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Fruit and vegetable wastes as co-substrates in anaerobic co-digestion: Effect of storage temperature on physicochemical properties and biogas production

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ABSTRACT

Global population growth has led to a significant increase in food waste, including Fruit and Vegetable Waste (FVW). Anaerobic co-digestion offers a sustainable way to valorise FVW, especially when combined with Municipal Sewage Sludge (MSS) to mitigate imbalances in their mono-digestion. This study investigates the effects of storage temperatures (10 °C and 25 °C, which represent Mediterranean climates with an Atlantic influence like Portugal) on the degradation of apple, carrot, and banana peels. Changes in physicochemical properties were assessed and anaerobic co-digestion batch assays with purées of fresh and stored FVW alongside MSS were performed. Results indicated that apple peels purées, at a 1:2 peel-to-water ratio, achieved over the double of volatile solids concentration compared to MSS, with each FVW type having a C/N ratio above 40. Storage at 10 °C significantly reduced the degradation of total and volatile solids, as well as chemical oxygen demand, with apple peels retaining the highest carbohydrate concentrations. Anaerobic co-digestion with fresh FVW boosted biogas and CH_4 production by 19.5% and 15.2%, respectively. FVW storage at 10 °C further enhanced CH_4 yield and decreased H_2S content by 71% relative to MSS mono-digestion. These findings demonstrate that mild storage temperatures can improve biogas quality and yield by facilitating controlled FVW degradation.

1. Introduction

The global population is expected to reach 9.7 billion by 2050 [1], which could exacerbate the paradigm of waste generation, creating negative impacts on sectors like the food industry and sanitary engineering activities, namely in what concerns Wastewater Treatment Plants (WWTP). Regarding the food sector, estimates suggest that approximately 14% of post-harvest food is lost, representing 400 billion USD, with an additional 17% loss through retail and consumer channels [2]. Of the worldwide municipal solid waste (MSW), 33% is composed of food waste, including fruit and vegetable waste (FVW), amounting to 1.3 billion tons annually [3]. One of the most common final destinations for FVW is landfilling [4], which could lead to soil and aquatic pollution, besides greenhouse gas (GHG) emissions due to its high biodegradability. These issues have repercussions on some of the 2030 Sustainable Development Goals (SDG), namely the Goals: 6, 11, 12, and 13 which are related to clean water and sanitation, sustainable cities and cummunities, responsible consumption and production, and climate action respectively [5]. Portugal has proactively aligned its waste management policies with European Union directives through the implementation of key national frameworks, such as the Decree-Law nbr. 24/2024, from 26th March [6], the National Plan for Waste Management (PNGR 2030) [7], and the Plan for Urban Waste Management 2030 (PERSU) [8]. These initiatives reflect Portugal's commitment to meeting the EU's environmental objectives, which are outlined in European Directives like (EU) 2018/851 [9]. The overarching goals of these measures include enhancing waste reduction, promoting recycling, and driving the transition towards a circular economy by using more sustainable approaches. Anaerobic Digestion (AD) has been widely used as a successful technology for managing organic waste while reducing the

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negative impacts of landfilling [10]. During AD, a consortium of microorganisms feeds on the organic matter, breaking it down into monomers, ultimately producing biogas and biodigestate [11].

FVWs are mainly composed of water and carbohydrates, making them a rapidly biodegradable source of organic matter [12]. Besides, it contains essential nutrients like N, P, and K, vital for microbial activity and growth [13]. Usually, FVW have low concentrations of inhibitory substances like heavy metals or toxic compounds. Also, FVW have higher C/N ratios and intrinsically acidic pH, which could increase the production of volatile fatty acids (VFAs) and inhibit biogas production, making them unsuitable for use as a sole substrate for AD [14]. On the other hand, approximately 380 billion m^3 of municipal wastewater is produced annually worldwide, with a 51% increase expected by 2050 [15]. Their treatment is supported by physical, chemical, and biological technologies to remove pollutants, generating about 45 million tons of dried sewage sludge in 2017 [16], a figure that continues to grow, and their current management, including landfilling and combustion, is often unsustainable [17]. Since sludge handling accounts for nearly 50% of the WWTP's costs [18], alternative methods like AD are needed. However, mono-digestion of municipal sewage sludge (MSS) faces challenges such as fluctuations in organic content and contaminants, foaming, low C/N ratios, and slow degradation due to high levels of cell walls in biological sludge [19].

Anaerobic co-digestion (AcoD) emerges as a solution to correct the imbalances resulting from mono-digestion by combining two or more substrates, exploiting the best properties of each substrate to increase biogas and biomethane production [20]. Consequently, it is hypothesized that the co-digestion of MSS with FVW will enhance biogas and CH_4 production relative to MSS mono-digestion due to the increment in carbohydrate content and favourable nutrient profile of FVW. Supporting this, recent research highlights the positive impact of FVW's carbohydrate-rich composition on microbial degradation rates and biogas output when co-digested with sewage sludge [21,22,23], studying and testing distinct aspects such as ratios of both substrates, temperature ranges, single-stage *vs* multi-stage processes, among other conditions, achieving promising results and paving the way for further research.

In the context of sustainability, the paradigm shift from the linear economic model to a circular one is of major importance, essentially due to its direct link to renewable energies and symbiosis between industries [24]. AcoD is a methodology that actively contributes to the circular economy and promotes synergies between industries. However, using co-substrates in AcoD may require their storage before use under limited controlling conditions, which may alter their physicochemical properties. The storage temperature is one of the most important environmental variables that can influence the degradation of organic co-substrates and it is hypothesized that different storage temperatures may create different physicochemical characterization profiles of the FVW, resulting in possible differences in the anaerobic co-digestion process and consequently differences in the production of biogas as well as in its composition [25]. Intending to generate biogas from substrates that not only lack economic value but also pose an environmental threat, this work aims: (1) to select different fruits and vegetables that are widely produced and consumed worldwide, (2) to perform the physicochemical characterisation of selected FVW and a MSS sample from a WWTP, (3) to study the effect of two storage temperatures (simulating the average Summer and Winter temperatures in Mediterranean climates with Atlantic influence like the mainland region of Portugal) in the FVW physicochemical properties, and (4) to study the effect of adding the FVW purées, under a short storage period at two different temperatures, to the MSS, in biogas and biomethane production in batch mode AcoD.

2. Material and methods

An overall flowchart of the methodology and experimental trials, including the characterization analysis is presented in Fig. 1.

2.1. Composition and origin of municipal sewage sludge (MSS)

The MSS (represented by letter "a" in Fig. 1) consists of a mixture of Primary Sludge (PS) and Secondary Sludge (SS) in a blending ratio of 60:40% v/v, respectively. It was obtained from a WWTP located in Lisbon (Portugal) which serves approximately 211,000 inhabitants-equivalent and produces an average flow of treated wastewater of 52,500 m^3/day. The PS results from the primary sedimentation tank after a gravitational thickening process, and the SS is the excess activated sludge from the biological treatment process after undergoing an air flotation thickening process.

2.2. Selection of fruit and vegetable waste (FVW) and preparation of purées

The selected FVW (represented by letter "b" in Fig. 1) were peels from apples, bananas, and carrots, which are among the most produced and consumed worldwide. According to FAO [26], banana is the most-produced fruit worldwide, apples are the third most-produced fruit, and carrots are the sixth most-produced vegetable. All these fruits and vegetable are widely available year-round in markets and food-related industries. In Portugal, approximately 300,000 tons of apples, 134,000 tons of carrots, and 27,000 tons of bananas (mostly from Madeira Island) are produced annually, with exports of approximately 66,000 tons of apples and 28,000 tons of carrots (with bananas being mostly for the domestic market) [27]. The selected varieties were "Gala" apples, "Cavendish" bananas, and "Nantes" carrots, with fresh peels collected by the authors in a canteen at the beginning of the experimental trials.

A mechanical pre-treatment was required since these peels cannot be used directly in AD wet digestion. This mechanical pre-treatment consisted of blending them with water through three different peel:water ratios of 1:1, 1:2, and 1:4 (Figure S1, supplementary material). The mixtures were chopped by using a kitchen food chopper (Hoffen, model PSP-H151, 350 W) for two minutes. The chopping time was based on previous experimental trials performed within the scope of food waste valorisation in AD [23]. The following purées (represented by letter "c" in Fig. 1) were obtained: apple peel purée (APP); banana peel purée (BPP); carrot peel purée (CPP). The most appropriate ratio was selected by considering the best results in AcoD, physicochemical characterisation, and reduced impact as possible on water consumption to produce the purées.

