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Does seasonality of feedstock affect anaerobic digestion?

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Abstract

The feedstock seasonality has been poorly studied in the anaerobic digestion process. The seasonality could disturb the digestion process stability, mainly for fruit and vegetable waste. In this study, three seasonal waste mixtures generated in wholesale markets were reduced to 10, 6, and 4 mm to assess the influence of seasonality and particle size reduction on anaerobic biodegradability. The methane yield ranged between 298 and 465 mL CH_4 g VS^{-1} (volatile solids). Waste mixtures produced in spring at 10-mm particle size presented higher methane production than in autumn/winter and summer, i.e., 32% and 61%, respectively. Methane production decreased with reducing particle size for waste produced in spring from 482 ± 12 to 310 ± 1 mL CH_4 g VS^{-1}. In contrast, waste produced in autumn/winter and summer did not show high differences among different sizes. Despite these differences, mixtures with the smallest particle size presented the highest methane production rate.

Keyword Bioprocess · Fruit and vegetable waste · Mechanical treatment · Valorization · Wholesale market

1 Introduction

According to the Food and Agriculture Organisation of the United Nations, at least one-third of the food produced for human consumption is globally lost or wasted annually. Horticultural waste is the main produced waste, reaching up to 60% of the total food waste [1]. The high quantity of horticultural waste results from various stages, such as harvesting, transport, storage, marketing, and processing. A high percentage of fruit and vegetable waste (FVW) occurs in wholesale fresh food markets [2]. For instance, according to the study carried out by Zia et al. [3], the fruit and vegetable wholesale markets of New Delhi and Mumbai in India had an annual arrival of 3,120,000 and 2,920,000 tons, respectively, of fruit and vegetables in the year 2018. Of these, 181,000 and 170,000 tons of fruit and vegetables, respectively, were wasted, i.e., around 6% of the total volume. Overall, 18–30% is discarded worldwide as FVW in fruit and vegetable markets [4].

The production and composition of FVW generated in these markets vary considerably depending on the season. The storage during certain times of the products prior to their selling on the market can also influence the FVW production. Fruits and vegetables are rapidly degraded due to the high moisture and readily biodegradable organic matter they contain, i.e., mainly sugar and hemicellulose, and, to a lesser extent, cellulose, lignin, and other nutrients [3, 5]. The putrefaction of the FVW can even be accelerated when they show signs of mechanical damage or overripeness [6]. Another influential factor would be the climate of the area, as depending on the ambient

Highlights
- The seasonality and particle size influence of FVW on the AD process were evaluated.
- The SpM had higher methane production at the same particle size than the AWM and SM.
- Mixtures with the smallest particle size had the highest methane production rate.
- Methane production decreased with reducing particle size only for SpM.

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temperature, the putrefaction of the product might be accelerated [7]. Despite the FVW management challenge, FVW waste could also be an opportunity for wholesale markets. Considerable and well-localized quantities of FVWs are generated in these markets, which would favor the ease of separate collection. Therefore, it would provide an excellent opportunity to develop more efficient management technologies [8].

In recent years, technologies such as composting, incineration, pyrolysis, anaerobic digestion, and enzymatic treatments, among others, have been proposed to treat fruit and vegetable waste generated in wholesale markets widely used for other organic waste such as agro-industrial waste or food waste [9, 10]. Among all these techniques, composting and anaerobic digestion (AD) would be the most environmentally safe [10]. Although composting is a widely used, simple, and effective technology, its implementation requires a large land area, whereas the end product, i.e., compost, has a low economic interest [11]. Conversely, AD is a promising technology for segregated organic fractions treatment of food waste and fruit and vegetable market waste [12–15]. AD allows the conversion of organic substrates into biogas, which can be used as a renewable energy source while allowing the recovery of nutrients and other materials used as organic soil amendments [6, 16].

Some studies have already investigated applying the AD process to FVWs generated in wholesale markets [4, 15, 17–20]. In these studies, constraints associated with the AD process application to FVW have been reported, such as the variability in composition due to the high dependence on seasonality, the need for pre-treatment to reduce the size of the constituent products for a homogeneous feeding particle size, or the accumulation of a high concentration of soluble organic matter because of fast hydrolysis of fruit, among others.

