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# EXTRACTION OF PHENOLIC COMPOUNDS AND PRODUCTION OF BIOMETHANE FROM STRAWBERRY AND RASPBERRY EXTRUDATES

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## Abstract

This study proposes a biorefinery approach system to treat two berry extrudates generated by the berry-tasted products industry. The berry extrudates studied were strawberry extrudate (SE1 and SE2) and raspberry extrudate (RE), both of them processed in the same industrial plant. The proposed biorefinery approach consists in the extraction of bioactive compounds after hydrothermal pre-treatment followed by anaerobic digestion of the remaining biomass after extraction. A high concentration of valuable phenolic compounds was extracted from each extrudate through the absorption-desorption processes, i.e. 876, 392 and 2,402 mg of gallic acid equivalents/kg extrudate in SE1, SE2 and RE, respectively. Anaerobic digestion of the remaining biomass after extraction led to high methane production, between 371 and 503 mL $CH_4$/g VS. The economical evaluation showed that the proposed biorefinery

approach would offer higher benefits than just anaerobic digestion of the untreated extrudate, although this last option would be economically feasible as well.

**Keywords:**
Anaerobic digestion; economical assessment; hydrothermal pre-treatment; phenol recovery; valorization.

## 1. Introduction

Strawberry and raspberry are a non-climacteric fruit with an attractive color and a delicious taste, being among the most commonly consumed berries both as fresh dessert fruit and processed [1,2]. During the 2016 season, more than 12 million tons of berries were obtained in the world [3]. Currently, a large variety of berries is produced, among which are: strawberries, raspberries, blueberries, cranberries, blackberries, etc., but the berries with the highest worldwide production are strawberries (9 million tons) and raspberries (795,250 tons) [3]. Most of production of berries is sold in the fresh market. Nevertheless, another use is the production of berry concentrates, used to produce transformed products such as jam, juice or yogurt among others. The production of these concentrates entails the generation of a residual fraction called berry extrudates formed by the fibrous part and the achenes. Currently, 21 and 5% of strawberries and raspberries production, respectively, are destined to the manufacture of transformed products [4,5]. At present, strawberry and raspberry extrudates are dumped in landfills.

Strawberry and raspberry extrudates still contain most of the compounds present in the corresponding berry, many of which are bioactive compounds. These berries are an important source of many nutrients, including essential minerals, vitamin C, fatty acids, sugars, as well as a wide range of phenolic compounds [2,6,7]. The phenolic anthocyanins and ellagitannins are the major antioxidant phytochemicals present in strawberries and raspberries [7,8]. Other compounds present in these berries are uronic acids, which indicates the existence of acidic carbohydrates or pectins [9]. Out of all compounds contained in the berries, phenolic compounds are the most interesting due to their strong antioxidant capacity [10]. Therefore, an interesting management option might be the recovery of these phenolic bioactive compounds still present in the strawberry and raspberry extrudates.

Solubilization of the phenolic compounds from the extrudates is necessary in order to recover them. Several methods have been recently proposed to extract phenolic

compounds from strawberry such as high hydrostatic pressure extraction, microwave hydro-diffusion and gravity, Pulsed Electric Field with solvent and hydrothermal treatments [13, 15]. Hydrothermal pre-treatment has been previously proposed for solubilization and extraction of phenols from other agro-industrial waste such as olive mill solid waste [11], the bagasse of the wine [12] or strawberry extrudate [13]. It should be kept in mind that most phenolic compounds present in strawberry and raspberry extrudates are thermosensitive [14], therefore, after the hydrothermal pre-treatment phenolic compounds might be degraded to other compounds of greater or lesser interest. It is also important to mention that hydrothermal pre-treatment at high temperatures could release soluble-sugar derived byproducts such as furfural or 5-hydroxymethylfurfural (5-HMF), which can be inhibitory for the anaerobic digestion processes at certain concentrations [11-13].

The phenolic compounds represent a minor percentage of the total volume of the berry extrudate, therefore, a further stabilization of the remaining biomass would be still required for a complete treatment. A very promising option is the combination of the extraction of phenolic compounds with a further anaerobic digestion of the remaining biomass. The stabilized digestate produced after anaerobic digestion might be used as fertilizer component [15]. The biogas produced in the anaerobic digestion might supply the energy needed to carry out the hydrothermal pre-treatment, thus closing the cycle of use of this waste. The combination of the extraction of phenolic compounds followed by anaerobic digestion of the remaining biomass can be considered as a very promising biorefinery approach.

The aim of this study was to evaluate the valorization of one raspberry and two different strawberry residual extrudates. These extrudates were very different among them but generated in the same industrial plant. The proposed biorefinery approach consisted in a hydrothermal pre-treatment, followed by extraction of phenolic compounds and subsequent anaerobic digestion process of the remaining biomass.

## 2. Materials and methods

### *2.1. Strawberry and raspberry extrudates*

The company supplying the strawberry and raspberry extrudates used in the assays was “HUDISA S.A.” located in Lepe (Huelva, Spain). Strawberry extrudate was obtained in two different campaigns. Strawberry Extrudate 1 (SE1) in 2016-2017 season and Strawberry Extrudate 2 (SE2) in 2017-2018 season, while Raspberry Extrudate

(RE) was obtained in the 2017-2018 season. In the industrial process, SE1 was sieved with a 1.5 mm sieve, while SE2 and RE with a 0.5 mm sieve, and SE2 was subjected to pasteurization. Strawberry and raspberry extrudates were kept under freezing conditions (-20 ºC) before their use in order to prevent their self-fermentation and deterioration.

The different mesh size used and the occasional use of pasteurization was due to the different requirements of the final products that the berry processing company was producing at each moment. The present study used different berries with different mesh size to have a broader screening of the potential of the proposed biorefinery approach for different by-products derived from the same berry processing industry.

*2.2. Hydrothermal pre-treatment*

Hydrothermal pre-treatments were performed using a steam treatment batch reactor (100 L) and can reach temperatures up to 190 ºC and 1.2 MPa of maximum pressure. Strawberry and raspberry extrudates were heated directly by steam injection and indirectly by a heating jacket. Samples (12.59 kg) were treated at 150 ºC in the reactor for 60 min. After the pre-treatments, samples were cooled to 25 ºC and then centrifuged at 4700g/1450 rpm (Comteifa, S. L., Barcelona, Spain). After centrifugation, a Solid Phase (SP) and a Liquid Phase (LP) were separated from each pre-treated extrudate. Samples were stored at 4 ºC before characterization.

*2.3. Extraction of phenolic compounds*

Phenolic compounds extraction from 2 liters of LP was carried out using a column of 4.5 cm in diameter and 140 cm in height, filled with 100 mL of Amberlite XAD16 adsorbent resin dissolved in water, with a bed of 12 cm. After extraction, a De-phenolized Liquid Phase (DLP) was obtained. The compounds retained in the resin were extracted with 200 mL ethanol 80% (v/v) and 40 mL ethanol 96%.

*2.4. Anaerobic inoculum*

Sludge from the anaerobic treatment of wastewater from "HEINEKEN SPAIN, S. A." (Seville, Spain) beer industry was used as an inoculum source. Two samples of the same sludge were taken at different times, which were called Inoculum 1 and Inoculum 2. The main anaerobic inoculum characteristics were for Inoculum 1: pH = 7.1 ± 0.1; alkalinity = 2,505 mg $CaCO_3$/L; VS = 55,585 ± 2,690 mg/kg; and for Inoculum 2: pH = 7.8 ± 0.1; alkalinity = 2,490 mg $CaCO_3$/L; VS = 35,610 ± 280 mg/kg. Inoculum 1 was used for the test with SE1, while Inoculum 2 was used in the tests with SE2 and RE.