2.3. Physicochemical characterisation of FVW purées and MSS

The physicochemical characterisation of the FVW purées comprised the following parameters: pH; Electrical Conductivity (EC); Total Solids (TS) and Volatile Solids (VS); Total and Soluble Chemical Oxygen Demand (COD_T and COD_S, respectively); Total Nitrogen (TN); Protein content; Mineral content; Elemental Analysis (EA); and Carbohydrate content, which was quantified through three different methods, namely (i) Brix degree, (ii) sugars in the soluble fraction, and (iii) total sugars from the fresh sample. MSS sample was characterised by the following parameters: pH; EC; TS and VS; COD_T and COD_S; Total Kjeldahl Nitrogen (TKN); heavy metals; and EA. pH and EC were assessed using Hanna Edge pH (Hanna Instruments) and Orion Star A215 (Thermo Scientific) electrodes, respectively. TS, VS, COD_T, COD_S, and TKN were quantified following the methodologies of the American Public Health Association [28]. TN and Protein content was determined by the Dumas method (Thermo Quest NA 2100 Nitrogen and Protein Analyser, Interscience, Breda, the Netherlands), using a protein-to-nitrogen conversion factor of 6.25. The Brix degree was determined using a manual refractometer (ATAGO MASTER–M.2313). Sugars in the soluble fraction (expressed as galactose, glucose, xylose, fructose, and sucrose) were assessed through

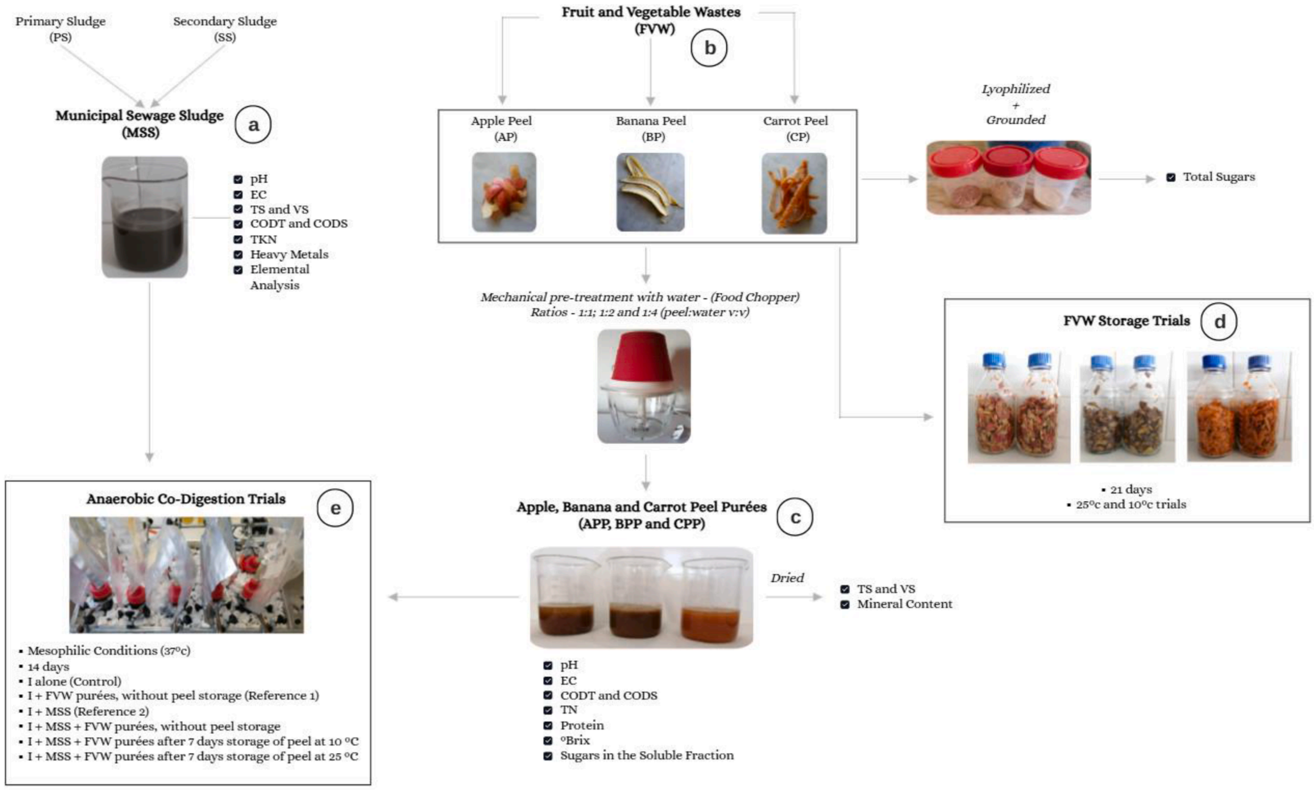

Fig. 1. Flowchart og the methodology and experimental trials, including characterization analysis.

sequential centrifugation of the purées, for 20 min, at 1000 *g*, and 30 min, at 5000 *g* (Sigma 4K15C centrifuge), and the supernatant was analysed through High-Performance Liquid Chromatography (HPLC) with electrochemical detection (Dionex ICS-3000 HPLC; column: Thermo Carbopac MA1 250×4 mm, with a pre-column; eluent: 480 mM NaOH, at a flow of 0.4 mL/min; injection volume: 10 μL; temperature: 30 °C).

The fresh FVW purées and MSS were previously dried in an oven, at 105 °C, for mineral content and EA. The quantification of heavy metals and semi-metals comprised the quantification of Cd, Cu, Ni, Pb, Zn, Hg, Cr, Fe, and Al by ICP-AES (Horiba Jobin-Yvon, France, Ultima) following the European Standard EN 15,290. Mg, Ca, K, and P were also quantified by ICP-AES using the same methodology as for heavy metals and semi-metals. The EA encompassed the quantification of CHNS (Thermo Finnigan-CE Instruments Flash EA 1112 CHNS series elemental analyser).

Regarding the total sugar content, fresh FVW were lyophilized, ground in a centrifugal mill (Retsch SM2000) with a mesh of 0.5 mm, and then used for the determination of Klason lignin following the TAPPI 13m-54 standard. This standard indicates the percentage of residue (acidic solid residue) and total sugars (expressed as galactose, glucose, xylose, arabinose, rhamnose, fructose, and sucrose), determined through HPLC in the same equipment and under the same conditions as defined for sugars in the soluble fraction. In addition to this procedure, a test was conducted to assess the percentage of residue and total sugars by performing only the acidic pre-treatment and allowing the samples to react with H_2SO_4 for 2, 4, and 6 hours before filtration. The main aim was to evaluate the effectiveness of the chemical pre-treatment compared to the thermochemical pretreatment defined in the Klason lignin method.

2.4. *FVW storage trials*

The trials (represented by letter “d” in Fig. 1) aimed to assess the evolution of some physicochemical parameters of FVW, for 21 days, simulating the temperature and storage conditions that the peels would be subjected to at a WWTP located in a Mediterranean climate with Atlantic influence. The long duration of storage trials (21 days) was established to allow a more comprehensive analysis of how FVW degrades over time, including potential late-stage degradation processes for a long storage period, even if it is not foreseen that FVW would be stored for so long time. Two temperatures were tested: 25 °C, simulating the average temperature during the Summer season in a Mediterranean climate with a strong Atlantic influence [29], and 10 °C simulating the average temperature during the Winter season in the same climate [30]. All trials were performed in triplicates for each experimental run. After storage, FVW would be used as co-substrates in the AcoD process.

FVWs were stored in closed Schott flasks, with air inside, and kept at a constant temperature of 25±1 °C in a water bath (Nüve NB 20) controlled by a thermostat (VELP SCIENTIFICA OCB – F40300240) and of 10±1 °C in a thermostat cabinet (Aqualytic model AL 186). Each Schott flask contained 300 g of peels (Figure S2, supplementary material) and pre-selected days were chosen to assess their degradation: 0 (reference), 1, 3, 7, 14, and 21 days. On each sampling day, approximately 50 g of peels were withdrawn and subjected to the same mechanical pre-treatment as in Section 2.2, for 2 minutes, with 100 g of water. The peel:water ratio of 1:2 was selected considering the results obtained from the physicochemical characterisation and the amount of water needed to produce fluidised liquid purées. The parameters pH, EC, TS, VS, COD_T, COD_S, Brix, sugars in the soluble fraction (sucrose, fructose, glucose), VFAs (formic, acetic, lactic, propionic acids), and ethanol were quantified. The gas produced during the storage process was neither collected nor analysed as this was not the aim of this study. The parameters were analysed following the methodology of the American Public Health Association [28]. Sugars and VFAs were quantified through HPLC (equipment and conditions as defined in Section 2.3).

2.5. Anaerobic co-digestion batch assays

To assess the effect of the two storage temperatures in the AcoD of FVW purées blended with MSS, batch trials (represented by letter "e" in Fig. 1) using stored FVW and fresh MSS were carried out using 500 mL Schott flasks tightly sealed and connected to Tedlar bags for collecting biogas. The working volume for the trials was 2/3 of the flask's full capacity (333 mL). Bioreactors were incubated in a water bath under mesophilic conditions (temperature: 37±1 °C) and gently manually stirred two times a day.

The anaerobic inoculum (I) was collected from an anaerobic digester of a WWTP performing anaerobic digestion of municipal mixed (primary and secondary) sludge, under mesophilic conditions. Six different batch tests were prepared in triplicate:

- I alone (Control).
- I + FVW purées, without peel storage (Reference 1).
- I + MSS (Reference 2).
- I + MSS + FVW purées, without peel storage.
- I + MSS + FVW purées after 7 days storage of peel at 10 °C.
- I + MSS + FVW purées after 7 days storage of peel at 25 °C.

The FVW submitted to a storage step were stored for only 7 days due to the following main reasons: (i) Most of the degradation of TS, VS, COD_T, and COD_S occurred within the first week of the trial at 25 °C; (ii) It is not supposed that FVW will be stored in a WWTP for a longer period.