In recent years, some authors have assessed the variability of the composition of FVW generated in wholesale markets throughout the year and its influence on methane production by anaerobic digestion. For instance, Edwiges et al., Arhoun et al., Mozhiarasi et al., and Zia et al. have shown that the generation of FVW strongly depends on seasonality in terms of quantity and composition [2, 3, 8, 19], whereas, about methane production, Edwiges et al. [2] reported variation in methane production for different seasons of up to 40%. At the same time, Arhoun et al. [8] concluded that the differences in methane production found between the FVWs generated in each season were relatively small. In all these experiments, the authors have assessed the influence of seasonality by reducing the particle size to a unique particle size. However, Jain et al. [21] and Rocamora et al. [22] have reported particle size reduction might positively or negatively affect methane production depending on the waste or waste mixture composition. Some studies have shown that particle size reduction could release compounds considered inhibitory to the anaerobic digestion process [23–25]. Therefore, the influence of particle size reduction for such variable substrate compositions could be crucial.

The main novelty of this research is to assess whether seasonal changes in composition would influence methane production over a range of particle sizes. For that, this study will evaluate the FVW generated in wholesale markets as substrate. Thus, this research would help to provide a sustainable management method for the huge volume of waste generated in these markets, whose variable characteristics make its treatment a challenge.

2 Materials and methods

2.1 Definition and composition of fruit and vegetable waste

A total of three mixtures as model substrates of FVW, i.e., autumn and winter mixture (AWM), spring mixture (SpM), and summer mixture (SM) were used, according to the seasonal variation in the waste generated by the wholesale markets. Autumn and winter mixtures were considered one since no significant difference were observed in the waste generated during these seasons [26]. These mixtures represented the generation of FVW in the wholesale markets of Sfax (Tunisia) and Amman (Jordan) and were previously defined by Papirio et al. [26]. The compositions of the different mixtures are shown in Table 1. The products were purchased in local markets in Seville city (Spain) for mixture preparation and mixed in an adequate proportion.

2.2 Particle size reduction of fruit and vegetable wastes

Three different particle sizes have been studied, i.e., 4, 6, and 10 mm, due to the previously reported relation between the particle size and the variation of the anaerobic digestion behavior [27]. For that, the FVW mixtures were chopped to the desired particle size in the laboratory. For larger particle sizes (10 and 6 mm), a multi-functional slicer (*Seehoom*, model: B436-09) with several blades was used to cut it into cubes. For smaller particle sizes (4 mm), a 0.5-L capacity mincer equipped with three stainless steel blades was used. Each FVW was chopped for 3 min using the turbo speed function (*Moulinex*, Multi moulinette AT714G32). Then, the obtained substrates were stored in plastic bags in the freezer at − 20 °C until their use.

2.3 Anaerobic digestion experimental procedure

The anaerobic biodegradability of nine conditions, i.e., three seasonal FVW mixtures (AWM, SpM, and SM) with three

Table 1 Composition in mass percentage (% w/w) of three FVW mixtures (based on [26])

Fruit	% w/w			Vegetable	% w/w		
	AWM[a]	SpM[b]	SM[c]		AWM[a]	SpM[b]	SM[c]
Apple	5.0	5.0	5.0	Broccoli	4.0	3.0	–
Apricot	–	7.0	–	Carrot leaves	8.0	5.0	2.0
Cherry	–	–	6.0	Cauliflower leaves	5.0	4.0	2.0
Grape	–	–	6.0	Celery	3.0	2.0	1.0
Grapefruit	5.0	–	–	Coriander	2.0	2.0	1.0
Kiwi	–	–	5.0	Courgette/zucchini	3.0	3.0	3.0
Lemon	2.0	3.0	4.0	Cucumber	2.0	3.0	5.0
Loquat	–	7.0	–	Eggplant	2.0	2.0	4.0
Melon	–	–	6.0	Fennel leaves	6.0	3.0	1.5
Mandarin	6.0	–	–	Green beans	2.5	–	–
Orange	12.0	–	–	Green cabbage leaves	4.0	3.0	2.0
Peach	–	4.0	7.0	Lettuce leaves	–	3.0	2.0
Pear	4.0	6.0	6.0	Onion leaves	8.0	5.0	2.0
Pomegranate	1.5	–	–	Parsley	4.0	3.0	1.5
Strawberry	–	7.0	–	Pea (with green cover)	–	4.0	–
Tomato	7.0	8.0	14.0	Pepper	2.0	2.0	4.0
Watermelon	–	4.0	8.0	Potato	2.0	2.0	2.0
Fruit (%)	42.5	51.0	67.0	Vegetable (%)	57.5	49.0	33.0