*2.5. Anaerobic digestion experimental procedure*

The anaerobic digestion of untreated strawberry and raspberry extrudates and the mixtures of the phases obtained after the pre-treatment and the extraction of phenolic compounds (SP+DLP) was evaluated by biochemical methane potential (BMP) tests. The mixtures SP+DLP were made in relation to the mass generated of each phase after the separation of solid and liquid phases, with a ratio of 64:36, 74:26 and 85:15 in volatile solids (VS) in SE1, SE2 and RE, respectively. BMP tests were carried out in 250 mL Erlenmeyer flasks using a working volume of 240 mL. In all cases, an inoculum/substrate ratio of 2:1 based on VS was used. BMP reactors were sealed, and the headspace of each flask was flushed with nitrogen at the beginning of the assay. All reactors were submerged in a thermostated bath under mesophilic conditions (35 ºC), and continuously stirred by magnetic bars to favor mass transfer between inoculum and substrate. All assays were carried out in triplicate. The produced biogas was passed through a 2 N NaOH solution to capture $CO_2$ and to let methane go through. The volume of methane was measured daily by liquid displacement. The BMP tests were carried out in the time interval required (c.a. 24-day period) to exhaust methane production.

*2.6. Kinetic study*

The kinetic parameters and the mathematical adjustment for the anaerobic processes were determined from the experimental data obtained, by means of a non-linear regression using the software SigmaPlot (version 11.0). Two kinetics models were used for the different substrates, the first of them is the model of the Transfer Function (TF) (eq. (1)), which has been applied by other authors [16–18] using the following expression:

$$B = B_m * \left(1 - exp\left[\frac{R_m(t-\lambda)}{B_m}\right]\right) \qquad \text{Equation (1)}$$

where $B$ (mL $CH_4$/g VS) is the cumulative specific methane production, $B_m$ (mL $CH_4$/g VS) is the ultimate methane production, $R_m$ is the maximum methane production rate (mL $CH_4$/ (g VS/d)), $t$ (d) is the time and $\lambda$ (d) is the lag time. The second kinetics model used is the Logistic model (Sigmoidal parameter 4) (eq. (2)), which has been applied by other authors [16,19,20] using the following expression:

$$B_2 = B_0 + P/\left[1 + exp\left(-4 \cdot R_m \cdot (t - \lambda)/(P + 2)\right)\right] \qquad \text{Equation (2)}$$

where $B_2$ is the cumulative methane production during the second stage (mL $CH_4$/g VS), $B_0$ is the cumulative methane production at the star-up of the second stage (mL

$CH_4$/g VS) and should approximately coincide with the value of $B_m$ obtained at the end of the first stage, $P$ is the maximum methane production obtained in the second stage (mL $CH_4$/g SV), $R_m$ is the maximum methane production rate (mL $CH_4$/g SV d) and $\lambda$ (d) is the lag time. Additionally, $r^2$, error (%) and standard error of estimate ($\sigma_{est}$) were determined to evaluate the fit and precision of the results. Error was defined as the difference in percentage between the experimental accumulated final methane production and $B_m$ (TF) or $P+B_0$ (logistic model). In this study, it is only possible to compare the maximum methane production between the substrates and not the $R_m$ values, because of the experiments were not carried out at the same time and different inoculums were used. $R_m$ was used to compare the maximum methane production rate between the untreated and pre-treated extrudates, as the BMP of each extrudate, untreated and pre-treated, were carried out with the same inoculum at the same time.

*2.7. Chemical analyses*

The succeeding chemical analyses were used for the characterization of the strawberry and raspberry extrudates and inoculum as well as for the effluents from each BMP test at the end of the process. The concentration of total solids (TS), volatile solids (VS) and mineral solids (MS), and pH, alkalinity and elemental C and N were determined according to the recommendations of the Standard Methods of APHA [21]. pH was analyzed using a pH-meter model Crison 20 Basic. Alkalinity was determined by titration to 4.3. C and N were determined through a LECO CHNS-932 (Leco Corporation. St Joseph, MI, EEUU) elemental analyser. Chemical Oxygen Demand (COD) was determined using the method described by Raposo et al [22], while soluble COD (sCOD) was determined by the closed digestion and the colorimetric standard method 5220D [21].

2.7.1. Total phenols content

Content of total phenols was determined by Folin-Ciocalteu spectrophotometric method [23] after an extraction with methanol/water solution (80:20) at 70 ºC. Samples preparation included either centrifugation at 400g during 5 min and subsequent filtration through 0,45 µm filters [24]. Results were expressed as milligrams of gallic acid equivalents per kilogram of extrudate.

2.7.2. Total sugars and uronic acids

Antrone colorimetric method was used for determining total sugars [25] using a spectrophotometer (Biorad iMark Microplate Reader, USA). Samples preparation

included either centrifugation at 400g during 5 min and subsequent filtration through 0,45 µm filters. Results were expressed as milligrams of glucose equivalents per kilogram of extrudate.

M-Hydroxybiphenyl Chromogen Method, as described by Blumenkrantz and Asboe-Hansen [26] was used for quantifying uronic acids. Results were expressed as grams of galacturonic acid equivalents per kilogram of extrudate.

2.7.3. Individual neutral sugars

Using a method described by Lama-Muñoz, Rodríguez-Gutierrez, Rubio-Senent, and Fernández Bolaños [27] individual neutral sugars were analyzed from duplicate samples of solubilized fractions with and without initial trifluoroacetic acid (TFA) hydrolysis before to reduction, acetylation, and analysis by gas chromatography (GC).

*2.8. Antioxidant capacity*

To determine the antioxidant capacity, the following tests were carried out: antiradical activity (2,2-diphenyl-1-picrylhydrazyl (DPPH)) and reducing power (RP). The antiradical activity shows the ability to scavenge the DPPH free radical. Antiradical activity is expressed as the concentration of extract (in mg per mL) necessary to decrease the initial concentration of DPPH by 50% ($EC_{50}$) [13]. Therefore, low $EC_{50}$ values represent high antioxidant capacity. Reducing power was expressed as milligram of Trolox equivalents per mL, high reducing power indicating a high antioxidant capacity.

*2.9. Economic assessment*

A preliminary economic assessment was carried out in order to estimate the minimum sales price of the phenol extracted which allows a positive incoming costs balance. Six different cases were included. Three of them corresponding to only anaerobic digestion of the untreated extrudates. The other three include pre-treatments, phenols extraction and anaerobic digestion of each of the extrudates. In all cases, the generated biogas is used in a cogeneration system for simultaneous generation of heat and electricity. The net benefit of the different options was defined as the economic balance between operational costs and incomings from sales. Minimum sales price for the phenol extracted (expressed as €/g gallic acid equivalents) has been calculated imposing a value of zero to the net benefit. The following considerations were assumed for the economic assessment:

- Anaerobic digester and co-generation. The energy production in the anaerobic digester was obtained applying a scale-up factor of 0.85 to the experimental methane production values obtained for each case [11]. Lower calorific power of the methane was equal to 35,793 J/L [28]. The efficiency in the energy obtained through a cogeneration biogas engine was considered as 33% and 55% for electricity and thermal energy, respectively [29]. In order to reach the required operating temperature (35 ºC) a specific heat of 4.18 kJ/kg·ºC was considered to obtain the thermal energy requirement to increase the waste temperature from 20 to 35 ºC, including thermal loss of 10%. The electricity consumption was estimated employing values of $1.8 \cdot 10^3$ kJ/m$^3$ and $3.0 \cdot 10^2$ kJ/m$^3$ of reactor for pumping and stirring, respectively [30].
- Hydrothermal pretreatment. Enthalpy values of 104.9 (water 20 ºC and 1 kg/cm$^2$) and 2,745.7 kJ/kg (steam 150 ºC and 5 kg/cm$^2$) were employed to calculate the thermal energy requirement. The amount of steam was obtained experimentally for each waste: 1.93 (SE1), 0.44 (SE2) and 2.93 (RE) kg of steam/kg of extrudate. When the energy requirement is higher than the thermal energy generated in the anaerobic digester, a methane supply is used to produce the amount of consumed steam. A heat recovery system was included considering a thermal efficiency of 80% [29].
- Phenols extraction. The electricity consumption was calculated as the electricity employed for pumping using the same approach described for anaerobic digestion. Other costs for phenols extraction involve 0.50 €/kg of extrudate [11]. The phenol extraction efficiency was obtained from the reduction of gallic acid observed during the experiments in the liquid phase after the extraction process respect the original extrudate without pre-treatment. Theses efficiencies were: 16% (SE1), 18% (SE2) and 55% (RE).
- Prices. Electricity: 0.104 €/kWh [31]. Methane: 0.04 €/kW [15].