FVW purées were prepared following the same process described in Section 2.2, with the peel:water ratio of 1:2 (m:m), blended for two minutes. The ratio of I:MSS was 2:1 (v:v) and in the mixtures with FVW purées, the percentage of FVW added corresponded to 10% of the MSS volume used in Reference 2 (I + MSS). All the FVW purées prepared consisted of 33.3:33.3:33.3% w/w (apple:banana:carrot peels). In this study, no changes in the FVW purée ratios were tested as this will be the aim of a future study.

The biogas production was daily measured, through water displacement using an acidic water column. Biogas composition was assessed periodically by GC-TCD (Thermo Trace CG Ultra) for CH_4, CO_2, and H_2S. The duration of the batch assays was 14 days aiming to achieve the plateau of biogas production, *i.e.* biogas volume in 3 consecutive days was less than 5% of the total volume. Physicochemical characterization was performed at the beginning and end of each assay, for each AD and AcoD assay, comprising pH, EC, TS, VS, COD_T, and COD_S.

The modified Gompertz kinetic model (Eq. (1)) was fitted to the accumulated biogas production curves:

$$P(t) = P \times \exp\left\{ - \exp\left[\frac{R_{max} \times \exp(1)}{P} \times (\lambda - t) + 1\right]\right\} \qquad \text{(Eq.1)}$$

where $P(t)$ is the accumulated biogas volume at time t (mL), P is the final accumulated biogas volume (mL), R_{max} is the maximum daily biogas production rate (mL/d), λ is the lag phase duration (days), and t is the time of assay (days).

The kinetic model was adjusted by using the Solver function in MS Excel 365 to find the optimised values for P, R_{max}, and λ constants that minimise the sum of squared errors. GRG non-linear model was used by restricting the constants to positive values.

2.6. Statistical analysis

Statistical analysis was conducted to determine whether significant differences were obtained in the physicochemical characterisation between the beginning and end of each storage trial (within trials) and between the two storage temperatures (between trials). Using GraphPad Prism software (version 5.0), a two-way analysis of variance (ANOVA) was performed, applying the Tukey test to compare the samples with a 95% degree of confidence (p-value: 0.05). Differences were considered significant for p-values lower than 0.05.

3. Results and discussion

3.1. Physicochemical characterisation of FVW and MSS

Table 1 shows the physicochemical characterisation of the apple, banana, and carrot peels after being converted into purées with three different peel:water ratios (1:1, 1:2, and 1:4), where Brix degree in the MSS was not quantified since it is usually applied to assess the concentration of soluble solids (particularly sugars) in food. Regarding the nitrogen content, TN and Protein were not quantified in MSS because the apparatus used to quantify the parameters is only applied to food, so it was not possible to evaluate nitrogen in this way and the determination was carried out using TKN for the MSS.

APP (apple) exhibited the lowest pH values for the three different peel:water ratios tested (ranging between 4.20±0.33 and 4.90±0.34), compared to BPP (banana) and CPP (carrot), which showed very similar pH values among them (ranging between 5.40±0.03 to 6.10±0.04, for banana, and 5.00±0.27 to 6.20±0.27, for carrot). Low pH values could inhibit microbial activity during the methanogenesis phase, thereby reducing biogas yield [31]; therefore, adding these co-substrates in controlled percentages combined with the main substrate is mandatory to avoid bioreactor acidification. MSS exhibited acidic pH values of 5.70 ±0.03.

Regarding EC, BPP displayed the highest values (ranging between 4.30±0.36 and 6.30±0.61 mS/cm), while the APP and CPP presented values between 0.70±0.04 to 1.00±0.04 and 2.30±0.16 to 3.20±0.48 mS/cm, respectively. For the MSS, the EC value (2.6±0.17 mS/cm) was closer to the CPP. Concerning TS, VS, COD_T, and COD_S, APP exhibited the highest values for the three different water ratios compared to banana and carrot ones.

All the purées exhibited similarly low protein content (ranging between 0.7 to 0.9%) and TKN (0.1%), which is common among this type of biowastes [32]. For sugars in the soluble fraction, only xylose and sucrose were detected in the APP. This biowaste is richer in fructose (≈57%) and sucrose (≈25%), followed by glucose (≈17%), xylose (≈0.76%), and galactose (≈0.24%). BPP is richer in fructose (≈64%), followed by glucose (≈35%) and galactose (≈1%). CPP is also richer in fructose (≈62%), followed by glucose (≈36%) and galactose (≈2%).

Concerning metals (Table S1, supplementary material), only Fe and Al were detected in small quantities above the limit of detection in FVW (apple peel: 0.011 mg Fe/g dw; carrot peel: 0.019 mg Al/g dw). Regarding MSS, Fe and Al were also detected, along with Cu (0.087 mg/g dw), but in the case of Fe, the concentration was significantly higher (12.9 mg/g dw). This Fe concentration in MSS can be attributed to ferric chloride used as a coagulant in the wastewater treatment process, a common procedure in large-scale WWTPs, which helps decrease the concentration of H_2S in the biogas [33].

For the EA, the overall content for each element was similar for the three peels, ranging from 39.0–43.8% for C, 5.6–6.9% for H, 0.3–1.0% for N, and <0.01% for S. C/N ratios of around 146 for apple peel, 42 for banana peel, and 49 for carrot peel were obtained, demonstrating the positive impact these substrates can have on improving the AD of MSS as, in contrast, the elemental analysis of MSS showed around 44.1% C, 6.8% H, 7.2% N, and 0.6% S, dealing a C/N ratio of only 6, significantly below the optimal ratio of 20–30 currently cited in the literature [34].

For total sugars quantified according to the Klason lignin method (Table S2, supplementary material), the percentages of residue and total sugars were 19.3 and 80.8% for apple peel, 24.1 and 75.9% for banana peel, 19.0 and 81.0% for carrot peel, respectively. Only the presence of monosaccharides was detected, and the main sugars were glucose and fructose, with percentages varying from 12.9–16.6% to 14.7–38.9%, respectively, and with the highest percentages observed in the apple peel. These values are slightly higher than those reported by [35], who indicated that approximately 75% of sugar and hemicellulose are in

Table 1
Physicochemical characterisation of the apple, banana, and carrot peels after being converted into purées with three different peel:water ratios (1:1, 1:2, and 1:4).

	APP			BPP			CPP			Municipal Sewage Sludge (MSS)
	Peel:water ratio			Peel:water ratio			Peel:water ratio			
	1:1	**1:2**	**1:4**	**1:1**	**1:2**	**1:4**	**1:1**	**1:2**	**1:4**	
pH	4.20±0.33	4.30±0.20	4.90±0.34	5.40±0.03	5.70±0.12	6.10±0.04	5.00±0.27	5.40±0.21	6.20±0.27	5.70±0.03
EC (mS/cm)	1.00±0.04	0.90±0.04	0.70±0.04	6.30±0.61	5.30±0.23	4.30±0.36	3.20±0.48	2.90±0.36	2.30±0.16	2.60±0.17
Brix (°Bx)	6.2 ± 0.20	4.1 ± 0.23	2.4 ± 0.20	2.7 ± 0.58	1.6 ± 0.53	0.8 ± 0.35	1.9 ± 0.31	0.9 ± 0.50	0.4 ± 0.20	n.q.
TS (g/L)	79.5 ± 3.12	53.9 ± 2.93	31.2 ± 2.17	43.8 ± 4.23	32.7 ± 3.74	21.9 ± 2.89	32.1 ± 3.54	25.8 ± 2.76	18.9 ± 2.05	26.0 ± 1.98
VS (g/L)	78.5 ± 2.24	53.4 ± 2.35	30.9 ± 1.86	37.6 ± 3.42	27.8 ± 3.06	19.2 ± 2.18	26.6 ± 2.69	22.8 ± 2.13	16.9 ± 1.72	22.2 ± 1.42
VS/TS (%)	98.7	99.1	99.0	85.8	85.0	87.7	82.9	88.4	89.4	85.4
COD_T (g O_2/L)	84.0 ± 5.66	46.7 ± 3.41	30.0 ± 1.96	52.0 ± 3.87	21.3 ± 2.34	16.4 ± 1.51	27.3 ± 2.54	15.3 ± 2.02	10.6 ± 1.38	38.4 ± 2.94
COD_S (g O_2/L)	78.0 ± 3.59	43.0 ± 3.10	27.3 ± 1.53	33.3 ± 1.89	11.4 ± 1.46	9.6 ± 1.13	24.7 ± 1.89	13.2 ± 1.24	9.8 ± 0.89	3.8 ± 0.20
COD_S/COD_T (%)	92.9	92.1	91.0	64.0	53.5	58.5	90.5	86.3	92.5	9.9
TN (%)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	n.q.
TKN (g/L)	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	n.q.	1.77±0.28
Protein (%)	0.7	0.7	0.7	0.7	0.8	0.9	0.8	0.8	0.8	n.q.
Carbohydrates										
Galactose (mg/L)	35	367	31	135	34	32	46	42	38	n.q.
Glucose (mg/L)	3073	2688	1669	2788	734	743	1647	1106	529	n.q.
Xylose (mg/L)	140	116	79	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	n.q.
Fructose (mg/L)	10,337	8556	6265.2	3459	1919	1443	2687	1783	1006	n.q.
Sucrose (mg/L)	4567	3387	2888.6	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	< 1 (LOD)	n.q.