[a]*AWM* autumn and winter mixture, [b]*SpM* spring mixture, [c]*SM* summer mixture.

different particle sizes each (10, 6, and 4 mm), were evaluated. Biochemical methane potential (BMP) tests were conducted in the anaerobic biodegradability study under mesophilic conditions (35 ± 2 °C), according to the methodology described by Raposo et al. [28]. The BMP tests were performed in Erlenmeyer flasks (total volume of 250 mL). The FVW mixtures were added to the reactors in a ratio of 2:1 (inoculum:substrate) in grams of volatile solids (VS), and enough distilled water was added to reach a working volume of 240 mL. Blanks containing only inoculum were included in triplicate to take into consideration the endogenous methane production. The reactors were immersed in a water bath with a circulation thermostat (*JULABO*) to maintain the operating temperature and hermetically sealed with a rubber stopper after nitrogen flashing to ensure the anaerobic conditions. The methane production was measured using 1-L gasometers submerged in 2 N NaOH solutions. Due to NaOH property of chemically absorbing the CO_2 present in the biogas, a correct methane volume measurement can be obtained by liquid displacement. Based on methane production, the biodegradability was calculated against the theoretical maximum methane production that would be stoichiometrically produced, i.e., 1 g COD = 382 mL CH_4 at 25 °C and 1 atm [27].

A fresh sludge from an industrial anaerobic reactor from "COPERO" (Seville, Spain) wastewater treatment plant was used as an inoculum source. The main anaerobic inoculum characteristics were pH = 7.8 ± 0.1; alkalinity = 8220 ± 260 mg $CaCO_3$ L^{-1}; total solids (TS) = 35 ± 0 g kg^{-1}; and volatile solids (VS) = 19 ± 0 g kg^{-1}.

2.4 Kinetic study

The mathematical adjustment and the kinetic parameters for the anaerobic processes from the experimental data obtained were determined through a non-linear regression using the software SigmaPlot (version 14.5). The BMP tests of the nine FVW mixtures were simultaneously performed to ensure that the initial activity of the inoculum was similar in all the cases. A first-order kinetic model for the different substrates was used, according to the following expressions (Eqs. (1) and (2)) [29, 30]:

$$G = G_{max} \bullet \left(1 - e^{-kt}\right) \tag{1}$$

$$R_m = G_{\max} \times k \tag{2}$$

where G (mL CH_4 g VS^{-1}) is the cumulative specific methane production, $G_{\max}$ (mL CH_4 g VS^{-1}) is the ultimate specific methane production, k (day^{-1}) is the specific rate constant or apparent kinetic constant, t (day) is the time, and R_m (mL CH_4 g VS^{-1} day^{-1}) is the methane production rate.

2.5 Chemical analyses

The following chemical analyses were applied for the nine FVW mixtures characterization and inoculum, just as for the final effluents from each BMP test. The determination of pH, alkalinity, the concentration of total solids (TS), mineral solids (MS) and volatile solids (VS), total chemical oxygen demand (tCOD), and soluble chemical oxygen demand (sCOD) following the recommendations of the APHA were carried out [31]. Anthrone colorimetric method was used to determine total water-soluble carbohydrates using a spectrophotometer [32]. Results were expressed as a gram of glucose equivalents per kilogram of FVW mixture. A previous water extraction widely used for soluble compounds analysis in composted materials was applied to analyze soluble COD and total water-soluble carbohydrates [33]. Elemental C and N were determined through a combustion carbon and nitrogen determinator (*LECO* CN828) by Dumas's method and following the recommendations of the APHA [31]. Prior to the determination, the samples were dried. The biogas composition of reactors (CH_4, CO_2, O_2, N_2, and H_2) was analyzed using a gas chromatograph *Shimadzu* GC-2014. The gas chromatograph was equipped with a packed column ShinCarbon ST 100/120 (*RESTEK*) of 2 m×1 mm of 1/16″ OD Silco and a thermal conductivity detector (TCD) at 200 °C. The oven temperature gradually increased from 50 to 110 °C at a rate of 14° C min^{-1} and from 110 to 156 °C at 6.8 °C min^{-1}, being 11.05 min, the total time of the method applied. Helium was used as carrier gas with a 10 mL min^{-1} flow. Each biogas sample was taken from the BMP flask using 1-mL plastic syringes fitted with a special Mininert valve for Luer-Lock (*Supelco*) for gases.