## 3. Results and discussion

### *3.1. Characterization of the untreated extrudates.*

The physicochemical characterization of untreated strawberry and raspberry extrudates is showed in Table 1. SE1 had more than double total phenols than SE2 and RE. This fact may be due to the difference in the particles size caused by the larger sieve used for SE1, in which some phenolic compounds might remain adherent to other

larger molecules or compounds. Based on the initial phenol concentration, SE1 might be a better source for phenol recovery. However, a high concentration of phenols can be inhibitory for the microorganisms of the subsequent anaerobic digestion [32–35].

The three extrudates had a pH value around 3 (Table 1), and this low pH could affect the anaerobic digestion, causing an acidification. This effect was shown with strawberry digestion in the study of Arhoun et al [36]. SE1 and SE2 had 85% moisture, whereas the RE had 75% moisture. This moisture difference may be due to the fact that raspberry has more fibrous than strawberry, which are compounds with lower moisture [2]. It was also observed in three berry extrudates that around 96% of the TS were VS, which correspond to the organic matter susceptible to be biodegraded during the anaerobic digestion, and from which energy could be obtained. Also, in relation to the biodegradable organic matter, the determined $COD_S$/COD ratio, i.e. 33 %, 24% and 10% for SE1, SE2 and RE, respectively, indicated that most of the organic matter was not initially in soluble form, being SE 1 the substrate with the largest amount of soluble organic matter. The soluble matter is usually more easily digestible by the microorganisms during the anaerobic digestion. The C/N ratio values were 23, 24 and 28 for SE1, SE2 and RE, respectively (Table 1). C/N ratios varying between 10 and 30 are considered to be suitable for anaerobic digestion, with an optimum between 15 and 30, the C/N ratios of the three berry extrudates being in this optimal range [37]. SE1 contained a higher concentration of total sugars compared to SE2 and RE (Table 1). As was described for total phenols, this behaviour may be due to the difference in the particle sizes caused by the larger sieve used for SE1. Finally, Table 1 shows that RE was the substrate with the least amount of uronic acids, while SE2, which was pasteurized in the industrial process, contained the greatest quantity of uronic acids. Uronic acids can be released from hemicellulose at high pressure and temperatures [38]. Uronic acids in SE2 were probably generated by oxidation of monosaccharides during the pasteurization process.

*3.2. Hydrothermal pre-treatment of extrudates and phenol extraction.*

Hydrothermal pre-treatment of SE1, SE2 and RE allowed the separation by centrifugation of two phases, i.e. SP (Solid Phase) and LP (Liquid Phase). Extraction of phenols from LP was carried out by an adsorption-desorption column, resulting in a DLP (De-phenolized Liquid Phase) and a phenolic extract.

*3.2.1. Effect of the hydrothermal pre-treatment on extrudates composition.*

Table 2 shows the physicochemical characteristics of the pre-treated extrudates and each obtained phase. pH values in all cases but for DLP from SE2 increased with respect to untreated berry extrudates after hydrothermal pre-treatment, pH in the case of DLP from SE2 was similar to SE2. Moisture in SP from SE1 and SE2 was not modified with respect to untreated berry extrudates, while in the case of SP from RE the moisture decreased by approximately 20% with respect to untreated berry extrudate. The largest amount of organic matter, expressed as VS, was retained in the SP for all extrudates, i.e. 57%, 66% and 71% in SE1, SE2 and RE, respectively (Table 2). Minor losses of VS during the hydrothermal pre-treatment occurred, i.e. 9%, 10% and 15% with respect to untreated SE1, SE2 and RE, respectively. COD values confirmed that the largest amount of organic matter was retained in SP, except for SE1 where the largest amount of COD was retained in LP.

It could be observed that after hydrothermal pre-treatment most of the total sugars per kg of extrudate were mainly transferred to LP for all cases (Fig. 1). This was expected as most of sugars are soluble in water [13]. After the hydrothermal pre-treatment, total sugars markedly increased in SE2 and RE. By contrast, total sugars did not increase in SE1. During the extraction of phenolic compounds by the adsorption-desorption column, total sugars were not extracted, being concentrated in the DLP. Total sugars contained in DLP could be used for example as a fermentable source for the production of wine or vinegar [39] or as a biodegradable substrate in anaerobic digestion as done in this study. The major sugar present in LP in all cases was glucose, followed by mannose (Table 3). Sugars contained in cellulose and hemicellulose were most likely produced and solubilized during the hydrothermal pre-treatment, as seen in other studies [13,40,41]. As counterpart of the hydrothermal pre-treatment, part of the sugars is known to form Hydroxymethylfurfural (HMF) (Table 3). This compound is known to damage the microbial cells by selectively altering the permeability of the membrane, which causes leakage of the intracellular components and the inactivation of essential enzymatic systems [42,43]. It should be also noted that HMF has recently been identified within natural extracts with high antioxidant properties that can be used to prevent the oxidation of edible oils, enhancing the commercial life up to four times for sunflower oils [13]. HMF was mainly detected in the LP. The maximum concentration of HMF was obtained in the LP of SE2. The extraction by an adsorption-desorption column resulted in the retention of 65%, 65% and 57% of HMF in SE1, SE2 and RE, respectively. This retention should be beneficial of the further anaerobic digestion of the

remaining biomass. Uronic acids are released from hemicellulose at high pressure and temperatures and, therefore, it is an indication of hemicellulose degradation [38]. Fig. 2 shows the uronic acids expressed as g galacturonic acid per kg extrudate. After the application of the hydrothermal pre-treatment, it was observed that uronic acids increased, being the majority retained in LP of SE1 and RE, while in SE2 were retained in SP.

*3.2.2. Effect of the hydrothermal pre-treatment on phenolic compounds extraction.*

After hydrothermal pre-treatment, it was observed that in SE1 and RE the greatest amount of total phenols per kg of berry extrudate was contained in LP compared to SP and DLP, while in SE2 the greatest quantity was retained in SP (Fig. 3). It was observed that LP from RE presented higher total phenols per kg of berry extrudate than untreated RE, indicating that a certain production of phenols by the hydrothermal pre-treatment occurred, this might be caused by the breakdown of achenes in RE, which are known to have a high concentration of phenols inside their structure [44]. The hydrothermal pre-treatment applied to SE1 and RE generated a LP with significantly higher antiradical activity than the untreated extrudates, while when applied to SE2 resulted in a LP with lower antiradical activity than the untreated extrudate (Fig. 4A). Similarly, LP from SE1 and RE showed lower reducing power than untreated extrudates, while LP from SE2 showed higher reducing power than the untreated extrudate.

The extraction of phenols by an adsorption-desorption column resulted in the recovery of a phenol extract that accounted for 876, 392 and 2,402 mg of gallic acid equivalents/kg extrudate in SE1, SE2 and RE, respectively. Therefore, The recovery efficiencies for phenols, expressed as the percentage of phenols recovered respect the total phenol in the pretreated substrate, for SE1, SE2 and RE were 33%, 63% and 82%, respectively. The antioxidant capacity related to the extracted phenols was evaluated through the antiradical activity and reducing power. After the extraction of phenolic compounds, antiradical activity increased in DLP, indicating a lower antioxidant capacity compared to LP (Fig. 4A). Similarly, reducing power in DLP decreased compared to LP (Fig. 4B). The difference between the antiradical activity and the reducing power of LP and DLP in all cases indicate that the extracted phenols have a significant antioxidant capacity.