APP: Apple peel purée; BPP: Banana peel purée; CPP: Carrot peel purée; n.q.: not quantified; LOD: Limit of detection.

FVW.

The results obtained in the alternative chemical pre-treatment assay with H_2SO_4, for 2 hours, and without thermal pre-treatment (Table S3, supplementary material) showed a significant reduction in the percentage of extracted sugars from the apple, banana, and carrot peels (15.3, 29.3, and 23.5%, respectively). Another significant aspect concerns the presence of sucrose, which is a disaccharide composed of fructose and glucose, highlighting the significance of thermal

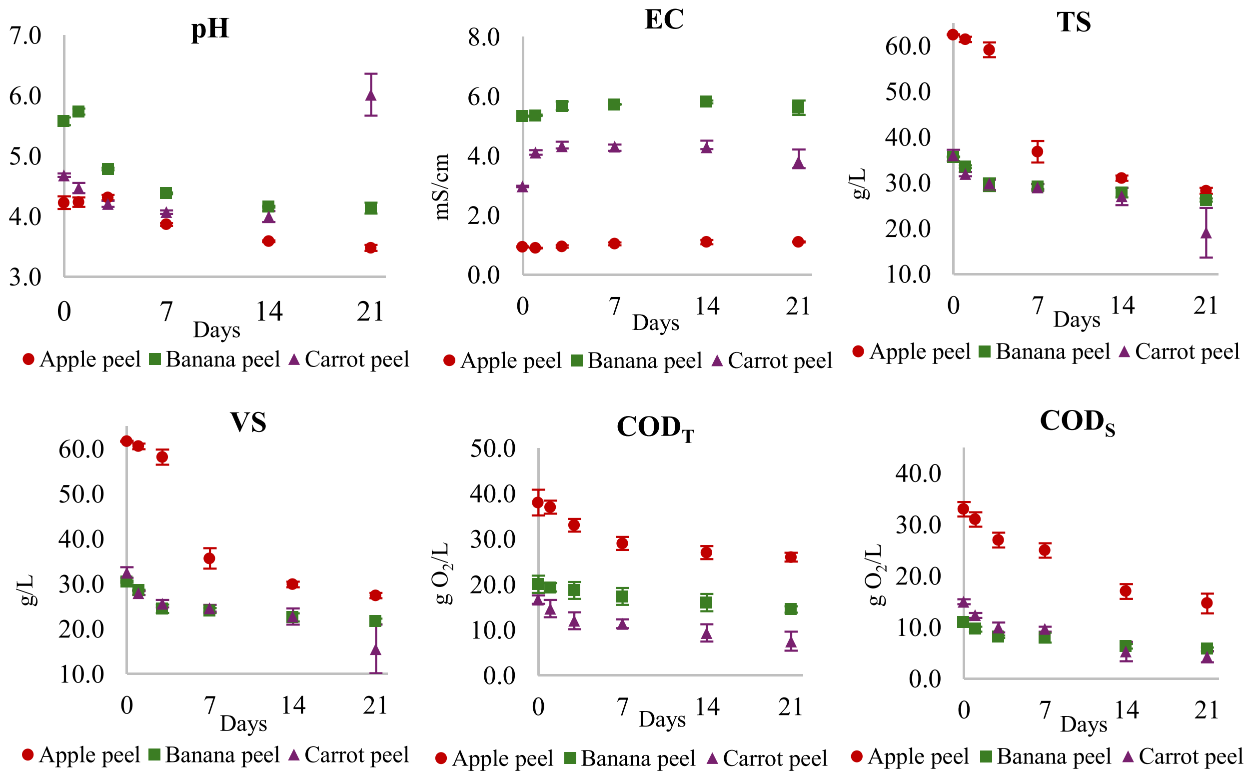

Fig. 2. Evolution of pH, EC, TS, VS, COD_T, and COD_S over the 21 days of storage at 25 °C for apple, banana, and carrot peel purées ($\overline{X} \pm \sigma$; $n = 3$).

pretreatment to degrade complex sugars into monosaccharides. This is proven by the reduction of fructose and glucose from the complete methodology comprising the thermochemical pre-treatment compared to only the 2 hours acidic reaction. Once again, the sugars presenting the higher concentrations were fructose and glucose.

Regarding the 4 and 6 hours acidic reaction times (Tables S4 and S5, supplementary material), the differences in the extracted sugars compared to the 2 hours acidic treatment were low, with only increments between 0.4% and 2.3% among the three FVW samples. After 6 hours of the acidic treatment, galactose was detected in the carrot peel, although in a very small percentage (0.4%), proving that even with the increase in the acidic reaction time, the results did not improve considerably. This allows concluding that thermal pre-treatment is one of the most effective processes to hydrolyse and solubilise organic compounds, namely carbohydrates, from several organic substrates with similar properties to those used in this work [36,37,38,39].

3.2. Physicochemical evolution of FVW during the storage trials

To the authors' best knowledge, no literature exists on the degradation of FVW under different storage conditions matching the specific waste type and circumstances examined in the present study. Consequently, the findings herein are compared with those from studies bearing approximate similarities.

3.2.1. ***Storage trial at 25 °C***

During the storage trial at 25 °C, BPP started with the highest pH and EC (Fig. 2 and Table S6, supplementary material), with values of 5.58 ±0.06 and 5.33±0.02 mS/cm, respectively. After 21 days, it ended up with a pH of 4.14±0.09 and an EC of 5.61±0.24 mS/cm. Regarding APP (Table S7, supplementary material), pH decreased from 4.23 to 3.48 when comparing the beginning and end of the trials. CPP (Table S8, supplementary material) presented a different behaviour from the 2nd to the 3rd weeks, where the initial tendency was to register a decrease in pH (4.68 to 4.00 from time 0 to the 2nd week) and increase in EC (2.97 to 4.28 mS/cm from time 0 to the 2nd week, despite the slight decrease from the 1st week with 4.32±0.06 mS/cm to 4.28±0.23 mS/cm in the 2nd week). Between the 2nd and 3rd weeks, pH increased from 4.00 to 6.02, and EC decreased from 4.28 to 3.81 mS/cm. This can be explained by the marked deterioration of the carrot peels on the last day, which originated a smoother and more homogeneous purée, providing better mixing with the water. Another noteworthy factor to highlight in the carrot peel was the visible presence of white fungi after 72 hours of storage (Figure S3, supplementary material), which may explain its pronounced degradation.

The three samples of FVW purées presented a continuous decrease in TS and VS throughout the trial (Fig. 2). The most evident drop was observed in the APP between the 72-hour and 1 week of the experiment, where TS had a 38% reduction and VS of 39%, compared to the 1.3 and 1.6% drop in the BPP and the 2.9 and 4.0% in the CPP. Overall, the decrease in TS and VS from the first day until the last day was 54.8 and 55.6% for the APP, 26.4 and 28.9% for the BPP, and 47.0 and 52.1% for the CPP, respectively. BPP presented the lowest reduction since it has more hemicellulose and lignin compounds, as well as more fibres, like pectin, which provides more rigidity to the fruit cell walls, making it harder to degrade.

A continuous decrease also occurred in COD_T and COD_S (Fig. 2), albeit with a more gradual reduction during the trial. The APP presented reductions of COD_T and COD_S of 31.6 and 55.5%, BPP of 27.5 and 47.7%, and CPP of 55.0 and 71.7%, respectively. This high reduction in COD, mainly in the soluble fraction, can be attributed to the conversion into volatile organic compounds as seen in ethanol and VFA productions. It is worth noting that the COD_S of the CPP exhibited the highest variation.

Regarding the Brix degree (Figure S4, supplementary material) and when comparing the reference day (time 0) with the last day of the assay, BPP showed the most stable evolution, with a decrease of only 25.0% compared to the 59.0% decrease observed in APP and 66.7% decrease in CPP.

Concerning carbohydrates (Fig. 3), APP showed the highest concentrations of the three identified saccharides (glucose, fructose, and sucrose). All FVW purées exhibited a consistent degradation pattern throughout the storage experiment, with most of the degradation occurring within the first 7 days. None of the samples presented detectable values after 14 days for sucrose, suggesting this carbohydrate may have converted into fructose and glucose during that period.

In the case of CPP, all the carbohydrates were degraded within the first 72 hours. By the end of the trial, the APP was the only sample with detectable concentrations of glucose and fructose, despite experiencing 82 and 83% reductions in both sugars over the 21 days. It is notorious a pattern for the evolution of glucose and fructose in the APP, starting with their degradation during the first 24 hours, followed by a slight increase between the 24 and 72 hours. This can be explained by the degradation of the sucrose into the two monomers. Bas-Bellver et al. [40] observed the same variations in sucrose while studying the carbohydrate content of upcycled vegetable waste during 4-month storage. At the end of the trial, part of the carbohydrates was converted into final products (namely, VFAs and ethanol) but the majority could be converted into new biomass of microorganisms involved in the biodegradation of FVW.

VFAs, namely acetic and lactic acids, were detected (Fig. 3). Acetic acid began to be detected after 72 hours in the CPP sample and after 1 week in both APP and BPP.