3 Results and discussion

3.1 Influence of seasonality and particle size on substrate composition

The physicochemical characterizations of the FVW mixtures, i.e., AWM, SpM, and SM, are shown in Table 2. The pH values of the FVW mixtures ranged between 3.5 and 4.5, with no marked differences. These acid pH values were a consequence of the composition of the FVW mixtures, where the low pH of the fruits, some of their citrus, are the main contributors to such acid pH (Table 1). Due to high water content, the moisture values of the FVW mixtures ranged between 86 and 92%. The VS/TS ratio values of the FVW mixtures ranged between 90 and 92% (Table 2). Values in the same pH range and VS/TS ratio were reported for other fruit and vegetable waste mixtures. For instance, samples collected from Malaga's fruit and vegetable wholesale market (Spain) at four different seasons showed average values of 4.1 ± 0.5 and 93.8 ± 2.0% of pH and VS/TS ratio, respectively [8]. Similarly, samples collected monthly from Municipal Central Supply of Foz do Iguaçu (Brazil) showed average values of 4.2 ± 0.2 and 92.0 ± 1.3% of pH and VS/TS ratio, respectively [2].

C/N ratios were similar for AWM and SpM mixtures, with values around 23 (Table 2). The lowest nitrogen concentration in SM resulted in a C/N ratio slightly higher than the other two mixtures, ranging from 27 to 30 (Table 2). The C/N ratio values of the FVW mixtures were within the optimal range for the anaerobic digestion process, i.e., 17–32 [34]. It advocates mixing of the different fruit and vegetable waste generated in each season, since some of them alone present C/N ratio values out of the desirable range. For instance, carrot leaves, lettuce, onion leaves, cabbage, and pepper usually present low C/N ratio values, i.e., 7, 10, 11, 12, and 15, respectively, whereas potato tops, whole carrots, potatoes, cucumber, and tomato present high C/N ratio values, i.e., 25, 27, 35, 68, and 152, respectively [35–38]. In general, vegetable waste from leaves and stems seems to have higher N percentages than fruit wastes.

Table 2 Physicochemical characterization of the fruit and vegetable waste mixtures

Season	AWM[a]			SpM[b]			SM[c]		
Particle size (mm)	≤ 4	≈ 6	≥ 10	≤ 4	≈ 6	≥ 10	≤ 4	≈ 6	≥ 10
pH	4.5 ± 0.1	3.7 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	3.6 ± 0.1	3.7 ± 0.1	4.2 ± 0.1	3.9 ± 0.1	4.2 ± 0.1
Moisture (%)	88.4 ± 0.3	89.8 ± 0.1	86.7 ± 0.6	88.9 ± 0.3	90.9 ± 0.1	89.5 ± 0.1	89.6 ± 0.4	90.5 ± 0.6	91.4 ± 0.3
Total solid (g kg^{-1})	116 ± 3	102 ± 1	133 ± 6	111 ± 3	91 ± 1	105 ± 1	104 ± 4	92 ± 1	86 ± 3
Total mineral solid (g kg^{-1})	11 ± 0	9 ± 1	11 ± 1	9 ± 0	7 ± 0	9 ± 1	6 ± 0	7 ± 0	7 ± 0
Total volatile solid (g kg^{-1})	105 ± 3	94 ± 1	121 ± 5	102 ± 4	83 ± 1	97 ± 2	98 ± 4	85 ± 0	80 ± 3
tCOD[d] (g O_2 kg^{-1})	151 ± 1	159 ± 2	176 ± 4	127 ± 1	107 ± 2	136 ± 3	115 ± 1	166 ± 5	123 ± 3
sCOD[e] (g O_2 kg^{-1})	63 ± 1	54 ± 1	56 ± 0	62 ± 1	57 ± 0	63 ± 1	75 ± 1	74 ± 1	73 ± 1
sCOD[e]/tCOD[d] ratio	0.4	0.3	0.3	0.5	0.5	0.5	0.6	0.4	0.6
Carbohydrates (g glucose eq. kg^{-1})	44 ± 1	16 ± 0	29 ± 0	40 ± 1	21 ± 0	35 ± 1	55 ± 0	36 ± 1	48 ± 1
C (%)	44.8 ± 0.3	46.9 ± 0.2	46.5 ± 0.3	46.0 ± 0.3	47.0 ± 0.1	47.3 ± 0.3	46.9 ± 0.4	48.9 ± 0	49.3 ± 0.5
N (%)	2.34 ± 0.05	2.02 ± 0.0	1.85 ± 0.1	1.98 ± 0.03	2.14 ± 0.0	2.38 ± 0.1	1.72 ± 0.04	1.77 ± 0.0	1.65 ± 0.0
C/N ratio	19	23	25	23	22	20	27	28	30