*3.3. Assessment of digestion stability, methane yield and methane production rate after hydrothermal pre-treatment and subsequent phenolic compounds extraction.*

This section evaluates the digestion stability, methane yield and methane production rate of the remaining biomass after phenol extraction, i.e. the mixture of SP and DLP from each extrudate. All these parameters were compared to the anaerobic digestion of the untreated extrudates. Table 4 shows the analytical characterization of the effluents obtained after the BMP tests of the untreated extrudates and the mixture of SP and DLP from each extrudate. In all cases the pH values were kept within the recommended range for an adequate methanogenic activity, i.e. 7.3-7.8 [45]. The high alkalinity observed in all cases was sufficient to dampen possible pH variations. The values of HMF in all BMP tests were always lower than 80 mg/L (Table 3). These values were in all cases markedly lower than the reported inhibition concentration of HMF for anaerobic digestion process, i.e. 800 mg/L [42].

The maximum methane productions for untreated SE1 and the mixture of SP and DLP from SE1 were 391 ± 55 and 503 ± 20 mL $CH_4$/g VS, respectively (Fig. 5 A, B), which entails an increase of 28.6% when the substrate is thermally pre-treated and the phenolic compounds are extracted. The maximum methane productions for SE2 and the mixture of SP and DLP from SE2 were 324 ± 6 and 386 ± 26 mL $CH_4$/g VS, respectively (Fig. 5 A, B), which entails an increase of 19 % when the substrate is thermally pre-treated, and the phenolic compounds are extracted. The maximum methane productions for RE and the mixture of SP and DLP from RE were 334 ± 15 and 371 ± 0 mL $CH_4$/g VS, respectively (Fig. 5 A, B), which entails an increase of 11% when the substrate is thermally pre-treated, and the phenolic compounds are extracted. Table 5 shows the values of methane production rate, $R_m$, obtained by the transference function model applied to the BMP test of untreated SE1 and the mixture of SP and DLP from SE1 and by the logistic model (Sigmoidal 4 parameters) applied to the BMP test of untreated SE2, RE, mixture of SP and DLP from SE2 and mixture of SP and DLP from RE. The $r^2$ values were higher than 0.98 in all cases (Table 5). Likewise, the low values of the errors and standard errors of estimates also indicated a good fit of the experimental data to the proposed models in all cases tested (Table 5). $R_m$ for the untreated SE1 was 14.3% higher than that obtained for the mixture of SP and DLP from SE1. Opposite to this, $R_m$ for the mixture of SP and DLP from SE2 was 34.1% higher than that obtained for untreated SE2 (Table 5), similar to the $R_m$ for the mixture of SP and DLP from RE, which was 7.8% higher than that obtained for untreated RE.

The sieving difference in the industrial process to obtain the extrudates, 1.5 mm for SE1 and 0.5 mm for SE2 and RE, could have influence on the matter digestibility by

403 the microorganisms, since it was observed that the substrates that produced the greatest
404 amount of methane were those of larger particles (SE1 and the mixture of SP and DLP
405 from SE1, sieve 1.5 mm). Theoretically, the smaller the size of the particles, the greater
406 the degradability of the matter by the microorganisms [46], since, logically,
407 microorganisms can more easily degrade particles of smaller size and more hydrolyzed
408 than particles with a larger size, which they must break down and previously degrade.
409 The result obtained in this study was already described by other authors [47], who
410 pointed out that smallest particles contained a higher concentration of recalcitrant
411 compounds (compounds resistant to biodegradation) than particles with a larger size.
412 *3.4. Economic assessment.*
413 The minimum sales price of phenol extract for a zero net benefit, expressed as
414 €/g gallic acid equivalents, was calculated for each of the studied extrudates,
415 corresponding to cases 2, 4 and 6 of Table 6. The most economically favorable case
416 corresponded to the RE, from 0.203 €/g gallic acid equivalents, thus indicating that the
417 proposed biorefinery approach would be economically feasible. This was 63.5 % lower
418 than in the case of SE1, where the minimum phenol sales price for profitability was
419 0.556 €/g gallic acid equivalents. In the case of SE2, the minimum sales price of the
420 phenols was calculated to be 1.23 €/g gallic acid equivalents, several times higher than
421 the most favorable case of RE. This may be due to the lower initial amount of phenolic
422 compounds present in SE2, compared to the other two cases (Table 1).
423 Although the steam consumed in RE, 2.93 kg steam/kg extrudate, was much
424 higher than in the cases of SE1 and SE2, 1.93 and 0.44 kg steam/kg extrudate,
425 respectively, the extracted phenolic compounds are also much higher in RE, 2.40 g
426 gallic acid equivalents/kg extrudate versus 0.88 g gallic acid equivalents/kg extrudate in
427 SE1 and 0.39 g gallic acid equivalents/kg extrudate in SE2. In addition, the methane
428 yield of RE was higher than in SE1 and SE2 (Table 6). According to the above, the
429 higher steam consumption of the RE case was largely compensated by a higher methane
430 yield and a better phenol extraction.
431 The high methane production and phenol recovery of any of the three
432 substrates, make them viable substrates for the proposed biorefinery approach. So, in
433 the same industrial plant could be treated without the need to make changes in the
434 operational process. Likewise, although pre-treated extrudates generate greater methane
435 production than untreated extrudates, if the extraction would not be possible, either due
436 to a possible lowering of the value of the extracted compounds or to any technical

difficulty that would involve the implementation of the process, just the anaerobic digestion of any of the extrudates without pre-treatment would also give a high methane production and net benefit as seen in cases 1, 3 and 5 of Table 6.

## 4. Conclusions

Hydrothermal pre-treatment enhanced the subsequent extraction of valuable phenolic compounds from the three studied extrudates, i.e. 876, 392 and 2,402 mg of gallic acid equivalents/kg extrudate in SE1, SE2 and RE, respectively. Anaerobic digestion of the remaining biomass after extraction showed high methane production, ranging between 371-503 mL $CH_4$/g VS. The economical assessment showed that the proposed biorefinery approach would offer higher benefits than just anaerobic digestion of the untreated extrudate, although this last option would be economically feasible as well.

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**Table 1.** Physicochemical characterization of untreated strawberry and raspberry extrudates.

| | | Strawberry Extrudate 1 (SE1) | Strawberry Extrudate 2 (SE2) | Raspberry Extrudate (RE) |
|---|---|---|---|---|
| **pH** | | 2.8 ± 0.1 | 3.7 ± 0.1 | 3.0 ± 0.1 |
| **Moisture** | **%** | 85.4 ± 0.7 | 85.5 ± 2.4 | 74.0 ± 1.7 |
| **TS** | **mg/kg** | 146,095 ± 1,210 | 144,680 ± 3,985 | 260,415 ± 5,895 |
| **MS** | **mg/kg** | 5,765 ± 260 | 5,345 ± 520 | 4,645 ± 200 |
| **VS** | **mg/kg** | 140,325 ± 955 | 139,335 ± 4,425 | 255,770 ± 5,925 |
| **COD** | **mg $O_2$/g VS** | 1,210 ± 45 | 1,440 ± 65 | 985 ± 45 |
| **$_S$COD** | **mg $O_2$/g VS** | 400 ± 5 | 340 ± 10 | 100 ± 5 |
| **($_S$COD/COD) ratio** | **%** | 33 | 24 | 10 |
| **C/N ratio** | | 22.6 | 24.2 | 27.8 |
| **Total sugars** | **mg Glucose/g VS** | 267.0 ± 10.9 | 14.5 ± 0.1 | 9.1 ± 0.4 |
| **Total phenols** | **mg Gallic Acid/g VS** | 40.2 ± 1.5 | 15.7 ± 0.7 | 16.9 ± 0.4 |
| **Uronic acids** | **mg Galacturonic Acid/g VS** | 0.0267 ± 0.0004 | 0.0451 ± 0.0017 | 0.0048 ± 0.0002 |