In contrast, lactic acid was detected on the reference day (day 0) for the BPP and CPP but only after a week for the APP. The highest concentration of lactic acid (5.10±0.71 mg/mL) was observed in the CPP on day 14, while acetic acid (4.31±1.23 mg/mL) was detected in the APP on the last day of the trial.

Additionally, propionic acid was detected in vestigial concentrations on the APP during the 2- and 3-week marks (0.07±0.02 and 0.09±0.03 mg/mL, respectively). Apart from VFAs, ethanol was also produced during the experimental assay. Notably, the highest concentration of ethanol (13.03±0.37 mg/mL) was registered in the APP on day 14. In contrast, the second highest concentration (1.83±0.38 mg/mL) was observed in the CPP after 72 hours.

Lü et al. [41] performed a storage trial of food waste (including vegetables, and cooked rice, among other food wastes) for 12 days, at 35 °C, and observed a reduction of the pH from 6.33 on the first day to 3.92 on the last day, with the appearance of lactate 24 hours after the beginning of the trial, and obtained an increment of more than 3.5 times until the end of the experiment (from 286.5 to 1022.6 mg C/L).

Påledal et al. [42] studied the storage at two different temperatures (6 and 22 °C), during 21 days, of the organic fraction of municipal solid waste (OFMSW) in plastic bags and paper bags to simulate the conditions of storage and transport of this type of waste from home to biogas plant in Sweden. In the trial with the OFMSW storage in plastic bags (closest to anaerobic conditions), at 22 °C, the authors also observed a decrease in the pH from approximately 5.3 to 4.5, along with an approximate 10% decrease in the VS.

Degueurce et al. [25] performed two different storage trials (at small and large scales) of food waste from a collective restaurant, for 14 days, at an approximately average temperature of 20 °C, and obtained reductions in the pH from 4.69 to 4.50, along with TS and VS reductions of approximately 6.18 and 4.58%, respectively.

Despite the operational conditions of the presented studies are not the same as those of this work, they present a similar degradation pattern for the parameters discussed. Degueurce et al. [25] and Lü et al. [41] concluded that the best storage time for the substrates to subsequent anaerobic digestion would be approximately one week, more specifically 7 days and between 5 and 7 days, respectively.

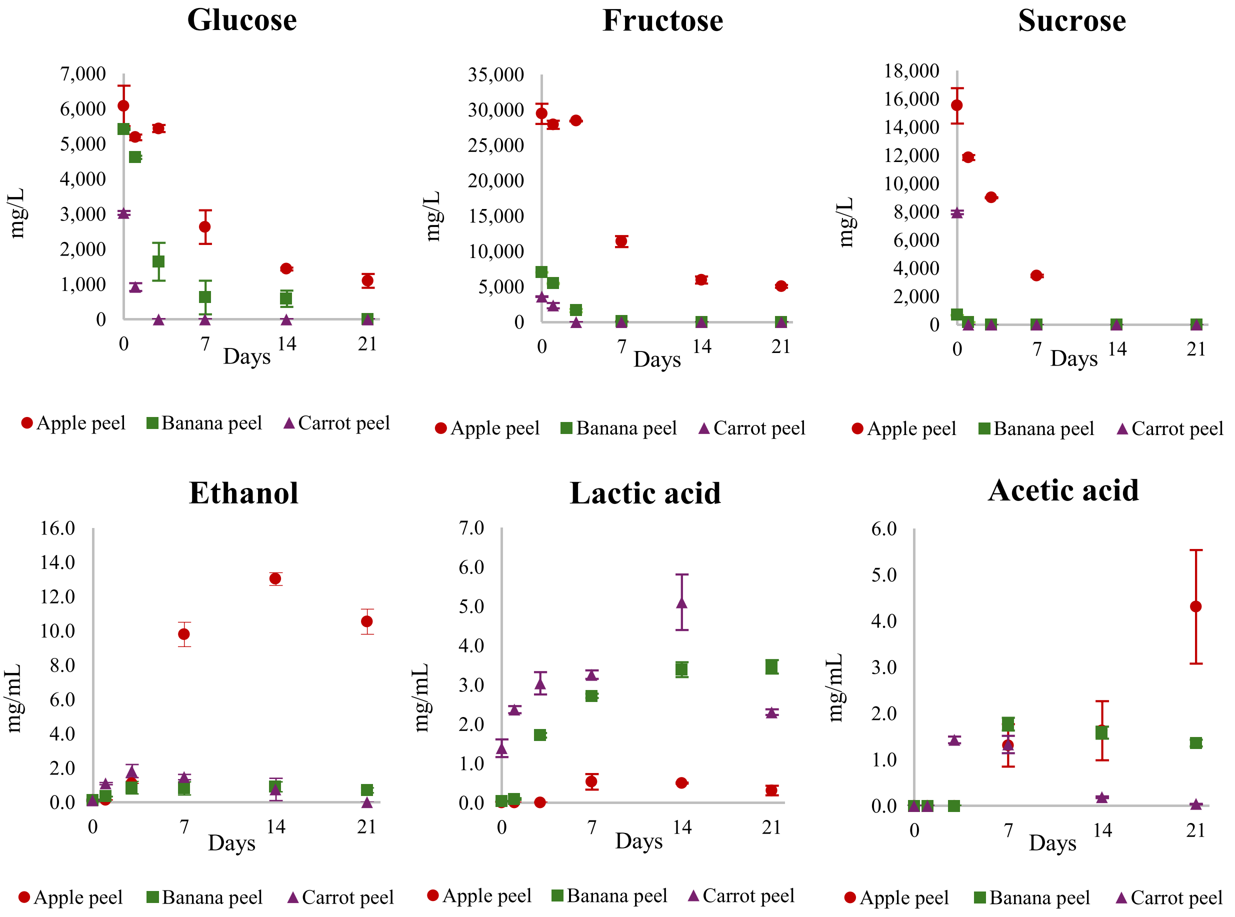

Fig. 3. Evolution of carbohydrates, VFAs, and ethanol over the 21 days of storage at 25 °C for apple, banana, and carrot peel purées ($\overline{\boldsymbol{X}} \pm \sigma$; $n = 3$).

3.2.2. *Storage trial at 10 °C*

In this experiment, the sample with the highest initial pH was the CPP (6.02±0.08) (Fig. 4) (Table S9, supplementary material). Similarly, to the 25 °C trial, the pH tended to decrease as the test progressed. However, there was a steep increment between the days 14 and 21 (from 4.65±0.21 to 5.63±1.51). This could be explained by a phenomenon similar to what occurred with the carrot peel in the previous trial at 25 °C.

Regarding the BPP, they started with a similar pH value (5.82±0.08) as in the previous trial at 25 °C (5.58±0.06) and ended with a pH of 4.69 ±0.09 (Fig. 4) (Table S10, supplementary material), which is a smaller variation compared to the 25 °C trial. The APP (Table S11, supplementary material) started with a pH of 4.42±0.05 and presented a reduced variation, ending with 3.92±0.16, which is also smaller than the previous storage assay.

The BPP was once again the sample with the highest EC value at the beginning of the trial (5.53±0.06 mS/cm), followed by CPP (3.07±0.04 mS/cm) and APP (0.96±0.01 mS/cm), with the carrot being the FVW with the highest variation (almost a 30% increment from reference day until the last day of the assay). APP was the most stable for EC, with only a 6.3% maximum variation. Ene-Obong et al. [43] obtained reductions in the pH between 6.7% (after 3 weeks in 12 °C storage) and 40.0% (after 3 weeks in 29–32 °C storage) while assessing the impact of storage duration and temperature on the physicochemical properties of two monkey cola fruit jams.

Concerning TS and VS (Fig. 4), the most noticeable difference between the 25 and 10 °C trials is the APP. In the 25 °C trial, between the 72 hours and the one-week mark, TS and VS decreased by 38 and 39%, respectively, while in the 10 °C trial, these parameters remained relatively stable with only 11.8 and 11.9% decrease from the reference until the last day of the trial. For BPP, there was a 22.7 and 25.6% reduction in TS and VS, respectively, and for CPP, the reduction was 31.8 and 35.3%, respectively. Compared to the 25 °C experiment, where reductions for TS and VS were 26.4 and 28.9%, for BPP, and 47.0 and 52.1%, for CPP, respectively, these parameters also had smaller variations in the 10 °C trial over the 21 days. Ene-Obong et al. [43] observed reductions between 5.9 and 47.1% in the TS of the two jams, similar to the range obtained in this trial.

For COD_T and COD_S (Fig. 4), banana peel and apple peel purées exhibited the most stable behaviour, with reductions of 24.4 and 40.8% for BPP and 28.6 and 35.0% for APP, respectively. Comparing these results with the previous experiment, the reduction in COD_S for APP was the parameter with the highest disparity, as the reduction was 55.5% in the 25 °C trial. In contrast, CPP showed the highest variation over the 21 days (41.4 and 45.3% for COD_T and COD_S, respectively). However, compared with the 25 °C trial (55.0% and 71.7%), the reductions were smaller, especially regarding COD_S. This suggests that in the lowest temperature, mitigating a depletion of 26.4% in COD_S is possible.