[a]*AWM* autumn and winter mixture, [b]*SpM* spring mixture, [c]*SM* summer mixture, [d]*tCOD* total chemical oxygen demand, [e]*sCOD* soluble chemical oxygen demand.

Higher soluble organic matter concentrations, measured as sCOD and water-soluble carbohydrates, were observed for SM compared to SpM and AWM (Table 2). SM had an sCOD mean value of 74 ± 1 g O_2 L^{-1}, while AWM and SpM had average values of 58 ± 1 g O_2 L^{-1} and 61 ± 1 g O_2 L^{-1}, respectively. The differences observed in the soluble organic matter concentration of the FVW mixtures could be due to the higher percentage of fruit in the waste mixture of SM compared to the SpM and AWM, i.e., 67.0%, 51.0%, and 42.5%, respectively (Table 1). Fruits contain higher concentrations of soluble organic matter, such as simple and highly biodegradable carbohydrates, than vegetables [6]. The particle size reduction process solubilized 15% of the organic matter, measured as sCOD, in the AWM mixture. This behavior was not observed for the other two mixtures (Table 2). Similarly, Izumi et al. [24] reported a 40% improvement in the solubilization of sCOD after particle size reduction through a beads mill for food waste.

3.2 Influence of seasonality and particle size on the anaerobic digestion process

3.2.1 Methane production

The accumulated methane production (mL CH_4 g VS^{-1}) during the experimental time for each FVW mixture is shown in Fig. 1A, B, and C. These figures show no lag phase for neither the mixtures nor any particle sizes studied. All FVW mixtures had enough readily biodegradable soluble material to start methane production instantly. Although vegetables (including leaves and stems) have a high lignin content [36], fruits have a greater quantity of readily biodegradable compounds such as carbohydrates, i.e., 75% of its composition [3]. The initial degradation of fruits, present in all cases, causes a negligible lag phase.

According to Fig. 1, the seasonality of the substrates and particle size reduction resulted in differences in methane production. For the 10-mm particle size (Fig. 1A), a marked difference was observed in the methane production of the seasonality mixtures, reaching the highest value for SpM, i.e., 482 ± 12 mL CH_4 g VS^{-1}. This methane production was 32% and 61% higher than the values obtained for SM and AWM, respectively (Fig. 1A). This methane production difference was not observed for 6- and 4-mm particle sizes (Fig. 1B, C), with less than 15% differences among the mixtures. Similar studies have reported contradictory results on the influence of seasonality on methane production [2, 8].

The influence of particle size on methane production was mainly observed for SpM. Reducing the particle size of SpM from 10 to 6 and 4 mm, methane production decreased by 18% and 56%, respectively (Fig. 1). This decrease might be attributed to the release of undesirable compounds at reducing the particle size of the mixture. Some authors