**Table 2.** Physicochemical characterization of untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenolic compounds.

| Phases | pH | TS (mg/kg Ext.) | MS (mg/kg Ext.) | VS (mg/kg Ext.) | %Moist. | COD (mg $O_2$/kg Ext.) | sCOD (mg $O_2$/kg Ext.) |
|---|---|---|---|---|---|---|---|
| **SE 1** | 2.8 ± 0.1 | 146,095 ± 1,210 | 5,765 ± 260 | 140,325 ± 955 | 85.4 ± 0.7 | 169,900 ± 6,230 | 56,030 ± 315 |
| **SP** | 3.0 ± 0.1 | 83,830 ± 488 | 2,410 ± 135 | 81,420 ± 510 | 81.9 ± 0.5 | 111,605 ± 4,755 | 9,475 ± 370 |
| **LP** | 3.3 ± 0.1 | 49,655 ± 1,150 | 3,145 ± 180 | 46,510 ± 1,325 | 98.0 ± 2.3 | 59,800 ± 1,555 | 50,370 ± 435 |
| **DLP** | 3.3 ± 0.1 | 40,730 ± 625 | 2,900 ± 125 | 37,825 ± 555 | 98.3 ± 1.5 | 50,200 ± 785 | 42,055 ± 615 |
| **SE 2** | 3.7 ± 0.1 | 144,680 ± 4,605 | 5,345 ± 600 | 139,335 ± 5,105 | 85.5 ± 2.7 | 200,365 ± 7,730 | 47,235 ± 390 |
| **SP** | 4.4 ± 0.1 | 95,695 ± 3,940 | 4,550 ± 2,025 | 91,145 ±3,945 | 82.1 ± 3.4 | 142,305 ± 2,600 | 16,595 ± 350 |
| **LP** | 3.9 ± 0.1 | 34,295 ± 365 | 1,685 ± 825 | 32,610 ± 775 | 96.2 ± 1.0 | 43,815 ± 900 | 44,450 ± 1,510 |
| **DLP** | 3.6 ± 0.1 | 31,695 ± 465 | 2,275 ± 580 | 29,415 ± 665 | 96.5 ± 1.4 | 32,860 ± 125 | 39,100 ± 1,280 |
| **RE** | 3.0 ± 0.1 | 260,410 ± 5,895 | 4,645 ± 200 | 255,770 ± 5,925 | 74.0 ± 1.7 | 252,225 ± 9,365 | 26,205 ± 665 |
| **SP** | 3.7 ± 0.1 | 184,105 ± 7,390 | 1,585 ± 260 | 182,520 ± 7,155 | 50.9 ± 2.0 | 232,420 ± 8,725 | 2,635 ± 65 |
| **LP** | 3.9 ± 0.1 | 36,340 ± 1,075 | 3,080 ± 660 | 33,265 ± 435 | 99.0 ± 2.9 | 45,750 ± 485 | 43,135 ± 1,235 |
| **DLP** | 4.0 ± 0.1 | 29,845 ± 665 | 2,660 ± 485 | 27,190 ± 815 | 99.2 ± 2.2 | 34,540 ± 545 | 40,440 ± 165 |

**Table 3**. Glycoside composition (g sugar/kg extrudate), total monosaccharides (Total MS) and total oligosaccharides (Total OS) and hydroxymethylfurfural (HMF, mg/kg extrudate) of the strawberry and raspberry extrudates (SE 1; SE 2; RE), solid phase (SP), liquid phase (LP) and de-phenolized liquid phase (DLP). The analysed sugars are **Rhamnose (Rha), Fucose (Fuc), Arabinose (Ara), Xylose (Xyl), Mannose (Man), Galactose (Gal) and Glucose (Glu).** n.d.: non-detected.

| Phases | Rha | Fuc | Ara | Xyl | Man | Gal | Glu | Total OS | Total MS | HMF |
|---|---|---|---|---|---|---|---|---|---|---|
| **SE1** | n.d | n.d | n.d | 2.40 ± 0.07 | 9.48 ± 1.17 | n.d | 25.30 ± 2.82 | 2.47 ± 2.06 | 37.18 ± 3.06 | n.d |
| **SP** | n.d | n.d | n.d | 0.33 ± 0.02 | 1.57 ± 0.01 | n.d | 3.22 ± 0.70 | 0.45 ± 0.12 | 5.12 ± 0.70 | 611 ± 10 |
| **LP** | n.d | n.d | 0.31 ± 0.05 | 0.72 ± 0.09 | 7.20 ± 0.38 | 0.14± 0.03 | 22.28 ± 2.67 | 1.44 ± 0.33 | 30.65 ± 2.70 | 2,411 ± 10 |
| **DLP** | n.d | n.d | 0.21 ± 0.02 | 0.58 ± 0.07 | 5.88 ± 0.28 | 0.21± 0.18 | 18.20 ± 0.03 | 1.06 ± 0.31 | 25.08 ± 0.34 | 838 ± 10 |
| **SE2** | 0.11 ± 0.01 | n.d | 0.34 ± 0.01 | 0.80 ± 0.03 | 3.68 ± 0.07 | n.d | 0.22 ± 0.01 | 5.76 ± 0.47 | 5.14 ± 0.08 | n.d |
| **SP** | 0.07 ± 0.00 | n.d | 0.26 ± 0.00 | 0.36 ± 0.02 | 1.04 ± 0.12 | n.d | 0.20 ± 0.05 | 1.97 ± 0.13 | 1.93 ± 0.13 | n.d |
| **LP** | n.d | n.d | 0.14 ± 0.01 | 0.38 ± 0.04 | 3.89 ± 0.19 | 0.14 ± 0.12 | 12.04 ± 0.02 | 2.08 ± 0.14 | 16.6 ± 0.23 | 6,359 ± 10 |
| **DLP** | 0.06 ± 0.00 | n.d | 0.28 ± 0.01 | 0.50 ± 0.01 | 4.37 ± 0.18 | n.d | 8.09 ± 0.26 | 1.23 ± 0.20 | 13.35 ± 0.32 | 2,195 ± 10 |
| **RE** | 0.08 ± 0.00 | n.d | 0.31 ± 0.00 | 0.34 ± 0.01 | 0.28 ± 0.02 | 0.09 ± 0.00 | 0.41 ± 0.02 | 1.28 ± 0.10 | 1.52 ± 0.05 | n.d |
| **SP** | 0.02 ± 0.00 | n.d | 0.08 ± 0.00 | 0.06 ± 0.00 | 0.12 ± 0.03 | 0.01 ± 0.00 | 0.12 ± 0.01 | 0.08 ± 0.06 | 0.41 ± 0.03 | 315 ± 10 |
| **LP** | 0.11 ± 0.00 | n.d | 0.80 ± 0.00 | 0.42 ± 0.00 | 3.43 ± 0.27 | 0.14 ± 0.01 | 10.85 ± 0.01 | 1.95 ± 0.28 | 15.79 ± 0.27 | 3,269 ± 10 |
| **DLP** | 0.09 ± 0.00 | n.d | 0.68 ± 0.00 | 0.36 ± 0.01 | 2.47 ± 0.13 | 0.10 ± 0.01 | 8.17 ± 0.63 | 1.83 ± 0.18 | 11.88 ± 0.64 | 1,418 ± 10 |

**Table 4.** Physicochemical characterization of the effluents of the anaerobic digestion process at the end of the BMP tests.