Regarding the Brix degree (Figure S4, supplementary material), APP was the most stable sample in this experiment, with a reduction of only 15.8%, compared to the 40% reduction observed for CPP and 45.0% reduction for BPP, over the 21 days. Comparing both experiments, banana peel had the most stable purées in the previous temperature trial, but in this 10 °C assay, it was the least stable (with a 20% higher reduction). Apple reduced its natural degradation concerning Brix by around 43% (from a 59.0% depletion, in the 25 °C test, to a 15.8% depletion, in the 10 °C test). CPP also reduced the variation throughout the 21 days (with a 40.0% depletion from the reference until the last

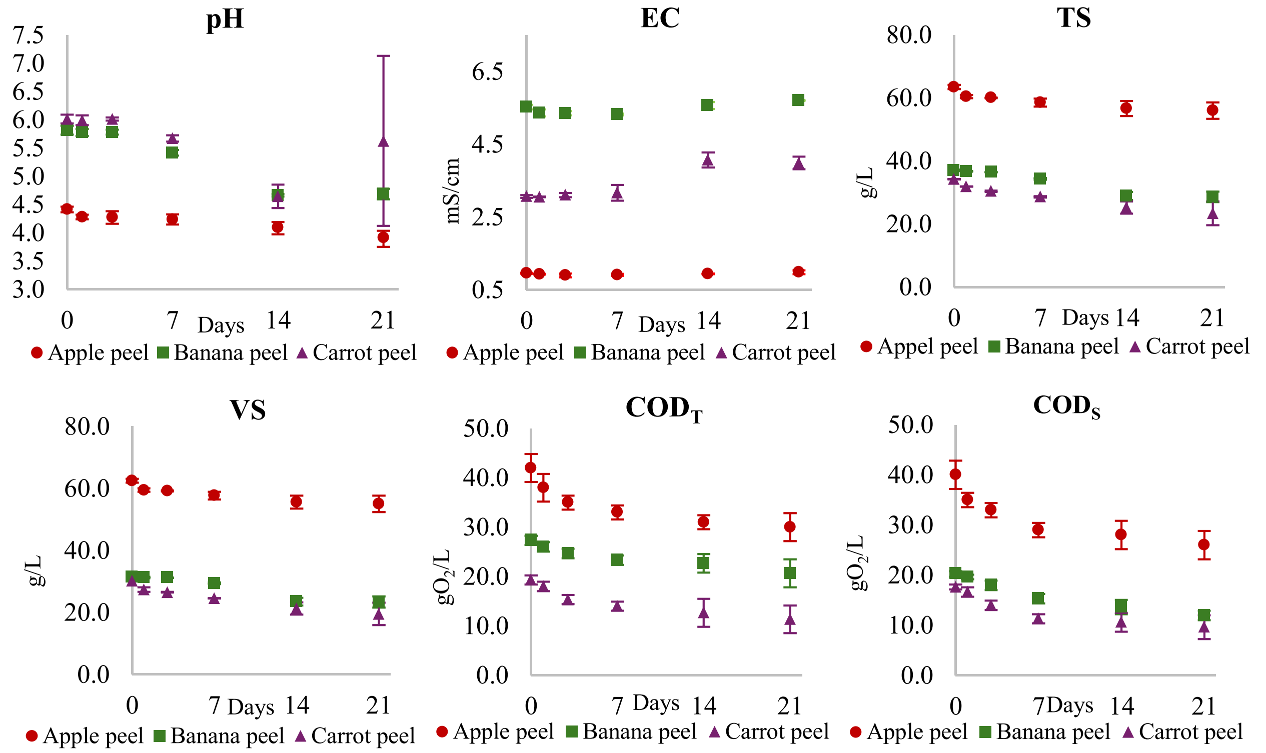

Fig. 4. Evolution of pH, EC, TS, VS, COD$_T$, and COD$_S$ over the 21 days of storage at 10 °C for apple, banana, and carrot peel purées ($\overline{X} \pm \sigma$; $n = 3$).

day, compared with a 66.7% depletion in the previous trial).

Compared with the previous trial, significant differences were observed in the degradation of carbohydrates (Fig. 5). In APP, fructose remained stable throughout the 21 days, whereas in the 25 °C experiment, it degraded by 83%. CPP kept all saccharides until after the 2-week mark, contrasting with the complete degradation observed

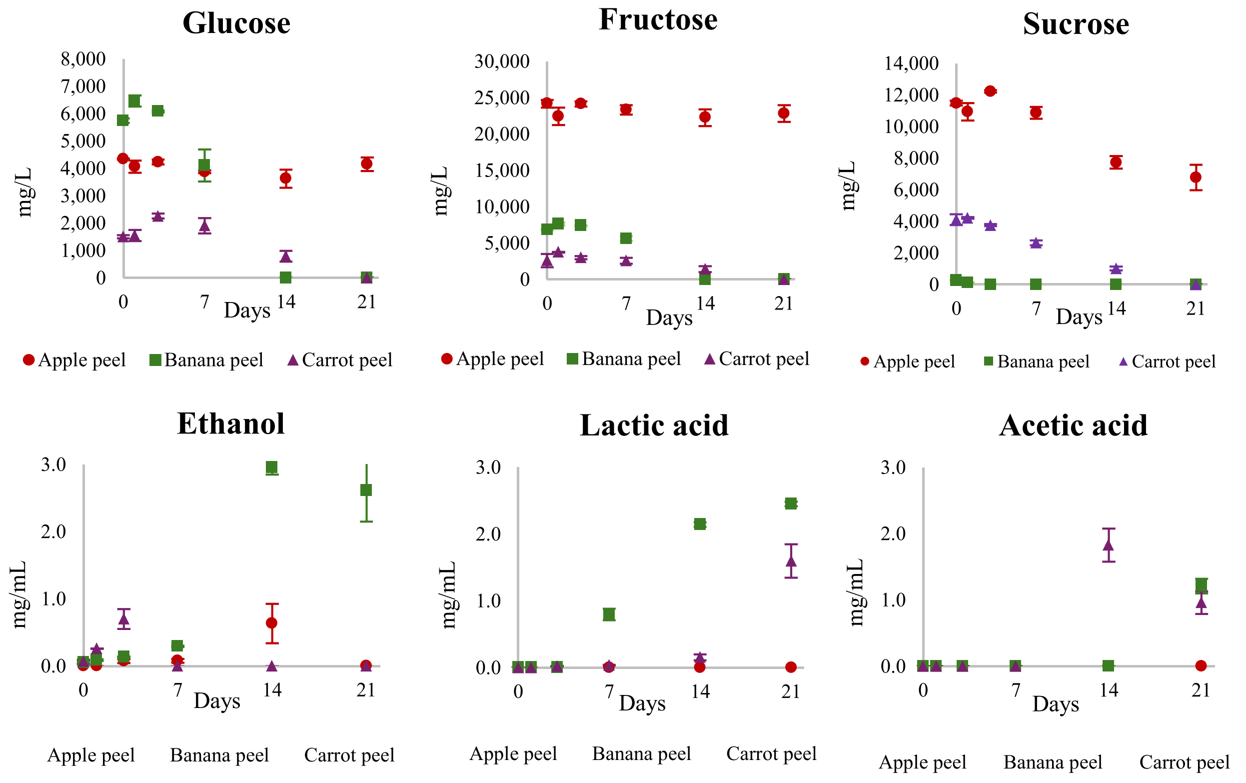

Fig. 5. Evolution of carbohydrates, VFAs, and ethanol over the 21 days of storage at 10 °C for apple, banana, and carrot peel purées ($\overline{X} \pm \sigma$; $n = 3$).

within the first three days at the 25 °C trial. BPP exhibited the most pronounced degradation, with a steep decrease in glucose and fructose during the first 14 days.

A significant reduction in ethanol production was observed (Fig. 5), with the highest concentration being recorded for BPP (2.95 mg/mL), contrasting with the 13.03 mg/mL from APP in the 25 °C trial. Regarding the apple and carrot peel purées, the highest concentrations were lower than 0.70 mg/mL.

The concentrations of acetic and lactic acids also decreased and were detected later in this lowest temperature trial compared to the 25 °C experiment, indicating the effectiveness of colder temperatures in inhibiting the generation of VFAs. The highest lactic acid concentration (2.45 mg/mL) was detected in BPP on day 21, while the highest concentration of acetic acid (1.83 mg/mL) was observed in CPP on day 14. In this trial, APP did not produce detectable levels of both acids over the 21 days, showing only a small ethanol production from 72 hours until the last day. Additionally, a small concentration of propionic acid (0.26 mg/mL) was detected on the last day for CPP, and a residual concentration of formic acid (0.03 mg/mL) was observed on the last day for CPP too.

In the storage trial at 6 °C, Påledal et al. [42] also observed smaller variations between the beginning and end of the experiment. Regarding the pH, the value increased from 5.3 to 5.7 and the VS content decreased by only approximately 5% when compared with the approximate 10% decrease in the 22 °C trial.

3.2.3. Statistical analysis

Table S12 (supplementary material) shows the results of the statistical analysis (two-way ANOVA) of the experimental data obtained for some of the physicochemical parameters analysed between days 0 (reference) and 21 of the storage trials. Each FVW and physicochemical parameter were analysed individually, which means that the comparison of the letters is independent between samples and parameters. Different letters indicate significantly different results within each assay and between the storage assays, at the two tested temperatures.

Concerning pH, CPP was the only sample with no significant differences, while for EC, BPP was the FVW for which no significant differences between and within trials were detected.