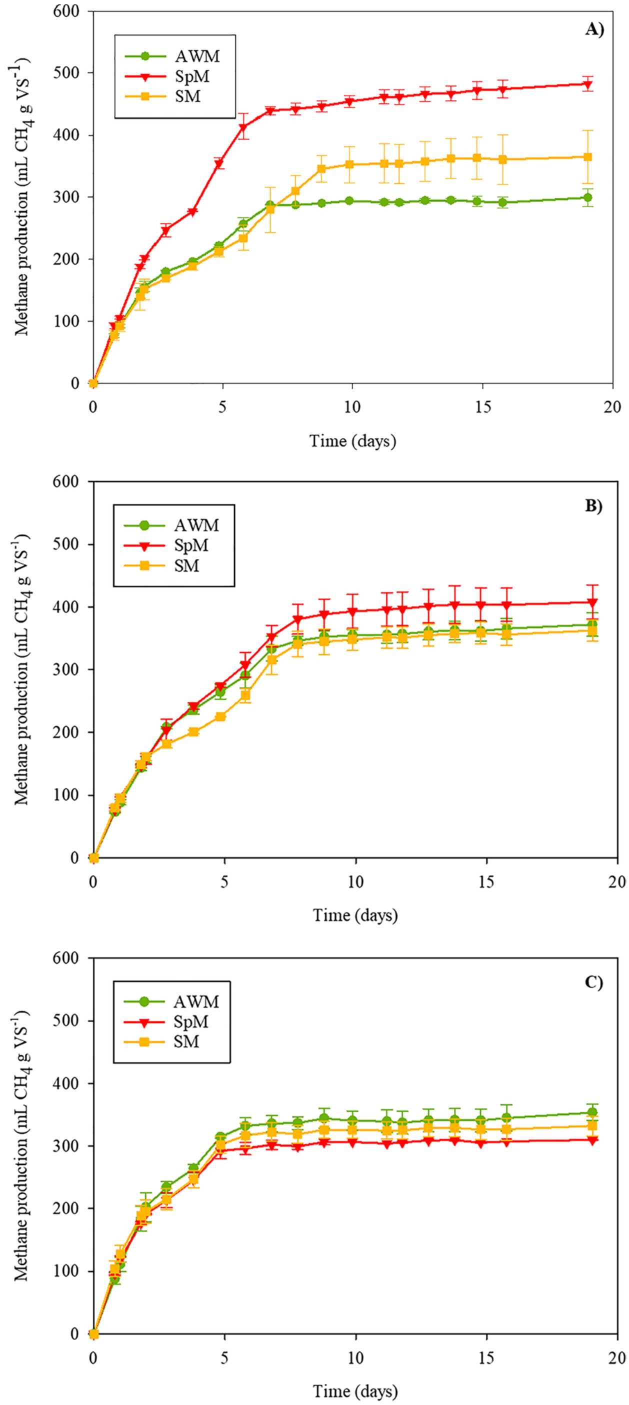

Fig. 1 Methane production (mL CH_4 g VS^{-1}) of the three FVW mixtures (AWM, SpM, and SM) according to the particle size **A** 10 mm, **B** 6 mm, and **C** 4 mm (AWM, autumn and winter mixture; SpM, spring mixture; and SM, summer mixture)

have reported that a high reduction in particle size leads to the release of inhibitory compounds, resulting in a decrease in methane production [22]. SpM mixture had a balanced composition of fruits and vegetables, i.e., 51.0 and 49.0% (Table 1). However, some fruits in SpM as apricot, loquat, or strawberry were not present in AWM and SM. These fruit wastes contain a high amount of bioactive compounds such as carotenoids (e.g., lycopene), polyphenols (e.g., phenolic acids and flavonoids), and volatile compounds (e.g., linalool, limonene, α-terpineol, or menthone), which are responsible for the typical aroma of these fruits [39–42]. Many of these bioactive compounds exhibit potent antibacterial, antimicrobial, and antioxidant activities [40, 41, 43], among which some have been identified as potential inhibitors of anaerobic digestion [23, 44, 45]. Besides, particle reduction can accelerate the hydrolysis and acidogenesis steps, resulting in excessively high organic loading in the anaerobic digestion reactor [46]. Izumi et al. [24] reported that excess particle size reduction to smaller than 0.7 mm caused an accumulation of volatile fatty acids in biogas production from food waste. A similar problem was also described by Ruiz and Flotats [25] with orange peel waste. Grinding the citrus peel released limonene, a terpene compound, into the medium and increased its inhibitory effect.

Based on these results, it could be deduced that the observed difference in methane production for SpM with decreasing particle size would be due to the release of compounds that could act as inhibitors of the process. In contrast, methane production for SM and AWM did not show high differences due to the variation of the particle size, i.e., the average methane production value for the three particle sizes was 353 ± 18 mL CH_4 g VS^{-1} and 342 ± 38 mL CH_4 g VS^{-1}, respectively (Fig. 1). For AWM, even though the difference in methane production was minor than SpM, an improvement in methane production of about 24% was observed when reducing the particle size from 10 to 4 mm. It was supposed that AWM had a higher content of lignocellulosic matter, which would be provided by the higher vegetable and citrus fruit waste (Table 1). Methane production improved for AWM, as it did for sCOD, reducing the particle size from 10 to 4 mm. According to Atelge et al. [47], the negative effect on anaerobic digestion of lignocellulosic material can be enhanced by applying a decrease in particle size. Using a particle size reduction process would increase the availability of microorganisms and provide a higher specific surface area of the substrates, thus enhancing methane production [27]. As previously reported by Jain et al. [21] and Rocamora et al. [22], and corroborated in the present research, particle size can positively or negatively affect methane production depending on the composition of the waste or waste mixture.

