extraction process did not significantly affect the lignin content, and that lignin was retained in the extracted biomass due to its insolubility in hot water.

Table 5 Characterization of sawdust from Monti, Finland

Component (%)	Sawdust	Sawdust after hot water treatment
Moisture	41.4 ± 0.7	39.1 ± 0.6
Ash	0.3 ± 0.2	0.2 ± 0.1
Starch	0.1 ± 0.1	0.0 ± 0
Pectin	1.0 ± 0.6	0.2 ± 0.0
Protein	0.0 ± 0.0	0.0 ± 0.0
Lipids	7.1 ± 0.0	3.3 ± 0.9
Free sugars	0.0 ± 0.0	0.0 ± 0.0
Organic Acids	0.0 ± 0.0	0.0 ± 0.0
TPC	0.5 ± 0.1	1.0 ± 0.0
Lignin	27.2 ± 0.1	31.7 ± 0.0
Glucan	43.2 ± 0.2	52.0 ± 1.0
Hemicellulose	20.4 ± 0.2	11.0 ± 0.9
Xylan	*10.0*	*7.6*
Galactan	*0.9*	*0.9*
Arabinan	*0.0*	*0.0*
Mannan	*9.5*	*2.5*

Overall, the comparison between hot-water extracted sawdust and sawdust highlights the impact of the extraction process on the composition of the material.

5. Conclusions

Seasonal analysis of organic waste fractions from MercaMadrid, households, and supermarkets revealed distinct compositional differences throughout the year. Moisture content was highest during the winter, reaching up to 83.9% in MercaMadrid samples. This likely reflects the seasonal availability of fresh produce and its higher moisture retention during colder months. MercaMadrid samples also consistently showed the highest levels of lipids and free sugars, particularly fructose, along with increased starch content in autumn and winter. These trends can be attributed to the higher proportion of fresh fruits and vegetables in these waste streams, especially during colder months when products may spoil or remain unsold. In contrast, household biowaste displayed a more consistent composition year-round, with protein levels typically higher than in MercaMadrid samples. This is likely due to the greater variety of waste, including kitchen scraps and expired food. Supermarket waste showed higher starch content, particularly in autumn and spring, which may be linked to the larger quantities of packaged and processed foods in these streams. The levels of organic acids, primarily lactic acid, also varied seasonally, with the highest concentrations observed in supermarket samples during spring and winter, likely reflecting greater spoilage or fermentation. Across all seasons, pectin was a significant component in MercaMadrid samples, while glucan content remained relatively stable across all waste fractions. The lignin content also varied, with supermarket waste consistently exhibiting the highest levels, likely due to the presence of packaging and other plant-based materials.

Overall, these finding demonstrate that seasonal fluctuations significantly influence the composition of organic waste, with moisture, starch, lipids, and free sugars showing the most notable variations. MercaMadrid samples are more strongly influenced by the type and availability of fresh produce, while supermarket and household waste streams exhibit more consistent compositions across seasons, reflecting the broader variety of products and waste generated in these sectors. These insights are essential for improving waste management strategies and optimizing resource recovery across different waste streams and seasons.

Regarding the sawdust samples, a comparison between hot-water extracted sawdust and regular sawdust reveals notable compositional differences driven by the extraction process. Both materials are predominantly composed of cellulose, hemicellulose, and lignin, with regular sawdust generally containing higher concentrations of hemicellulose and lipids. In contrast, hot-water extracted sawdust is richer in cellulose, as the extraction process effectively reduces non-cellulosic components like pectin, hemicellulose, and lipids. Despite these variations, both sawdust types contain minimal amounts of starch, proteins, organic acids, and free sugars. These compositional changes are particularly relevant to the enzymatic hydrolysis of sawdust for fermentable sugar production (WP3, WP4), as the higher cellulose content in hot-water extracted sawdust may facilitate more efficient hydrolysis, which is critical for bioprocess development.

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