Concerning TS and VS, the concentrations were considered similar for day 0 (reference) in both storage trials at two different temperatures, for the three FVW samples. However, in the case of APP, there were significant differences in the values on day 21 between 10 and 25 °C trials. For COD, COD_T, and COD_S, there were significant differences between the start and end of both storage trials with apple and carrot peels. In the case of BPP, the same is true for COD_S. There were no significant differences between the beginning and end of each trial for COD_T.

Regarding the Brix degree, all the values differed within each trial and between the storage trials for APP. For BPP, the value from the last day of the trial at 10 °C and the value from the last day of the trial at 25 °C were similar. For CPP, only the reference values from both trials were considered similar.

Overall, the results of the two-way ANOVA showed some significant differences in the parameters analysed within each trial and between the two trials. This means that for the same temperature, the degradation processes differ among FVW, and between storage temperatures, 25 °C has a significant impact on altering some of the physicochemical characteristics of FVW, which must be considered in industrial-scale storage operations due to the effect it can cause in AcoD.

3.2.4. Key parameters to enhance anaerobic co-digestion of MSS and FVW

To assess the benefits of FVW co-substrates to improve AD of MSS, three parameters were analysed as they are the most relevant: pH, VS/TS ratio, and COD_S/COD_T ratio.

The results (Table S13, supplementary material) demonstrate that the pH varied from 3.48, for APP, to 6.02, for CPP, on the last day at 25 °C trial. These values are lower than the optimal range for anaerobic digestion (6.8 to 7.5) [44], meaning that a precise dosage of these co-substrates must be made to avoid acidification of the biodigester. In any case, these acidic values are normal for FVW; for example, Slopiecka et al. [36] observed a range from 3.93 to 7.40 while assessing the physicochemical properties of FVW.

Regarding the VS/TS ratio, Slopiecka et al. [44] obtained an average percentage of 87.74% for FVW, being higher than the values obtained in this study on the last day, for both 10 °C and 25 °C trials, in the BPP (81.66 and 82.62%, respectively) and CPP (83.53 and 81.46%, respectively). Nevertheless, for APP, the values were higher at the beginning and end of both trials (98.44% on day 0 and 98.30% on day 21, for the 10 °C trial; 98.77% on day 0 and 97.02% on day 21, for the 25 °C trial).

Concerning the COD_S/COD_T, APP had the highest ratios especially in the 10 °C trial (95.24%, at day 0) when compared to the BPP (74.20%, at day 0, for 10 °C trial) and CPP (91.36%, at day 0, for 10 °C trial). These results demonstrate the potential of using these FVW as co-substrates in the AcoD process to enhance eventually the anaerobic digestion of MSS, but under controlled dosages to avoid acidification of the anaerobic biodigester.

3.3. Anaerobic digestion and co-digestion batch assays: physicochemical characterisation and biogas/biomethane production

Table S14 (supplementary material) presents the physicochemical composition of the 4 different mixtures studied in the AcoD batch assays. The results for the control assays containing the anaerobic inoculum (I) alone and I + FVW fresh purées without previous storage are not shown as biogas production was limited, especially for I alone.

Reference 2 (I + MSS) was the assay with the highest pH (6.52±0.02) and lowest EC (5.50±0.07 mS/cm) when compared with the three mixtures which contained the FVW purées (whose values ranged between 6.35±0.03 to 6.44±0.04 for pH and 5.71±0.12 to 5.86±0.10 mS/cm for EC). Reference 2 was also the assay with the lowest TS, VS, and COD_S concentrations since the other three blends had 10% more co-substrates, which is reflected in slightly higher concentrations of the initial organic matter.

After the batch assays (Table S15, supplementary material), the blends with the purées with FVW stored for 7 days presented the highest pH values (6.99±0.01 and 6.92±0.01 for 10 °C and 25 °C, respectively), meaning that the natural degradation for one week allowed to increase the pH values closer to the neutrality. The storage of FVW at 25 °C also allowed achieving a lower final COD_T (15.00±0.33 g/L) when compared with Reference 2 (I + MSS) (15.01±0.34 g/L). For TS, VS, and COD_S, Reference 2 (I + MSS) was the experimental condition with the lowest values when compared with the other three mixtures.

By analysing Table S16 (supplementary material) showing the removal efficiencies of each physicochemical parameter, it is possible to verify that the blend with the highest removal efficiencies for TS, COD_T, and COD_S was the one composed by I + MSS + FVW purées without peels storage (fresh FVW), with 30.56±0.34; 42.59±2.14 and 75.88 ±0.02 % removal, respectively, representing increases of 0.62, 3.51, and 1.38% for each parameter in comparison with the Reference 2 (I + MSS) (second assay with the highest removal efficiency). Kumari and Chandel [45] achieved VS removal efficiencies between 55 and 65%, during the anaerobic co-digestion of sewage sludge with OFMSW without storage (80:20 SS:OFMSW ratio), which is higher than the VS removal efficiency achieved in the present work (38.79±0.03 %) and that could be explained by the addition of a smaller percentage of co-substrate in this experimental trial.

These highest conversion percentages are also reflected in the production of accumulated biogas (Table 2), where the I + MSS + FVW purée blend, without previous storage of the peels, achieved the highest accumulated biogas volume (2295 mL). This biogas accumulated volume was 19.5% higher than Reference 2 (I + MSS), 10.2% higher than the blend with the FVW stored for 7 days at 10 °C, and 14.6% higher

Table 2
Average accumulated biogas volumes for AD and AcoD assays after 14 days of mesophilic incubation ($n = 3$).

Mixtures	Accumulated Biogas Volume (mL)	Accumulated Methane Volume (mL)
I alone (Control)	180	n.q.
I + FVW purées without peel storage (Reference 1)	435	n.q.
I + MSS (Reference 2)	1920	1271
I + MSS + FVW purées (without peel storage)	2295	1464
I + MSS + FVW purées (after 7 days storage of peel at 10 °C)	2083	1410
I + MSS + FVW purées (after 7 days storage of peel at 25 °C)	2003	1304

I: Inoculum; MSS: Municipal sewage sludge; FVW: Food and Vegetable Wastes; n.q.: not quantified as biogas volume was very low.

than the mixture with the FVW stored for 7 days at 25 °C. When assessing the differences between the two blends with the stored FVW for 7 days, the one with the FVW stored at 10 °C achieved a biogas production 4.0% higher than the one with the FVW stored at 25 °C.

The modified Gompertz model fittings for the AD and AcoD assays are presented in Figure S5 (supplementary material) and the obtained kinetic parameters with correlation coefficients (R^2) are shown in Table 3.

AD and AcoD assays presented lag phases (λ) lower than 1 day and the five correlation coefficients were within the range of 0.951–0.989, which demonstrates the good fit of the model. Kumari and Chandel [45] achieved similar results for the trial with 80:20 (SS:OFMSW without storage) with correlation coefficients ranging between 0.94 and 0.99 with lag phase inferior to 1 day also. In Santos et al. [46] work, λ values reached 9.40 days while correlation coefficients were between 0.96 and 0.99, when studying the anaerobic co-digestion of industrial sludge and sewage sludge with different co-substrates like orange bagasse, passion fruit peel, and cashew bagasse.

Regarding the kinetic parameters, the highest accumulated biogas volume, P, was obtained in the blend of *I* + MSS + FVW purées without peel storage, and the second highest value was from the blend with the FVW purées with peels stored for 7 days at 10 °C, approximately 5.4% lower than for *I* + MSS + FVW purées without peel storage. The lowest value was obtained in the mixture with only inoculum, followed by Reference 1 (*I* + FVW purées without peel storage).

Comparing Reference 2 (*I* + MSS) and the blend *I* + MSS + FVW purées without peel storage, P increased by 20.2% from the former to the latter. Concerning the two mixtures with the stored peels for 7 days, the one with the peels stored at 10 °C showed a P value 4.3% higher than the one with the peels stored at 25 °C.

For the maximum biogas daily production rate, R_{max}, the blend with *I* + MSS + FVW purées without peels storage was again the one with the highest value (2.7% higher than Reference 2 (*I* + MSS)). Among the blends with the stored peels, the difference was only 0.3% (290 and 289 mL/d for the blends with peels stored at 10 °C and 25 °C, respectively).

Table 3
Modified Gompertz model kinetic parameters and correlation coefficients (R^2) of the AD and AcoD batch assays ($n = 3$).

Mixtures	P (mL)	R_{max} (mL/d)	λ (d)	R^2
I alone (Control)	166	31	<1	0.959
I + FVW purées without peel storage (Reference 1)	396	131	<1	0.951
I + MSS (Reference 2)	1829	314	<1	0.980
I + MSS + FVW purées (without peel storage)	2199	323	<1	0.977
I + MSS + FVW purées (after 7 days storage of peel at 10 °C)	2081	290	<1	0.989
I + MSS + FVW purées (after 7 days storage of peel at 25 °C)	1994	289	<1	0.989

I: Inoculum; MSS: Municipal sewage sludge; FVW: Food and Vegetable Wastes.

Biogas and methane cumulative productions, with the respective composition profiles for CH_4, CO_2, and H_2S are shown in Figs. 6 and 7. Assays composed by the I alone and *I* + FVW purées without peel storage are not shown due to the very low accumulated biogas volumes obtained.