The biogas composition, for all conditions, was similar, with around 50:50 CH_4 and CO_2 ratio (Table 3). According to Schnürer and Jarvis [48], the biogas composition depends on the digested material and the operation of the process. Still, biogas typically has a CH_4 content between 45–85% and 15–45% CO_2, indicating that the substrate degradation process was carried out under stable conditions.

3.2.2 Stability and substrate biodegradability

Table 3 shows the results obtained in the characterization of the final effluents of the BMP test for the FVW mixtures. After the anaerobic digestion process, the pH values ranged between 7.2 and 7.7 for all the tested conditions. These values were optimal for the anaerobic digestion process, according to Mozhiarasi et al. [10], in particular for

Table 3 Characterization of the final effluents of the BMP tests

Season	AWM[a]			SpM[b]			SM[c]		
Particle size (mm)	≤ 4	≈ 6	≥ 10	≤ 4	≈ 6	≥ 10	≤ 4	≈ 6	≥ 10
pH	7.3 ± 0.1	7.5 ± 0.0	7.7 ± 0.4	7.3 ± 0.1	7.6 ± 0.0	7.4 ± 0.1	7.2 ± 0.0	7.6 ± 0.0	7.7 ± 0.1
Alkalinity (mg $CaCO_3$ L^{-1})	6 610 ± 150	6 885 ± 210	7 065 ± 50	5 855 ± 155	7 160 ± 375	7 015 ± 225	6 260 ± 55	6 775 ± 160	6 990 ± 120
Total solid (TS) (g kg^{-1})	21 ± 1	22 ± 1	21 ± 0	21 ± 2	22 ± 1	21 ± 1	20 ± 1	21 ± 1	22 ± 1
Volatile solid (VS) (g kg^{-1})	12 ± 0	12 ± 0	11 ± 1	12 ± 1	12 ± 1	11 ± 0	11 ± 0	11 ± 0	12 ± 1
sCOD[d] (mg O_2 kg^{-1})	525 ± 20	580 ± 40	565 ± 20	470 ± 40	555 ± 20	710 ± 65	490 ± 35	650 ± 60	565 ± 5
Exp. production (ml CH_4 g VS^{-1})	354 ± 13	372 ± 19	299 ± 15	310 ± 1	408 ± 27	482 ± 12	332 ± 16	362 ± 17	365 ± 43
Teo. production (ml CH_4 g VS^{-1}; based on COD)	551	645	552	475	492	536	451	745	587
Biodegradability (%; based on COD)	64	58	54	65	83	90	74	49	62
Biogas composition (%; CH_4:CO_2)	52:48	51:49	63:37	50:50	53:47	53:47	53:47	53:47	56:44

[a]*AWM* autumn and winter mixture, [b]*SpM* spring mixture, [c]*SM* summer mixture, [d]*sCOD* soluble chemical oxygen demand.

the methanogenic activity, which requires a pH between 6.5 and 8.0 to optimize their function [4]. The alkalinity values of the reactors were between 5800 and 7100 mg $CaCO_3$ L^{-1} (Table 3). This concentration is high compared to the concentration recommended in the literature, which would be between 2000 and 4000 mg $CaCO_3$ L^{-1} for plant-based waste [49]. The initial alkalinity provided by the inoculum, i.e., 8000 mg $CaCO_3 \cdot L^{-1}$, was the reason for the high alkalinity concentration in the final BMP test. The pH and alkalinity values provided by inoculum and substrates ensured that the reactor conditions remained stable even though the system was not doped with a buffer solution at the beginning of the test.