| | | SE1 | SE1 Mixture (SP and DLP) | SE2 | SE2 Mixture (SP and DLP) | RE | RE Mixture (SP and DLP) |
|---|---|---|---|---|---|---|---|
| **pH** | | 7.4 ± 0.1 | 7.4 ± 0.1 | 7.7 ± 0.1 | 7.8 ± 0.1 | 7.6 ± 0.1 | 7.8 ± 0.1 |
| **Alkalinity** | **mg $CaCO_3$/L** | 6,979 ± 390 | 7,526 ± 391 | 7,468 ± 97 | 7,477 ± 137 | 6,072 ± 88 | 6,857 ± 28 |
| **TS** | **mg/kg** | 22,960 ± 625 | 23,760 ± 690 | 24,090 ± 1,140 | 24,690 ± 315 | 14,750 ± 560 | 18,015 ± 510 |
| **MS** | **mg/kg** | 8,095 ± 160 | 8,040 ± 445 | 10,420 ± 940 | 11,220 ± 290 | 6,445 ± 900 | 9,775 ± 440 |
| **VS** | **mg/kg** | 14,865 ± 655 | 15,720 ± 600 | 12,940 ± 295 | 13,470 ± 345 | 7,940 ± 490 | 8,460 ± 375 |
| **$COD_S$** | **mg $O_2$/L** | 1,775 ± 170 | 1,055 ± 190 | 700 ± 95 | 935 ± 45 | 525 ± 10 | 925 ± 180 |
| **Total Phenols** | **mg Gallic acid/g VS** | 42 ± 2 | 57 ± 3 | 96 ± 2 | 108 ± 6 | 38 ± 3 | 63 ± 2 |
| **Theoretical Production** | **mL $CH_4$/g VS** | 463 | 518 | 549 | 552 | 377 | 486 |
| **Experimental production** | **mL $CH_4$/g VS** | 391 ± 55 | 503 ± 20 | 324 ± 6 | 386 ± 26 | 334 ± 15 | 371 ± 0 |

**Table 5.** Values of the parameters obtained from the **Transference Function model and Logistic model (Sigmoidal 4 parameters)** for the different substrates studied.

| | | SE1 | SE1 Mixture (SP and DLP) | SE2 | SE2 Mixture (SP and DLP) | RE | RE Mixture (SP and DLP) |
|---|---|---|---|---|---|---|---|
| $B_m$ | **mL $CH_4$/g VS** | 376 ± 3 | 490 ± 5 | - | - | - | - |
| $P$ | **mL $CH_4$/g VS** | - | - | 256 ± 4 | 311 ± 2 | 329 ± 3 | 349 ± 3 |
| $B_0$ | **mL $CH_4$/g VS** | - | - | 70 ± 3 | 75 ± 1 | 10 ± 1 | 21 ± 2 |
| $R_m$ | **mL $CH_4$/ (g VS · d)** | 144 ± 5 | 126 ± 5 | 41 ± 1 | 55 ± 2 | 38 ± 2 | 41 ± 1 |
| $\lambda$ | **d** | $2.2 \cdot 10^{-9}$ | $3.5 \cdot 10^{-9}$ | 9.34 ± 0.06 | 9.44 ± 0.02 | 12.01 ± 0.06 | 9.78 ± 0.07 |
| $r^2$ | | 0.9884 | 0.9849 | 0.9981 | 0.9997 | 0.9983 | 0.9970 |
| **Error*** | **%** | 3.8 | 2.5 | 1.8 | 3.1 | 1.2 | 5.5 |
| **S.E.E**** | | 11.27 | 17.57 | 4.62 | 2.15 | 5.28 | 7.78 |

*Error $((B_{m\ experimental} - B_{m\ model}) / B_{m\ experimental}) \cdot 100$

**S.E.E.: Standard error of estimate

**Table 6.** Net benefits for the different cases.

| **Case** | | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|---|
| **Waste** | | **SE1** | **SE1** | **SE2** | **SE2** | **RE** | **RE** |
| **Pre-treatment** | | **NO** | **YES** | **NO** | **YES** | **NO** | **YES** |
| **Mass balance (per kg of extrudate)** | | | | | | | |
| Methane yield (L) | | 46.64 | 50.98 | 38.37 | 39.56 | 72.61 | 66.13 |
| Initial phenols compounds (g gallic acid) | | 5.64 | 5.64 | 2.19 | 2.19 | 4.34 | 4.34 |
| Extracted phenols compounds (g gallic acid) | | 0.00 | 0.88 | 0.00 | 0.39 | 0.00 | 2.40 |
| Consumed steam (kg) | | 0.00 | 1.93 | 0.00 | 0.44 | 0.00 | 2.93 |
| **Benefits (€/kg extrudate)** [*1] | | | | | | | |
| Methane avoided | | 0.010 | 0.0 | 0.008 | 0.006 | 0.015 | 0.0 |
| Electricity | | 0.015 | 0.016 | 0.012 | 0.012 | 0.022 | 0.020 |
| | ***Total*** | ***0.025*** | ***0.016*** | ***0.020*** | ***0.018*** | ***0.037*** | ***0.020*** |
| **Costs (€/kg extrudate)** | | | | | | | |
| Methane Consumed | | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.007 |
| Phenols Extraction | | 0.000 | 0.500 | 0.000 | 0.500 | 0.000 | 0.500 |
| | ***Total*** | ***0.000*** | ***0.503*** | ***0.000*** | ***0.500*** | ***0.000*** | ***0.507*** |
| **Net Benefit (€/kg extrudate)** | ***Total*** | ***0.025*** | ***- 0.487*** | ***0.020*** | ***-0.482*** | ***0.037*** | ***-0.487*** |
| ***Minimum prices for phenols extract (for positive Net Benefit)* (€/g gallic acid)** | | ***-*** | ***0.556*** | ***-*** | ***1.23*** | ***-*** | ***0.203*** |

*[1] Excluding incoming from phenols extract sales

**Figure Captions**

**Figure 1.** Total sugars expressed as milligrams of glucose equivalents per kilogram of extrudate for untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenolic compounds.

**Figure 2.** Uronic acids expressed as grams of galacturonic acid per kg of extrudate for untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenolic compounds.

**Figure 3.** Total phenols expressed as milligrams of gallic acid equivalents per kg of extrudate for untreated strawberry and raspberry extrudates and different fractions obtained after the hydrothermal pre-treatments and extraction of phenolic compounds.

**Figure 4.** Antioxidant capacity determined by antiradical activity (DPPH) **(A)** and reducing power **(B)** methods of each LP obtained after hydrothermal pre-treatments and of each DLP obtained after extraction of phenolic compounds.

**Figure 5.** Methane production (mL $CH_4$/g VS) of untreated extrudates **(A)** and of pre-treated extrudates **(B).**

**Figure 1.**

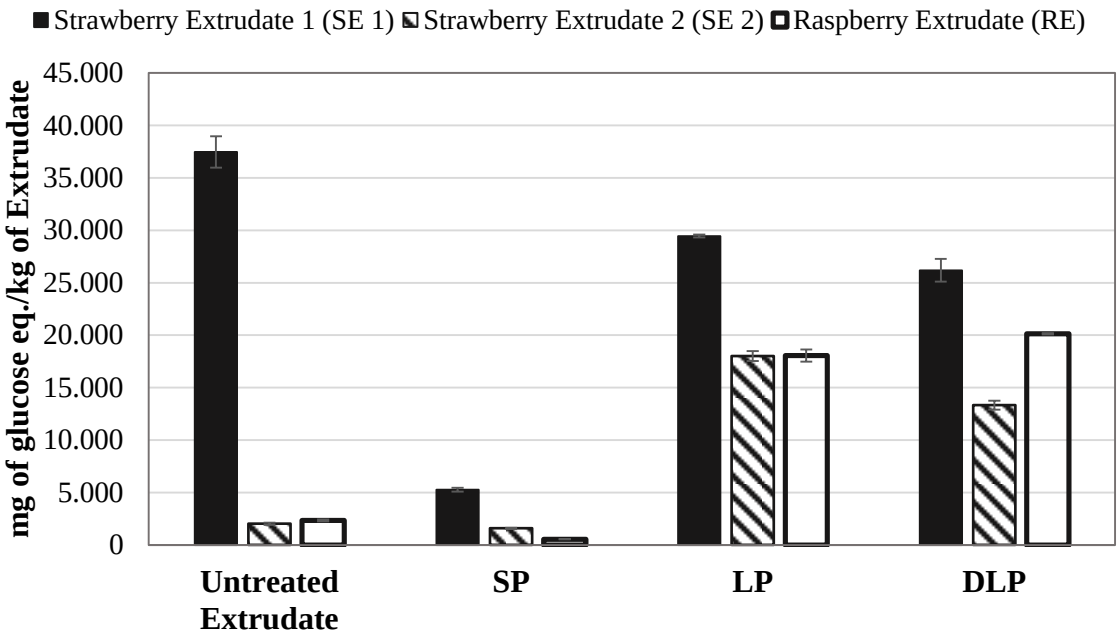

Strawberry Extrudate 1 (SE 1)
Strawberry Extrudate 2 (SE 2)
Raspberry Extrudate (RE)
mg of glucose eq./kg of Extrudate
45.000
40.000
35.000
30.000
25.000
20.000
15.000
10.000
5.000
0
Untreated Extrudate
SP
LP
DLP