The blend with the highest CH_4 percentage was the one with the peels stored at 10 °C (67.7% v/v), which was 1.5% higher than Reference 2 (*I* + MSS). The mixture with *I* + MSS + FVW purées without peel storage obtained a CH_4 percentage of 63.8% (v/v), but considering the accumulated biogas production, the total volume of CH_4 produced was 1464 mL. The blend with the peels stored at 10 °C obtained a total volume of CH_4 of 1410 mL. When comparing the blends with stored peels, the one stored at 10 °C achieved a CH_4 production 8.1% higher than the one stored at 25 °C (1410 *vs* 1304 mL, respectively). Kumari and Chandel [45] only obtained a CH_4 percentage of approximately 60% (v/v) after 20 days in the 80:20 (SS:OFMSW without storage) at 7.5% TS, while Santos et al. [46] achieved 69% (v/v) CH_4 in the trials with sewage sludge + orange bagasse and sewage sludge + passion fruit peel, which is slightly higher than the highest percentage obtained in this work (67.7% v/v).

Regarding H_2S, it was found that the three AcoD assays containing FVW purées (peels stored at 10 and 25 °C and not stored) had much lower concentrations of H_2S than Reference 2 (*I* + MSS). For instance, the lowest value of H_2S observed in Reference 2 was 105 ppmv, and for the blends with FVW purées, the H_2S values ranged between 20 and 40 ppmv.

Globally, these results reflect the positive impact of adding FVW, mainly under fresh conditions, to the anaerobic digestion of MSS on both biogas and biomethane production, as well as on the reduction of H_2S.

4. Conclusions

The main cocnlusions of this work indicate that among the three co-substrates (apple, banana, and carrot peel purées), apple peel purées had the highest concentration of carbohydrates, predominantly monosaccharides, and higher VS and COD, but exhibited the lowest pH values. Conversely, banana peel purées and carrot peel purées had higher pH values, and all three FVW exhibited C/N ratios over 40. Notably, during the storage trials, one of the most significant reductions occurred at 25 °C, where the TS and VS of the APP decreased by 38% and 39%, respectively, between the 3 and 21 d marks. In general, the 10 °C trial yielded significantly better preservation results, especially regarding the carbohydrates, proven by the stability of fructose in APP. The lowest storage temperature also allowed for the reduction of the concentration of VFAs and delayed their production. The anaerobic co-digestion batch assays demonstrated the potential that FVW purées, mainly under fresh conditions, have to improve the AD process of MSS by increasing the biogas production and total volume of biomethane by up to 19.5% and 15.2%, respectively. In any case, the storage of the peels for 7 days, at 10 °C, increased the biomethane production by up to 11% when compared with the mono-digestion of MSS, while reducing by up to 71%, approximately, the concentration of H_2S. These results thus prove the hypotheses initially established according to which the addition of FVW would increase biogas and methane production and different storage temperatures would affect some of the physicochemical parameters of FVW and consequently create differences in the production of biogas and methane.

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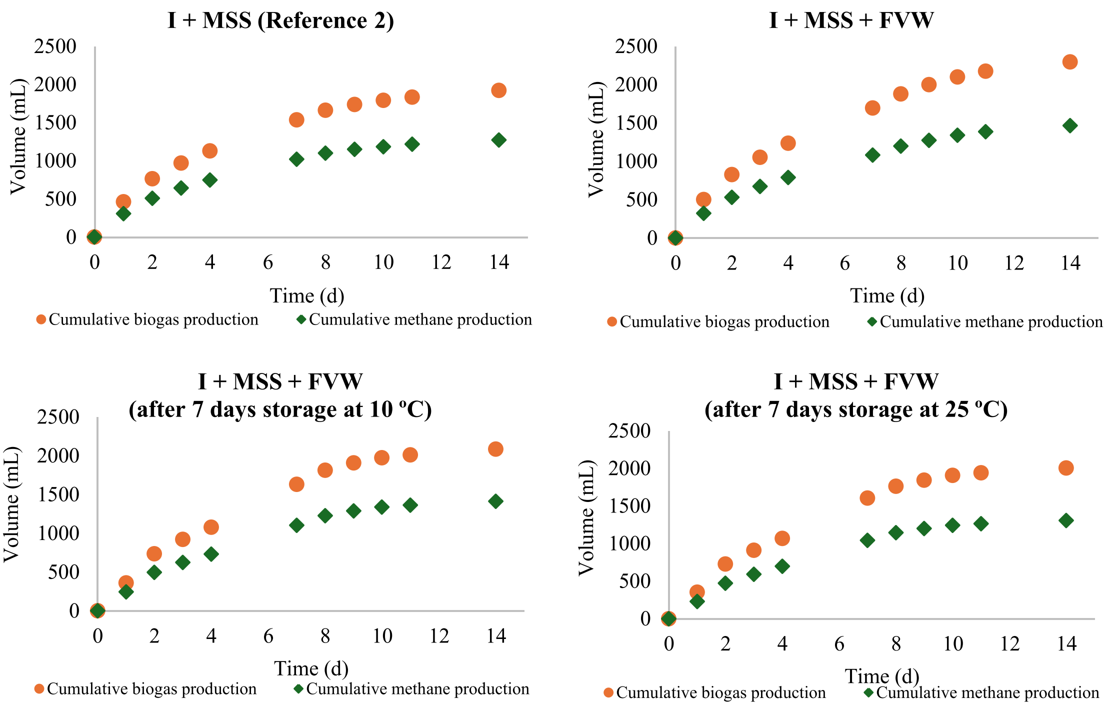

Fig. 6. Biogas and methane cumulative productions of the AD and AcoD batch assays (I: Inoculum; MSS: Municipal sewage sludge; FVW: Fruit and Vegetable Wastes) (*n* = 3).

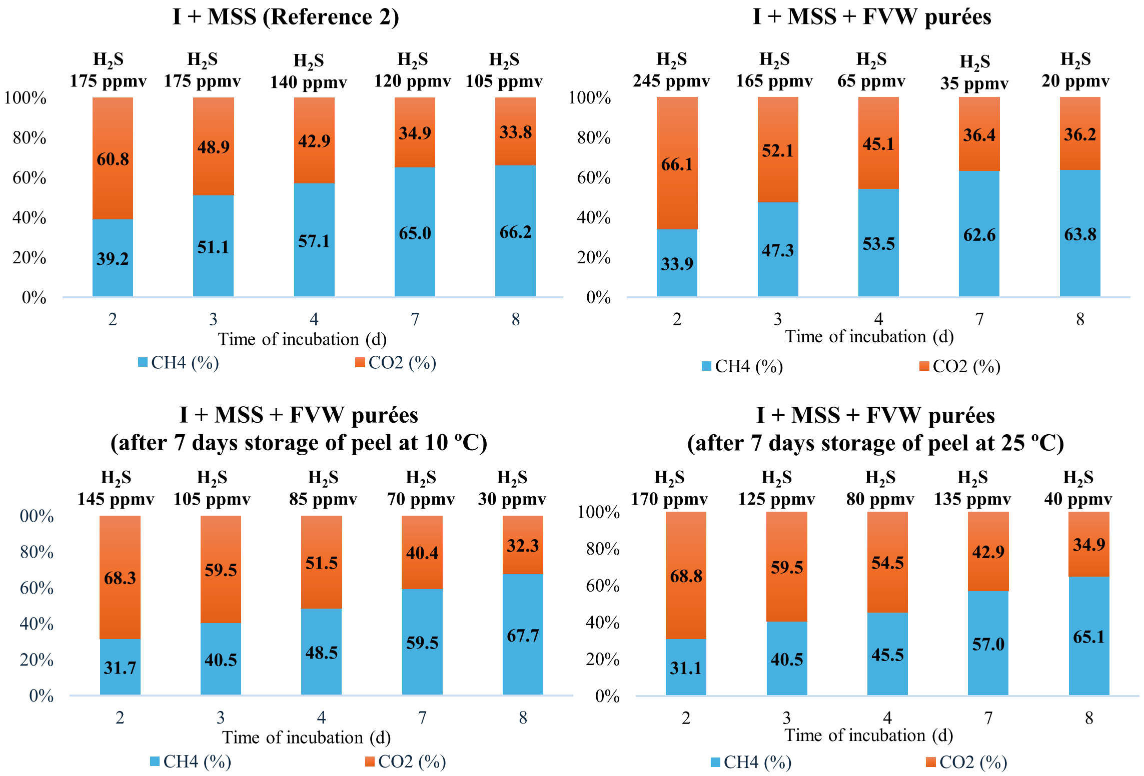

Fig. 7. Average biogas composition (% v/v for CH_4 and CO_2; ppmv for H_2S) of the AD and AcoD batch assays (I: Inoculum; MSS: Municipal sewage sludge; FVW: Fruit and Vegetable Wastes) (*n* = 3).

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Declaration of generative ai in scientific writing

During the preparation of this work, the authors did not use any AI tool to manage experimental data or to write the paper.

CRediT authorship contribution statement

André Azevedo: Writing – original draft, Resources, Methodology, Investigation, Conceptualization. **Nuno Lapa:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Margarida Moldão:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Jorge Gominho:** Writing – review & editing, Resources, Methodology, Conceptualization. **Elizabeth Duarte:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nexus.2024.100354.

Data availability

The data that has been used is confidential.

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