The low concentration of sCOD at the end of the BMP test would also corroborate that the hydrolyzed compounds were effectively converted into methane instead of accumulated in the effluents (Table 3). The average sCOD value concentrations at the end of the BMP test for AWM, SM, and SpM were 557 ± 28, 568 ± 80, and 578 ± 122 mg O_2 L^{-1}, respectively (Table 3). The substrates' seasonality and particle size reduction resulted in differences in the biodegradability values. The anaerobic biodegradability of the substrates strongly varied between 49 and 90% (Table 3). For the 10-mm particle size, a marked difference can be observed in the seasonality mixtures' biodegradability (Table 3). The highest values of biodegradability corresponded with SpM, reaching up to 45 and 67% higher than the obtained for SM and AWM, respectively. For 4-mm particle size, the difference of the FVW mixtures was less marked, the biodegradability variation range being less than 16% (Table 3). Similar biodegradability results by Edwiges et al. [2] for FWV substrates generated in a Brazilian wholesale market were reported, which showed minimum and maximum values of 63 and 98%, respectively, with a mean value of 79 ± 12%. The lowest biodegradability values corresponded to samples with the highest lignocellulosic content. The influence of particle size on anaerobic biodegradability was observed mainly for SpM, as it was observed for methane production (Table 3, Fig. 1). Reducing the particle size of SpM from 10 to 4 mm decreased anaerobic biodegradability by 39%. By contrast, biodegradability increased by reducing particle size from 10 to 4 mm by around 20% for SM and AWM.

3.2.3 Kinetics of methane production

Figure 2 shows the values of methane production rate (R_m, mL CH_4 g VS^{-1} day^{-1}) for each FVW mixture. The results in Fig. 2 indicate an increase in R_m values with decreasing particle size for AWM and SM. However, for SpM, this tendency did not occur. Furthermore, R_m in the FVW mixtures with smaller particle sizes, i.e., 4 mm, had a higher methane production rate (Fig. 2). This behavior could be due to the hydrolysis stage being often accelerated by providing a pre-treatment to the substrates [13]. The increase in specific surface area due to a reduction in particle size improved the accessibility of the microorganisms to the substrate and, thus, facilitated microbial activity [27]. Similar results were also obtained with other organic solid wastes; i.e., the methane production rate improved with decreasing particle size. For instance, De la Rubia et al. [50] studied the effects of mechanical pre-treatment of organic fraction of municipal solid waste (OFMSW) after grinding and screening on anaerobic digestion using BMP tests. They reported that the maximum methane production rate, R_m, was 2.4 times higher for ground plus screened OFMSW than the value for the untreated OFMSW.

The condition that did not follow the described trends was SpM at 10 mm, as it had a value of 132 ± 6 mL CH_4 g VS^{-1} day^{-1}, which was higher than SpM at 6 mm. As previously described for methane production, this fact could be due to the release of compounds that could inhibit the AD process [22].

4 Conclusions

The influence of seasonality and particle size reduction on substrate composition and the anaerobic digestion process of Mediterranean fruit and vegetable markets waste was evaluated. The evaluation of the seasonal mixtures showed that waste mixtures produced in spring had higher methane production than AWM and SM, i.e., 32% and 61%, respectively, at larger particle sizes. Methane production decreased with reducing particle size for waste mixtures produced in spring from 482 ± 12 to 310 ± 1 mL CH_4 g

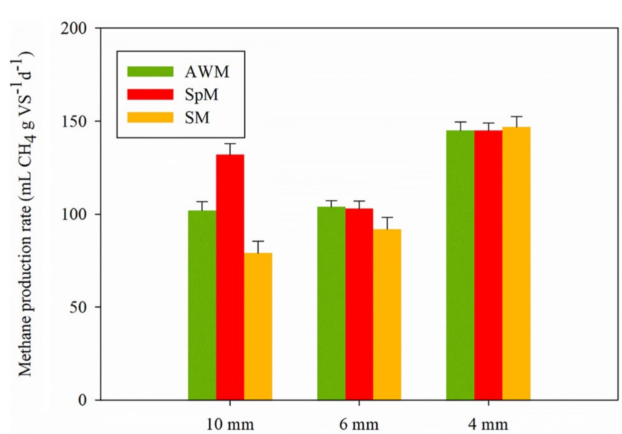

Fig. 2 Methane production rate (mL CH_4 g VS^{-1} $day^{-.1}$) of the three FVW mixtures according to the particle size (AWM, autumn and winter mixture; SpM, spring mixture; and SM, summer mixture)

VS^{-1}, whereas waste mixtures produced in autumn/winter and summer did not show high differences.

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Data availability The data that support the findings of this study are available from the corresponding author, A. Trujillo-Reyes, upon reasonable request.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

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