**Figure 2.**

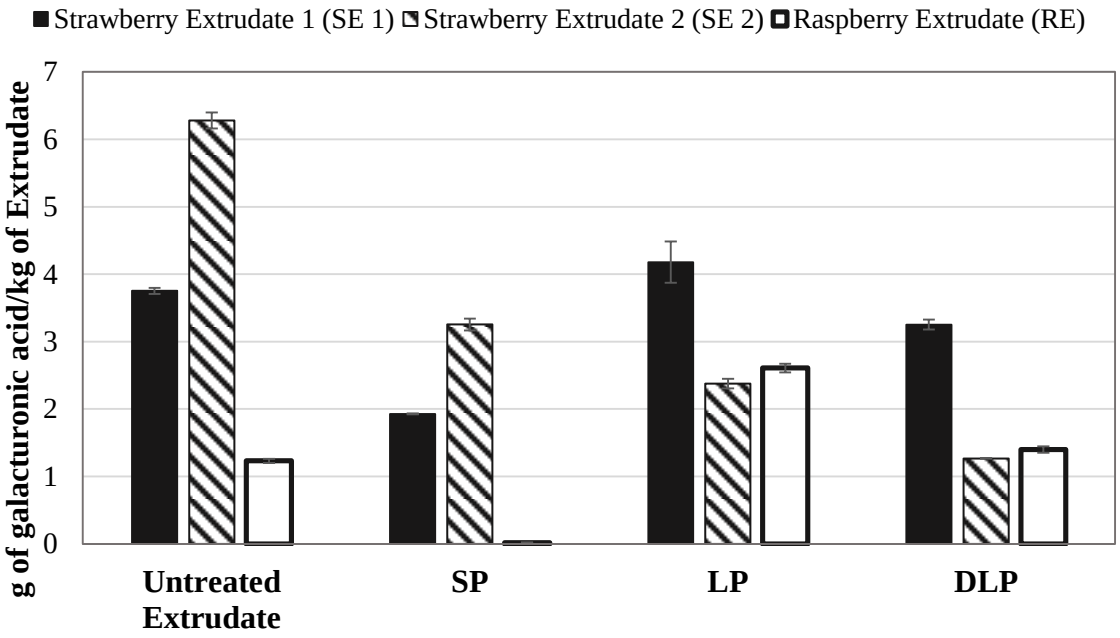

Strawberry Extrudate 1 (SE 1)
Strawberry Extrudate 2 (SE 2)
Raspberry Extrudate (RE)
g of galacturonic acid/kg of Extrudate
0
1
2
3
4
5
6
7
Untreated Extrudate
SP
LP
DLP

**Figure 3.**

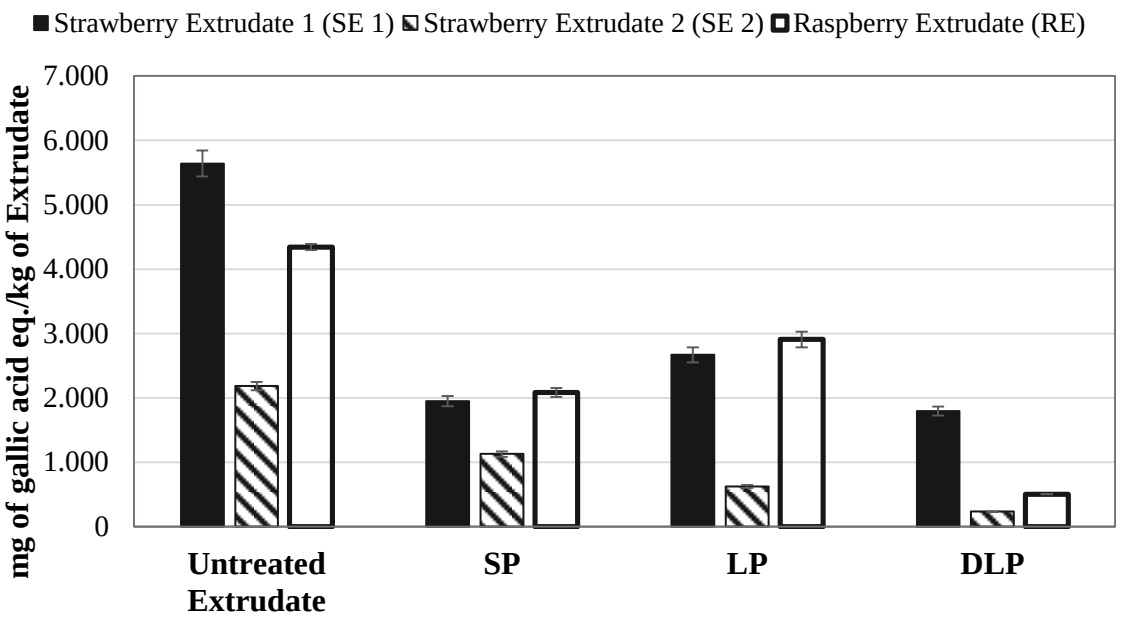
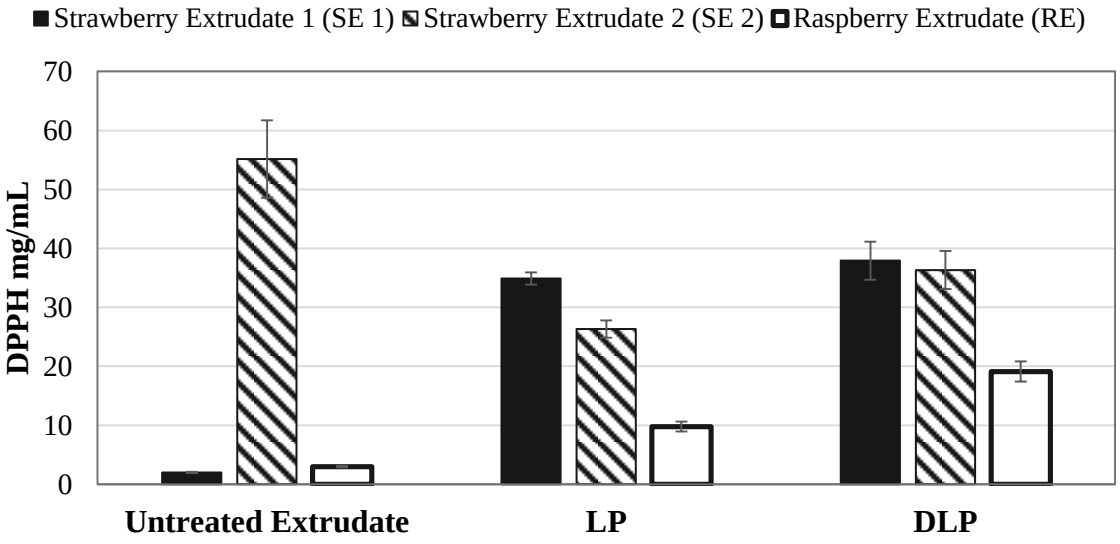

Strawberry Extrudate 1 (SE 1)
Strawberry Extrudate 2 (SE 2)
Raspberry Extrudate (RE)
mg of gallic acid eq./kg of Extrudate
7.000
6.000
5.000
4.000
3.000
2.000
1.000
0
Untreated Extrudate
SP
LP
DLP

**Figure 4.**

**A)**


Strawberry Extrudate 1 (SE 1)
Strawberry Extrudate 2 (SE 2)
Raspberry Extrudate (RE)
DPPH mg/mL
70
60
50
40
30
20
10
0
Untreated Extrudate
LP
DLP

**B)**

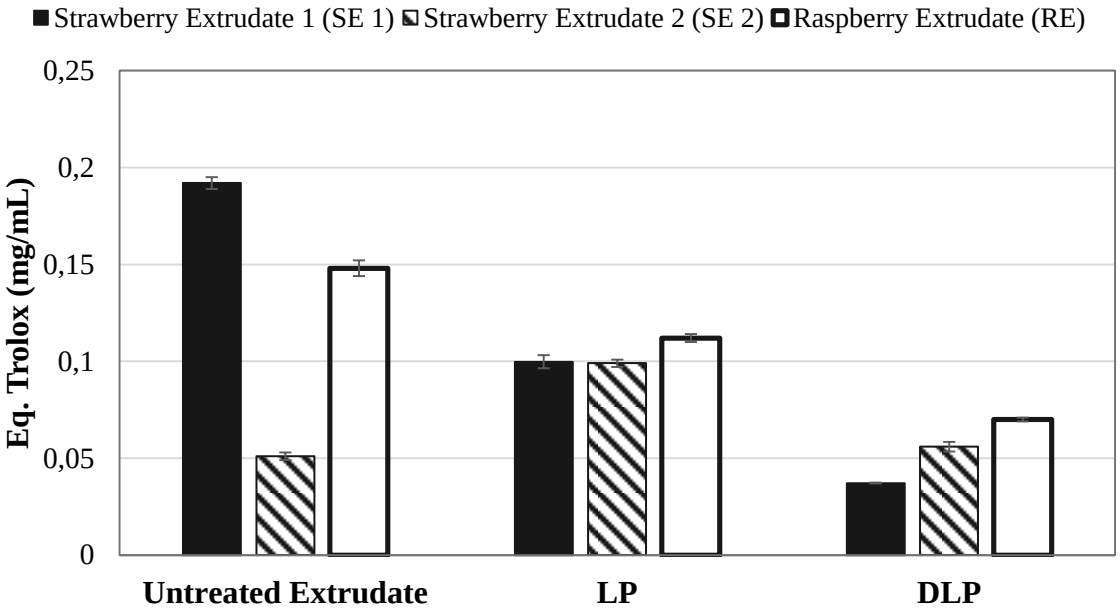
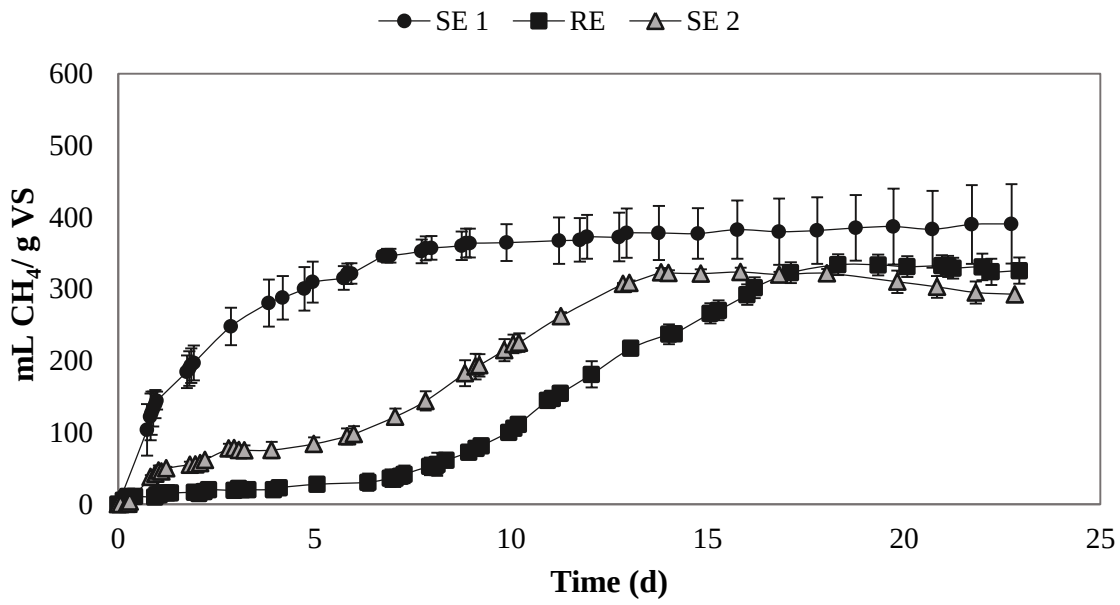
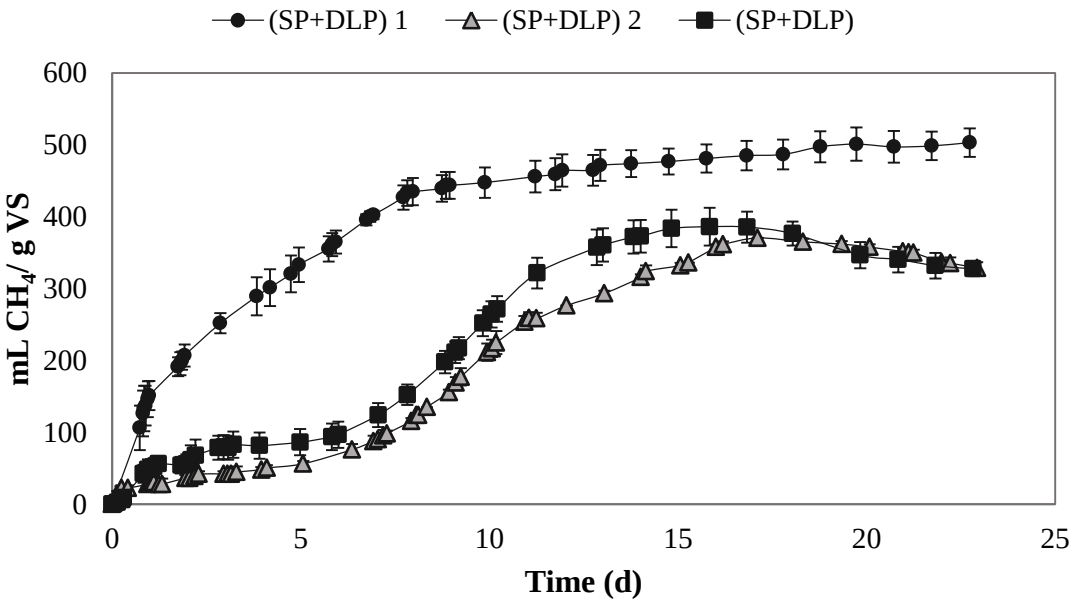

Strawberry Extrudate 1 (SE 1)
Strawberry Extrudate 2 (SE 2)
Raspberry Extrudate (RE)
Eq. Trolox (mg/mL)
0,25
0,2
0,15
0,1
0,05
0
Untreated Extrudate
LP
DLP

**Figure 5.**

**A)**


SE 1
RE
SE 2
600
500
400
300
200
100
0
mL CH4/ g VS
0
5
10
15
20
25
Time (d)

**B)**


(SP+DLP) 1
(SP+DLP) 2
(SP+DLP)
600
500
400
300
200
100
0
mL CH4/ g VS
0
5
10
15
20
25
Time (d)